CHARACTERIZATION OF THE D-GLUCOSE TRANSPORT SYSTEM AND THE REGULATION OF METABOLITE TRANSPORT BY CYTOKININS AND CITRATE IN A WATER MOULD

by

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TO MY PARENTS

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Abbreviations

BAP	benzylaminopurine
cAMP	adenosine 3':5' cyclic monophosphate
CCCP	m-chlorophenylcarbonylcyanidehydrazone
DMSO	dimethylsulfoxide
DTT	dithiothreitol
EDTA	ethylene diamine tetra-acetic acid
EGTA	ethylene glycol-bis-(aminoethyl ether)-N,N'-tetra-acetic acid
GA	gibberellic acid
HAP	hexylaminopurine
IAA	indole-3-acetic acid
6ipAde	N^6-(Δ^2-isopentenyl)adenine
6ipAdo	N^6-(Δ^2-isopentenyl)adenosine
K	kinetin
KR	kinetin riboside
NEM	N-ethylmaleimide
ONPG	o-nitrophenyl-β-D-galactoside
PHA	phytoheamagglutinin
PhenylAP	phenylaminopurine
Tris	trihydroxymethylaminomethane
Z	zeatin

ABSTRACT

D-glucose and 2-deoxy-D-glucose were transported by an active process into the cells of the water mould, Achlya. D-Fructose and glycerol failed to be transported by D-glucose grown cells. Metabolic inhibitors such as 2,4-dinitrophenol, cyanide, azide and CCCP (m-chlorophenylcarbonylcyanidehydrazone) inhibited transport markedly at micromolar concentrations, the most potent of these inhibitors being CCCP.

Competition studies revealed that D-galactose (K_i=1.55 x 10^{-3}M), D-mannose (K_i=5.8 x 10^{-4}M), 2-deoxy-D-glucose (K_i=1.7 x 10^{-4}M) and D-xylose (K_i=3.7 x 10^{-3}M) inhibited competitively D-glucose transport. Other sugars tested showed very slight to no inhibition, indicating the existence of a fairly specific D-glucose transport system. The uptake of D-glucose was pH and temperature dependent, each with a fairly well defined optimum. Sulfhydryl group reactive reagents such as I_2 (0.5 to 5.0 μM), Hg^{++} (1 to 200 μM) and NEM (1 to 500 μM) inhibited D-glucose uptake. All these results suggest that a protein carrier may mediate D-glucose

transport in Achlya.

Citrate, at concentrations of 1 mM or greater, inhibited D-glucose transport (K_i=8.2 x 10^{-3}M). Data are presented which indicate that citrate act by chelating Ca^{++} and so deprive the fungus of this cation which is absolutely essential for growth and active transport processes.

Indirect experimental observations support the idea that phosphorylation of D-glucose during transport may be essential for uptake. A similar phosphorylation process has been detected inyyeast.

Cytokinins (plant growth hormones), the most effective being 6ipAde (50 to 500 µM, K_i=1.88 x 10^{-4}M) and hexylaminopurine were found to inhibit D-glucose transport. These N^6-adenine derivatives appear to play important regulatory roles throughout the life cycle of Achlya in a variety of transport activities.

INTRODUCTION

Transport of metabolites through biological membranes and hormonal regulation of intracellular processes through hormone-membrane interaction are two major fields of research.

To a cell, the membrane is not only the window to the world but also acts as a protective barrier. Like intracellular enzymes, the activities of protein mediated transport systems located on cell membranes are regulated in very intricate fashions for the maximum economy to the cell. Tumour cells and certain viral transformed cell lines have been observed to take up D-glucose at faster rates when compared to normal cell lines. Understanding this anomaly may give some insight to the process of tumour formation. In the same light, differences in the topography of membranes between normal and tumour cells have been reported. It could well be, as suggested, that signals for regulating normal cell metabolism and proliferation cannot be transmitted to the cells owing to the absence or alteration of specific membrane located binding sites for such signals in tumour cells.

