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Glucose induces two amino acid transport systems in *Chlorella*

(active uptake/arginine/proline/6-deoxyglucose)

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ABSTRACT **In autotrophically grown *Chlorella* cells, glucose induces a hexose transport system but, at the same time, the synthesis of two amino acid transport systems is also induced. Thus, the rates of uptake of glycine, L-alanine, L-proline, and L-serine, all of which compete with each other for entry into the cells, increase more than 100-fold when the algae are pretreated with glucose. The rates of L-arginine and L-lysine uptake increase by a factor of 25 to 50. The accumulation of proline and arginine within the cells amounts to 200- and 600-fold, respectively. Glucose does not cause the positive effect on amino acid uptake by serving as metabolic substrate because the nonmetabolizable 6-deoxyglucose also acts as inducer. Cycloheximide prevents the induction. The induced transport system for the four neutral amino acids has a turnover with a half-life of 7 hr, which corresponds closely to the half-life of the hexose transport system. The transport system for the basic amino acids, on the other hand, disappears with a half-life of 25 hr.**

Since the classical work of Rickenberg *et al.* (1) on the induction of the *lac* permease in *Escherichia coli*, many inducible transport systems have been described for bacteria (2–4). In a few cases this phenomenon has also been observed for eukaryotic cells (5, 6). Wherever induction has been found, however, the inducing substrates were either transport substrates and analogues of these or, like *allo*-lactose (7), a physiological substance closely related to the transport substrate.

It has been surprising, therefore, to find that glucose and nonmetabolizable glucose analogues act as inducers for two specific amino acid uptake systems in the unicellular alga *Chlorella vulgaris*. This autotrophically growing alga rapidly takes up various hexoses once a specific hexose transport system is induced by monosaccharides (6, 8). This transport of hexoses is an active one and proceeds as proton symport (9, 10).

This paper reports that *Chlorella* induced for sugar transport also shows an uptake rate for basic and neutral amino acids that is increased more than 100-fold compared to cells not pretreated with hexoses.

MATERIALS AND METHODS

The strain of *C. vulgaris* and the growth conditions were as described (8).

L-[^{14}C]Arginine, L-[^{14}C]alanine, and L-[^{14}C]proline were obtained from New England Nuclear; all other radioactive L amino acids were a gift of A. Böck. 6-Deoxyglucose was tritiated by Amersham. Cycloheximide and the nonradioactive amino acids were obtained from Serva (Heidelberg, Federal Republic of Germany), and 6-deoxyglucose was from Koch-Light (Colnbrook, England).

Uptake of Amino Acids. The screening for amino acid uptake that might be stimulated by glucose was performed with 0.1 mM labeled amino acid in an algal suspension of 50 μl of packed cells per ml in the presence or absence of 15 mM glucose. The disappearance of radioactivity from the medium was determined by centrifugation of the cells in intervals (2 or 5 min) and measurement of residual radioactivity in the medium. When the uptake of an amino acid was strongly stimulated by glucose, a lesser algal density (10 μl of packed cells per ml) and a higher concentration of amino acid (e.g., 1 mM) had to be used to determine an accurate uptake rate.

For competition and K_m determinations, cell density had to be decreased even further. For determining the K_m for L-arginine uptake, 0.3 μl of packed cells per ml was used. In these cases, the uptake of labeled amino acids was measured by filtering the incubation mixtures at 30-sec intervals and measuring radioactivity in the cells in a scintillation cocktail.

All incubations were performed under aerobic conditions in 25 mM sodium phosphate buffer (pH 6.0) at 27°C in a rotary shaker.

Analysis of Amino Acid Metabolism. The uptake of amino acids (e.g., L-arginine or L-proline), was followed up to a steady state, which was reached after about 1 hr. Samples of the suspension were filtered and extracted in distilled H_2O at 100°C for 5 min. The percentages of extractable and insoluble radioactivity were determined. Then the extract was separated by paper chromatography [butanol/acetic acid/ethyl acetate/water, 40:25:30:40 (vol/vol)] and the percentages of nonmetabolized amino acid and of metabolic products were determined. The same chromatographic procedure was also applied to samples of the supernatant of the algal suspension. It was ascertained by summation of supernatant and cell radioactivities that no significant amount of radioactivity was lost during incubation.

