AN ATTEMPT TO ISOLATE AND PURIFY DEVELOPMENT-SPECIFIC SURFACE PROTEINS FROM DEVELOPING CELLS OF *MYXOCOCCUS XANTHUS*

by

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ABSTRACT

Surface proteins of vegetative and developing (24 hr) cells of Myxococcus xanthus were labeled with ^{125}I and analyzed by one-dimensional electrophoresis and autoradiography. A comparison of ^{125}I-labeled vegetative and developmental cell extracts indicated that the cell surface was altered during development. Four surface proteins with approximate molecular weights of 84,000, 67,000, 47,000, and 31,000 were accessible to ^{125}I-labeling and exclusively associated with the developing cells. An attempt to isolate and purify these iodinated development-specific surface proteins was undertaken using column chromatography. Each purification step was monitored by one-dimensional electrophoresis and autoradiography. The non-ionic detergent Triton X-100 was used to extract the iodinated proteins from the cell envelope. Triton X-100 could not completely solubilize the iodinated proteins, but did solubilize many of the unlabeled proteins. The majority of the iodinated proteins were Triton X-100 insoluble. However, faintly iodinated proteins which appeared to be Triton X-100 soluble were detected, but the results from chromatofocusing indicated that the amount was not suitable for purification. Chromatofocusing of the Triton X-100 insoluble fraction was moderately successful, with only partial separation of the iodinated proteins from the unlabeled proteins. Additional purification by DEAE-Sephacel ion-exchange chromatography suggested that individual iodinated proteins were being separated, but proof of this was not available due to a lack of detectable protein. As a result, the complete purification of the iodinated development-specific surface proteins from the Triton X-100 insoluble fraction was not achieved. The iodinated proteins were also

insoluble in the detergents CHAPS and SDS. Only after being boiled in the presence of SDS for a few minutes did the iodinated proteins become completely solubilized. The tight association of the iodinated proteins with the insoluble components of the cell envelope suggests that strong interactions exist between these surface proteins and the cell envelope in developing cells of M. xanthus.

To my parents

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TABLE OF CONTENTS

Table of Contents (continued)

LIST OF FIGURES

List of Figures (continued)

LIST OF ABBREVIATIONS

BSA	bovine serum albumin
CHAPS	3-[(3-cholamidopropyl)dimethyl-ammonio]-1-propanesulfonate
CPM	counts per minute
DEAE-Sephacel	diethylaminoethyl-Sephacel
DNase	deoxyribonuclease I
EDTA	ethylenediamine tetraacetate
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
PMSF	phenylmethylsulfonylfluoride
SDS	sodium dodecyl sulfate
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TEMED	N,N,N',N'-tetramethylethylenediamine
Tris-HCl	tris(hydroxymethyl)-aminomethane-hydrochloride

An Attempt to Isolate and Purify Development-Specific Surface Proteins from Developing Cells of *Myxococcus xanthus*

INTRODUCTION

INTRODUCTION

Myxococcus xanthus is one of many gram-negative prokaryotes, collectively known as the myxobacteria, that exhibit a complex cycle of vegetative and developmental growth. Under nutritionally limiting conditions on solid surfaces, a developmental program consisting of cellular aggregation and sporulation is initiated. During aggregation, large numbers of cells move toward aggregation centers to produce multicellular structures known as fruiting bodies. During sporulation, a portion of the vegetative cells within the fruiting bodies convert to resting cells known as myxospores. In the aggregation phase, intercellular cooperation in the form of complex cell-cell interactions take place. In order for this to occur, some means of cell-cell recognition must exist. In the eukaryotic slime molds, which display a similar but more complex life cycle, studies have shown that changes in the cell surface components are involved in some aspects of cell development and interaction. Many changes in the synthesis of soluble and membrane proteins have been observed in developing cells of M. xanthus, including the production of new development-specific proteins. These proteins may act as signals and/or receptors in the cellular interactions that accompany development. One such protein, known as myxobacterial hemagglutinin, displays lectin-like activity, and is located in the periplasmic space and on the outer surface of the cell. Its precise role is still undefined, but it may be involved in end-to-end cellular interactions during aggregation. Major alterations in the surface structure of developing cells have also been observed. Certain proteins become exposed to the outer cell surface during development. It is quite likely that new cell surface

proteins are involved in the cell-cell interactions that occur during cellular aggregation. In this investigation, an attempt was made to isolate and purify one of these development-specific surface proteins.

HISTORICAL

HISTORICAL

In 1892, while in search of fungal specimens, Roland Thaxter noticed an unusual form of life which he later described in the Botanical Gazette as "...bright orange-colored growth occurring upon decaying wood, fungi and similar substances..." (Thaxter, 1892). This marked the discovery and first description of the complex group of organisms commonly known as the myxobacteria. These gram-negative, rod-shaped bacteria are found in soils in various regions of the world. They often develop on decaying vegetation, animal dung, and the bark of both living and dead trees (Dworkin, 1966; McCurdy, 1974; Reichenbach and Dworkin, 1981). Myxobacteria are nonphotosynthetic and strictly aerobic chemo-organotrophs which utilize a respiratory metabolism, rather than fermentative (McCurdy, 1974). They are also mesophilic, tending to grow within a temperature range of between 6 and 38^{o}C, with optimal growth at 34^{o}C (Reichenbach, 1984).

All myxobacteria belong to the single order Myxobacterales. According to McCurdy (1974), the order is subdivided into four families: Myxococcaceae, Archangiaceae, Cystobacteraceae, and Polyangiaceae. Recently, however, a revised taxonomic scheme was proposed by Reichenbach and Dworkin (1981). In the revised scheme, the order is subdivided into two suborders: Cystobacterineae and Sorangianeae. Each suborder is further subdivided into families. Cystobacterineae consists of the three families, Myxococcaceae, Archangiaceae, and Cystobacteraceae, whereas Sorangianeae consists of the one family, Sorangiaceae.

Although myxobacteria are considered typical gram-negative prokaryotes, they are distinguished from the majority of other gram-negative organisms on the combined basis of three unique characteristics: (i) a developmental life cycle, (ii) gliding motility, and (iii) bacteriolytic activity (Dworkin, 1966; Kaiser et al., 1979).

Myxobacteria are unique among other bacteria in that they exhibit a vegetative and developmental cycle of growth that closely resembles that of the eukaryotic slime molds. Two levels of morphogenesis occur in myxobacterial development which is induced by nutrient depletion. The first, called cooperative morphogenesis, involves the aggregation of thousands of individual vegetative cells towards a central location by gliding motility resulting in the formation of a multicellular, macroscopic structure known as the fruiting body. The second, called cellular morphogenesis, involves a morphogenetic change within the fruiting body which results in the conversion of the rod-shaped vegetative cells to spherical or oval resting cells known as myxospores (Reichenbach, 1984). Based on the study of mutants, it has been suggested that the developmental program consists of an aggregation pathway and a sporulation pathway (Morrison and Zusman, 1979).

Myxobacteria lack flagella and, as a result, are unable to swim in liquid culture. However, they exhibit a slow but steady gliding movement on moist solid surfaces (McCurdy, 1974; Zusman, 1980) that is always associated with the production of a polysaccharide-containing slime material (Reichenbach, 1984). The gliding movement also tends to follow the stress lines of the agar surface on which the myxobacteria are located (Stanier, 1942). The actual mechanism of

gliding motility is still unknown, although several theories have been proposed and reviewed as possible explanations for this unique phenomenon (Kaiser et al., 1979; Zusman, 1980).

Movement of myxococcal cells appears to be controlled by two multigene systems. The first, known as gene-system "A" (for adventurous), is chiefly responsible for the control of single cell movement. The second, known as gene-system "S" (for social), is responsible for the control of movement of groups of cells. Motility by gene-system "A" requires the expression of 23 loci, whereas motility by gene-system "S" requires the expression of more than 10 loci. Piliation and cell-cell contact seem to be associated with S motility, but not with A motility. It has been demonstrated that the majority of S motility mutants were unable to form fruiting bodies, whereas A motility mutants were unaffected in their ability to produce fruiting bodies. It was suggested that developmental aggregation was dependent primarily on the S motility system, whereas both the A and S motility systems appear to be involved in food gathering during vegetative growth (Kaiser et al., 1979; Zusman, 1980; Dworkin and Kaiser, 1985).

While in their natural environment, myxobacteria subsist on insoluble macromolecules and other microorganisms through the production of a variety of hydrolytic exoenzymes such as proteases, nucleases, lipases, and several cell wall lytic enzymes. The production of these enzymes permits the lysis of both prokaryotic and eukaryotic organisms, followed by the degradation and utilization of the released contents (McCurdy, 1974; Kaiser et al., 1979; Reichenbach and Dworkin, 1981). Many of the enzymes have been

purified and analyzed for substrate-specificities (Kaiser et al., 1979). Although the majority of myxobacteria display bacteriolytic activity, there are some notable exceptions. For example, the species Polyangium cellulosum is cellulolytic and thus depends on the decomposition of cellulose, rather than on the degradative products of other microorganisms (Kaiser et al., 1979; Reichenbach and Dworkin, 1981).

Myxobacteria are social or communal organisms which tend to be closely associated with one another during their entire life cycle. Vegetative cells have the capacity for independent growth, but usually grow on solid surfaces in dense populations known as swarms. Individual cells may move away from the edge of the swarm, but only for a brief period. Cells quickly return to the swarm where the mass of cells moves as a coherent unit. Within each swarm, the cells are able to increase the local concentration of digestive enzymes. This facilitates an increase in the rate of nutrient release and more rapid growth. Thus, the swarm ensures that the myxobacteria possess the critical cell mass required for the attack and digestion of other microorganisms and/or the degradation of complex organic matter (White, 1975; Kaiser et al., 1979; Zusman, 1980).

Myxococcus xanthus (Myxococcaceae) is one of the most intensively studied species of myxobacteria. The vegetative cells are long, slender, flexible rods (0.5-1.0 by 4-10 μm) with tapering or rounded ends (McCurdy, 1974). The genome size was estimated to be approximately 5,700 kilobases (Yee and Inouye, 1982). Some strains can be grown in a dispersed state in liquid medium of

either defined or complex mixture. Cells divide by binary transverse fission and grow with a generation time of approximately 200 to 250 minutes while in complex media (Wireman and Dworkin, 1975).

Several groups have investigated the nutritional requirements for the vegetative growth of M. xanthus. In the study by Rosenberg et al. (1977), vegetative cells of M. xanthus were cultivated on media containing purified casein. In addition, Dworkin (1962) had earlier reported the growth of M. xanthus on media containing a defined mixture of 17 amino acids and salts. Using a liquid medium containing pyruvate and aspartate as sources of carbon and energy, supplemented with leucine, isoleucine, valine, and methionine or vitamin B_{12}, Bretscher and Kaiser (1978) demonstrated the slow but continuous growth of M. xanthus, and also established a defined minimal medium capable of supporting vegetative growth. For over twenty years, however, the standard medium for growing M. xanthus vegetatively has been a complex medium consisting of casitone (Difco, hydrolyzed casein), supplemented with potassium phosphate buffer and magnesium sulfate (Dworkin, 1962).

Amino acids serve as excellent sources of carbon and energy in M. xanthus. Some amino acids, such as leucine, valine, and methionine are essential for growth since their absence results in the cessation of vegetative growth. Other amino acids, such as aspartate, serve mainly as sources of carbon, nitrogen, and energy, and thus can be replaced by an alternative source without severely affecting vegetative growth (Bretscher and Kaiser, 1978). Watson and Dworkin (1968) found that a number of the tricarboxylic acid (TCA) cycle intermediates, such as fumarate, oxalacetate, and

succinate were oxidized to carbon dioxide by M. xanthus. Bretscher and Kaiser (1978) reported that these TCA cycle intermediates, along with acetate and pyruvate, could be utilized as possible sources of carbon and energy. However, pyruvate appeared to be the most effective in terms of providing carbon and energy to the cells. Several studies have also suggested that carbohydrates (hexoses and pentoses) do not act as sources of carbon or energy in M. xanthus (Watson and Dworkin, 1968; Bretscher and Kaiser, 1978).

As in all gram-negative organisms, the cell wall in vegetative cells of M. xanthus is trilaminar in appearance; consisting of the peptidoglycan layer, and the outer and cytoplasmic (inner) membranes (White, 1984). White et al. (1968) reported that the peptidoglycan in M. xanthus is similar in composition to that observed in other gram-negative bacteria, however, some differences in quantity and structure exist. White et al. (1968) found that only 0.6% of the dry weight of the vegetative cell consisted of peptidoglycan; less than the content determined for cells of E. coli. In addition, it was also found that the peptidoglycan layer existed as a series of patches held together by a non-peptidoglycan interpatch material sensitive to trypsin and sodium dodecyl sulfate (SDS), rather than as a discrete continuous sheet as observed in most eubacteria. These differences may help to explain the flexibility of the vegetative cells and their capacity for direct conversion to myxospores (Kaiser et al., 1979).

Orndorff and Dworkin (1980) found that the outer membrane of M. xanthus exhibited a lower bouyant density than the cytoplasmic membrane; a phenomenon which is reversed in other gram-negative

organisms such as E. coli. It was suggested that this condition is partially due to the unusually high phospholipid content of the outer membrane. The presence of lipopolysaccharides in the outer membrane of M. xanthus has been established (Sutherland and Smith, 1973). Analysis of the sugar components of the lipopolysaccharides revealed an overall composition similar to that of most gram-negative bacteria, although heptose, a monosaccharide commonly found in bacterial lipopolysaccharides, was absent.