A better understanding of the regulation of sugar transport processes and the metabolism of such transported metabolites will undoubtedly contribute to the development of more efficient commercial fermentation processes. These are but some of the examples of membrane-hormone-transport related problems. Research into such fundamental processes may enable man to understand better the intricacies of cell growth.

The purpose of this study is two fold: first, to define some of the characteristics of the sugar transport system in the Oömycete, Achlya; and second, to examine some possible regulatory features of transport related to the growth of this fungus.

HISTORICAL

Plant Cytokinins

Cytokinins, a class of 'hormonal' compounds, exhibit a multitude of biological effects not only in plants but also in organisms as diverse as bacteria, fungi, algae and probably mammals as well. Some of these effects are summarized in a table (I). Since the isolation of kinetin by Miller et al in 1954 (67), similar purine compounds have been isolated or synthesized, the more significant ones being 6ipAde, zeatin [N^6-(trans-γ-hydroxymethyl-γ-methyl-allyl)adenine] and N^6-methyl and dimethyl-aminopurine. Structurally, an intact purine ring with an N^6-substituent of moderate size is necessary for a compound to exhibit high cytokinin activity (97). However certain exceptions do exist of which the urea derivatives like N,N' diphenylurea and N-3chlorophenyl-N'-phenylurea and 8-azakinetin are the more outstanding examples (29).

Cytokinins are intimately involved in nearly every facet of plant development. Their effects on metabolism vary from inducing cell division and cell enlargement to delaying senescence. Enzyme activities and their rates of biosynthesis and degradation have

been also shown to be affected by these hormones. RNA and DNA synthesis are also affected likewise. Table I summarizes some of the biological phenomena known to be influenced by cytokinins.

Cytokinins are not exclusively found in plants. Such compounds have been isolated and characterised from certain bacteria and fungi (39, 50, 51, 65, 78, 101). The pathogenic symptoms exhibited by plants infected with such pathogens can be mimicked by singular addition of cytokinins. Cytokinins have also been shown to be constituents of tRNA from organisms as diverse as bacteria and mammals (7, 36). These purine derivatives are all found situated adjacent to the 3' end of the anticodon on the tRNA (35). Biochemical and genetical studies have shown that alterations or deletions of the substituted side chain(s) resulted in a reduction in the ability of such tRNAs to bind to ribosomes in the presence of the appropriate messenger. However, the charging process of these modified tRNAs with their specific amino acids is not affected (25, 31, 66). Whether the unique position of these cytokinins on tRNAs has any important relationship to their biological activities remains to be elucidated.

Cytokinins may also play an important role in plant tumourigenesis. Unlike normal cells in tissue culture, cells which are transformed are capable of autonomous growth in the absence of exogenously supplied cytokinins (112). This indicates that such tissues are capable of synthesizing their own cytokinin requirements in culture, a process which was repressed before, Braun et al have suggested that cytokinesins (glucose containing 3,7-dialkyl-2-alkylthio-6-purinone compounds) are the primary factors which induce cytokinesis (113). Also, cytokinesins are synthesized persistently in crown gall tumour cells. Normal plant cells require kinetin to induce cytokinesin synthesis. Wood and Braun have also demonstrated that cytokinesins are strong inhibitors of both plant and animal adenosine 3':5'-cyclic monophosphate phosphodiesterases (114, 115).

Despite the multitude of information concerning the biological activities of cytokinins, no satisfactory explanation has yet been given relating cytokinin activity to cytokinesis and cellular differentiation.

TABLE (I)

EFFECTS OF CYTOKININS ON PLANTS, MICRO-ORGANISMS AND ANIMALS

(A) IN PLANTS

SYSTEM	SPECIFIC EFFECT	CYTOKININ SPECIFICITY	LITERATURE REFERENCE
CELL GROWTH			
bean leaf	promoted leaf expansion	K,BAP	Scott, R.A. and Liverman, J. L. (1956) Plant Physiol. 31, 321-322. and Miller, C.O. (1956) Plant Physiol. 31, 318-319.
lettuce seeds	stimulation of germination related to cellular expansion	K	Haber, A.H., and Luippold, H.J. Plant Physiol. (1960) 35, 168-173.
sunflower hypocotyls	increased fresh and dry weight without elongation	K	de Ropp, R.S. (1956) Plant Physiol. 31, 253-254.
radish leaf discs	expansion promoted	general	Kuraishi, S. (1959) Sci. Papers Coll. Gen. Educ. Uni. Tokyo, 9, 67-104.
Lemna minor fronds	increase in frond area stimulated at low concentrations (10^{-6}M); inhibited at high concentrations	K	Tasser de Jong, J.G. and Veldstra, H. (1971) Plant Physiol. 24, 235-238.

