UNIVERSITY OF MANITOBA

STRUCTURAL STUDIES ON *RHODOSPIRILLUM RUBRUM* AND *RHODOPSEUDOMONAS PALUSTRIS* CELL-FREE PROTEIN SYNTHESIZING SYSTEMS

by

SUDARSHAN DEVARASHETTY

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements for the
Degree of
Master of Science

Department of Microbiology
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ABSTRACT

Several protein-containing fractions, including the total, the PS-60, the S-100 and the ribosome fractions, have been prepared from both phototrophically and heterotrophically grown Rs. rubrum and Rp. palustris cells. Analyses by isoelectric focusing polyacrylamide gel electrophoresis of the first three fractions as well as the ^{35}S-labeled Rs. rubrum S-100 fractions have revealed that there are no significant differences between the phototrophic and the heterotrophic samples, however, the latter may contain a higher content of acidic proteins.

In addition to the isoelectric focusing PAGE system, the ribosomal proteins have also been analyzed by the SDS and the two-dimensional PAGE systems in which similar number of protein bands (about 18 in SDS gels) or protein spots (about 32 in two-dimensional gels) has been observed for both phototrophic and heterotrophic Rs. rubrum or Rp. palustris samples. The intensity of several acidic protein bands is, however, much higher in the heterotrophic ribosome samples than in the phototrophic ribosome samples, as demonstrated in the isoelectric focusing gels. The possible association of the higher quantity of acidic proteins in the heterotrophic ribosomes and the generally higher protein-synthesizing activity of the heterotrophic bacterial ribosomes will be discussed.

To my brother-in-law, Hanmiah

my sister, Bharathi

and

my fiancee, Sitara

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LIST OF ABBREVIATIONS

A_{260}	absorbance at 260 nm
dpm	disintegrations per minute
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
EDTA	ethylenediamine tetraacetic acid
mRNA	messenger RNA
PAGE	polyacrylamide gel electrophoresis
PMSF	phenylmethyl sulfonylfluoride
pI	isoelectric point
RNA	ribonucleic acid
RNase	ribonuclease
SDS	sodium dodecyl sulfate
S	Svedburg's unit
TCA	trichloroacetic acid
Tris	tri (hydroxymethyl) aminomethane
TEMED	N, N, N', N' - tetramethylene diamine

H I S T O R I C A L

AND

I N T R O D U C T I O N

The phototrophic bacteria are represented by a large number of species of different morphology, pigmentation and physiological-biochemical properties. These bacteria are encountered in almost every body of water as well as in the soil, but they are most abundant in stagnant bodies of water where organic material is undergoing decay and hydrogen sulfide is present.

The terminology and classification of phototrophic bacteria have changed considerably in recent years. Prior to 1974, the phototrophic bacteria were placed in the sub-order Rhodobacterineae of the order Pseudomonadales, with three families, i.e., Thiorhodaceae (the purple sulfur bacteria), Athiorhodaceae (the purple non-sulfur bacteria), and Chlorobacteriaceae (the green bacteria). However, according to the more recent classification system, as described in the eighth edition of Bergey's manual of Determinative Bacteriology (Pfenning and Trüper 1974), all phototrophic bacteria with the exception of Cyano-bacteria have been placed in the order Rhodospirillales which is described into two suborders, the Rhodospirillineae and the Chlorobiineae. These two suborders can be easily distinguished on the basis of the physical location of their photosynthetic pigments. In the Rhodospirillineae, the

pigments are always located in the intracytoplasmic membrane system which is continuous with the cytoplasmic membrane, whereas members of Chlorobiineae have their pigments located in non-unit-membrane bound, cigar-shaped organelles, known as "chlorobium vesicles", that underlie the cytoplasmic membrane.

Based on the ability to use elemental sulfur as an electron donor, the suborder Rhodospirillineae is further divided into two families, the Rhodospirillaceae, formerly Athiorhodaceae, and the Chromatiaceae, formerly Thiorhodaceae. As suggested by their old but more descriptive names members of the former and the latter families are, respectively, unable and able to use elemental sulfur. The second suborder Chlorobiineae also consists of two families, the Chlorobiaceae, formerly Chlorobacteriaceae and the recently discovered Chloroflexaceae (Trüper 1974). The species of Chloroflexaceae are differentiated from those of the Chlorobiaceae by their filamentous, flexible appearance and by their gliding motility on solid surfaces.

The metabolic activity exhibited by the Rhodospirillaceae members differs from the rest of the phototrophic bacteria in their preference for photoorganotrophic growth (Pfenning and Trüper 1971). Most strains in this family require one or more vitamins as growth factors and are unable to grow with sulfide as the sole photosynthetic electron donor. Furthermore, none of them can utilize elemental sulfur as an electron donor. Their internal

photosynthetic membrane system is continuous with the cytoplasmic membrane and is vesicular, lamellar or tubular in appearance. Their storage materials include polysaccharides, poly-β-hydroxy butyrates and polyphosphates.

Certain phototrophic bacteria of the family Rhodospirillaceae afford an unusual opportunity for the study of the molecular events involved in the control mechanism of gene expression, because they are able to grow both phototrophically and heterotrophically, and during such physiological conversions different proteins are produced. According to the current models, protein synthesis in bacteria is believed to be regulated primarily at the level of transcription. The properties of the messenger RNA species in facultative phototrophs, mainly Rs. rubrum and Rp. sphaeroides, have therefore been investigated. Using DNA-RNA hybridization competition techniques which detect differences in RNA base sequences, Yamashita and Kamen (1968), and later Chow (1976a) have demonstrated that there is little qualitative difference between the RNA species of the phototrophically and the heterotrophically grown Rs. rubrum. In another report, Yamashita and Kamen (1969) have shown that after the transfer from heterotrophic to phototrophic growth conditions, light exerted a stimulatory effect on uracil incorporation in Rs. rubrum cultures and from the pulse and chase results, they have also concluded that this light stimulating effect is associated with the synthesis of chromatophores and bacteriochlorophylls. Based on these

findings, the above investigators have suggested that the regulation of protein synthesis in Rs. rubrum is probably at the translational rather than at the more widely accepted transcriptional level. Similar proposals have been made for another facultative phototroph, Rp. sphaeroides, by Witkin and Gibson (1972).

The concept of translational control has also been proposed for many other groups of microorganisms, including E. coli (Shine and Dalgarno, 1974), Staphylococcus aureus (Martin and Landolo, 1975) and Bacillus subtilis (Legault-Demare and Chambliss 1975, Guha and Szulmajster, 1977). The most interesting findings are, perhaps, in the differentiating, spore-forming Bacillus species. From several antibiotic-resistant mutants of B. subtilis 168, Graham and Bott (1975) have isolated a sub-class of mutants that is conditionally asporogenous. Mutants of this sub-class are resistant to erythromycin, kanamycin, spectinomycin and streptomycin during vegetative growth but become sensitive to these antibiotics after initiation of sporulation. Their results have demonstrated that this antibiotic sensitivity is due to alterations in the structure of the 30 S and the 50 S ribosomal subunits in sporulating cells. This conclusion has also been supported by the results of many other investigators (Smith et al., 1969; Kimura et al., 1972; Hanson and Cocoran, 1969; Tanaka et al., 1973), especially by Fortnagel's group (1973 and 1975) who have demonstrated differences in electrophoretic mobilities

between vegetative and sporulating B. subtilis ribosomal proteins. Another interesting finding has recently been reported by Tipper et al (1977). Using erythromycin resistant and temperature sensitive B. subtilis mutants, they have shown that these cells are not able to sporulate at elevated temperatures, and, at the same time, their 50 S ribosomal protein L17 is either missing or shows an altered electrophoretic mobility. Furthermore, the ribosomes of these mutants bind to erythromycin at a rate much lower (less than 1%) than those of the wild-type cells. Ribosomes of the Spo^+ revertants have, however, regained the original L17 protein and the erythromycin-binding rate. From these results, they have concluded that the ribosomal protein L17 has a special function in sporulation.

On the other hand, Guha et al (1975) have obtained some different results when analyzing the ribosomal proteins of vegetative and sporulating B. subtilis 168 M cells by two-dimensional acrylamide gel electrophoresis techniques. They have identified 22 protein species in the 30 S subunits and 28 protein species in the 50 S subunits in both the vegetative and the sporulating cells and these proteins are all identical in their electrophoretic mobility. The discrepancy between these sets of results is, at present, difficult to explain, however according to Guha et al (1975), it may result from the protease activity in the sporulating cells and the different techniques employed in preparing the ribosomes.

As mentioned earlier, translational control has also been proposed for Rs. rubrum (Yamashita and Kamen, 1968 and 1969, Chow, 1976a) and Rp. sphaeroides (Witkin and Gibson, 1972). Thus far most of the studies on the phototrophic bacterial ribosomes have been concentrated on the determination of their sedimentation constants. According to Taylor and Stork (1964), the ribosomes of Rs. rubrum, Rp. palustris, Rp. sphaeroides and Rp. gelatinosa have a sedimentation constant of 66 S, and the subunits of Rp. palustris (Bhatnagar and Stachow, 1972) and Rp. sphaeroides (Friedman et al, 1966) ribosomes are of 29 S and 46 S types. Preliminary analyses of the purified phototrophic Rp. palustris 29 S and 46 S ribosomal subunit proteins by urea-polyacrylamide gel electrophoresis have revealed 23 protein bands for the former and 28 protein bands for the latter (Bhatnagar and Stachow, 1972). Examination of the Rp. palustris ribosomes by sucrose gradient sedimentation techniques (Mansour and Stachow, 1975) has shown that structural changes in ribosomal subunits occur after shifting the cells from heterotrophic growth conditions to phototrophic growth conditions. From these results, Mansour and Stachow (1975) have concluded that Rp. palustris cells contain two distinct populations of ribosomes, one for the phototrophic cells and the other for the heterotrophic cells. Detailed comparison between these two ribosomal populations and their proteins has, however, not been made.

In this study, we have analyzed the ribosomal proteins of both phototrophic and heterotrophic Rs. rubrum and Rp. palustris cells by several different electrophoresis systems. Similar studies on the bacterial S-100 and PS-60 fractions have also been carried out in attempt to achieve a better understanding of the protein synthesizing system and the possible translational control mechanisms in these cells.

MATERIALS AND METHODS

Organisms

The organisms used throughout this study were as follows:

1. Rhodospirillum rubrum strain VF, a varient strain isolated in this laboratory.
2. Rhodopseudomonas palustris ATCC 17002, obtained from Dr. S. Kaplan of the University of Illinois.
3. Escherichia coli B, a stock culture from the Department of Microbiology, University of Manitoba.

Growth Media

a) The growth medium for Rs. rubrum was of the following composition (Chow 1976a):

l - Glutamic acid	4.0 g
dl - Malic acid	3.5 g
Sodium citrate	0.8 g
$MgSO_4.7H_2O$	0.2 g
KH_2PO_4	0.12 g
K_2HPO_4	0.18 g
$CaCl_2$	33.0 mg
Biotin	5.0 µg
Yeast extract	2.0 g

Distilled, deionized water up to 1000.0 ml

The pH was adjusted to 6.8 with a saturated solution of sodium hydroxide. This medium will hereafter be referred to as the A1 medium in this thesis.

b) The medium used to grow Rp. palustris was a modified, semisynthetic medium of Lascelles (1956) with the following composition:

l - Glutamic acid	1.47 g
dl - Malic acid	2.70 g
KH_2PO_4	0.50 g
K_2HPO_4	0.50 g
$(NH_4)_2HPO_4$	0.80 g
$MgSO_4.7H_20$	0.20 g
$CaCl_2.2H_20$	53.00 mg
$MnSO_4.7H_20$	0.85 mg
$FeSO_4.7H_20$	2.78 mg
Na_3 Nitriloacetic acid	1.00 mg
Thiamine HCl	1.00 mg
Nicotinic acid	1.00 mg
Biotin	10.00 µg
Yeast extract	2.00 g

Distilled, deionized water added up to 1000.0 ml

The pH was adjusted to 6.8 with a saturated solution of sodium hydroxide. This medium will hereafter be referred to as the B2 medium in this thesis.

c) Escherichia coli was grown in the casamino acids (0.2%) - minimal salts - glucose medium of Kurland (1966), containing:

KH_2PO_4	3.0 g
$Na_2HPO_4.H_20$	6.8 g
NH_4Cl	2.0 g
Casamino acids	2.0 g
$MgCl_2.6H_20$	0.2 g
Glucose	5.0 g

Distilled, deionized water up to 1000.0 ml

The pH was adjusted to 7.4 with a saturated solution of sodium hydroxide.