Extending from the poles of vegetative cells of M. xanthus are slender threadlike projections known as pili or fimbriae. Two studies reported that these appendages were glycoprotein in composition (Dobson and McCurdy, 1979; Kaiser, 1979). Studies have also suggested that pili may be involved in cell-cell adhesion and gliding motility (White, 1984).

Vegetative cells of myxobacteria are also surrounded by an extracellular slime layer. Gnosspelius (1978) found that the slime layer of M. virescens consisted of a protein-polysaccharide-lipid complex. The precise function of the slime layer is still unknown, although several possible roles have been suggested (White, 1984).

In order for fruiting body formation to occur in M. xanthus, three basic conditions must exist: (1) nutritional deprivation, (2) a solid surface, and (3) a high cell density. Fruiting bodies are often brightly colored and their macroscopic appearance ranges from simple mounds of soft or hardened slime and cells as in the genus Myxococcus to elaborately stalked-structures of characteristic shape and size as in the genus Stigmatella (Dworkin, 1966; McCurdy, 1974; Kaiser et al., 1979; Reichenbach and Dworkin, 1981; Dworkin

and Kaiser, 1985). During the formation of fruiting bodies in M. xanthus, approximately 60 to 80% of the vegetative cells lyse and die. This leaves a minimum of about 20% of the vegetative cells to survive as myxospores (Wireman and Dworkin, 1975). Within each fruiting body there exists about 40,000 or more myxospores which under favorable conditions, may germinate into vegetative cells (White, 1975).

The induction of fruiting body formation and the role of nutritional control in the formation of fruiting bodies in M. xanthus has been extensively studied, however, the exact biochemical mechanism still remains in doubt. In the study by Dworkin (1963), fruiting was induced by eliminating phenylalanine and tryptophan from a chemically defined growth medium, thus revealing that amino acid starvation can lead to fruiting body formation. Hemphill and Zahler (1968) found that the limitation of certain amino acids from the growth medium, rather than complete elimination, also resulted in fruiting body formation. Limitation of both the essential and nonessential amino acids gave rise to the fruiting response. Manoil and Kaiser (1980) showed that fruiting can also be induced by starvation for carbon, energy, and inorganic phosphate.

The induction of fruiting body formation can also be stimulated by the presence of a certain compound. Campos and Zusman (1975) reported that the presence of adenine-containing nucleotides, particularly ADP and cyclic AMP, stimulated and greatly enhanced fruiting body formation in M. xanthus.

Nutrition-controlled suppression of fruiting body formation has also been documented in a number of studies. Hemphill and

Zahler (1968) found that high concentrations of methionine, phenylalanine, and tryptophan inhibited fruiting body formation on media that normally permits the fruiting response. Rosenberg et al. (1973) also observed that high concentrations of methionine and isoleucine suppressed fruiting.

Myxospores of M. xanthus are optically refractile spheres about 2.0 μm in diameter (McCurdy, 1974). Sudo and Dworkin (1969) reported that myxospores displayed a greater resistance towards a number of destructive agents i.e., desiccation, sonic vibration, ultraviolet irradiation, and heat, than the vegetative cells. Since M. xanthus is normally confined to the top regions of soil, the capacity to resist these harsh conditions aids in the survival of the organism during periods of environmental stress.

Under natural conditions, the conversion of vegetative cells to myxospores occurs within the fruiting body (Reichenbach and Dworkin, 1981). Dworkin and Gibson (1964), however, found that the addition of 0.5 M glycerol to dispersed growing, liquid cultures of M. xanthus resulted in the rapid and synchronous production of myxospores without fruiting body formation. Sadler and Dworkin (1966) later found that a number of other compounds i.e., dimethyl sulfoxide and several compounds containing primary and secondary alcohol groups, also induced myxospore formation, although not as effectively or rapidly. Close to 100% of the vegetative cells are converted to myxospores in the glycerol-induced system (Dworkin and Sadler, 1966). The conversion requires normal vegetative growth media and continuous aeration, and is complete within 120 minutes (Dworkin and Gibson, 1964). Glycerol must be continuously present during the morphogenesis

since removal of glycerol reverses the process and results in the production of vegetative cells (Dworkin and Sadler, 1966). Glycerol-induced myxospores closely resemble fruiting body myxospores in gross morphology and resistance properties (Kottel et al., 1975), however, there are notable differences between the two types of myxospores (White, 1975).

The precise mechanism through which glycerol induces myxosporulation is still unknown. However, theories involving unregulated transcription (Zusman, 1980) and membrane perturbation (Sadler and Dworkin, 1966) have been proposed as possible mechanisms of glycerol induction. In any case, the net result of glycerol induction is the synchronous expression of a number of the sporulation genes (Zusman, 1984).

During myxosporulation, the myxospore synthesizes an extracellular spore coat (White, 1975). In the study by Inouye et al. (1979b), electron microscopy of thin sections of glycerol-induced myxospores showed that the spore coat consists of a single layer 20 nm thick. The spore coat of fruiting body myxospores, however, is thicker and consists of three layers: (1) an electron dense cortex about 45 nm thick, (2) a less electron dense intermediate coat about 55 nm thick, and (3) an even less electron dense surface coat about 30 nm thick.

The spore coat of glycerol-induced myxospores has been isolated and chemically characterized (Kottel et al., 1975; White, 1975). Kottel et al. (1975) found that the spore coat of glycerol-induced myxospores consisted of, on a dry weight basis, approximately 75% polysaccharide (50% galactosamine and 25% glucose), 14% protein,

8% glycine, and less than 1% organic phosphorous. In the case of fruiting body myxospores, coats have been isolated, but only partially characterized (Kottel et al., 1975). The composition of coats from fruiting body myxospores appears to be similar in polysaccharide content to that of coats from glycerol-induced myxospores. The content of protein and phosphorous has not been determined.

Much of the glucose and galactosamine found in the myxospore coat is in the form of separate polymers. Sutherland and MacKenzie (1977) reported that the glucose portion of the myxospore coat was mainly present as an α-1,3-glucan. Based on studies involving lectin binding and enzyme degradation, it was suggested that the glucan forms part of the surface layer of the spore coat. The galactosamine-containing polymer, however, appears to be located beneath the coat surface since lectin binding was not detected.

White et al. (1968) reported that the cell wall of the myxospore contained roughly the same amount of peptidoglycan as found in the vegetative cell. Furthermore, the overall composition of peptidoglycan in the myxospore was similar to that of the vegetative cell. However, the structure of peptidoglycan appears to change to a continuous sheath during myxospore formation. Johnson and White (1972) observed an 11% increase in the amount of peptidoglycan crosslinkages. It has been suggested that the changes occurring in peptidoglycan structure and in cell shape during myxosporulation may be linked together.

During glycerol-induced myxospore formation, numerous changes in enzyme activity take place. Orlowski et al. (1972) reported that two enzymes of the glyoxylate cycle, isocitrate lyase and

malate synthase, exhibited a sharp increase followed by a gradual decrease in specific activity during myxospore formation. It was suggested that these changes in enzyme activity are associated with the development of the myxospore. Filer et al. (1977) found that six of the seven enzymes responsible for the conversion of fructose 1,6-diphosphate to uridine 5'-diphosphate (UDP)-N-acetyl galactosamine, a critical precursor in spore coat biosynthesis, increased in specific activity during myxospore formation. The six enzymes exhibited a 4.5- to 7.5-fold increase in specific activity following two hours of glycerol or dimethyl sulfoxide induction. After four hours of induction, the specific activity returned to levels typical of vegetative cells. It was suggested that the enhanced activity of the six enzymes are responsible for the rapid synthesis of the spore coat during myxospore formation.

A number of studies have measured the changes in macromolecular synthesis during glycerol-induced myxospore formation in M. xanthus. Sadler and Dworkin (1966) found that the net synthesis of DNA, RNA, and protein increased at a linear rate, but in a two-step manner. Increased synthesis of the three macromolecules appeared to cease for a short time during the early stages of shape change. Synthesis continued to increase after this lag period, but began to level off after myxospores became optically refractile. The content of RNA, DNA, and protein exhibited an overall increase of 20, 25, and 30%, respectively. Rosenberg et al. (1967) reported that during glycerol-induced myxospore formation, DNA continued to be synthesized until the completion of chromosome replication, however, new initiation of chromosome replication was blocked. Bacon and Rosenberg (1967)

discovered that the net synthesis of RNA terminated immediately after glycerol induction; a finding which contradicted the results found earlier in the study by Sadler and Dworkin. It was also found that extensive RNA turnover occurred during myxospore formation; most of which was ribosomal RNA. Through hybridization competition experiments, Okano et al. (1970) found that certain species of mRNA are synthesized only during myxospore formation, and thus considered unique to the glycerol-induced myxospores.

The germination process in M. xanthus has not been thoroughly investigated, however, a basic outline of events has been established. During germination, the myxospore slowly loses optical refractility and becomes phase dark. The spherical inner cell of the myxospore changes to a rod-shaped cell, and then penetrates the spore coat, leaving an empty shell of the myxospore behind (Voelz and Dworkin, 1962). Dworkin (1973) observed that glycerol-induced myxospores undergo germination in distilled water when the cell density exceeds 5×10^9 cells per ml. Under this condition, cells excrete inorganic phosphate, a possible chemical signal for triggering the germination process. Germination of glycerol-induced myxospores can also occur at low cell densities, but only while in phosphate buffer. In the case of fruiting body myxospores, germination will not occur in phosphate buffer, but will readily occur in complete growth media (White, 1975).

Many studies have shown that several phenomena in myxobacteria are largely based on cell interactions. For example, the swarming of cells (S motility) and the feeding process heavily rely on the interaction of a large number of cells. Furthermore, the

aggregation of cells during fruiting body formation and the germination of myxospores are also dependent upon cell-cell interactions, i.e., high cell densities. It has been suggested that cell-cell signaling may be involved in many of these events, however, the identification of both a signal and cell surface receptor has not yet been made. The signal for these events may require cell-cell contact or may simply be a diffusible substance. The nature of signal and receptor recognition may be based on mutual differences as in hormone-receptor interactions or lectin-ligand interactions, or on similarities as in the self-recognition systems between homotypic cells (Dworkin, 1984).

In the slime mold, Dictyostelium discoideum, several studies have reported that the plasma membrane plays an important role in certain aspects of cell development and interaction. Studies have shown that some changes in protein content occur on the cell surface during developmental growth. A number of membrane proteins and glycoproteins have been linked to the phenomenon of cell adhesion in Dictyostelium (Murray, 1982).

Inouye et al. (1979a) reported that several biochemical changes in soluble and membrane proteins occurred during fruiting body formation in M. xanthus. At least 25% of the 30 major soluble proteins exhibited substantial changes in the rate of synthesis during developmental growth. In addition, a number of changes in the synthesis of membrane proteins were also observed. These changes included the increased and decreased production of certain vegetative proteins and the production of new development-specific proteins. In the study by Orndorff and Dworkin (1982), the change in the rate

of synthesis of several membrane proteins during the aggregation phase was investigated, with special emphasis on the increased synthesis of five major developmental membrane proteins. As a result of the significant changes in the cell surface of M. xanthus during development, several studies have investigated the possibility of development-specific proteins acting as the signals and/or receptors in the various cell interactions. The two most widely studied development-specific proteins are protein S and myxobacterial hemagglutinin (MBHA).

Protein S is a monomeric protein with an apparent molecular weight of 23,000 daltons (Inouye et al., 1979a). Purification, characterization, and crystallization of protein S has been carried out. Inouye et al. (1981) found that protein S had a very high content of β-structure with less than 1% α-helical content. Hexose and amino sugars were not detected, suggesting that protein S is not a glycoprotein. Protein S is also an acidic protein with an apparent isoelectric point of 4.5.

Electron microscopy revealed that protein S corresponds to the outer 30 nm surface layer of fruiting body myxospores (Inouye et al., 1979b). Approximately 2.5×10^6 molecules of protein S are bound per myxospore. Inouye et al. (1979b) determined that the presence of calcium ions is specifically required for the assembly of protein S onto the outer surface of the myxospore. During the early stages of development i.e., mound formation, protein S appeared to be loosely bound to the cell, however, during the later stages of development i.e., spore maturation, protein S became firmly bound to the myxospore.

Inouye et al. (1979a) found that during the early stages of fruiting body formation (3 to 6 hours), protein S rapidly increased in synthesis until reaching a maximum of 15% of the total soluble protein synthesis. This elevated level of synthesis was maintained until development was completed. Inouye et al. (1979b) determined that protein S is initially synthesized as a soluble protein and then transported through the cytoplasmic and outer membranes to the outermost layer of the myxospore and subsequently incorporated into the spore surface coat. Nelson and Zusman (1983) determined that protein S was translocated through the cell envelope without the cleavage of a signal peptide. Studies have also revealed that protein S is not synthesized during glycerol induction of myxosporulation (Inouye et al., 1979a; Komano et al., 1980).

Recently, the structure of the gene responsible for the production of protein S has been investigated. Inouye et al. (1983b) discovered that two genes in the chromosome of M. xanthus were capable of coding for protein S. The duplicated genes are an upstream located gene 1 (or the ops gene) and a downstream located gene 2 (or the tps gene). The two genes are tandemly repeated in the same direction and separated by 1.2 to 1.4 kilobases. Downard and Zusman (1985) found that the tps gene was expressed early in development, while the ops gene was expressed much later in development. Inouye et al. (1983a) sequenced the region of DNA covering the two genes (3,692 base pairs) and also determined the corresponding amino acid sequences of both gene products. The amino acid sequences of the two gene products displayed approximately 88% homology. Comparison of the amino acid sequence of protein S with the DNA sequences of the two genes for

protein S revealed that only the tps gene significantly produced protein S during development.