Table (I) Continued

pea stem segments	elongation inhibited	Z	Witham, F.H. and Miller, C.O.,(1965) Plant Physiol. 18, 1007-1017.
MITOSIS AND CELL DIVISION			
tobacco pith tissue	stimulated mitosis and cytokinesis	K	Das, N.K., Patau, K., and Skoog, F. (1956) Plant Physiol. 9, 640-651.
unorganized tobacco tissue	stimulated cell division	K(auxin)	Skoog, F. and Miller, C.O. (1957) Symp. Soc. Exptl. Biol. II, 118-131.
mature pea root tissue	induces mitosis	K	Torrey, J.G. (1961) Exptl. Cell Res. 23, 281-299.
intact Allium cepa roots	mitosis inhibited	K	McManus, M.A. (1960) Nature 185, 44-45.
DIFFERENTIATION			
Shoot, Root and Bud Development and Initiation			
leaf squares of Peperomia sandersii	inhibits root and bud generation	K	Harris, G.P., and Ennid, M.H.H. (1964) Ann. Bot. N.S. 28, 509-526.
root segments of Isatis tinctoria and Convolvulus arvensis	initiated regeneration of shoots	K	Danckwardt-Lilliestrom, C. (1957) Physiol. Plant 10, 794-797 and Torrey, J.G. (1958) Plant Physiol. 33, 258-263.

Table (I) Continued

leaf cuttings of *Saintpaulia ionantha*	increased budding	K	Plummer, T.H. and Leopold, A.C. (1957) Proc. Am. Soc. Hort. Sci. 70, 442-444.
leaf discs of *Begonia rex*	induced shoot formation inhibited root growth	K	Schraudolf, H. and Reinert, J. (1959) Nature (Lond.) 184, 465-466.
Begonia leaf cuttings	root induction inhibited at high concentrations, promoted at low concentrations	K	Heide, O.M. (1965) Physiol. Plant 17, 789-804.
Neutralization of Apical Dominance			
Alaska pea stems (and other sources)	released axillary buds from either apical dominance or auxin treatment	K	Wickson, M. and Thimann, K.V. (1958) Physiol. Plant II, 62-74 and Sachs, T. and Thimann, K.V. (1964) Nature (Lond.) 201, 939-940.
Specialized Tissue Formation			
pea stem segments	functional xylem induced	K	Sorokin, H.P. and Thimann, K.V. (1958) Protoplasmia 59, 326-
N. tabacum	tracheid formation	K	Bergmann, L. (1964) Planta 62, 221-254.
pith parenchyma (Romaine lettuce)	induced to differentiate into tracheary elements	K(IAA)	Torrey, J.G., Fosket, D.E., Hepler, P.K. (1971) Am. Sci. 59, 338-352.
Abscission			
Phaseolus	retarded when applied to abscission zone; enhanced when applied elsewhere	K	Osborne, D.J., and Moss, S.E. (1963) Nature 200, 1299-1301.