Solid agar media were prepared by adding 15 g to Bacto Agar in every liter of the above liquid media.

Buffers

1. Standard Buffer (Modelell, 1971): This buffer was used for preparations of various fractions of the in vitro protein-synthesis system and had the following composition:

10 mM	Tris-HCl pH 7.8
60 mM	NH_4Cl_2
10 mM	Mg-Acetate
6 mM	2-Mercaptoethanol

2. KCl-PMSF Standard Buffer (Legualt-Demare, L., and G.H. Chambliss, 1974; Chow, 1976b): This buffer was used for washing Rs. rubrum and Rp. palustris cells. It contained 3.54 mM of PMSF and 1.0 M KCl in the standard buffer.

A number of other buffers have also been used in this study, mainly for gel electrophoretic analyses, and will be described in later sections.

Growth Conditions

Both Rs. rubrum and Rp. palustris were routinely subcultured as stab cultures every seven days in the phototrophic mode of growth (Chow, 1976a), because there was a less chance of contamination when the cultures were maintained under semi-anaerobic conditions. The stab cultures were incubated in five inch screw capped tubes in a Conviron Model E7 incubator which was equipped with both incandescent and fluorescent lamps. Growth was allowed to proceed at 28°C for two to three days after which time the tubes were stored in a refrigerator at 4°C.

To grow these organisms phototrophically in large quantities, the following procedure was used: a needleful of bacteria was inoculated into a five inch screw capped tube filled with the liquid medium up to the neck; the tube was incubated in the light chamber at 28°C until the cells had reached mid-logarithmic phase of growth which was determined by measuring optical density (about 200 Klett units). The mid-logarithmic cultures were then transferred as an inoculum (7% v/v) into one-liter Roux bottles filled with about 700 ml of medium. The Roux bottle cultures were similarly incubated and at

mid-logarithmic phase they were transferred into 10 or 15 liter glass carboys. The cultures in the carboys were again incubated under the phototrophic condition until mid-logarithmic phase of growth.

For heterotrophic growth, the initial inoculating cultures were prepared by the same way as described above, i.e., growing in five inch screw capped tubes under phototrophic condition. However, these tube-grown cultures were transferred into one-liter flasks containing 200 ml of medium instead of into Roux bottles. These flasks were wrapped with aluminum foil to prevent exposure to light and were shaken on a shaker in a dark 28°C incubation room until the culture had adapted to heterotrophic growth. When the cultures had reached mid-logarithmic phase of growth (about 130 Klett units), they were used to inoculate 10 or 15 liter glass carboys as a 3% (v/v) inoculum. Similarly, these carboys were wrapped with aluminum foil and incubated with vigorous aeration in a dark 28°C incubation room until mid-logarithmic phase.

Escherichia coli cultures were grown under the standard conditions and were harvested when they had reached an optical density about 200 Klett units.

Harvesting Cells

All cultures were harvested in mid-logarithmic phase of growth. The cells were collected by centrifugation

in a Sharples steam-driven centrifuge at 40 lbs/in.^{2} and at a flow rate of 200 ml/min. The packed cells were first washed in the standard buffer by centrifugation at 12,000 x g for 10 minutes. Thus prepared bacterial pellets were weighed and stored at -76^{o}C. The average yields (wet weight) of various bacterial cultures were:

Phototrophic	Rs. rubrum	1.0	g/l
Heterotrophic	Rs. rubrum	1.2	g/l
Phototrophic	Rp. palustris	1.3	g/l
Heterotrophic	Rp. palustris	1.5	g/l
	E. coli	2.5	g/l

Preparation of S-30, PS-60, S-100 and ribosome fractions

All the following extraction and purification steps were carried out at 4^{o}C. Frozen cell pellets were thawed slowly and ground with 2X (w/w) of alumina until the mixture was homogeneous. The pastes were extracted with 2 volumes of the standard buffer. The alumina and cell debris were then sedimented twice by centrifugation at 27,000 x g for 20 minutes. Thus obtained supernatant fluids were the S-30 fractions.

The PS-60 and S-100 fractions were prepared by the procedure of Chow (1976 c) with some minor modification. The PS-60 pellets were obtained by centrifuging the S-30 fraction at 60,000 x g/50 minutes and the pellets were re-

suspended in small amounts of the standard buffer. Subsequently the supernatants were further centrifuged at 105,000 x g/2 hours and the top two-thirds of the supernatants were carefully aspirated, without disturbing the bottom layers of the fluids, and these samples were the S-100 fractions. All S-30, PS-60 and S-100 samples were stored in small aliquots at -76°C.

Ribosomes were purified from the S-30 extracts by the ammonium sulfate precipitation method of Kurland (1966) with the omission of the puromycin step. The final ribosomal pellets were resuspended in the standard buffer at a concentration of 300 A_{260} units per milliliter, divided into small aliquots, and stored at -76°C.

Extraction of Ribosomal Proteins

Ribosomal proteins were extracted by the acetic acid method of Hardy et al (1969). To one volume of ribosome samples, one-tenth volume of 1.0 M $MgCl_2$ and two volumes of glacial acetic acid were added in rapid succession with constant stirring. After stirring for 45 minutes in an ice bath, the mixture was centrifuged at 20,000 x g for 10 minutes. The RNA pellet thus obtained was washed with 67% acetic acid and recentrifuged at 20,000 x g for 10 minutes. The two supernatants which contained most of the ribosomal proteins were pooled together into which ammonium sulphate was added to a final concentration of 42% (w/v). The

sample was stirred at 4°C for 10 minutes and centrifuged at 25,000 x g for 10 minutes. The ribosomal protein pellet was resuspended in a very small amount of the standard buffer and after extensive dialysis against the standard buffer the sample was stored at -20°C.

Isoelectric Focusing-Polyacrylamide Gel Electrophoresis (PAGE) System of O'Farrell (1975) For Total (T), PS-60 and S-100

In the isoelectric focusing PAGE system two buffers were used for preparation of samples:

a) Sonication Buffer

10 mM Tris-HCl pH 7.4

5 mM $MgCl_2$

50 µg/ml Pancreatic RNase

b) Lysis Buffer

9.5 M Urea

2% (v/v) Ampholines (a mixture of 1.6% pH range 5 to 7 Ampholine and 0.4% pH range 3.5 to 10 Ampholine)

5% (v/v) 2-Mercaptoethanol

Preparation of Samples

One gram (wet weight) of bacterial cell pellet was mixed with 3 ml of the Sonication buffer and the mixture was sonicated in an Insonator, Model 1000 at a power setting of 2.5 for 6 x 30 seconds followed by a further

sonication step of 60 seconds. To the sonicated sample, pancreatic DNase was added to a final concentration of 50 µg/ml. This sample contained all of the bacterial proteins and was therefore called the total (T) fraction. A portion of the T-fraction was used to prepare the PS-60 and the S-100 fractions by the previously described high speed centrifugation technique. For preparation of isoelectric focusing samples, 500 mg of urea and 0.5 ml of the Lysis buffer were added to 0.5 ml of the total, the PS-60 or the S-100 fractions. The samples were either analyzed immediately or stored at -20°C.

Isoelectric Focusing Polyacrylamide Gels

Isoelectric focusing polyacrylamide gels with the following composition were prepared:

Urea	9 M
Acrylamide (w/v)	3.78%
Bis-acrylamide (w/v)	0.22%
Triton N-101 (v/v)	2.0%
Ampholine pH 5-7	1.6%
Ampholine pH 3.5-10	0.4%

The final gel solution contained a total of 4% acrylamide and was polymerized by adding 3.3 µl of a freshly prepared 10% ammonium persulfate solution and 3 µl of TEMED into every 10 ml of the gel solution. The gel tubes were prepared by adding 3.3 ml of the gel mixture into an acid washed glass

tube (130 x 6 mm, inner diameter). To flatten the gel surface, a small amount of 8 M urea solution was overlayed on top of the gel mixture and polymerization was allowed to proceed at room temperature for 1-2 hours. After polymerization, the overlaying urea solution was replaced by 20 µl of the Lysis buffer and the gels were let to stand for another period of 1-2 hours.

The polymerized gels were then electrophoresed without samples at 200 volts/15 minutes, 300 volts/30 minutes and 400 volts/30 minutes (the prerun step) in a Buchler Electrophoresis Apparatus with the anode chamber filled with 10 mM phosphoric acid and the cathode chamber filled with extensively degassed 20 mM NaOH. After the prerun, the gels were then loaded with protein samples, on top of which 10 µl of sample overlay solution (9 M urea, 1% Ampholines) was overlayed. The anode and the cathode chambers were again filled with fresh 10 mM phosphoric acid and 20 mM NaOH solutions, respectively, and electrophoresis was carried out at a constant voltage of 400 volts for 20 hours.

On the completion of isoelectric focusing, the gels were rimmed out, soaked in 10% trichloroacetic acid for 10 minutes and stained in a solution containing 0.2% coomassie Brilliant Blue, 25% ethanol and 10% acetic acid for 30 minutes. The stained gels were then destained and stored in 10% acetic acid solution.

For measurement of the pH gradient, the isoelectric focusing acrylamide gel was washed in water and cut into 5 mm sections after the prerun step. These sections were placed in individual scintillation vials with two milliliters of freshly prepared 9.2 M urea in degassed water. The vials were capped and shaken for 15 minutes; then the pH was measured.

Polyacrylamide Gel Electrophoresis of Ribosomal Proteins

In the present study, three PAGE systems had been used.

A. Isoelectric Focusing System of O'Farrell (1975)

Ribosomal protein samples prepared by the method of Hardy et al (1969) were mixed with an equal volume of the Lysis buffer and analyzed by the previously described methods.

B. The SDS PAGE System

The discontinuous SDS PAGE system of Laemmli (1970) modified by O'Farrell (1975) had also been used in this study for separating ribosomal proteins. Several buffers were used (O'Farrell, 1975):

a) Lower Gel Buffer: this buffer was used for the preparation of the lower running gel mixture. It contained

1.5 M Tris-HCl, pH 6.8 and 0.4% (w/v) SDS.

b) Upper Gel Buffer: this buffer was used for the preparation of upper stacking gel mixture and contained 0.5 M Tris-HCl, pH 6.8 and 0.4% (w/v) SDS.

c) SDS Running Buffer: this buffer was used as an electrode buffer in both the anode and the cathode chambers, it contained

25 mM Tris

0.192 M Glycine

0.1% (w/v) SDS

The slab gels contained a lower running gel and an upper stacking gel.