Protein S is not essential for myxospore viability and resistance since protein S-deficient and glycerol-induced myxospores withstood the harsh conditions of sonication and heat (Inouye et al., 1979b). As a result of finding fruiting body formation unaffected in the absence of protein S, Komano et al. (1984) suggested that protein S may be required during myxospore germination, rather than during fruiting body formation. Based on the observation that myxospores frequently become fused together via the protein S layer, Inouye et al. (1979b) suggested that protein S functioned as an adhesive to hold the myxospores together within the fruiting body.

Myxobacterial hemagglutinin (MBHA) has been purified and partially characterized (Cumsky and Zusman, 1981a). MBHA is a basic protein (pI = 8.3) with an apparent relative molecular weight of 28,000 daltons. MBHA is most likely non-glycoprotein since little (less than 0.05%) carbohydrate was detected in its composition. The secondary structure of MBHA contains a small amount of α-helix, but a large amount of β-sheet (50 ± 10%) and random coil (50 ± 10%). A large proportion of the amino acid content in MBHA consists of glycine (19%) and aromatic amino acids (9%) such as tyrosine, tryptophan, and phenylalanine, however, only a small proportion consists of charged amino acids.

In the study by Cumsky and Zusman (1979), lectin-like activity has been attributed to MBHA. MBHA was found to agglutinate sheep or guinea pig erthrocytes, but not ox, chicken, horse, or human erthrocytes. Furthermore, MBHA was detected in extracts of

aggregating cells, but not in extracts of vegetative cells, cells starved in liquid buffer, or in glycerol-induced cells.

Induction of MBHA synthesis occurs at about 6 to 8 hours of development and reaches a rate of about 5% of total protein synthesis. In addition, MBHA accounts for 1 to 2% of the total soluble protein at the completion of the aggregation phase. As developmental growth comes to an end, the amount of soluble MBHA and hemagglutinating activity begins to decline, however, MBHA continues to be synthesized (Cumsky and Zusman, 1979).

Nelson et al. (1981) reported that MBHA is located in the periplasmic space and on the outer surface of the cell. MBHA was only found on the surface of developmental cells; the presence of MBHA on vegetative cells was not detected. In addition, it was determined that MBHA was predominantly located at one or both of the cell poles. Some MBHA was located at multiple sites on the cell surface. Nelson et al. (1981) found no evidence to suggest that the translocation of MBHA across the cytoplasmic membrane is accomplished with the aid of a cleavable signal peptide even though the amino acid sequence of the NH_2 terminus of MBHA is hydrophobic.

MBHA appears to bind complex receptors containing a β-linked galactose residue (Cumsky and Zusman, 1979). Cumsky and Zusman (1981b) investigated the nature of MBHA-receptor binding in both vegetative and developmental cells of M. xanthus. Both types of cells could bind MBHA, however, the developmental cells appeared to bind substantially more MBHA and at a higher association constant. During developmental growth, there was a 4-fold increase in the number of binding sites per cell i.e., 2.1×10^4 sites per cell.

Cumsky and Zusman (1981b) suggested that a new developmentally regulated receptor for MBHA is incorporated into the outer surface of the cell during fruiting body formation.

The precise role that MBHA plays in the developmental growth of M. xanthus is still unknown, although the possible involvement in end-to-end cellular interactions during aggregation has been suggested (Nelson et al., 1981).

Recently, Maeba (1983) demonstrated that the protein composition of the cell surface of M. xanthus is dramatically altered during developmental growth. Using one- and two-dimensional SDS-polyacrylamide gel electrophoresis, the presence of numerous development-specific proteins was established. Through lactoperoxidase-mediated radioiodination labeling, it was found that several of these proteins were located on the surface of the cell. Using this labeling technique as a marker of surface proteins, an attempt will be made to isolate and purify one of these proteins.

MATERIALS AND METHODS

MATERIALS AND METHODS

Materials

Amino acids (L-isomers), leupeptin, pepstatin, Tris-HCl, HEPES, BSA, CHAPS, PMSF, vitamin B_{12}, dithiothreitol, and Triton X-100 were obtained from Sigma Chemical Company. Acrylamide, N,N'-bis-methylene acrylamide, TEMED, ammonium persulfate, and SDS were from Bio-Rad Laboratories. Coomassie brilliant blue R and Scintiverse scintillation fluid were obtained from Fisher Scientific Company and 2-mercaptoethanol was obtained from Eastman Kodak Company. DNase, lactoperoxidase, and glucose oxidase were from Boehringer Mannheim Corporation and carrier free $Na^{125}I$ was from Amersham Corporation. The molecular weight standard calibration kit, DEAE-Sephacel, and chromotofocusing buffers and exchangers were obtained from Pharmacia Fine Chemicals. Aquacide II was from Calbiochem. All other chemicals were of reagent grade and obtained from more than one source.

Centrifugation at 27,000 x g or less was carried out in the Sorvall Superspeed RC2-B automatic refrigerated centrifuge. Centrifugation at speeds greater than 27,000 x g was carried out in the Beckman model L ultracentrifuge. All centrifugations were carried out at approximately $4^{o}C$.

Bacteria and Media

Myxococcus xanthus strain DK1050 was obtained from D. Kaiser (Stanford University, California, USA) and was used exclusively throughout this study. Stock cultures were prepared by making mid-logarithmic cultures 0.5 M with sterile glycerol (6.8 M) and then

dispensing 2.0 ml aliquots into sterile glass vials for storage at -70°C.

Vegetative cells were grown in a medium of 2% Difco casitone containing 8.0 mM $MgSO_4$ and 1.0 mM KH_2PO_4-K_2HPO_4, pH 7.6, (Dworkin, 1962).

Developmental cells were grown on 1.5% Difco agar plates containing the A-1 medium of Bretscher and Kaiser (1978) modified by the absence of L-asparagine, L-phenylalanine, spermidine-3HCl, sodium pyruvate, and potassium aspartate. The final concentrations of the various ingredients (in distilled water) of the modified A-1 medium were as follows: 100 µg/ml L-isoleucine, 50 µg/ml L-leucine, 10 µg/ml L-methionine, 100 µg/ml L-valine, 10 µM $FeCl_3$, 10 µM $CaCl_2$, 0.5 mg/ml $(NH_4)_2SO_4$, 10 mM Tris-HCl (pH 7.6), 1.0 mM KH_2PO_4-K_2HPO_4 (pH 7.6), 8.0 mM $MgSO_4$, and 1.0 µg/ml vitamin B_{12}. The mixture of ingredients was sterilized by autoclaving for 20 minutes at 121°C. Vitamin B_{12} (sterilized by filtration) and $MgSO_4$ were added after the autoclaved medium had cooled to approximately 50°C. The medium was solidified by 1.5% Difco agar. Approximately 40 to 50 ml of the medium was poured per petri plate (15 cm in diameter) and then dried at room temperature for 2 to 5 days.

Growth

Vegetative Cells. Erlenmeyer flasks (250 ml) containing 40 ml of the casitone medium were inoculated with stock cultures of *M. xanthus* and incubated at approximately 30°C with vigorous shaking in a Gyratory shaker (New Brunswick Scientific Company). After reaching the mid-logarithmic phase (150 to 250 Klett units, No. 66 red filter), about 10 ml aliquots of the cultures were added to 2

liter Erlenmeyer flasks containing 400 ml of the casitone medium and incubated at approximately 30°C with vigorous gyratory shaking. Cells were grown to mid-logarithmic phase and then centrifuged at 11,000 x g for 20 minutes. Cell pellets were resuspended and washed with ice-cold HMSPLP buffer, pH 7.4, (20 mM HEPES, 10 mM $MgCl_2$, 0.15 M NaCl, 1.0 mM PMSF, 1.0 µg per ml leupeptin, and 1.0 µg per ml pepstatin) and then centrifuged at 27,000 x g for 15 to 20 minutes. Cell pellets were then stored at -70°C.

Developmental Cells. Vegetative cultures were grown to the mid-logarithmic phase in casitone medium (see above) and harvested by centrifugation at 11,000 x g for 20 minutes. Cell pellets were then resuspended in ice-cold sterilized TM buffer, pH 7.6, (10 mM Tris-HCl and 10 mM $MgCl_2$) and concentrated to a cell density of between 2.5 to 3.5 x 10^{10} cells per ml. The cell suspension was spotted on dried agar plates of modified A-1 medium with a peristaltic pump connected to Tygon tubing and a modified Pasteur pipette. Each plate received approximately 150 spots. Each spot had an average diameter of about 5 mm and an average volume of 5 to 10 µl. Spots were dried at room temperature and then incubated at 30°C for 24 hours. Using a razor blade, developmental cells were scraped from the agar plates and suspended in 10 to 15 ml HMSPLP buffer. The developmental cell suspension was blended (on ice) two times for 10 seconds at maximum speed in a Sorvall Omni-mixer and then centrifuged at 27,000 x g for 15 to 20 minutes. Pellets of the developmental cells were stored at -70°C.

Radioiodination

Fresh cell suspensions of M. xanthus (vegetative and developmental) in ice-cold HMSPLP buffer (see Growth) were blended for 15 seconds at maximum speed in a Sorvall Omni-mixer. The concentration of the cell suspension was then determined in a Klett-Summerson colorimeter with a red (No. 66) filter. A volume equivalent to 5.0×10^{10} cells per ml was placed into each of four 15 ml Corex centrifuge tubes and adjusted to 5.0 ml with HMS buffer, pH 7.4, (20 mM HEPES, 10 mM $MgCl_2$, and 0.15 M NaCl) and then centrifuged at 6,000 x g for 10 minutes. Cell pellets were then resuspended in 2.0 ml PBS buffer, pH 7.6, (50 mM KH_2PO_4-K_2HPO_4 and 0.15 M NaCl) containing 10 mM $MgCl_2$. The iodination process involved the addition (in order) of 50 µl lactoperoxidase (3.0 mg/ml), 50 µl glucose oxidase (2.0 mg/ml), and 25 µl carrier free $Na^{125}I$ (100 mCi/ml) to each of the four tubes of cell suspension. The iodination reaction was started by the addition of 50 µl glucose (8.0 mg/ml). The cell suspension was mixed and incubated for 20 minutes at room temperature. The reaction was stopped by the addition of 3.0 ml HMS buffer containing 20 mM dithiothreitol. The cell suspension was then centrifuged at 6,000 x g for 10 minutes. The iodinated cell pellets were resuspended and washed in 5.0 ml HMS buffer containing 25 mM KI and then centrifuged at 6,000 x g for 10 minutes. This process was repeated using 5.0 ml HMS buffer. The iodinated cell pellets were then stored at $-20^{\circ}C$.

Preparation of Crude Extracts

Crude extracts of ^{125}I-labeled vegetative and developmental

cells of M. xanthus were identically prepared. Iodinated cell pellets (see Radioiodination) were resuspended in 3.0 ml HMSPLP buffer containing 20 µg DNase and then sonicated (on ice) for three 60 second intervals with the small probe using a model 300 sonic Dismembrator (Fisher Scientific Co.) at the maximum setting. The sonicated cell suspension was then centrifuged at 6,000 x g for 10 minutes to remove unbroken cells and cellular debris. The resulting supernatant represented the crude extract.

Crude extracts of unlabeled developmental cells were prepared on a large scale for use in purification. Developmental cell pellets (see Growth) were weighed, slowly thawed, and resuspended in double their wet weight HMSPLP buffer containing 20 µg DNase. Breakage and disruption of cells was accomplished by passing the cell suspension through the Aminco French Pressure Cell Press at 700 atm. The cell suspension was diluted 1:1 with HMSPLP buffer after disruption. Centrifugation at 6,000 x g for 10 minutes was carried out to remove unbroken cells and cellular debris. The supernatant represented the crude extract.

Preparation of Developmental Crude Membranes

^{125}I-labeled crude extract was mixed with a large volume of unlabeled crude extract and centrifuged at 110,000 x g for 2 hours. The resulting pellet represented the crude membrane.

Electrophoresis

The protein content of M. xanthus was analyzed by one-dimensional SDS-polyacrylamide gel electrophoresis. Electrophoresis was carried out using the discontinuous buffer system of Laemmli

(1970) in a Protean I slab electrophoresis cell (Bio-Rad Laboratories). The separation gel was 0.75 mm thick and consisted of an 8 to 15% acrylamide gradient. In preparing the 15% acrylamide solution, 75% glycerol was used in place of distilled water. All samples for analysis were first lyophilized and then washed and precipitated with 85% ethanol (with or without 10 mM $MgCl_2$). Samples were dissolved in 0.1 ml 0.25 M Tris-HCl buffer (pH 6.8) containing 15% glycerol, 2% SDS, 10% 2-mercaptoethanol, and a small amount of bromophenol blue and then placed in a boiling water bath for 2 minutes. In most cases, the amount of protein applied to the gels ranged from 20 to 100 µg. The gels were run for 1300 volt hours (approximately 18.5 hours at 70 volts) such that the tracking dye was about 1 cm from the bottom of the gels. Also included in the electrophoresis was a series of protein markers of known molecular weight which included: α-lactalbumin, soybean trypsin inhibitor, carbonic anhydrase, ovalbumin, bovine serum albumin, and phosphorylase b, with relative molecular weights of 14,400, 20,100, 30,000, 43,000, 67,000, and 94,000, respectively.

Staining and Autoradiography

The gels were stained for 30 minutes in a solution of 0.03% Coomassie brilliant blue R, 30% ethanol, and 10% acetic acid with gentle agitation. The gels were destained in the following solutions: (1) 30% ethanol-10% acetic acid for 30 minutes, (2) 25% methanol-10% acetic acid for 1 hour, and (3) 20% methanol-10% acetic acid for 1 hour. Lastly, the gels were added to a solution of 7% acetic acid and 2% glycerol for 30 minutes. The gels were

dried for 1 to 2 hours onto thick filter paper in a Bio-Rad gel slab dryer.