Table (I) Continued

Phaseolus leaves	promotion at low concentrations; inhibition at high concentration		Chatterjee, S.K. and Leopold, A.C. (1964) Plant Physiol. 39, 334-337.
Fruit Production			
figs	parthenocarpy induced	SD 8339 (synthetic)	Crane, J.C. and van Overbeek, J. (1965) Science 147, 1468-1469.
Vitis vinifera L.	increased fruit size and fruit set	BAP	Weaver, R.J., van Overbeek, J. and Pool, R.M. (1965) Nature (Lond.) 206, 952-953 and Weaver, R.J. and van Overbeek, J. (1963) California Agric. 17, 12-
plum and apple fruitlets	fruit and seed development	(endog)	Letham, D. (1963) New Zeal. J. Bot. I, 336-350.
Flowering			
Cichorium intybus L.	flower formation induced in cold requiring plant grown under non-inductive conditions	K(+Vit.E)	Michniewicz, M. and Kamienska, A. (1964) Naturwiss 51, 295-296.
Arabidopsis thaliana	overcomes long day requirements	K(+Vit.E)	Michniewicz, M. and Kamienska, A., (1965) Naturwiss, 52, 623-
tomatoe and pea (intact plants)	application to roots-inhibited flowering in tomato plants, enhanced in pear	K	Wittwer, S.H. and Dedolph, R.R. (1963) Amer. J. Bot. 50, 330-336.

Table (I) Continued

Special Organelles and Structures			
tobacco pith tissue	induced maturation of proplastids to plastids	K	Stetler, D.A. and Laetsch, W.M. (1965) Science 149, 1387-1388.
Solanum tuberosum	induction of tuber formation	K	Palmer, C.E. and Smith, O.E. (1969) Plant and Cell Physiol. 10, 657-664.
tobacco roots	induction of pseudonodule formation in the root cortex	K(IAA)	Arora, N., Skoog, F. and Allen, O.N. (1959) Amer. J. Bot. 46, 610-613.
SYNTHESIS			
Lemna minor	starch synthesis stimulated at high concentrations, inhibited at low concentrations	K,BAP	Hillman, W.S. (1957) Science 126, 165-166 and Tasser de Jong, J.G. and Veldstra, H. (1971) Plant Physiol. 24, 235-238.
detached Impatiens petals	anthocyanin production stimulated	K	Klein, A.O. and Hagen, C.W. Jr. (1961) Plant Physiol. 36, 1-9.
tobacco tissue	lignin synthesis activated	K	Bergmann, L. (1964) Planta 62, 221-254.
tobacco tissue	thiamine synthesis activated	K	Digby, J. and Skoog, F. (1966) Plant Physiol. 41, 647-652.
soybean tissue	deoxyisoflavone synthesis stimulated	general	Miller, C.O. (1969) Planta 87, 26-35.

Table (I) Continued

Amaranthus seedlings	betacyanin production increased	general	Kohler, K.H. and Conrad, K. (1966) Biologische Rundschen 4, 36-40.
cucumber cotyledons	chlorophyll content increased (in light)	BAP BAP	Fletcher, R.A. and McCullagh, D. (1971) Can. J. Bot. 51, 1347-1354.
leaves of intact plants	patterns of lipid fatty acids and lipid bound sugars altered	K,Z	Kull, U. and Büxenstein, R. (1974) Phytochem. 13, 39-44.

DORMANCY AND GERMINATION

Seed Germination

beech, hazel and rowan seeds	stimulation of germination of intact seeds	K(thiourea, GA)	Frankland, B. (1961) Nature 192, 678-679.
irradiated lettuce seeds	stimulation of recovery of germination	K	Haber, A.H. and Luippold, H.J. (1960) Plant Physiol. 35, 168-173.
Xanthium seeds	effects of naturally occurring inhibitors of seed germination such as coumarin and xanthetin are reversed	K(+red light)	Khan, A.A. and Tolbert, N.E. (1965) Physiol. Plant 18, 41-43.
bean seeds	enhanced germination	K,BAP, Phenyl AP, HAP	Miller, C.O. (1956) Plant Physiol. 31, 318-319.