Composition of the Lower Running Gel

Lower gel buffer	12.5 ml
Acrylamide (w/v)	10.95%
Bis-acrylamide (w/v)	0.3%
Distilled, deionized water up to	50.0 ml
Freshly prepared 10% ammonium persulfate	0.17 ml
TEMED	25 µl

Composition of the Upper Stacking Gel

Upper gel buffer	2.5 ml
Acrylamide	4.38%
Bis-acrylamide	0.62%
Distilled, deionized water up to	10 ml
Freshly prepared 10% ammonium persulfate	30 µl
TEMED	10 µl

Both the lower running and the upper stacking gels were degassed prior to the addition of TEMED. The polymerized gel slabs had a thickness of 1.5 mm and a length of 12 cm of which 10.5 cm were the lower running gel and 1.5 cm were the upper stacking gel. The apparatus used for electrophoresis was purchased from the Medical Research Apparatus Corporation (Clear Water, U.S.A.). Ribosomal protein samples (Hardy et al, 1969) were loaded into 1.5 mm sample wells on top of the upper stacking gel together with a small amount of 0.1% bromophenol blue solution which was used as a tracking dye. Electrophoresis was carried at a constant current of 20 mA/gel slab. After electrophoresis, the gels were stained and destained by the same method as described earlier.

C. Kaltschmidt and Wittmann's Two-Dimensional PAGE System For Ribosomal Proteins (1970)

In this system, ribosomal protein samples prepared by the acetic acid method (Hardy et al 1969) were dialyzed against distilled water, concentrated by lyophilization and then analyzed by two successive steps.

i) The First Dimension

Two types of acrylamide gels were required in this system:

a) Sample gels:

Acrylamide	0.4 g
Bis-acrylamide	0.02 g
EDTA-Na_2	8.5 mg
Boric acid	32.0 mg
Urea	4.8 g

Distilled, deionized water up to 10.0 ml

The pH was adjusted to 8.6 with HCl.

b) Separation gel:

Acrylamide	0.6 g
Bis-acrylamide	0.02 g
EDTA-Na_2	0.12 g
Boric acid	0.48 g
Tris	0.73 g

Urea 5.4 g

Distilled, deionized water up to 15.0 ml

The pH was adjusted to 8.6 with HCl.

To polymerize these gels, 5 µl of 10% freshly prepared ammonium persulfate and 4 µl of TEMED were added into 10 ml of gel mixture.

For analysis, an acid-washed glass tube (130 x 6 mm inner diameter) was first filled with the separation gel up to 50 mm. The gel was allowed to polymerize at room temperature in the presence of a small amount of overlaying distilled water. After polymerization, a mixture of 1.5 mg of ribosomal proteins dissolved in 0.2 ml of the sample gel was overlayed on top of the flat surface of the separation gel, and allowed to polymerize again. Finally the gel tube was filled with the separation gel up to the top and polymerization of the gel proceeded as described above. Separation of ribosomal proteins in thus prepared gels was carried out at a constant current of 3mA/gel tube for 20 hours in a Buchler Electrophoresis Apparatus.

On the completion of electrophoresis, the gels were rimmed out and equilibrated in an equilibration buffer (8 M urea, 0.07% glacial acetic acid, 0.012 N KOH, pH 4.6) for two hours. The gel samples were then ready for analysis by the second dimension PAGE system.

The electrode buffer used in this system, for both the anode and the cathode chambers, contained in every 1500 ml the following components:

2.0 N NaOH	24.0 ml
EDTA-Na_2	3.6 g
Boric acid	14.4 g
Tris	21.8 g

Distilled, deionized water up to 1500.0 ml

The pH was adjusted to 8.6 with HCl.

ii) The Second Dimension

The acrylamide gel used in this system has the following composition:

Acrylamide	26.0 g
Bis-acrylamide	0.72 g
Glacial acetic acid	7.6 ml
5.0 N KOH	1.4 ml
Distilled, deionized water up to	140.0 ml

After adjusting the pH of the gel solution to 4.6 with HCl, 2.0 ml of 30% freshly prepared ammonium persulfate solution and 0.3 ml of TEMED were added to 140 ml of the gel solution for polymerization, and slab gels (194 x 182 x 1.5 mm) were prepared in an apparatus similar to that described by Kaltschmidt and Wittmann (1970).

The previously described first dimension gels, after equilibration, were placed on top of the gel slab, and electrophoresis was carried out at a constant current of 33 mA/gel slab for 20 hours. After completion, the slabs were stained with 0.6% amido black in 5% acetic acid for 15 minutes with occasional agitation and destained in 1% acetic acid for several days. The electrode buffer in the second dimension electrophoresis has the following composition:

Glycine	14 g
Glacial acetic acid	1.5 g
Distilled, deionized water up to	1000.0 ml

The pH was adjusted to 4.6 with NaOH

Protein Determination

Protein was determined by the method of Lowry *et al* (1951) with crystalline lysozyme as the standard.

Labeling of *Rs. rubrum* Proteins with ^{35}Sulfur

Rhodospirillum rubrum (VF) cells were labeled with ^{35}S in a modified Al medium in which 0.8 M $MgSO_4$ was replaced by 0.8 M $MgCl_2$. Phototrophic cells were grown in five inch screw capped tubes and heterotrophic cells were cultured in 125 ml Erlenmyer flasks. Under both conditions,

cells were grown in 10 ml of medium containing 0.5 mCi of carrier-free $^{35}SO_4^{=}$. After 40 hours of incubation, the labeled cells were harvested and washed with the standard buffer and the KCl-PMSF standard buffer, as described in the previous section.

Two-Dimensional Electrophoresis of ^{35}S-labeled S-100 Fraction

By the same method, as described earlier in the isoelectric focusing PAGE system, ^{35}S-labeled S-100 fractions were prepared. Under our conditions the phototrophic and the heterotrophic Rs. rubrum S-100 samples had about 10×10^6 and 9×10^6 d.p.m. per 500 µl, respectively. These samples (2×10^6 d.p.m. per load) were analyzed by the combination of the isoelectric focusing PAGE system (for the first dimension) and the SDS PAGE system (for the second dimension), as described by O'Farrell (1975). After the second dimension electrophoresis, the gel slabs were wrapped with Saran Wrap and covered with Kodak X-O mat RP films. Autoradiographs were prepared by the standard methods after an exposure period of 96 hours.

Chemicals

The following chemicals were purchased from the Sigma Chemical Company: l-glutamic acid, dl-malic acid, lysozyme (egg white), nicotinic acid, thiamine hydrochloride,

biotin, 2-mercaptoethanol, DNase I (from bovine pancreas), pancreatic RNase, trizma base, glycine, coomassie brilliant blue.

Acrylamide, bis-acrylamide and TEMED were purchased from the Eastman Kodak Company. The ultra pure ammonium sulfate and urea were from the Schwarz-Mann, Division of Becton, Dickinson and Company. The yeast extract was from the Difco Laboratories, SDS from the Fisher Scientific Company, amido black 10 B from the EM Reagents and the ampholines from the LKB.

RESULTS

Microorganisms and Their Fractions

Under our experimental conditions, the mean generation time for Rhodospirillum rubrum VF and Rhodopseudomonas palustris El was about 6.5 hours. From each liter of exponential cultures, we were able to harvest 1.0 - 1.5 grams of bacterial cells which were used for preparations of various fractions by the described methods. These fractions were generally pigmented and a detailed description of them is listed in Table 1.

Analysis of Proteins in Total (T), PS-60 and S-100 Fractions by Isoelectric Focusing Polyacrylamide Gel Electrophoresis Techniques

The principle of isoelectric focusing is rather simple: individual proteins will migrate to and focus at their own specific isoelectric point when electrophoresed in a preformed pH gradient consisting of a mixture of low molecular weight carrier ampholytes (Vesterberg, 1971). This technique has been proven to be extremely useful as an analytical tool for separation and characterization of proteins (O'Farrell, 1975) and has therefore been used in this study for analyzing various bacterial fractions which will be discussed below in separate sections.

Figure 1 shows the pH gradient in our gel system which, after electrophoresis, covered a pH range from 5.75 to 7.95, a result very similar to the published data of O'Farrell (1975).

Table 1. General properties of various Rs. rubrum and Rp. palustris fractions[a].

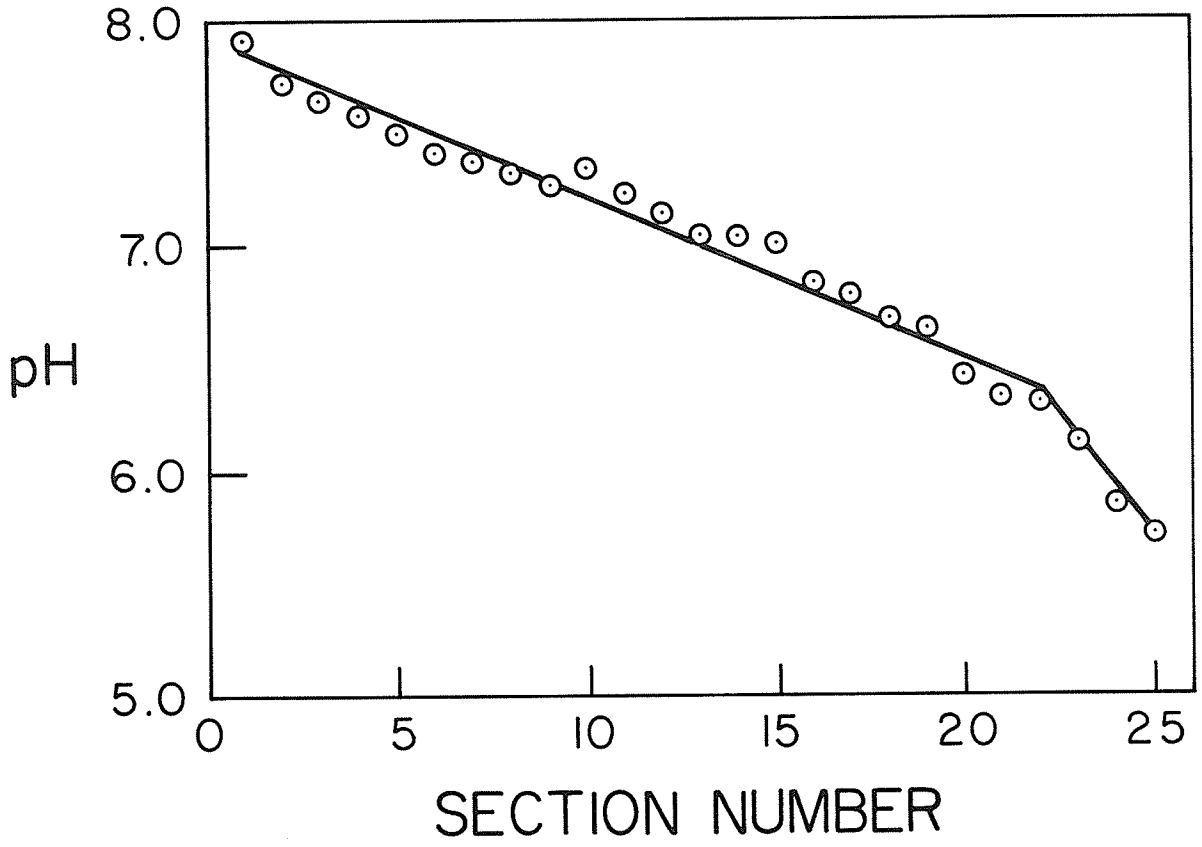
	P-Rs. rubrum	H-Rs. rubrum	P-Rp. palustris	H-Rp. palustris
Recovery per 10 g cells				
T (Total) fraction	30 ml	30 ml	30 ml	30 ml
PS-60 fraction	1 - 1.5 ml	1 - 1.5 ml	1 - 1.5 ml	1 - 1.5 ml
S-100 fraction	20 ml	20 ml	20 ml	20 ml
Ribosomal proteins	8.0 mg	8.6 mg	8.8 mg	8.4 mg
Color and pigment content/ml[b]				
T-fraction	deep red, 0.211 mg	beige pink,0.124 mg	deep red, 0.037 mg	beige pink, 0.022 mg
PS-60 fraction	deep red, 0.292 mg	beige pink,0.149 mg	deep red, 0.094 mg	beige pink, 0.042 mg
S-100 fraction	pink, 0.004 mg	yellow, 0.001 mg	pink, 0.0004mg	yellow, 0.0001mg
Ribosomal fractions	pink, 0 mg	milky white, 0 mg	milky white, 0 mg	milky white, 0 mg
Protein content/ml[c]				
T-fraction	25.0 mg	24.0 mg	20.0 mg	20.0 mg
PS-60 fraction	40.0 mg	38.0 mg	38.0 mg	35.0 mg
S-100 fraction	10.8 mg	8.9 mg	5.5 mg	5.8 mg
Ribosomal fraction	7.8 mg	8.0 mg	5.5 mg	6.0 mg

[a] The various fractions were prepared from exponential bacterial cells by the techniques described in Materials and Methods. P- and H- represent phototrophic and heterotrophic cells, respectively.