Autoradiographs were prepared by exposing the gels to Kodak RP-1 X-ray film in a Kodak X-omatic intensifying screen at -70°C. The time of exposure ranged from 3 days to 4 weeks depending upon the level of radioactivity present in the samples. Films were placed in a developing solution for 4 minutes and then a fixative solution for 6 minutes.

Radioisotope Counting

Radioactivity of iodine-125 was measured in the tritium spectrum with a Beckman LS-230 liquid scintillation counter. Microliter volumes of column fractions were counted in glass vials containing 5.0 ml of Scintiverse scintillation fluid.

Protein Determination

Protein was estimated for use in electrophoresis by the assay method of Schacterle and Pollack (1973), but modified by the replacement of distilled water with 1.0% SDS. Measurement of protein was determined in a Klett-Summerson colorimeter with a red (No. 66) filter. Protein measurement of column fractions was determined by the BCA protein assay (Pierce Chemical Company) with a Beckman DU 2400 spectrophotometer. Bovine serum albumin was used as the standard for both methods of protein determination.

RESULTS

RESULTS

Surface Proteins of Vegetative and Developmental Cells

Vegetative and developmental cells of M. xanthus were grown, harvested, and labeled with ^{125}I as outlined in Materials and Methods. Crude extracts of both the vegetative and developmental cells were prepared and then analyzed by one-dimensional electrophoresis and autoradiography (Materials and Methods).

Coomassie blue-stained gels (Fig. 1a) showed numerous protein bands, many of which appeared to be identical in both the vegetative and developmental cell extracts. However, a few differences were apparent. Some of the protein bands were only detected in the vegetative cell extract, while others were only detected in the developmental cell extract. An autoradiograph of the stained gel (Fig. 1b) revealed that a few of the proteins in both the vegetative and developmental cell extracts were iodinated. Several of the bands were found in both the vegetative and developmental extracts, however, four labeled bands with approximate molecular weights, (as determined by molecular weight standards), of 84,000, 67,000, 47,000, and 31,000 were exclusively found in the developmental cell extract (lane B, Fig. 1b). These bands represent development-specific proteins which are located on the cell surface and thus accessible to ^{125}I-labeling. Proteins with similar ^{125}I-labeling patterns and molecular weights had previously been reported by Maeba (1983).

As seen in Figure 1b, a large deposit of radioactivity was detected at the bottom of the lanes in the autoradiogram. This fast migrating radioactivity may represent lipid-bound ^{125}I.

Figure 1. a) Electropherogram of vegetative and developmental crude extracts. One-dimensional SDS-PAGE and coomassie blue-staining were carried out as described in Materials and Methods with 100 µg of protein.

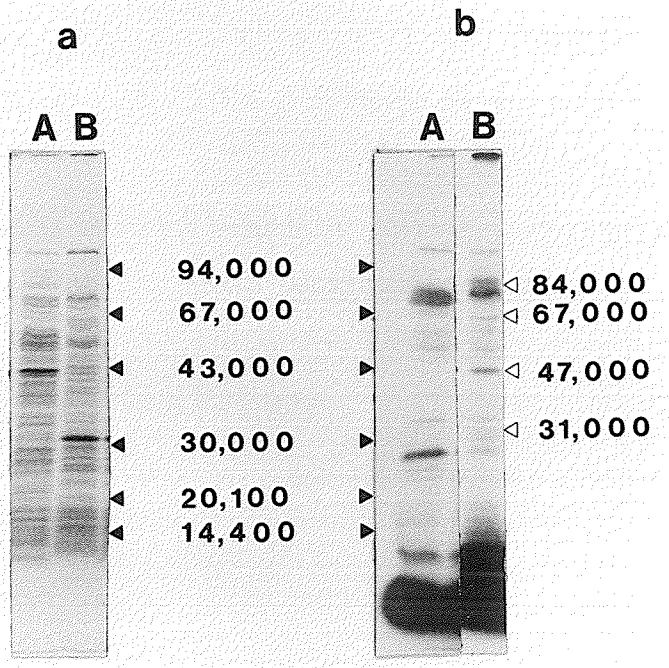
b) Autoradiogram of vegetative and developmental crude extracts. Autoradiography was carried out as described in Materials and Methods.

A. Vegetative crude extracts

B. Developmental crude extracts

Numbers indicate the molecular weights of standards (solid triangles) and iodinated bands specific for developmental cells (open triangles).

Fig. 1


a
b
A B
A B
94,000
67,000
43,000
30,000
20,100
14,400
84,000
67,000
47,000
31,000

The results of this experiment confirm earlier findings (Historical) in which some distinct changes in the outer cell surface occur during development. This can be seen by the differences in the protein and iodination patterns between the vegetative and developmental cell extracts (Figs. 1a and 1b).

Triton X-100 Extraction of Iodinated Membrane Proteins

Cells that had developed for 24 hours were harvested and a small portion was iodinated as described in Materials and Methods. The crude extract from the iodinated cells was mixed with the crude extract from 30 grams of unlabeled cells (Materials and Methods). The iodinated cells served as tracers for the isolation of development-specific surface proteins.

The crude membrane was pelleted by ultracentrifugation (110,000 x g for 2 hours) and resuspended in approximately 16 ml TEPLPT buffer (50 mM Tris-HCl, pH 7.8, containing 10 mM tetrasodium EDTA, 1.0 mM PMSF, 1.0 µg per ml leupeptin, 1.0 µg per ml pepstatin, and 2% Triton X-100). The suspension was stirred overnight at 4°C and then centrifuged at 110,000 x g for 1 hour. The supernatant was collected and represented the Triton X-100 soluble fraction (16 ml, 8.8 mg/ml protein). The pellet was dissolved in 2.0 ml of 1.0% SDS and represented the Triton X-100 insoluble fraction (9.8 mg/ml protein). Proteins of the crude membrane and the Triton X-100 soluble and insoluble fractions were then analyzed by electrophoresis and autoradiography.

Coomassie blue-stained gels (Fig. 2a) showed only minimal differences in the overall protein pattern between the crude membrane

and Triton X-100 soluble fraction. There were, however, more prominent differences in the overall protein pattern of the Triton X-100 insoluble fraction. There was a general reduction in the number of protein bands in the Triton X-100 insoluble fraction. Figure 2b represents autoradiograms of all three fractions. As shown in Figure 2b, the iodinated proteins were not extracted from the membrane by Triton X-100. In the Triton X-100 soluble fraction (lane B, Fig. 2b), only a few faint bands were detected. On the other hand, the Triton X-100 insoluble fraction (lane C, Fig. 2b) contained several intense bands. Four of these strongly labeled bands corresponded to the four development-specific proteins mentioned previously (Fig. 1b).

From these results, it appears that the majority of iodinated proteins are tightly associated with the membrane and not solubilized by Triton X-100.

Purification of Iodinated Developmental Membrane Proteins

Attempts were made to isolate iodinated proteins from both the Triton X-100 soluble and insoluble fractions obtained from the crude membranes. Although only faintly labeled proteins were detected in autoradiograms of Triton X-100 soluble fractions (Fig. 2b), it was possible that these bands would become more prominent as purification progressed since the specific radioactivities would be increased. This seemed likely since the soluble fraction contained a large variety of proteins. Since earlier work indicated that iodinated proteins exhibit a range of isoelectric points (Maeba, 1983), chromatofocusing was employed in their purification. The iodinated proteins were then identified by electrophoresis and autoradiography.

Figure 2. a) Electropherogram of the Triton X-100 extraction of iodinated membrane proteins from developing cells (see Text). One-dimensional SDS-PAGE and coomassie blue-staining were carried out as described in Materials and Methods with 50 µg of protein.

b) Autoradiogram of the Triton X-100 extraction of iodinated membrane proteins from developing cells (see Text). Autoradiography was carried out as described in Materials and Methods.

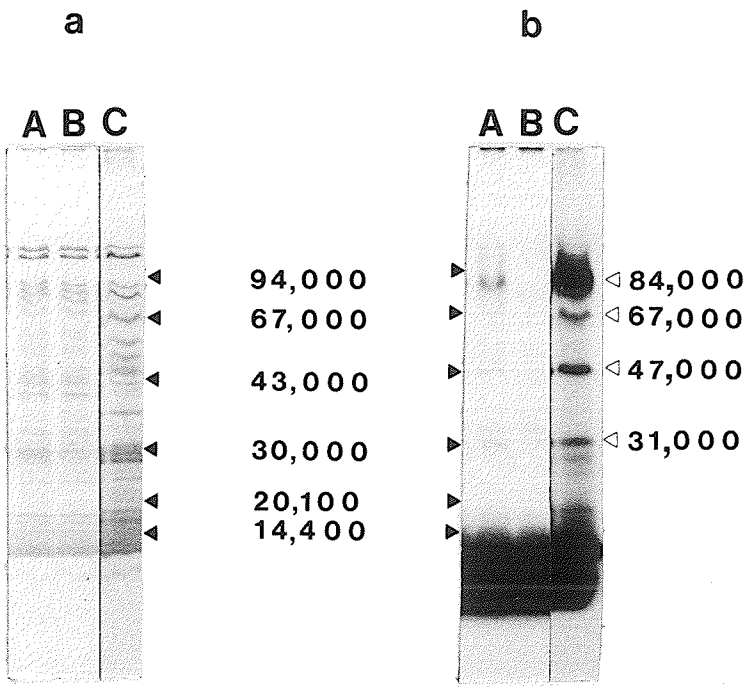
A. Crude membrane

B. Triton X-100 soluble fraction

C. Triton X-100 insoluble fraction

Numbers indicate the molecular weights of standards (solid triangles) and iodinated bands specific for developmental cells (open triangles).

Fig. 2


a
b
A B C
A B C
94,000
67,000
43,000
30,000
20,100
14,400
84,000
67,000
47,000
31,000

The Triton X-100 soluble fraction was adjusted to a volume of approximately 30 ml with TEPLPT buffer (pH 7.8) and then applied to a 60 ml bed volume column packed with polybuffer exchanger (Pharmacia, PBE 94) equilibrated with 0.025 M imidazole-HCl buffer (pH 7.4) containing 1% Triton X-100. The column was washed with about two bed volumes of equilibrating buffer and then eluted with 840 ml of 12.5% Polybuffer 74 (Pharmacia) containing 1% Triton X-100. The polybuffer, adjusted to pH 4.0, produced a linear pH gradient from 7.4 to 4.0. Material still bound to the column was removed by a stepwise pH gradient made from 0.1 M glycine-HCl buffer containing 1% Triton X-100 at pH values at 3.0, 2.0, and 1.2. Fractions (5.0 ml) were collected and measured for pH, radioactivity, and protein content.

Figure 3 presents the radioactivity and protein content of the various fractions collected from the chromatofocusing column as eluted by the linear and stepwise pH gradients. Several major and minor peaks of protein and radioactivity were present. The profile indicates that both protein and radioactivity were well resolved so that, as expected, the specific radioactivity of some fractions would be increased. Samples from representative fractions were analyzed by SDS-PAGE and the electropherograms (Fig. 4) show the effectiveness of the column in separating the proteins. To determine the proteins which were radioactive, the electropherograms were subjected to autoradiography (not shown), but despite exposures of up to 4 weeks at -70^{o}C, iodinated proteins were not detected. The results indicated that the peaks of radioactivity observed in Figure 3 were associated with the fast migrating radioactivity seen in previous autoradiograms (Figs. 1b and 2b) and probably represent lipid-bound ^{125}I. Since

Figure 3. Elution profile from chromatofocusing of the Triton X-100 soluble fraction. The Triton X-100 soluble fraction (30 ml) was loaded onto a 60 ml bed volume chromatofocusing column and eluted by a linear and stepwise pH gradient (solid line) as outlined in the text. Fractions (5.0 ml) were collected and measured for radioactivity (o——o) and protein content (●——●).