Table (I) Continued

Seed Dormancy Release			
apple seedlings	induces dormancy break	K	Pieniazek, J. (1964) Acta. Agrobot. 26, 157-169.
Xanthium seeds	dormancy of 'upper' seed broken, with RNA synthesis involved	K	Khan, A.A. (1966) Physiol. Plant 19, 869-874.
pear embryos	dormancy release	K	Khan, A.A. Plant Growth Substances 1970 pp. 207-215, Carr, D.J. (ed), Springer-Verlag Berlin, Heidelberg, New York. 1972.
DELAY OF SENESCENCE			
barley seedlings (etiolated)	retarded loss of synthetic ability of proteins and chlorophyll with aging	K	Stobart, A.K., Shewry, P.R. and Thomas, D.R. (1972) Phytochem. 11, 571-577.
oat leaf sections	chlorophyll retained	K,BAP (Z and i^6Ade ineffective)	Varga, A. and Bruisma, J. (1973) Planta 111, 91-93.
isolated discs of *Xanthium* leaves	yellowing delayed, RNA and protein levels maintained	K	Osborne, D.J. (1962) Plant Physiol. 37, 595-602.
corn seedling leaves	chlorophyll and protein preserved (senescence enhanced at low concentrations)	BAP	Tavares, J. and Kende, H. (1970) Phytochem. 9, 1763-1770.

detached Xanthium leaves	chlorophyll and protein degradation retarded	K	Richmond, A. and Lang, A. (1957) Science 125, 650-651.
intact bean plants	leaf senescence retarded	BAP	Fletcher, R.A. (1969) Planta 89, 1-8.

EFFECTS ON TRANSPORT

detached tobacco	glycine transported, against concentration gradient, from cell to cell to site of application	K	Mothes, K., Engelbrecht, L. and Kulaeva, O. (1959) Flora, 147, 445-464.
detached oat leaves	glycine and phosphate transported to application site	K	Gunning, B.E.S. and Barkley, W.K. (1963) Nature (Lond.) 199, 262-265.
corn leaves	phosphate transported to application site	K	Muller, K. and Leopold, A.C. (1966) Planta 68, 167-185, 186-205.
bean leaves	phosphate movementtto application site enhanced	K	Seth, A.K. and Waring, P.F. (1967) J. Exptl. Bot. 18, 65-77.
bean leaves	no observed increase in phosphorus uptake and translocation	K	Resnick, M.E. and Montaldi, E.R. (1968) Biol. Prod. Veg. 5, 99-111.
whole bean plants, senescing	no mobilization of ^{14}C and ^{32}P compounds in the whole plant	BAP	Adedipe, N.O. and Fletcher, R.A. (1970) J. Exptl. Bot. 21, 968-974.

Table (I) Continued

tobacco chloroplast	increased permeability of chloroplast membrane to ^{14}C-leucine	K	Richmond, A.E., Sachs, B. and Osborne, D.J. (1971) Physiol. Plant 24, 176-180.
leaf discs and detached cotyledons of sunflowers	increased uptake of K^+, Rb^+, Li^+ but not Na^+.	K	Richmond, (1971) Physiol. Plant 25, 230-233 and Ilan, I., Gilad, T. and Reinhold, L. (1971) Physiol. Plant 24, 337-241.
bean primary leaves	Na^+ absorption	BAP	Jacoby, B. and Dagan, J. (1970) Physiol. Plant 23, 397-403.

DEGRADATION AND SYNTHESIS PROCESSES

RNA and DNA

onion root nuclei	RNA content increased	K	Guttman, R. (1957) J. Biophys. Biochem. Cytol. 3, 129-131.
onion root tip cells	doubling of RNA levels, DNA levels reduced	K	Jensen, W.A. Pollock, E. G., Healy, P. and Ashton, M. (1964) Exptl. Cell Res. 33, 523-530.
excised soybean hypocotyl	inhibition of RNA synthesis, especially rRNA	K	Vanderhoef, L.N. and Key, J.L. (1968) Plant and Cell Physiol. 9, 343-351.
peanut cotyledons	RNA levels increased; no DNA increase	BAP	Carpenter, N.B. and Cherry, J. (1966) Biochim. Biophys. Acta 114, 640-642.