[b] The pigment content was determined by the methods of Cohen-Bazire et al.(1957) and the values listed here include bacterial chlorophylls, red carotenoids and yellow carotenoids.

[c] The amount of protein was measured by the method of Lowrey et al. (1951).

Figure 1. The pH gradient of the isoelectric focusing polyacrylamide gel. The gel (130 x 6 mm) was electrophoresed at 200 volts/15 min, 300 volts/30 min and 400 volts/30 min, and was then cut into 5 mm sections. Each of these sections was shaken in 2 ml of 9.2 M urea solution for 30 min and the pH was measured as described in Materials and Methods.


8.0
7.0
6.0
5.0
pH
0
5
10
15
20
25
SECTION NUMBER

(a) The Total (T) Fractions

About 0.5 mg of each of the bacterial total fraction protein samples was analyzed and the results are shown in Figure 2. From the P-Rs. rubrum, H-Rs. rubrum, P-Rp. palustris, and H-Rp. palustris samples, 27, 29, 18 and 23 protein bands were resolved, respectively. As compared with the E. coli total protein samples which gave as high as 70 protein bands in a similar system (O'Farrell, 1975), the low degree of resolution for the phototrophic bacterial samples was unexpected and was suspected to be caused by the following reasons: (1) most of the phototrophic bacterial proteins were basic proteins, with pI values higher than 8,0, and (2) the high content of photosynthetic pigments present in these samples rendered the proteins less soluble in our buffer system (Hui and Hurlbert, 1979). Because of the poor resolution, a detailed comparison between the P- and the H-samples has not been made at the present time, however, a general similarity between the P- and the H-samples was rather obvious, especially in the case of Rs. rubrum.

(b) The PS-60 Fractions

As mentioned earlier, these fractions contained a large amount of photosynthetic pigments (Table 1). When added into cell-free protein synthesis systems, they showed a profound stimulatory activity on the Rs. rubrum and Rp. Rp. palustris systems but rendered an inhibitory effect on

Figure 2. Separation of Rs. rubrum and Rp. palustris total fraction proteins by the isoelectric focusing PAGE system.
Total fraction protein samples were prepared by the described methods and applied to isoelectric focusing polyacrylamide gels at 0.5 mg protein per gel. After electrophoresis, the gels were fixed, stained and destained according to the methods described in Materials and Methods.

(A) P-Rs. rubrum

(B) H-Rs. rubrum

(C) P-Rp. palustris

(D) H-Rp. palustris


pH
7.9
A
B
C
D

the E. coli system (Table 2, the data listed in this table were summarized from Feenstra's M.Sc. thesis, Department of Microbiology, University of Manitoba, 1979). It is, therefore, of interest to examine the protein species present in these fractions.

In isoelectric focusing polyacrylamide gels, P-Rs. rubrum, H-Rs. rubrum, P-Rp. palustris and H-Rp. palustris PS-60 samples revealed, respectively, 24, 28, 19 and 16 protein bands (Figure 3). Again, the resolution was not very good, probably because of the same reasons described above. Since the stimulatory or inhibitory activity of the PS-60 fractions was dependent more on the nature of the cell-free protein synthesis rather than on the origin of the PS-60 fractions (Table 2), and because of the apparent lack of any common components in all of these four PS-60 fractions, it is impossible to assign any functions to these protein species at the present stage.

(c) The S-100 Fractions

The S-100 fraction supplies a number of important components for protein synthesis. When combined with various ribosome samples, the Rs. rubrum and the Rp. palustris S-100 fractions were all active (Table 3), but there was certainly a difference in protein-synthesizing activity between the phototrophic and the heterotrophic S-100 fractions. As shown in Table 3, the P-Rs. rubrum S-100 fraction was less

Table 2. Effect of PS-60 fractions on cell-free protein synthesis activity[a].

Cell-free protein synthesis system[b]	PS-60 Fraction[c]			
	P-Rs. rubrum	H-Rs. rubrum	P-Rp. palustris	H-Rp. palustri
P-Rs. rubrum	5.44	4.89	4.51	3.17
H-Rs. rubrum	1.73	1.56	1.24	1.18
P-Rp. palustris	3.59	1.48	1.98	2.51
H-Rp. palustris	1.16	1.14	1.95	2.10
E. coli	0.27	0.33	0.46	0.37

(a) The data were taken from Feenstra's M.Sc. thesis, University of Manitoba, 1979. The protein synthesizing activity by the various systems in the absence of any added PS-60 fraction was taken as 1.00.

(b) Each system contained 50 µg of S-100 proteins and 6.6 A_{260} units of ribosomes.

(c) Four hundred µg of the listed PS-60 fractions protein were added into the cell-free protein synthesis system.

Figure 3. Separation of Rs. rubrum and Rp. palustris PS-60 fraction proteins by the isoelectric focusing PAGE system.

PS-60 fraction protein samples were prepared by the described methods and were applied to isoelectric focusing polyacrylamide gels at 0.8 mg protein per gel. After electrofocusing, the gels were fixed stained and destained according to the methods described in Materials and Methods.

(A) P-Rs. rubrum

(B) H-Rs. rubrum

(C) P-Rp. palustris

(D) H-Rp. palustris


pH
7·9
5·7
A
B
C
D

Table 3. Cell-free protein synthesis by phototrophic and heterotrophic Rs. rubrum and Rp. palustris systems[(a)].

Ribosomes	S-100			
	P-Rs. rubrum	H-Rs. rubrum	P-Rp. palustris	H-Rp. palustris
P-Rs. rubrum	30,638	96,036	68,461	47,068
H-Rs. rubrum	108,502	140,251	158,807	120,485
P-Rp. palustris	153,573	46,215	92,878	114,787
H-Rp. palustris	100,693	23,818	144,691	136,835

(a) The figures represent dpm of ^{3}H-phenylalanine incorporated in a reaction mixture containing 50 µg of S-100 proteins and 6.6 A_{260} units of ribosomes (Chow, 1976c).

active than the H-Rs. rubrum S-100 fraction if combined with Rs. rubrum ribosomes whereas it showed 3-4 fold higher activity than its heterotrophic counterpart when used in combination with Rp. palustris rubosomes. The differences between the P- and the H-Rp. palustris S-100 fraction was, however, not as striking. These results together with the earlier findings of Chow (1976c) who reported some structural differences between the P- and the H-Rs. rubrum S-100 fractions in the urea PAGE system had led us to carry out the following analyses.

The phototrophic and the heterotrophic Rp. palustris S-100 fractions were electrophoresed in the isoelectric focusing polyacrylamide gels, and they had, respectively revealed 33 and 36 protein bands (Figure 4). The intensity of the protein bands, especially of those located near the middle regions of the gels (pI values about 7.1 - 6.8, see Figure 1) was relatively higher in the H- than in the P- sample.

The Rs. rubrum S-100 samples had also been analysed (Figure 5), and we had observed a lower intensity in the H-protein bands which were present mostly in the lower half of the gel. A number of protein bands which appeared in the upper part of the P-sample, with pI values about 7.8 - 7.5, were undetectable in the H-sample. The total number of protein bands revealed in the P-Rs. rubrum S-100 fraction was 33 and that in the H-sample was 31.

Figure 4. Separation of Rp. palustris S-100 fraction proteins by the isoelectric focusing PAGE system.

S-100 fraction protein samples were prepared by the described methods and were applied to isoelectric focusing polyacrylamide gels at 0.9 mg protein per load. After electrophoresis, the gels were fixed, stained and destained according to the methods described in Materials and Methods.