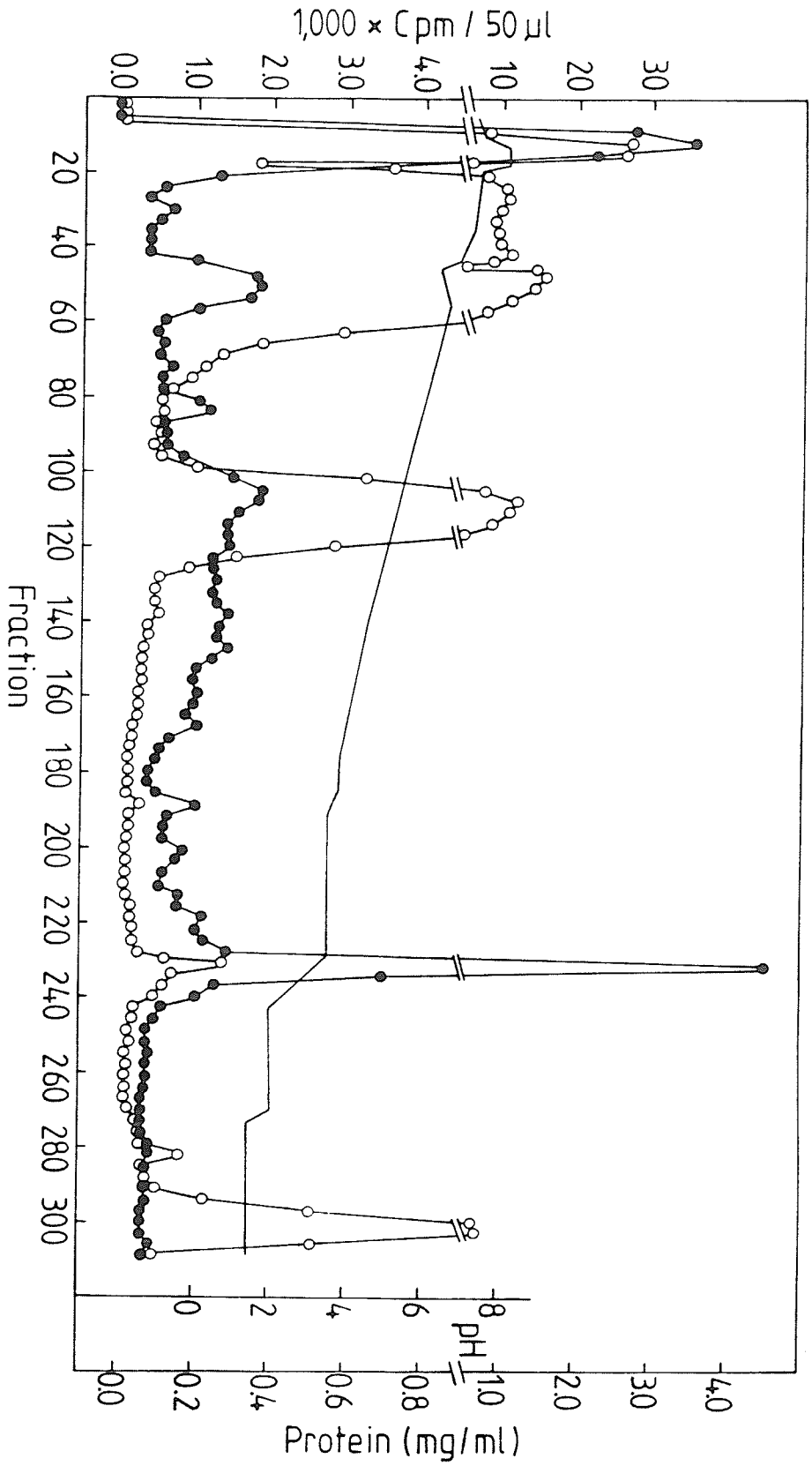
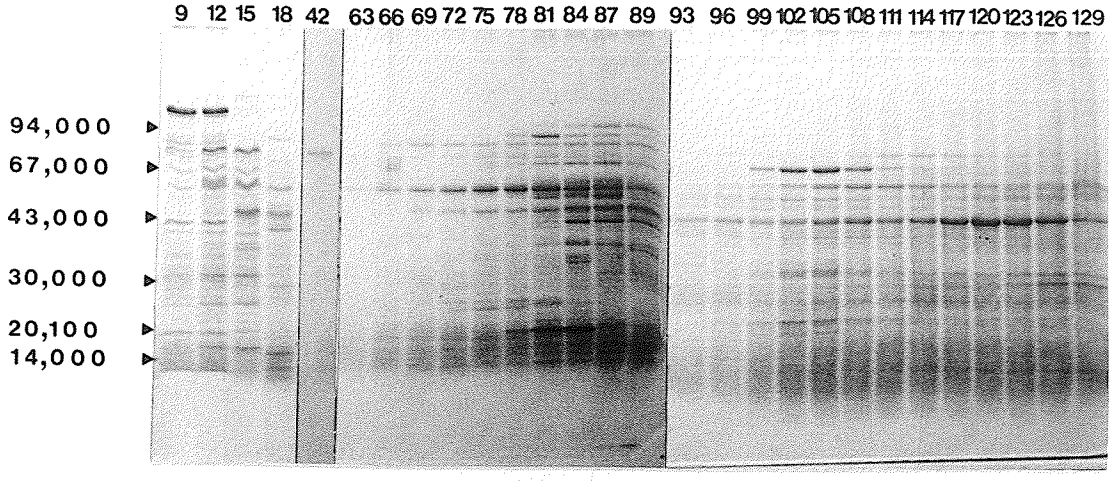
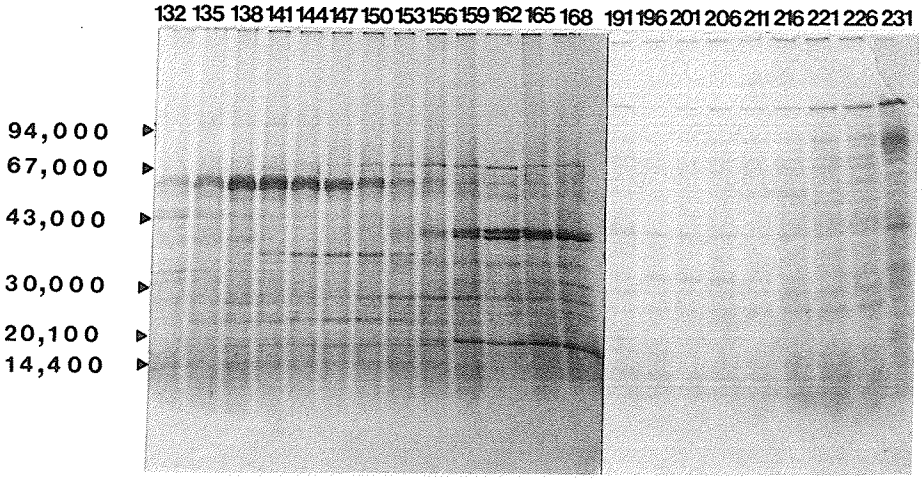

1,000 × Cpm / 50 µl
0.0
1.0
2.0
3.0
4.0
10
20
30
20 40 60 80 100 120 140 160 180 200 220 240 260 280 300
Fraction
0
2
4
6
8
pH
0.0
0.2
0.4
0.6
0.8
1.0
2.0
3.0
4.0
Protein (mg/ml)

Figure 4. Electropherogram of fractions collected from chromatofocusing of the Triton X-100 soluble fraction. The equivalent of 125 µl of sample from all indicated fractions, except for fractions 9, 12, and 15 (7.5 µl of sample) and fraction 231 (10 µl of sample), were processed as described in Materials and Methods for analysis by electrophoresis. One-dimensional SDS-PAGE and coomassie blue-staining were carried out as described in Materials and Methods. Numbers indicate the molecular weights of standards (solid triangles).

Fig. 4


9 12 15 18 42 63 66 69 72 75 78 81 84 87 89 93 96 99 102 105 108 111 114 117 120 123 126 129
94,000
67,000
43,000
30,000
20,100
14,000


132 135 138 141 144 147 150 153 156 159 162 165 168 191 196 201 206 211 216 221 226 231
94,000
67,000
43,000
30,000
20,100
14,400

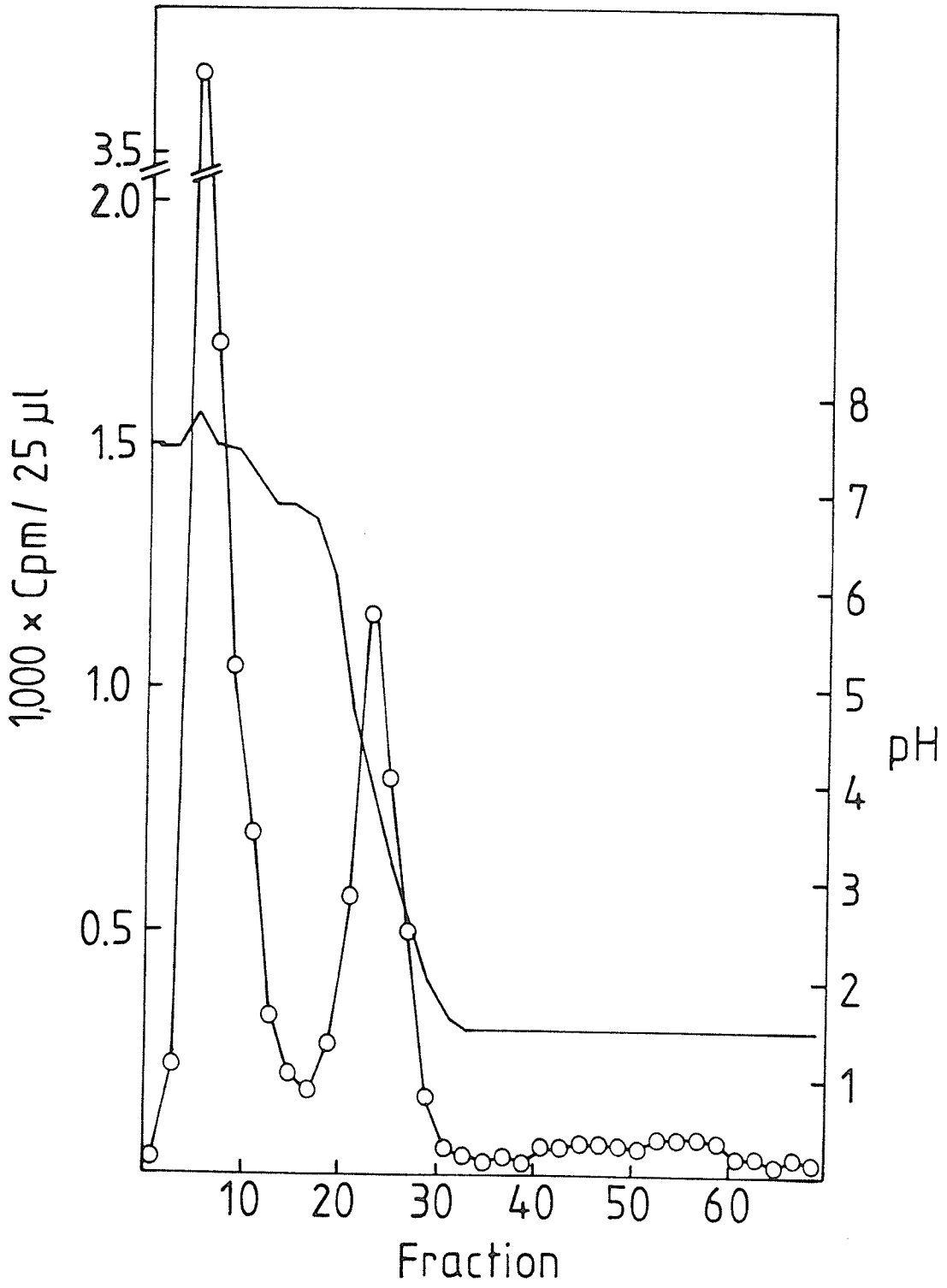
the autoradiogram of the Triton X-100 soluble fraction (Fig. 2b) showed only faint bands, this unsuccessful preliminary attempt at purifying the iodinated proteins was not totally unexpected.

Since partial success in separating the proteins in the Triton X-100 soluble fraction was obtained by chromatofocusing, this same technique was employed in the purification of the heavily iodinated proteins associated with the Triton X-100 insoluble fraction. Each purification step was then monitored by electrophoresis and autoradiography.

The Triton X-100 insoluble fraction (2.0 ml, 9.8 mg/ml protein) was adjusted to 5.0 ml with 10 mM HEPES buffer (pH 7.4) containing 1% Triton X-100 and then centrifuged at 12,000 x g for 5 minutes to remove debris that may have arisen from freezing during storage. The supernatant (4.5 ml) was applied to a 14 ml column packed with polybuffer exchanger (PBE 94) and equilibrated with 0.025 M imidazole-HCl buffer (pH 7.4) containing 1% Triton X-100. The column was washed with at least one bed volume of equilibrating buffer and then eluted with 210 ml of elution buffer (pH 1.5) made up of 5% Polybuffer 74, 1.4% Pharmalyte 2.5-5.0, and 1% Triton X-100. Pharmalyte (Pharmacia), a mixture of ampholines, was included in the polybuffer in an effort to extend the elution range to pH 1.5. Fractions (3.5 ml) were collected and assayed for pH and radioactivity.

The elution buffer failed to generate a pH profile that decreased regularly over the elution range (Fig. 5). Despite this, two well separated peaks of radioactivity were eluted from the column. Aliquots from representative fractions were concentrated and analyzed by SDS-PAGE (Materials and Methods). The gels were stained, dried,

Figure 5. Elution profile from chromatofocusing of the Triton X-100 insoluble fraction. The Triton X-100 insoluble fraction (4.5 ml) was loaded onto a 14 ml bed volume chromatofocusing column and eluted by a linear pH gradient (solid line) as outlined in the text. Fractions (3.5 ml) were collected and measured for radioactivity (o——o).


3.5
2.0
1.5
1.0
0.5
1,000 × Cpm / 25 µl
8
7
6
5
4
3
2
1
pH
10
20
30
40
50
60
Fraction

and used for autoradiography. Proteins were not detected in the early fractions (3-13, Fig. 5) that were eluted at approximately pH 7.5, and judging from the autoradiograms (not shown), the radioactivity was associated with the fast migrating unidentified material. A gel stained with coomassie blue (Fig. 6a) reveals that only fractions 19-29 contained protein. An autoradiograph of the gel (Fig. 6b) shows that a few of these proteins were iodinated, including three of the four development-specific proteins identified in Figures 1b and 2b. These fractions correspond to the smaller peak of radioactivity in Figure 5, strongly indicating that this peak (fractions 19-29) consists of iodinated proteins. As seen in Figure 5, fractions 19-29 were eluted from the column at pH's between 6.0 and 2.5, suggesting that these proteins have low isoelectric points.