Table (I) Continued

barley leaves, tobacco pith culture	mRNA synthesis increased or preserved	K	Srivastava, B.I.S. (1967) Ann. N.Y. Acad. Sci. 144, 260-278.
tobacco pith cells	rapid DNA increase	K(auxin)	Patau, K., Das, N.K. and Skoog, F. (1957) Physiol. Plant 10, 949-966.
Ribosome Levels			
detached wheat leaves (senescing)	levels maintained	K	Shaw, M. and Manocha, M. (1965) Can. J. Bot. 43, 747-755.
excised tobacco leaves	rRNA preserved	K	Srivastava, B.I.S. (1967) Ann. N.Y. Acad. Sci. 144, 260-278.
excised barley leaves	rRNA and ribosomes preserved	K	Srivastava, B.I.S. and Arglebe, C. (1968) Physiol. Plant 21, 851-857.
Chinese cabbage leaves	rRNA and ribosomes preserved	K	Berridge, M.V. and Ralph, R.K. (1969) Biochim. Biophys. Acta 182, 266-269.
Protein Levels			
tomato fruit locule plastids	increased amino acid incorporation	K(IAA)	Davis, J.N. and Cocking, E.C. (1967) Biochem. J. 104, 23-33.
isolated tobacco chloroplast	stimulation of synthesis (age dependent effect)	BAP	Kulaeva, O.N. and Romanko, E.G. (1967) Dokl. Akad. Nauk. SSSR (Bot. Sci. Sec.) 117, 464-467.

Table (I) Continued

mitochondria of *Vigna* seedlings	increase in protein specific activity	K	Bhattacharyya, J. and Roy, S.C. (1969) Biochem. Biophys. Res. Comm. 35, 606-610.
Tropaeolum majus (detached leaves)	decreased degradation	K	Mizrahi, Y., Amir, J. and Richmond, A.E. (1970) New Phytol. 69, 355-361.
darkened oat leaves	inhibition of proteolysis	K	Shibaoka, H. and Thimann, K.V. (1970) Plant Physiol. 46, 212-220.
corn seedling leaves	inhibition of proteolysis	BAP	Tavares, S. and Kende, H. (1970) Phytochem. 9, 1763-1770.
soybean chloroplast	increased amino acid incorporation in older leaf chloroplast; no response in those from younger leaves	K	Marchetti, S.E. and Baron, F.J. (1971) Adv. Frontiers of Plant Sciences 28, 397-404.
tobacco cultures	inhibition of uracil and leucine incorporation	K	Nudel, V. and Bamberger, E.S. (1971) Plant Physiol. 47, 400-403.
Lemna minor	alterations in rates of synthesis and degradation; influenced by medium.	BAP	Trewavas, A. (1972) Plant Physiol. 49, 47-51.
Cyclic AMP Levels			
soybean callus (cultured in liquid suspension)	increased levels of cyclic nucleotide with treatment	K	Brewin, N.J. and Northcote, D.H. (1973) J. Exptl. Bot. 24, 881-888.
tobacco tissue	addition results in cell division which is correlated to cAMP level changes	K	Lundeen, C.V., Wood, H.N. and Braun, A.C. (1973) Differentiation 1, 255-260.

Table (I) Continued

EFFECTS ON SPECIFIC ENZYMES

(Activities and Synthesis)

RNase			
excised barley leaves	reduced activity	K	Srivastava, B.I.S. and Ware, G. (1965) Plant Physiol. 40, 62-64.
tobacco leaves	reduced activity	K	Bagi, G. and Farkas, G.L. (1968) Experientia 24, 397-398.
detached oat leaves	inhibition of RNase I activity	K(protein synthesis inhibitors or auxins)	Udvardy, J. Farkas, G.L. and Marre, E. (1969) Plant Cell Physiol. 10, 375-386.
barley leaf discs	counteraction of increase in RNase and proteinase activities	K,BAP	Atkin, R.K. and Srivastava, B.I.S. (1969) Physiol. Plant. 22, 742-750.
darkened oat leaves	inhibits rise in RNase level	K	Shibaoka, H. and Thimann, K.V. (1970) Plant Physiol. 46, 212-220.
Pisum sativum (apical regions of epicotyls)	suppresses IAA stimulated RNase activity	BAP	Birmingham, B.C. and Maclachlan, G.A. (1972) Plant Physiol. 49, 371-375.
Other Enzymes			
barley roots	Tyramine methylpherase, specific increase in this but not four other enzymes	K	Steinhart, C., Mann, J.D. and Mudd, S.H. (1964) Plant Physiol. 39, 1030-1038.