(A) P-Rp. palustris

(B) H-Rp. palustris

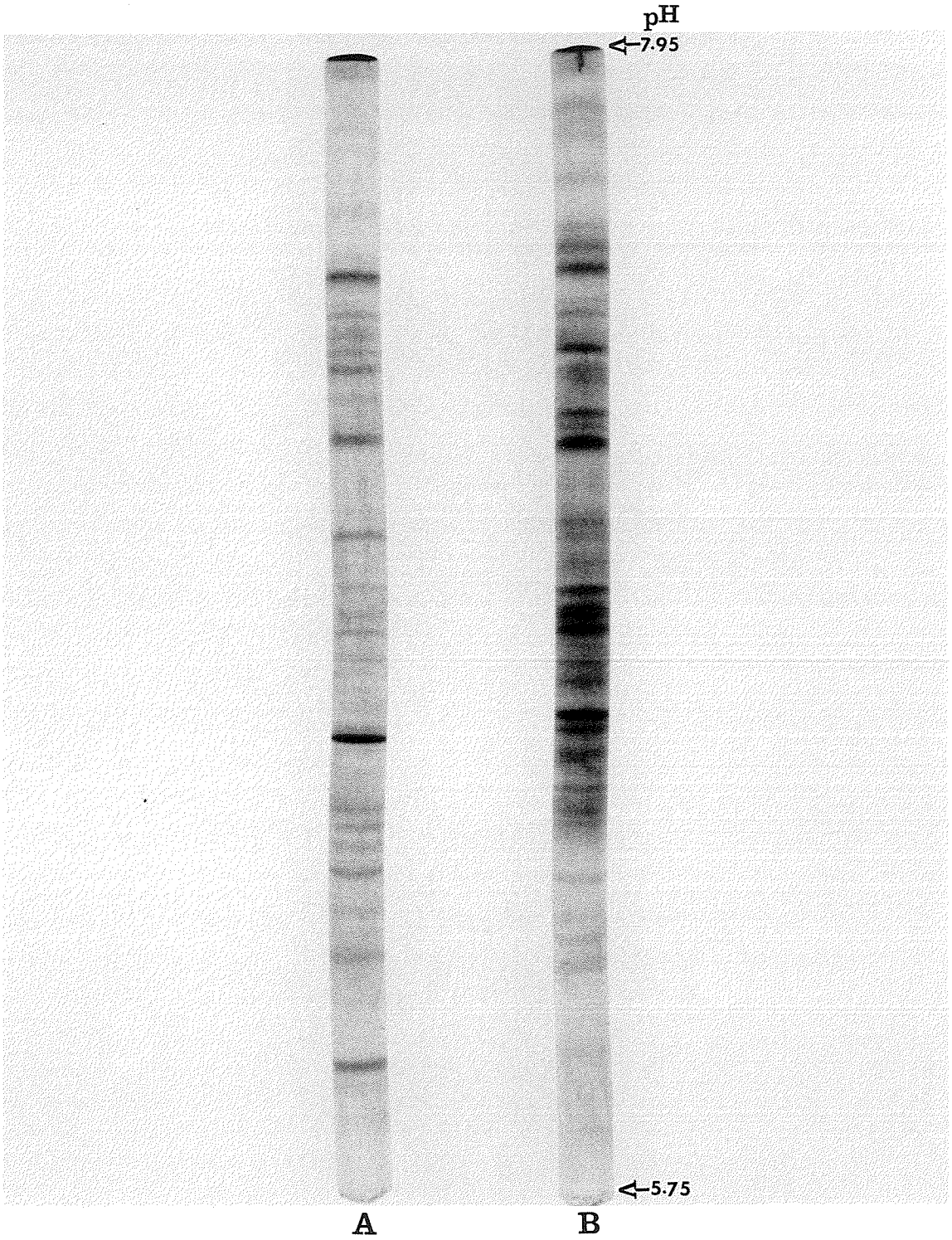
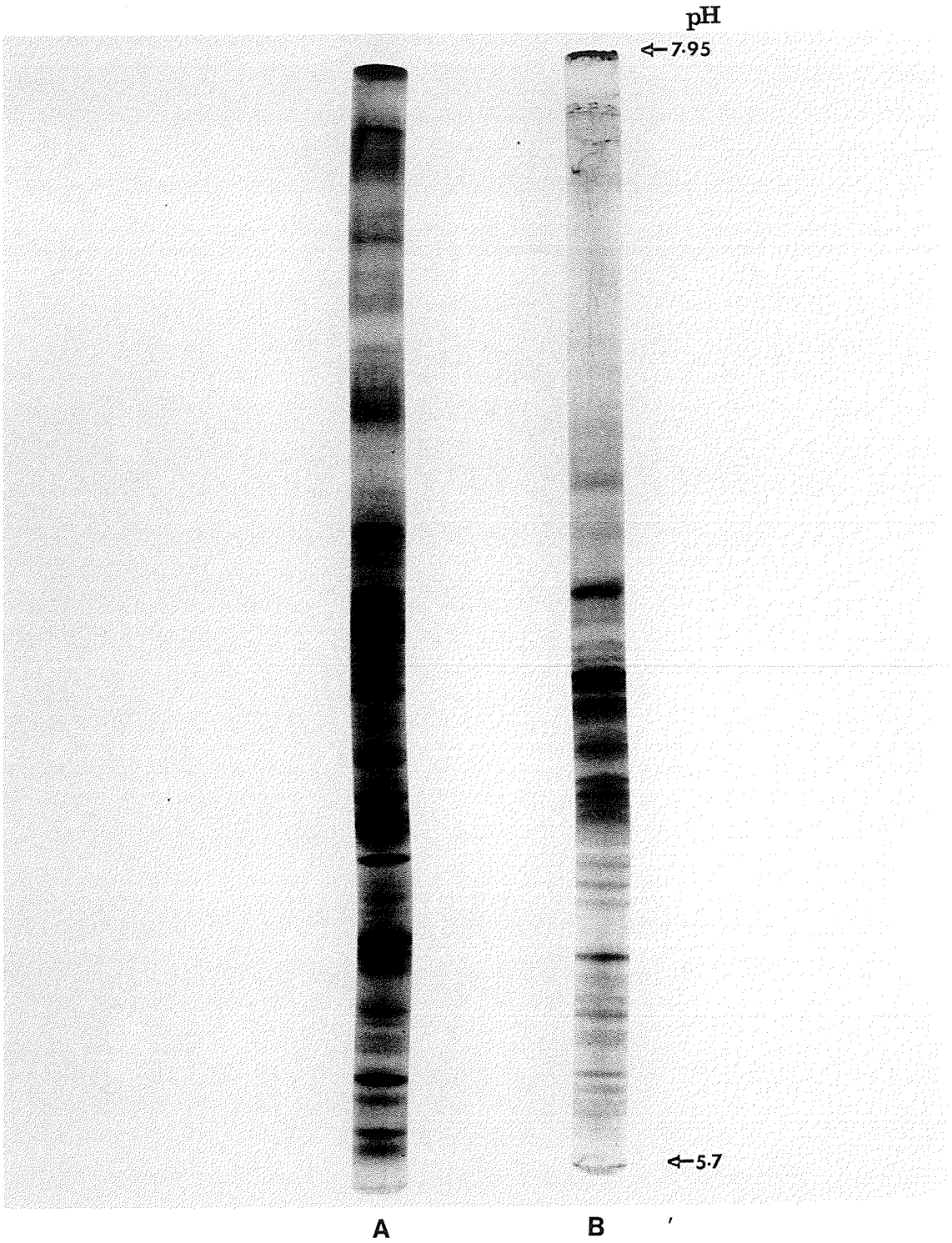

pH
7.95
5.75
A
B

Figure 5. Separation of Rs. rubrum S-100 fraction proteins by the isoelectric focusing PAGE system.

S-100 fraction protein samples were prepared by the described methods and were applied to isoelectric focusing polyacrylamide gels at about 0.9 mg protein per gel. After electrophoresis, the gels were fixed, stained and destained according to the methods described in Materials and Methods.

(A) P-Rs. rubrum

(B) H-Rs. rubrum


pH
7·95
5·7
A
B

Because of the larger number of protein bands observed in the S-100 samples (33-36 bands in Rp. palustris, Figure 4, 31-33 bands in Rs. rubrum, Figure 5) than in the total fraction samples (18-23 bands in Rp. palustris and 27-29 bands in Rs. rubrum, Figure 2), it was suspected that photosynthetic pigments were probably interfering with the solubility and the movement of the proteins in our PAGE system.

Two-Dimensional Electrophoresis of ^{35}S-labeled Rs. rubrum S-100 Fractions

Since there was a significant difference between the P- and the H-Rs. rubrum S-100 activities (Table 3), these fractions were further analyzed by the two-dimensional electrophoresis techniques of O'Farrell (1975).

Rhodospirillum rubrum cells were labeled with carrier-free $^{35}SO_4^=$ at a final concentration of 50 μCi/ml in a modified, low-sulfate medium as described in Materials and Methods. The incorporation rate of $^{35}SO_4^=$ into TCA insoluble materials was very similar for the phototrophic and the heterotrophic cells (Figure 6). Radioactive S-100 fractions were prepared from the labeled, mid-exponential phase cells and analyzed by electrophoresis, first in isoelectric focusing gels followed by SDS gels (O'Farrell, 1975). The resolution was not as good as expected (Figure 7), and less than 100 spots could be recognized in both samples. Application of more labeled samples per gel and longer exposure times,

Figure 6. Incorporation of $^{35}SO_4^=$ into Rs. rubrum cells.

Rhodospirillum rubrum cells were grown in 10 ml of modified A1 medium containing 50 μCi/ml of carrier-free $^{35}SO_4^=$. At indicated time intervals, 10 μl samples were removed from cultures and mixed with 5 ml of cold 5% TCA. After standing in the cold for at least 30 min the amount of TCA-insoluble counts in the mixture was determined by the standard filtration method.

(a) Phototrophic culture

(b) Heterotrophic culture

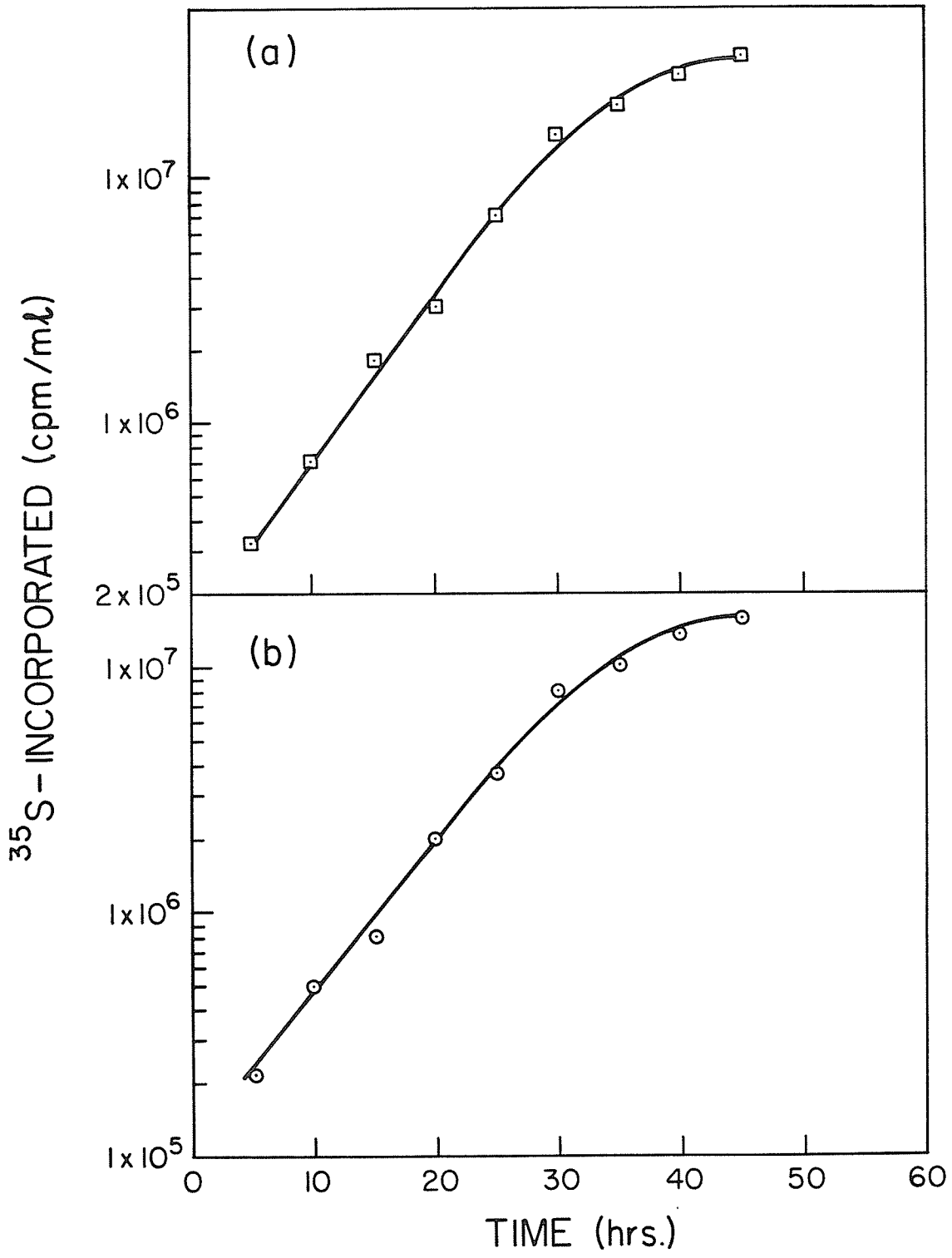

(a)
(b)
^{35}S-INCORPORATED (cpm/ml)
1x10^7
1x10^6
2x10^5
1x10^7
1x10^6
1x10^5
0
10
20
30
40
50
60
TIME (hrs.)

Figure 7. Two-dimensional autoradiograms of ^{35}S-labeled Rs. rubrum S-100 fraction.

^{35}S-labeled Rs. rubrum S-100 samples were electrophoresed according to the techniques of O'Farrell (1975) at $2x10^6$ dpm per gel. After completion, the (second-dimension) gel slabs were wrapped in Saran Wrap and exposed to Kodak X-Omat RP films for 96 hours.