Fractions 17-31 (50 ml) were pooled, concentrated with Aquacide II to 3.0 ml, and then dialyzed overnight against equilibrating buffer. The pooled fraction was passed through a second chromatofocusing column and eluted over a narrower pH range (pH 5.0 to 2.5). A 5.0 ml bed volume column was packed with polybuffer exchanger (PBE 94) and equilibrated with 0.025 M piperazine-HCl buffer (pH 5.5) containing 1% Triton X-100. The sample was applied to the column and washed with two bed volumes of equilibrating buffer. The sample was eluted with 210 ml of elution buffer (pH 2.5) consisting of 5% Polybuffer 74, 1.4% Pharmalyte 2.5-5.0, and 1% Triton X-100 to produce a linear pH gradient from 5.5 to 2.5. Fractions with volumes of 3.5 ml were collected and measured for pH and radioactivity.

Once again a gradual, regular decrease of pH over the elution range was not obtained (Fig. 7). Regardless, three peaks of

Figure 6. a) Electropherogram of fractions 19-29 collected after chromatofocusing of the Triton X-100 insoluble fraction. The equivalent of 100 µl of the indicated fractions were processed as described in Materials and Methods for analysis by electrophoresis. One-dimensional SDS-PAGE and coomassie blue-staining were carried out as described in Materials and Methods.

b) Autoradiogram of fractions 19-29 collected after chromatofocusing of the Triton X-100 insoluble fraction. Autoradiography was carried out as described in Materials and Methods.

Numbers indicate the molecular weights of standards (solid triangles) and iodinated bands specific for developmental cells (open triangles).

Fig. 6

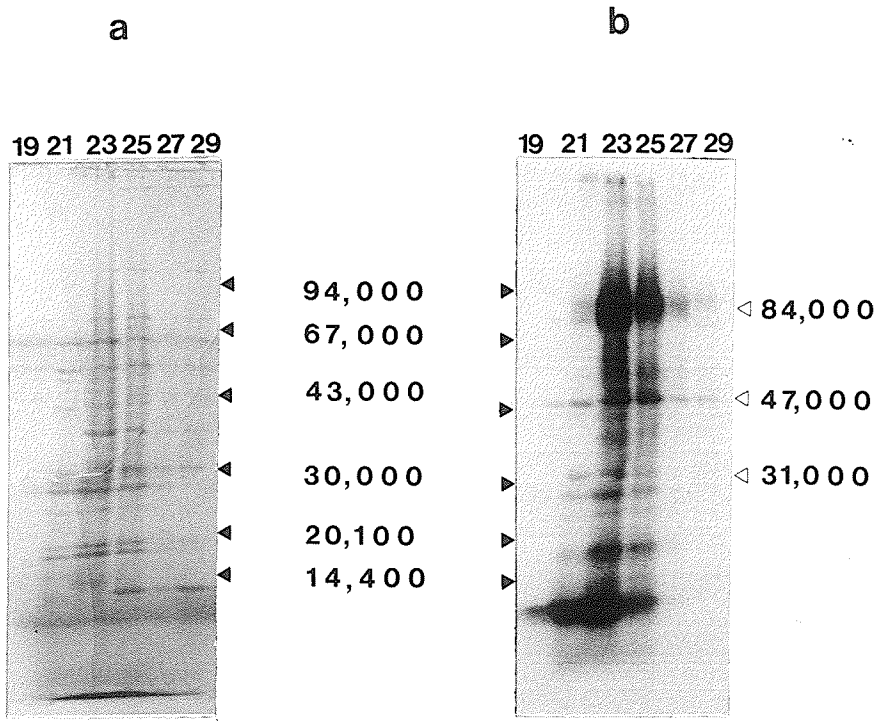
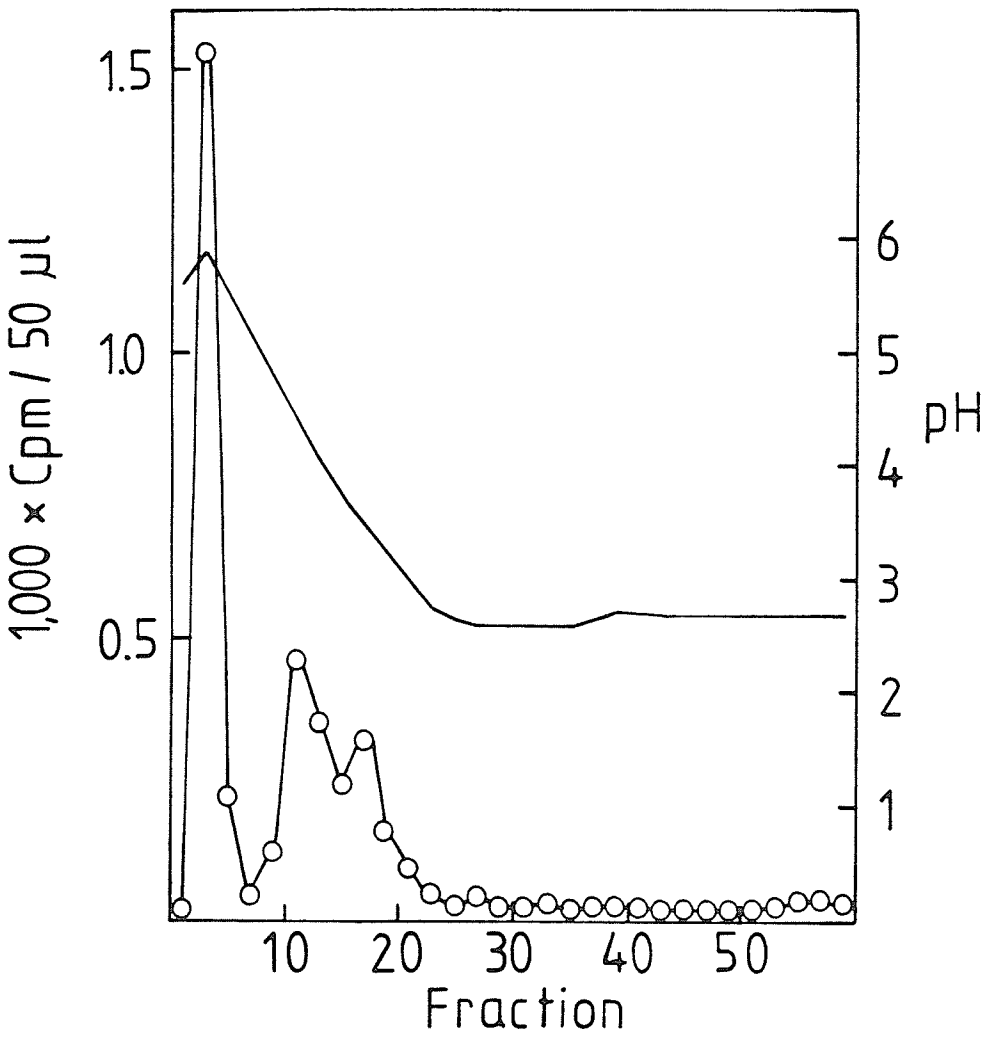

a
b
19 21 23 25 27 29
19 21 23 25 27 29
94,000
67,000
43,000
30,000
20,100
14,400
84,000
47,000
31,000

Figure 7. Elution profile from chromatofocusing of pooled fractions. Chromatofocusing fractions 17-31 (Fig. 5) were pooled, concentrated, loaded onto a second chromatofocusing column, and eluted over a narrower linear pH range (solid line) as outlined in the text. Fractions (3.5 ml) were collected and measured for radioactivity (o——o). Fractions 2-5, 9-14, and 16-19 were pooled for further study.


1,000 × Cpm / 50 µl
1.5
1.0
0.5
6
5
4
3
2
1
pH
10
20
30
40
50
Fraction

radioactivity were detected that eluted at approximately pH 5.5, 4.5, and 3.5, and corresponded to fractions 2-5, 9-14, and 16-19, respectively. The fractions were pooled, as indicated, and, after precipitation with 85% ethanol containing 10 mM $MgCl_2$, the protein was pelleted by low speed centrifugation. The pellet was then resuspended in 1.0 ml of 0.02 M sodium phosphate buffer (pH 7.0). The pooled samples were analyzed by electrophoresis and autoradiography.

The pooled fractions 16-19 exhibited few proteins in the electropherograms or iodinated bands in the autoradiograms (not shown). Pooled fractions 2-5 and 9-14 exhibited protein concentrations of 1.5 and 2.2 mg/ml, respectively. The coomassie blue-stained gel shows that the pooled fractions 2-5 (lane A, Fig. 8a) and 9-14 (lane B, Fig. 8a) differed significantly in protein content, although some protein bands in both fractions appeared to be identical. Fraction sample 2-5 contained far fewer proteins and indicates a significant amount of separation. The autoradiograms (Fig. 8b) show that most of the proteins in fraction sample 2-5 were iodinated, while relatively fewer of the proteins in fraction sample 9-14 were labeled. Although the labeling patterns were generally the same for both fraction samples (Fig. 8b), there were significant differences indicating the procedure separated some of the iodinated proteins.

The two major iodinated bands, marked by the open triangles in Figure 8b, appear to be enriched in only one of the fraction samples. The 84,000 molecular weight species predominates in fraction sample 9-14, although smaller amounts appear in fraction sample 2-5, while the reverse is true for the 47,000 molecular weight species. It may be that the species that appears in each fraction sample were

Figure 8. a) Electropherogram of pooled fractions 2-5 and 9-14. One-dimensional SDS-PAGE and coomassie blue-staining were carried out as described in Materials and Methods with 40 µg of protein.

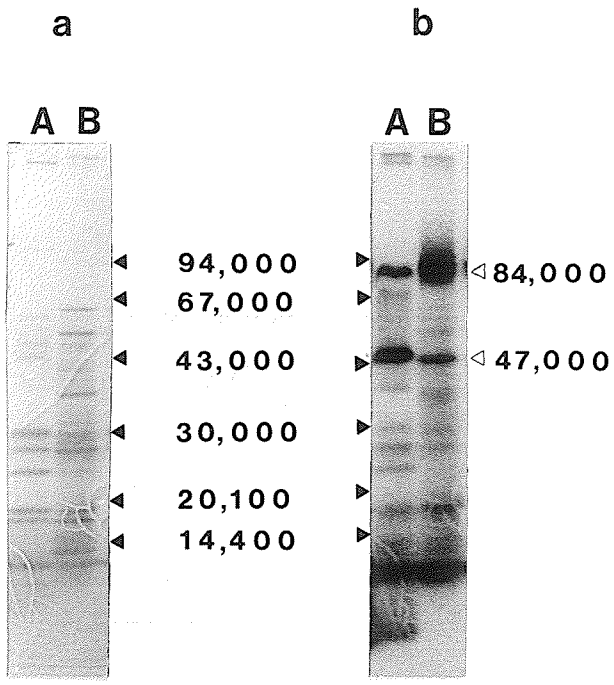
b) Autoradiogram of pooled fractions 2-5 and 9-14. Autoradiography was carried out as described in Materials and Methods.

A. Pooled fractions 2-5

B. Pooled fractions 9-14

Numbers indicate the molecular weights of standards (solid triangles) and iodinated bands specific for developmental cells (open triangles).

Fig. 8



different. This is indicated by the fact that their appearance in the autoradiograms differs in each fraction sample, appearing as diffuse bands in one and fairly sharp bands in the other. As well, the position of banding of the 47,000 molecular weight species was slightly different in fraction samples 2-5 and 9-14. One surprising result was that little or no stained band corresponding to these two heavily iodinated bands appeared in the electropherograms. This suggests that the two species may be glycoproteins which, in some cases, are known to stain poorly, if at all, with coomassie blue.

The purification steps to this point were able to remove most of the fast migrating, unidentified ^{125}I-labeled material. The removal of this material revealed the presence of a small molecular weight protein in fraction samples 2-5 and 9-14 that migrated just in front of the 14,400 molecular weight marker (Fig. 8a) and was heavily iodinated (Fig. 8b). This probably represents one of the smallest proteins that could be iodinated on the surface of M. xanthus. It is not known whether the protein is development-specific.

Since the results suggest that the iodinated proteins were acidic, DEAE-Sephacel ion-exchange chromatography was employed for the purification of the iodinated proteins. Triton X-100 was added to each of the fraction samples (2-5 and 9-14) to give a 2% solution. Sample volumes of 0.5 ml were diluted 1:1 with 0.02 M sodium phosphate buffer (pH 7.0) containing 2% Triton X-100 to give a final volume of 1.0 ml. The samples were then applied to a 3.0 ml bed volume column packed with DEAE-Sephacel equilibrated with the same buffer. The samples were eluted from the column by the addition of a series of progressively higher concentrated sodium phosphate

solutions (pH 7.0) containing 2% Triton X-100. Starting with a 0.02 M solution, solutions with concentrations of 0.2 M, 0.4 M, and 1.0 M were added in succession to produce a stepwise gradient. Fractions (1.0 ml) were collected and measured for radioactivity.

The elution profiles of fraction samples 2-5 and 9-14 from the DEAE-Sephacel column are presented in Figures 9 and 10, respectively. Since the autoradiograms of fraction samples 2-5 (lane A, Fig. 8b) and 9-14 (lane B, Fig. 8b) both exhibited three heavily iodinated bands, i.e., the 84,000 and 47,000 molecular weight species and the protein of low molecular weight (<14,400), and a few faintly iodinated bands, the elution of at least three major peaks of radioactivity from the column would be expected. As shown in Figure 9, only two major peaks of radioactivity were eluted when fraction sample 2-5 was run through the column. The two peaks, fractions 2-4 and 7-11, were eluted by a molarity of 0.02 M and 0.02 to 0.2 M, respectively. In the case of fraction sample 9-14 (Fig. 10), four major peaks of radioactivity were eluted from the column. Two of the four major peaks, fractions 7-10 and 11-16, were eluted by a molarity of 0.02 to 0.2 M and 0.2 M, respectively, while the remaining two peaks (fractions 19-21 and 22-25) were both eluted by a 1.0 M solution. Similarities and differences between the elution profiles of the two fraction samples (Figs. 9 and 10) were apparent. Both fraction samples exhibited the elution of a major peak of radioactivity by a molarity of 0.02 to 0.2 M. This common pattern of elution may reflect the presence of an identical protein species in the two fraction samples. However, the remaining peaks of the two fraction samples did not share common patterns of elution, and thus reflect the differences in

Figure 9. Elution profile from DEAE-Sephacel ion-exchange chromatography of pooled fractions 2-5. Chromatofocusing fractions 2-5 were pooled, concentrated, loaded onto a 3.0 ml bed volume DEAE-Sephacel column, and eluted by a stepwise gradient of sodium phosphate buffer, pH 7.0, (solid line) as outlined in the text. Fractions (1.0 ml) were collected and measured for radioactivity (o——o).