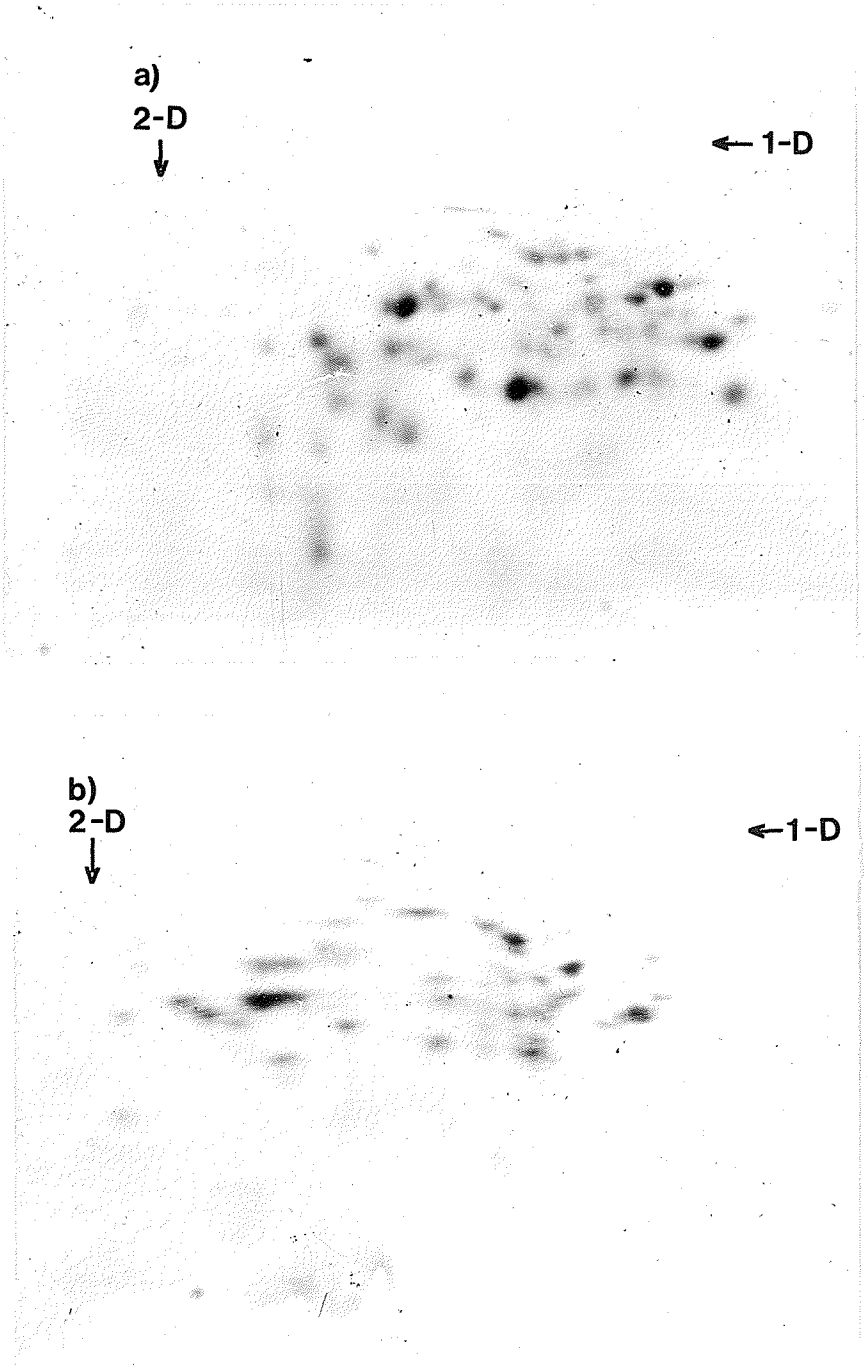
1-D-first dimension, isoelectric focusing gels

2-D-second dimension, SDS gels

Arrows indicated the direction of movement

(a) Phototrophic Rs. rubrum S-100 fraction

(b) Heterotrophic Rs. rubrum S-100 fraction


a)
2-D
1-D
b)
2-D
1-D

however, did not improve the quality of the autoradiograms. The only significant information we obtained from this exercise was probably that the H-sample contained relatively higher amounts of acidic proteins (left side in Figure 7) than the P-sample, a result consistent with our earlier findings (See Figure 5). Similar attempts on Rs. rubrum total and PS-60 fractions and on Rp. palustris total, PS-60 and S-100 fractions had also been made, but the results (not shown) were even of less success.

The failure in obtaining better resolution in these studies could be caused by a number of reasons, such as lack in experimental dexterity, presence of photosynthetic pigments in the samples, and, perhaps more likely, the presence of relatively large amounts of iron-sulfur-proteins which are different in both quality and quantity between phototrophic and heterotrophic cells (Yoch et al., 1977, Carithers et al., 1977, and Smith and Pinder, 1978) as well as the complex pathways in sulfur metabolism in Rs. rubrum (Truper, 1978).

Analyses of Rs. rubrum and Rp. palustris Ribosomal Proteins by Various PAGE Systems

Structural differences between the phototrophic and the heterotrophic ribosomal proteins have recently been demonstrated in Rp. palustris (Mansour and Stachow, 1975) and Rs. rubrum (Chow, 1976c). A detailed study on these proteins

with respect to their qualitative and quantitative properties is, however, still lacking. In this study we have analyzed these properties of Rs. rubrum and Rp. palustris ribosomal proteins by three different polyacrylamide gel electrophoresis systems, using the well characterized E. coli ribosomal proteins as a reference.

(a) The Isoelectric Focusing PAGE System of O'Farrell (1975)

Ribosomal protein samples from Rs. rubrum, Rp. palustris and E. coli were analyzed by the isoelectric focusing PAGE system and the results are presented in Figure 8. As shown earlier (Figure 1), the pH range in our gels was from 5.75 to 7.95, therefore, the proteins revealed in these gels were considered as mostly acidic proteins.

No major qualitative differences could be observed between the P- and the H-Rs. rubrum or Rp. palustris samples. Quantitatively, the protein bands in the H-samples were in general of higher intensity than those in the P-samples, especially in the case of Rp. palustris ribosomes. When compared with the E. coli ribosome sample which is known to contain mostly basic proteins (O'Farrell, 1975), a relatively higher number of acidic proteins was found in the phototrophic bacterial ribosomes. This is not totally unexpected, because phototrophic bacteria are generally considered to be more primitive than most other bacteria, and it has recently been reported that most 'primitive bacteria', such as the halophilic bacteria (Visentin et al., 1972) and the methane

Figure 8. Separation of Rs. rubrum, Rp. palustris and E. coli ribosomal proteins by the isoelectric focusing PAGE system.

Ribosomal protein were prepared by the acetic acid extraction method (Hardy et al. 1969) and were applied to isoelectric focusing polyacrylamide gels at 0.15 mg protein per gel. After electrophoresis, the gels were fixed, stained and destained according to the methods described in Materials and Methods.

(A) P-Rs. rubrum

(B) H-Rs. rubrum

(C) P-Rp. palustris

(D) H-Rp. palustris

(E) E. coli


pH
←7.95
←5.7
A
B
C
D
E

bacteria (A.T. Matheson, personal communication) do contain more acidic proteins in their ribosomes.

(b) The SDS PAGE System

In the SDS PAGE system, proteins are separated from each other because of different molecular weights.

Using this technique, we had separated the Rs. rubrum and the Rp. palustris ribosomal proteins to about 18 individual bands (Figure 9). The difference between the P- and the H-samples, for both Rs rubrum and Rp. palustris, was negligible, and the molecular weight range of the phototrophic bacterial ribosomal proteins (about 10,000 to 80,000) was very similar to that of E. coli.

(c) The Two-Dimensional PAGE System of Kaltschmidt and Wittmann (1970)

To achieve a better resolution and reproducibility for the separation of E. coli ribosomal proteins, Kaltschmidt and Wittmann (1970) had developed a two-dimensional PAGE system by which proteins could be separated based on the net charges they carried at pH 8.6 (for the first dimension) and at pH 4.6 (for the second dimension). By this technique they were able to separate E. coli ribosomal proteins into about 50 individual spots which were arranged over a wide area in a distinctive pattern.

This PAGE system had been adapted for our study of the phototrophic bacterial ribosomal proteins, and, an analysis on

Figure 9. Separation of Rs. rubrum and Rp. palustris ribosomal proteins by the SDS PAGE system.

Ribosomal proteins were prepared by the acetic acid extraction method (Hardy et al. 1969) and were applied to SDS polyacrylamide gels at 0.15 mg protein per load. After electrophoresis, the gels were fixed, stained and destained according to the methods described in Materials and Methods. Molecular weight was determined by co-electrophoresis of proteins with known molecular weights.

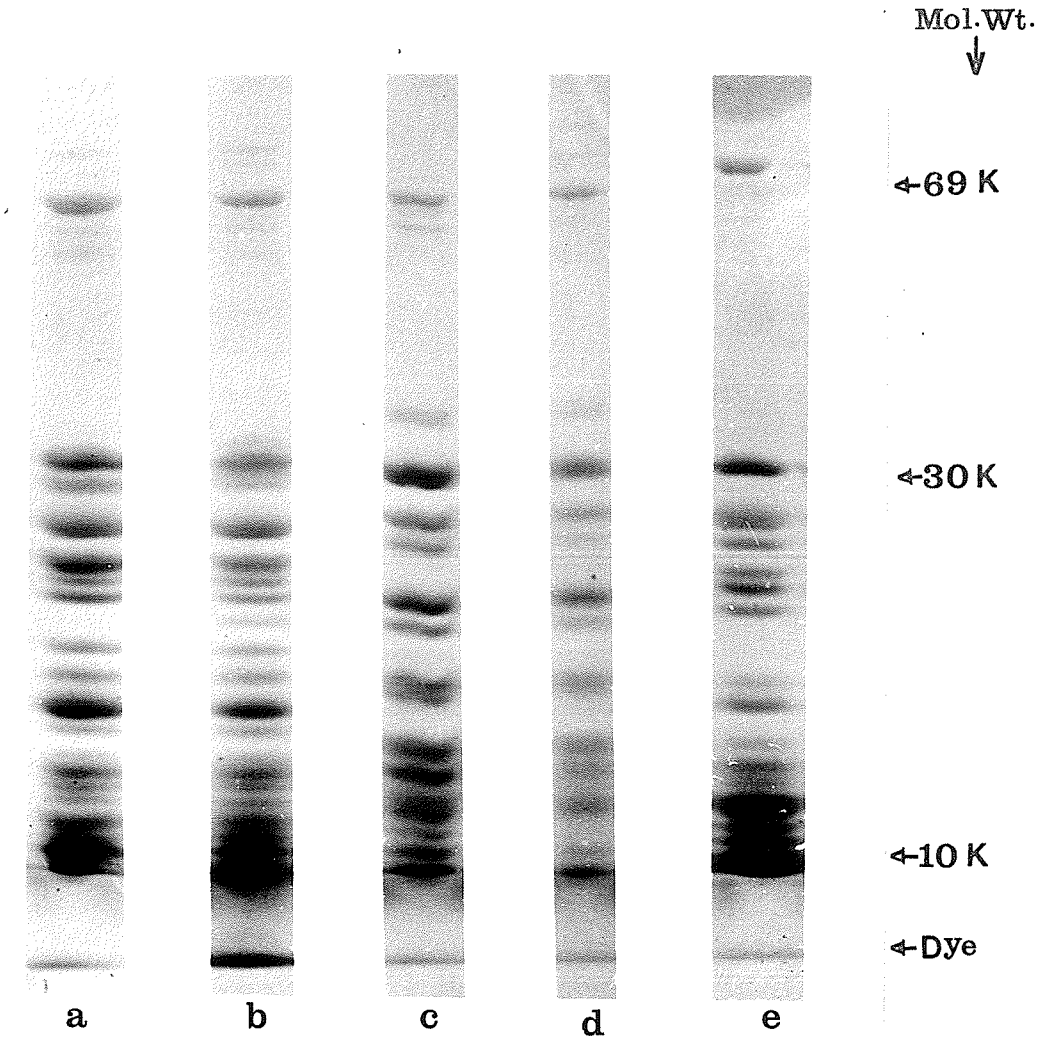
(a) P-Rs. rubrum

(b) H-Rs. rubrum

(c) P-Rp. palustris

(d) H-Rp. palustris

(e) E. coli


Mol.Wt.
69 K
30 K
10 K
Dye
a
b
c
d
e

the well characterized E. coli ribosomal proteins was first carried out in order to compare our results with the published data. As shown in Figure 10, the separation pattern of our E. coli 70 S ribosomal proteins was more or less comparable to that of Kalschmidt and Wittmann (1970), however, in our hands only about 32 protein spots could be readily identified.

The separation patterns of the phototrophic (Figure 11) and the heterotropic (Figure 12) Rs. rubrum ribosomal proteins were almost identical, both revealing about 34 protein spots.

On the other hand, a slight difference in the protein distribution pattern had been noticed between the phototrophic (Figure 13) and the heterotrophic (Figure 14) Rp. palustris samples. Further, we had identified 29 protein spots in the P-sample but 32 spots in the H-sample. Because of the apparent difference between the P- and the H-Rp. palustris protein distribution patterns in the second dimension, the distribution of the proteins in the first dimension gels had been examined. As shown in Figure 15, no obvious difference existed. Incidentally, the separation pattern of the Rp. palustris samples in this PAGE system was somewhat less reproducible than that of the Rs. rubrum samples.

Figure 10. Two-dimensional electrophoresis of *E. coli* ribosomal protein.

Ribosomal proteins were extracted with 67% acetic acid (Hardy *et al.* 1969) and 1.5 mg of the protein were analyzed by the bidimensional polyacrylamide gel electrophoretic system of Kaltschmidt and Wittmann (1970). The running conditions for the first dimension were 20 h at a constant current of 3 mA/gel tube at pH 8.6 and 20 h at a constant current of 30 mA/gel slab at pH 4.6 for the second dimension. Gel slab was stained with Amido Black and destained with 1% acetic acid as described in Materials and Methods.

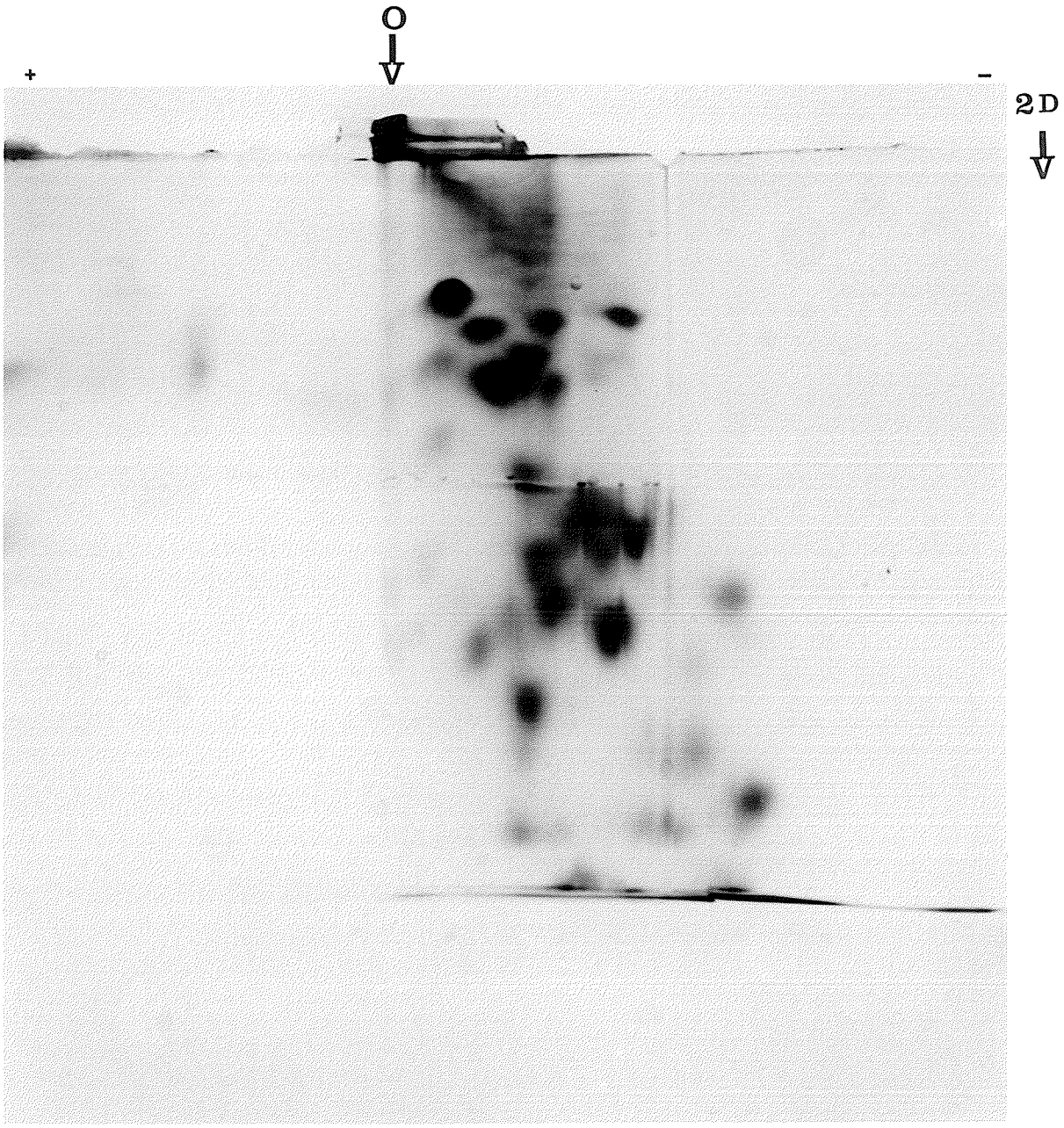

O
+
−
2D

Figure 11. Two-dimensional electrophoresis of P-Rs. rubrum ribosomal proteins.

Proteins were extracted from P-Rs. rubrum ribosomes and electrophoresed as described in the legend to Figure 10. Staining and destaining of gel slab was described in Materials and Methods.


O
+
−
2D

Figure 12. Two-dimensional electrophoresis of H-Rs. rubrum ribosomal proteins.

Ribosomal proteins were extracted and electrophoresed as described in the legend to Figure 10. Staining and destaining of gel slab was described in Materials and Methods.


O
+
−
2D

Figure 13. Two-dimensional electrophoresis of P-Rp. palustris ribosomal proteins.

Proteins were extracted from ribosomes and electrophoresed as described in the legend to Figure 10. Staining and destaining of gel slab was described in Materials and Methods.

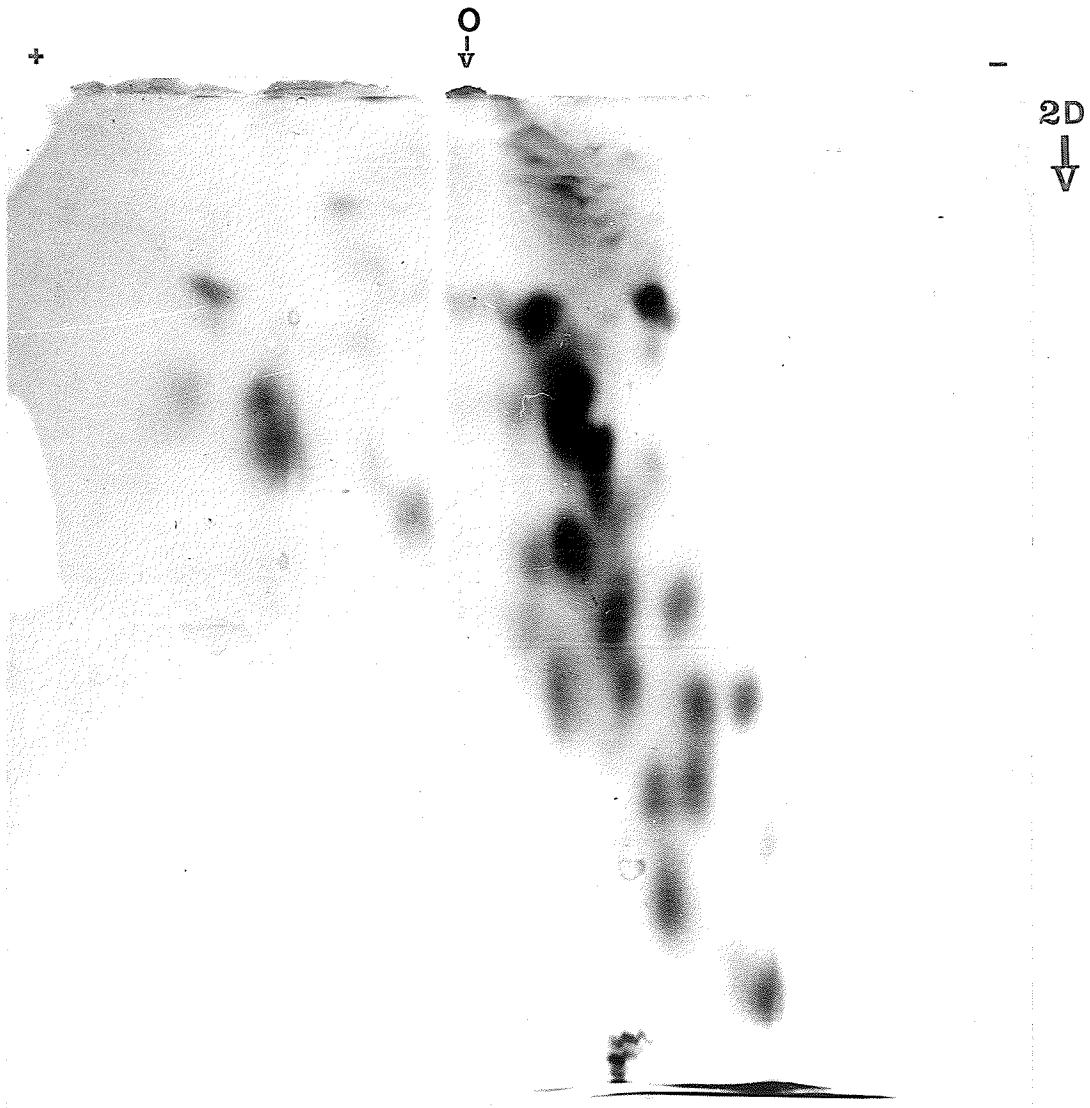

+
O
V
−
2D
V

Figure 14. Two-dimensional electrophoresis of H-Rp. palustris ribosomal proteins.

Ribosmal proteins were extracted and electrophoresed as described in the legend to Figure 10. Staining and destaining of gel slab was described in Materials and Methods.