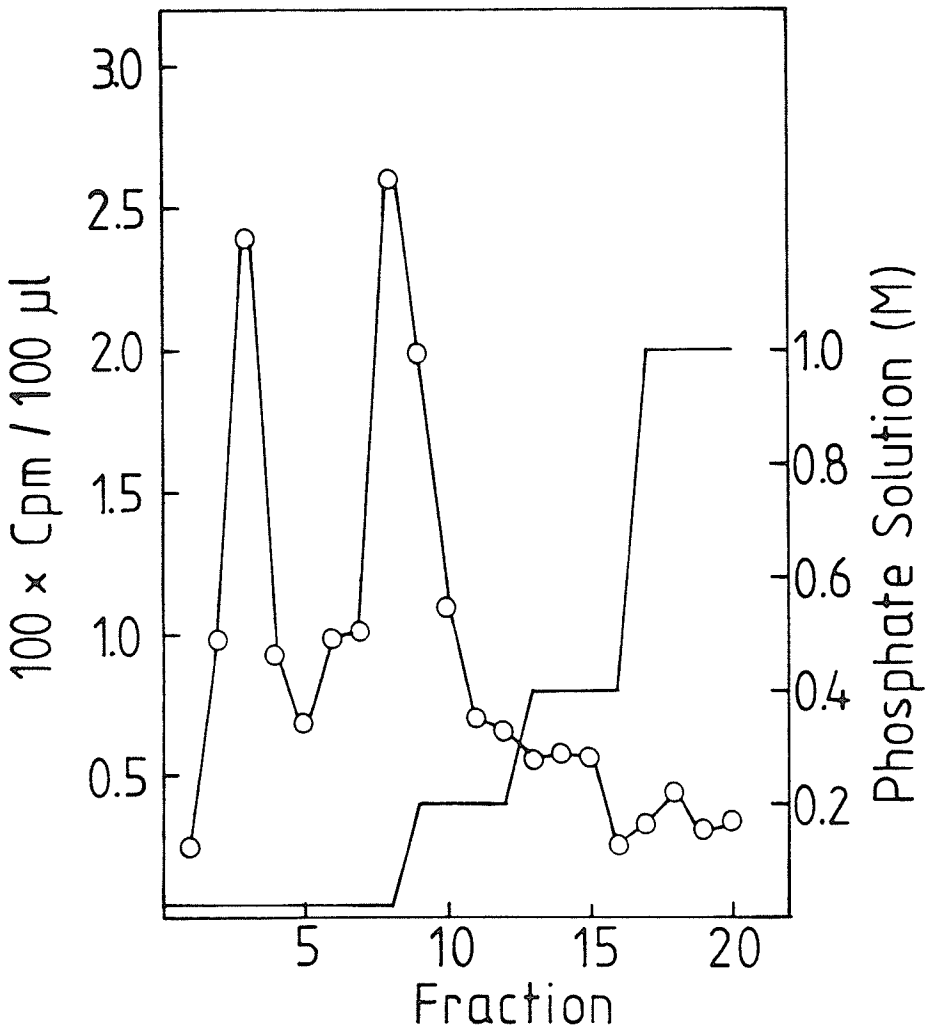
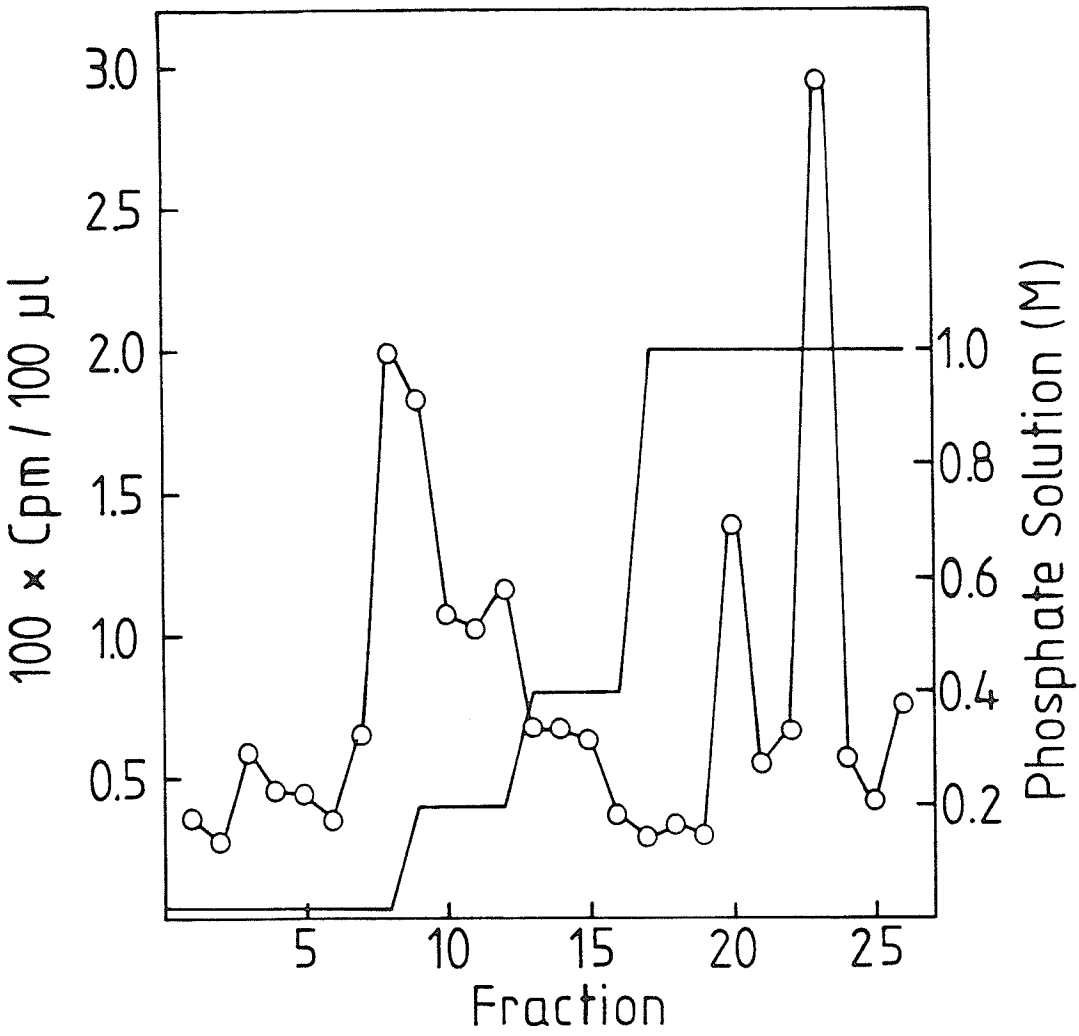

3.0
2.5
2.0
1.5
1.0
0.5
100 × Cpm / 100 µl
1.0
0.8
0.6
0.4
0.2
Phosphate Solution (M)
5
10
15
20
Fraction

Figure 10. Elution profile from DEAE-Sephacel ion-exchange chromatography of pooled fractions 9-14. Open circles represent radioactivity and the solid line the molarity of sodium phosphate used in elution.


3.0
2.5
2.0
1.5
1.0
0.5
100 × Cpm / 100 µl
1.0
0.8
0.6
0.4
0.2
Phosphate Solution (M)
5
10
15
20
25
Fraction

protein content between fraction samples 2-5 and 9-14. Based on the elution profile, it appeared that the DEAE-Sephacel column was capable of separating the iodinated proteins. However, proof of this separation was not obtained since proteins could not be detected by SDS-PAGE or autoradiography in fractions containing radioactivity (not shown). At this stage of purification, the quantity of protein and radioactivity was too low for detection by these procedures.

The results of this procedure indicate that the complete purification of any one of the iodinated development-specific proteins found in the Triton X-100 insoluble fraction was not achieved, but indicates the process may be modified for more successful results.

Extraction of Iodinated Membrane Proteins

Earlier findings revealed that Triton X-100 extraction of the iodinated proteins from crude membranes was unsuccessful (Fig. 2). The effectiveness of other methods of releasing the proteins from the membrane were tested. These methods included extraction by other detergents (CHAPS and SDS) and heating.

CHAPS and SDS Extraction of Iodinated Proteins. ^{125}I-labeled crude membranes were prepared and subjected to Triton X-100 extraction as previously stated (Materials and Methods). The pellet representing the Triton X-100 insoluble fraction (90 mg protein) was resuspended in 10 ml of 10 mM HEPES buffer (pH 7.4) containing 0.5% CHAPS. The suspension was stirred overnight and then centrifuged at 110,000 x g for 1 hour. The supernatant (10 ml) was collected and represented the CHAPS-soluble fraction. The pellet (CHAPS-insoluble fraction) was resuspended in 7.0 ml of 10 mM HEPES buffer (pH 7.4) containing 1.0% SDS and then centrifuged at 27,000 x g for 15 minutes. The

supernatant (7.0 ml) represented the SDS-soluble fraction and the pellet, resuspended in 5.0 ml of 10 mM HEPES buffer (pH 7.4) containing 1.0% SDS, represented the SDS-insoluble fraction. All samples were examined for iodinated proteins by electrophoresis and autoradiography.

An electropherogram (Fig. 11a) shows the effectiveness of the detergent extraction in releasing the proteins. Several proteins were associated with the CHAPS-soluble fraction (lane A, Fig. 11a) indicating that these proteins were solubilized by 0.5% CHAPS. However, several other proteins were not solubilized by 0.5% CHAPS (lane B, Fig. 11a) and remained associated with the CHAPS-insoluble fraction. A few of the CHAPS-insoluble proteins were solubilized by 1.0% SDS (lane D, Fig. 11a), but several others were not (lane C, Fig. 11a). Both the CHAPS and SDS extractions were successful in the removal of several proteins indicating that purification was obtained. Only a few of the proteins (between 8 and 11) were left in the SDS-insoluble fraction (lane C, Fig. 11a).

The effectiveness of the detergent extraction in releasing iodinated proteins is presented in Figure 11b. As shown in the autoradiogram (Fig. 11b), far fewer proteins were iodinated than had previously been detected (Fig. 8b). This indicates that the rapid nature of the procedure may have prevented proteolysis. Of the few iodinated proteins, the majority had already been described, including the four development-specific proteins identified in Figure 1b and the low molecular weight protein (<14,400) identified in Figure 8b. The autoradiogram (Fig. 11b) shows that the 0.5% CHAPS extraction proved to be ineffective in releasing iodinated proteins. Iodinated

Figure 11. a) Electropherogram of the CHAPS and SDS extraction of iodinated membrane proteins (see Text). One-dimensional SDS-PAGE and coomassie blue-staining were carried out as described in Materials and Methods with 100 µg of protein.

b) Autoradiogram of the CHAPS and SDS extraction of iodinated membrane proteins (see Text). Autoradiography was carried out as described in Materials and Methods.

A. CHAPS-soluble fraction

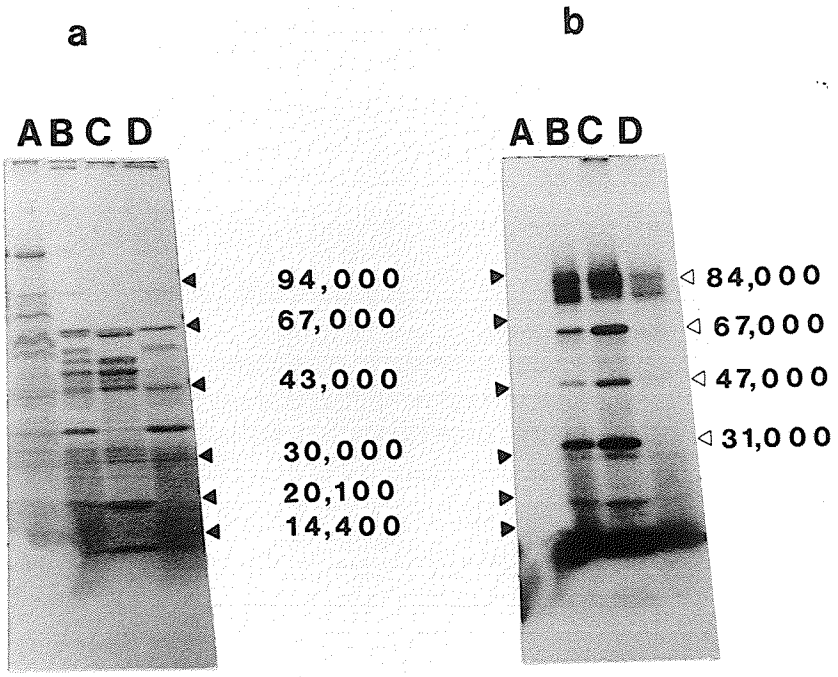
B. CHAPS-insoluble fraction

C. SDS-insoluble fraction

D. SDS-soluble fraction

Numbers indicate the molecular weights of standards (solid triangles) and iodinated bands specific for developmental cells (open triangles).

Fig. 11


a
A B C D
94,000
67,000
43,000
30,000
20,100
14,400
b
A B C D
84,000
67,000
47,000
31,000

proteins were not associated with the CHAPS-soluble fraction (lane A, Fig. 11b), but were associated with the CHAPS-insoluble fraction (lane B, Fig. 11b). 1.0% SDS was also ineffective in extracting the iodinated proteins. As shown in Figure 11b, the majority of the iodinated proteins remained in the SDS-insoluble fraction (lane C), although partial extraction of the 84,000 molecular weight species was achieved (lane D). As noted before (Fig. 8), there was no stained band in the electropherogram (Fig. 11a) which corresponded to the 84,000 band in the autoradiogram (Fig. 11b).

The results of the detergent extraction suggests that iodinated proteins are strongly and tightly bound in the Triton X-100 insoluble fraction.

Extraction of Iodinated Proteins by Heating. A 0.2 ml aliquot of the SDS-insoluble fraction (3.3 mg/ml protein) was diluted 1:3 with 10 mM HEPES buffer (pH 7.4) containing 1.0% SDS to produce a final volume of 0.6 ml. The sample was vortexed and then equally dispensed into three 1.5 ml Eppendorf tubes. The three tubes of sample were placed in a boiling water bath and removed at intervals of 1, 5, and 10 minutes. The samples were then centrifuged for 10 minutes in a Fisher Micro-centrifuge. Supernatants and pellets were then analyzed for iodinated proteins by electrophoresis and autoradiography.

As presented in the electropherogram (Fig. 12a), there were approximately eight proteins in the SDS-insoluble fraction (lane A) as detected by coomassie blue-staining. An autoradiograph of the electropherogram (Fig. 12b) shows that six proteins were iodinated including the four development-specific proteins and the low molecular weight protein (<14,400). Once again, the iodinated band

Figure 12. a) Electropherogram of the extraction of iodinated membrane proteins by boiling. One-dimensional SDS-PAGE and coomassie blue-staining were carried out as described in Materials and Methods with 75 µg of protein.

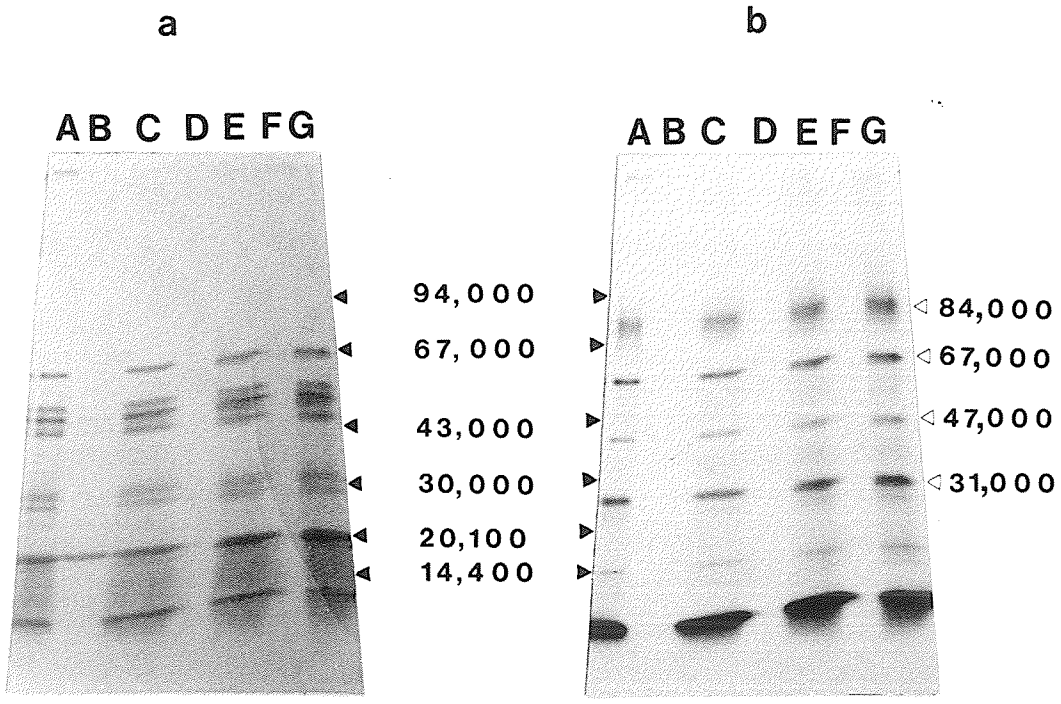
b) Autoradiogram of the extraction of iodinated membrane proteins by boiling. Autoradiography was carried out as described in Materials and Methods.

A. SDS-insoluble fraction
B. 1 minute pellet
C. 1 minute supernatant
D. 5 minute pellet
E. 5 minute supernatant
F. 10 minute pellet
G. 10 minute supernatant

Times indicate duration of boiling before separating into pellet and supernatant fractions by centrifugation (see Text).

Numbers indicate the molecular weights of standards (solid triangles) and iodinated bands specific for developmental cells (open triangles).

Fig. 12


a
b
A B C D E F G
A B C D E F G
94,000
67,000
43,000
30,000
20,100
14,400
84,000
67,000
47,000
31,000

representing the 84,000 molecular weight species was not stained by coomassie blue (Fig. 12a). In addition, the low molecular weight protein (<14,400) had become the most predominant species of iodinated proteins as exhibited by the prominently labeled band in the autoradiogram (Fig. 12b). One minute of boiling was sufficient for extracting all the proteins, with the exception of one protein (lane B, Fig. 12a) which required more than one minute for complete extraction. Nonetheless, these results indicate that boiling in the presence of SDS for a period greater than one minute is an effective method for extracting the iodinated proteins from the SDS-insoluble fraction. The need for such a harsh treatment reaffirms that these iodinated proteins are strongly and tightly bound in the detergent insoluble fractions.

DISCUSSION

DISCUSSION

Many events in the life cycle of M. xanthus are dependent upon cell-cell interactions, particularly development (Historical). As a result, the cell surface must play an integral role in the developmental process. In several studies (Historical) it has been shown that the cell surface is dramatically altered by the production of development-specific proteins. Maeba (1983) reported that a few of the development-specific proteins were accessible to ^{125}I labeling. Since these proteins appear to be exposed to the outer surface of the cell, they may have a possible role in the aggregation of cells during development.

In this investigation, an attempt was made to isolate and purify the development-specific proteins accessible to ^{125}I-labeling. Four development-specific proteins with approximate molecular weights of 84,000, 67,000, 47,000, and 31,000 were prominently iodinated. These proteins probably represent the g (82,000), c (62,000), d (46,000), and e (29,000) proteins identified and described in the study by Maeba (1983).

The non-ionic detergent Triton X-100 was employed in the extraction of the iodinated proteins from the crude membrane. Although most of the membrane proteins were solubilized by Triton X-100, the complete solubilization of the iodinated proteins was not achieved. Faintly iodinated proteins were detected in the Triton X-100 soluble fraction, but it was quite clear that the Triton X-100 insoluble fraction contained the majority of iodinated proteins. This suggested that the iodinated proteins were tightly associated with the insoluble components of the cell envelope, such as peptidoglycan. This was contrary to what was observed in the vegetative cells of M. xanthus. The

iodinated proteins of the vegetative cells were generally Triton X-100 soluble (Maeba, 1986), indicating that the tight association of the iodinated proteins with the cell envelope as found in the developmental cells did not exist. In a period of only 24 hours, the surface proteins accessible to iodination changed from Triton X-100 soluble to Triton X-100 insoluble. Thus, during the early stages of development there were different surface proteins present (Maeba, 1983) and changes in the properties of the cell surface. Morrison and Zusman (1979) proposed that the developmental program consisted of two parallel and independent pathways, i.e., the aggregation pathway and the sporulation pathway, both of which were initiated early in the developmental cycle. Since the aggregation pathway required cell-cell adhesion, the tight association of surface proteins with the cell envelope may be involved in the aggregation phase of development.

Attempts to purify the iodinated development-specific proteins from both the Triton X-100 soluble and insoluble fractions were carried out using column chromatography. Chromatofocusing of the Triton X-100 soluble fraction gave good separation of proteins, but none of the proteins appeared to be iodinated. The results suggested that the iodinated development-specific proteins were not associated with the Triton X-100 soluble fraction in amounts suitable for purification. In the case of the Triton X-100 insoluble fraction, which contained a greater proportion of the iodinated proteins, chromtofocusing proved to be moderately successful. Two chromto-focusing columns eluted over different pH ranges partially separated the iodinated proteins from the unlabeled proteins. However, a phenomenon of some significance was observed with respect to the

elution of iodinated proteins from both columns. All the iodinated proteins were eluted in one peak, suggesting that these proteins were in close association with one another possibly as a protein and/or protein-peptidoglycan complex. The insolubility of the complex in the detergent suggests the latter. Because of this, the columns were not able to separate the individual iodinated proteins on the basis of isoelectric points. Alternatively, the iodinated proteins may have been eluted in "bulk" form simply because the individual proteins share very similar isoelectric points. This would certainly result in the elution of the iodinated proteins within the same pH range. However, two-dimensional gels of the iodinated proteins (Maeba, 1983) indicate that this is highly unlikely.

Further purification by DEAE-Sephacel chromatography suggested that individual iodinated proteins were being separated, but confirmation of this was not obtained due to a lack of detectable protein. As a result, the complete purification of an iodinated development-specific protein from the Triton X-100 insoluble fraction was not achieved after chromatofocusing and DEAE-Sephacel chromatography. The purification was plagued by two problems. First, it was difficult to acquire large amounts of cells for protein purification. An average of 300 plates of developmental cells had to be scraped to yield approximately 30 grams wet weight of cells, much of which was comprised of capsular slime material that did not pack well with centrifugation. Consequently, little protein was available for purification and very little remained at the end of the purification procedure. The second problem was the sometimes inconsistent iodination patterns which impaired the interpretation of the results.

As the results have shown, the iodinated proteins were not solubilized by Triton X-100. Additional attempts to solubilize the iodinated proteins with the detergents CHAPS (0.5%) and SDS (1.0%) proved to be ineffective. Only after boiling the insoluble fraction in the presence of SDS for a few minutes did the iodinated proteins become solubilized. In Escherichia coli and Salmonella typhimurium, the same treatment has been used for the solubilization of the outer membrane proteins from the peptidoglycan complex (Lugtenberg and Van Alphen, 1983). The requirement for such a harsh treatment for solubilizing the iodinated proteins suggests that strong interactions exist between the iodinated proteins and the cell envelope.

Since E. coli and S. typhimurium are also gram-negative, similarities with respect to the outer membrane proteins may exist between these organisms and M. xanthus. A great deal of information has been compiled on the major outer membrane proteins of E. coli and S. typhimurium. Many of these proteins function as transmembrane channels (porins) that permit the selective passage of solutes through the cell envelope. The most extensively studied of these porins are the OmpC, OmpD, OmpF, and LamB proteins. In addition to their porin activity, these proteins also serve as phage receptors, indicating that they are exposed to the outer surface of the cell (Osborn and Wu, 1980; Lugtenberg and Van Alphen, 1983).

The iodinated proteins of M. xanthus are also located on the outer cell surface, suggesting that some of these proteins may be similar to the porins in E. coli and S. typhimurium and may function in the exchange of chemical signals between cells during the aggregation phase of development.

The porins of the enteric bacteria have molecular weights between 34,000 and 44,000 which is in the same range as the 31,000 and 47,000 molecular weight iodinated proteins of M. xanthus. In addition, the M. xanthus developing cell possesses 84,000 and 67,000 molecular weight species. Such high molecular weight proteins are not present in the enteric cell surface. Present in both vegetative and developing cells was a prominently iodinated and stained envelope protein with a molecular weight <14,400. That such a low molecular weight protein should stain so intensely indicates it may be present in large amounts. This suggests the protein may be similar to the 7,000 molecular weight lipoprotein of E. coli, a protein present in highest concentration in these cells, some covalently linked to peptidoglycan (Braun and Rehn, 1969). However, it is not known whether the M. xanthus protein has a lipid moiety associated with it and, as well, the lipoprotein in E. coli was not exposed to the outer surface (Lugtenberg and Van Alphen, 1983).

An interesting aspect of the iodinated proteins was their insolubility in detergents. Successive treatment with three different detergents (Triton X-100, CHAPS, and SDS) failed to solubilize the iodinated proteins, although the 84,000 development-specific protein was partially solubilized by SDS. The previously described E. coli and S. typhimurium porins were strongly but non-covalently bound to the peptidoglycan fraction after the cell envelope had been extracted with SDS (2.0%) at non-denaturing temperatures (e.g., 60°C). For this reason they have been collectively termed the "peptidoglycan-associated" proteins (Osborn and Wu, 1980; Lugtenberg and Van Alphen, 1983). Although the extraction of the iodinated proteins was not carried out in the same manner, the insolubility of the iodinated proteins in three

detergents and the need for the harsh treatment for complete solubilization suggested that these proteins may also fit into the category of "peptidoglycan-associated" proteins.

In conclusion, it is quite apparent that the purification protocol used in this investigation was not successful in completely isolating and purifying any one of the few iodinated development-specific surface proteins in M. xanthus. However, the results obtained with the three detergent extractions and boiling treatment were most promising. A large proportion of protein was removed from the iodinated proteins in the course of the extractions such that these steps may be of some benefit when incorporated into the previous protocol for a future attempt at purifying the development-specific surface proteins.

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