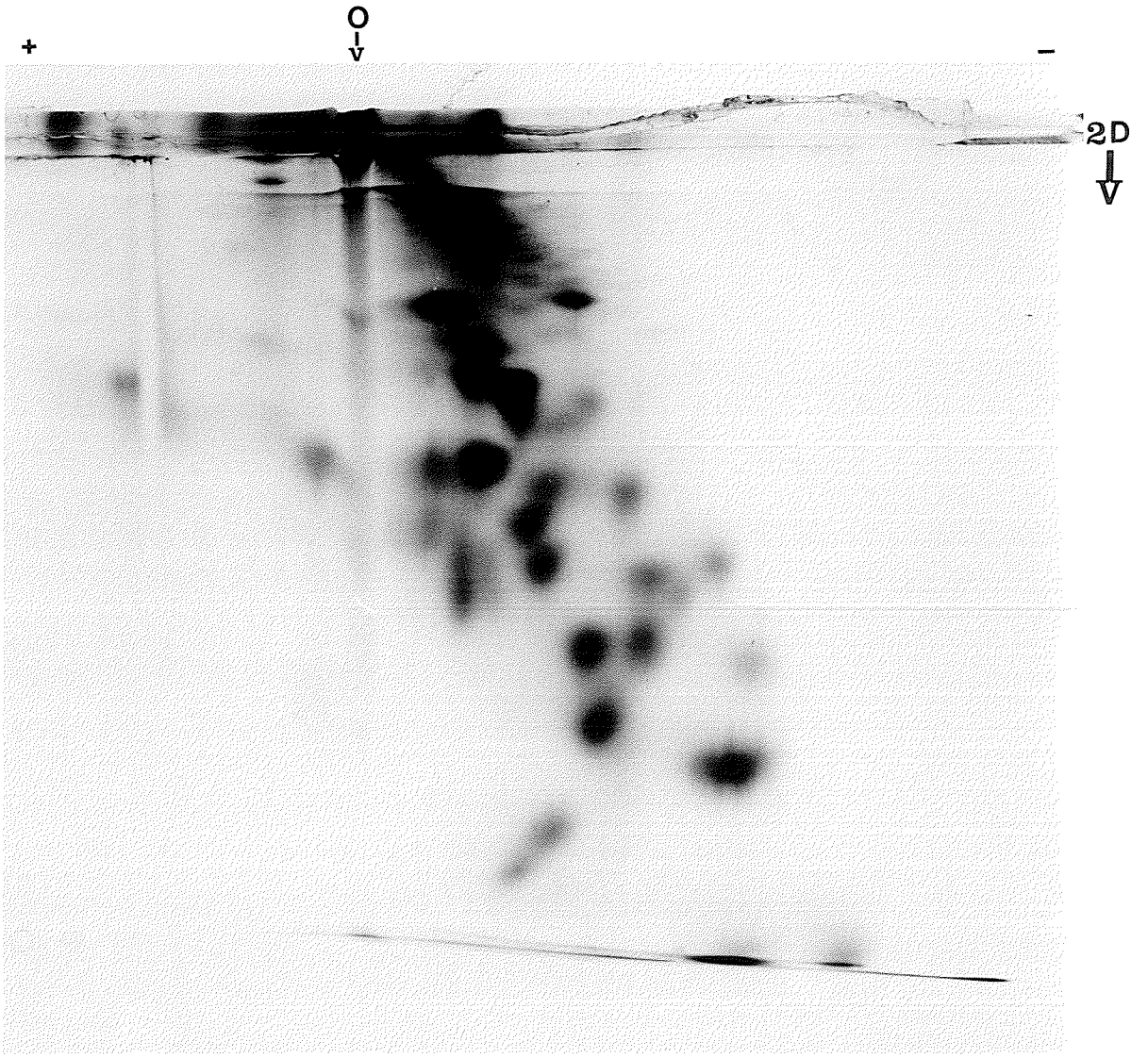
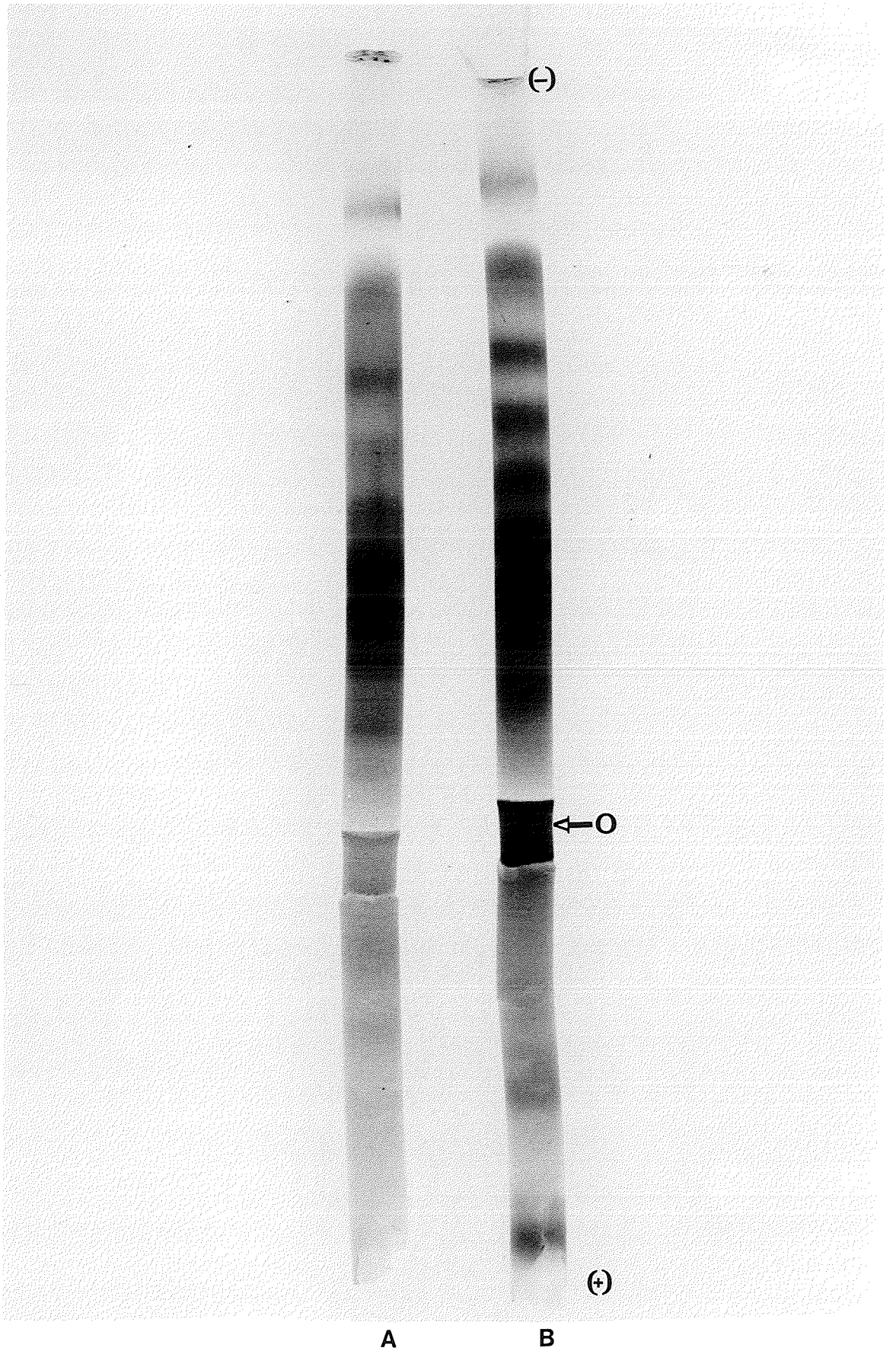

+
O
V
–
2D
V

Figure 15. Separation of Rp. palustris ribosomal proteins by the one-dimensional polyacrylamide gel electrophoresis.

Ribosmal proteins were extracted with 67% acetic acid (Hardy et al. 1969) and 0.15 mg of protein were analyzed with the one-dimensional polyacrylamide gel electrophoretic system (Kaltschmidt and Wittmann, 1970). Running time was 20 h at a constant current of 3 mA/gel tube at pH 8.6. Gels were fixed with TCA, stained with 0.1% coomassie blue and destained with 25% ethanol and 10% acetic acid mixture.

(A) P-Rp. palustris

(B) H-Rp. palustris


(-)
O
(+)
A
B

D I S C U S S I O N

This study was originally designed as an expansion of our earlier investigation (Chow, 1976 c) in which minor differences in protein species between the phototrophic and the heterotrophic Rs. rubrum S-100 and ribosome fractions had been demonstrated by urea and SDS polyacrylamide gel electrophoresis systems. By analyzing the total, the S-100 and the PS-60 fractions with the more advanced isoelectric focusing technique, especially with ^{35}S-labeled samples, it was expected that major differences between the P- and the H-samples, if they existed, should be readily recognizable. Our results, however, had failed to achieve this goal. The main difficulty in this type of study was probably caused by the presence of a relatively large amount of photosynthetic pigments in our samples. As recently shown by Hui and Hurlbert (1979) who had analyzed the chromatophore proteins of seven phototrophic bacteria including Rp. palustris but not Rs. rubrum, the mobility of these proteins in electrophoresis gels was variable, depending on a number of factors, such as the age of the samples, the composition of the solubilization buffers and the temperatures at which the samples were solubilized. Their findings together with our results that the low number of protein bands revealed as well as the accumulation of stainable materials on top of the gels (Figs. 2-5) have suggested to us that the separation of the proteins was

far from complete. Currently we are attempting to modify our systems by changing the composition of the solubilizing buffers (e.g., addition of organic solvents) and by expanding the pH range of the gels in order to obtain a better resolution.

Since phototrophic bacteria have a relatively high content of iron-sulfur-proteins which are quite different in both quality and quantity between the phototrophic and the heterotrophic cells (Yoch et al., 1977, Carithers et al., 1977, and, Smith and Pinder, 1978) and a complex pathway of sulfur metabolism (Trüper, 1978), it is rather questionable in the wisdom of using ^{35}S to label our samples, and, perhaps we should have used ^{14}C instead. This point will be investigated in the near future by the above-mentioned modified electrophoresis techniques.

Better (and more interesting) results were obtained for our ribosomal protein samples (Figs. 8-15), probably because of the absence of photosynthetic pigments in these samples (Table 1).

With respect to the acidic ribosomal proteins, although the qualitative difference between the P- and the H-Rs. rubrum (Fig. 8a and b) and the P- and the H-Rp. palustris samples (Fig. 8c and d) was negligible, the quantitative difference was rather obvious and worth mentioning. In general, the heterotrophic samples (especially that of Rp. palustris) showed a higher intensity in several of their major bands. Since the acidic proteins are generally considered to be

located on the surface of the ribosomes, their roles in selecting and translating natural mRNA has been suggested to be of utmost importance (for references, see Nomura et al., 1974). The higher activity of the heterotrophic ribosomes than their phototrophic counterparts, with the exception of Rp. palustris ribosomes + Rs. rubrum S-100 system (Table 3) could probably be explained by these findings, although more work with natural mRNA is necessary. Moreover, these acidic proteins should be analyzed further by the two-dimensional PAGE system of O'Farrell (1975).

As for the total ribosomal proteins, we had separated them in about 18 major protein bands in the SDS gels (Fig. 9) and to about 32 major spots in the two-dimensional gel slabs (Figs. 11-14). In the present study, however, we were not able to identify the "heavy" acidic proteins, corresponding to those protein bands mentioned above, in our H-samples. This can be easily explained for the SDS system, because in these gels proteins are separated based on their molecular weights rather than on charges, and it is a well known fact that many ribosomal proteins are of similar molecular weights. On the other hand, one should theoretically be able to recognize these "heavy" acidic proteins in the two-dimensional gels, since the protein spots are separated mainly because of the net charges they carry. The real reason(s) for this unsuccessful attempt is still not clear, but it is suspected that some acidic proteins were immobilized in the first dimension gels by yet unknown reasons, because certain

heavily stained bands had been noticed to be present in the first dimension gels of the H-samples and they had apparently never entered the second dimension slabs (Figs. 12 and 14, the extreme left sides of the first dimension gels).

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