RESULTS

Effect of Glucose on Arginine and Alanine Uptake by *Chlorella*. *Chlorella* cells took up L-[^{14}C]arginine and L-[^{14}C]alanine very slowly from the medium (<1 μmol/ml of packed cells per hr); however, the rate of uptake increased dramatically, when the cells were incubated in the presence of glucose (Fig. 1). This increased rate of uptake was observed after a lag period of about 30 min. Because this is the time required for full induction of the hexose transport system of autotrophically grown *Chlorella*, the data of Fig. 1 could mean that, once available for the cells, glucose is used as energy source to power active amino acid uptake.

This simple interpretation of the effect observed, however, is not correct because *Chlorella* contains a large amount of reserve starch and does not require external substrates for cellular activity. Cells induced for sugar uptake by the nonmetabolizable hexose 6-deoxyglucose are able to take up 6-deoxyglucose

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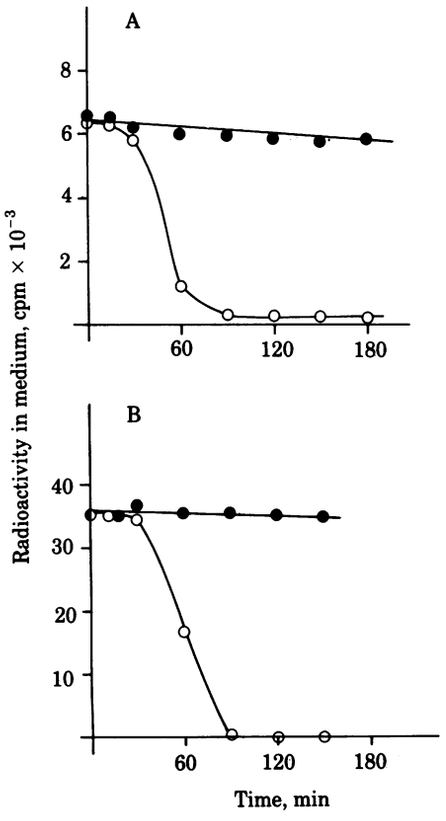

FIG. 1. Disappearance of L-arginine (*A*) and L-alanine (*B*) from the medium. Algae at a density of 80 μl of packed cells per ml were incubated with 1 mM L-arginine (specific activity, 35 nCi/μmol) or 2 mM L-alanine (specific activity, 90 nCi/μmol) in the absence (●) or presence (○) of 15 mM glucose. Samples (100 μl) of supernatant were assayed at intervals.

actively for hours and accumulate it more than 1000-fold (11). *Chlorella* treated with 6-deoxyglucose also shows an increased rate of amino acid uptake after a lag period of 30 min. Therefore, the glucose in the experiment of Fig. 1 definitely does not supply the energy for amino acid uptake. The results in Fig. 2 demonstrate that pretreatment of the cells with 6-deoxyglucose yields a rate of arginine uptake 58% of that achieved with glucose pretreatment.

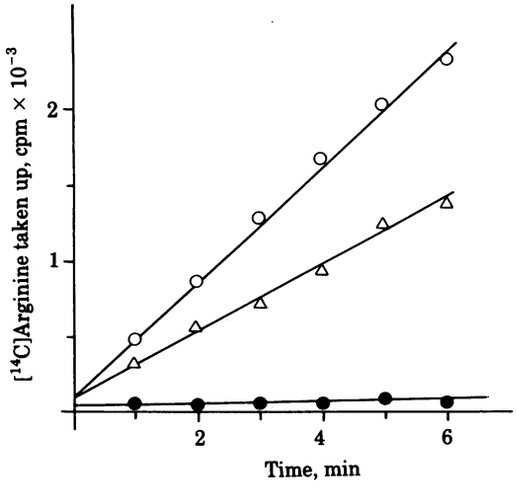

FIG. 2. Uptake of L-arginine by noninduced cells (●), glucose-induced cells (○), and 6-deoxyglucose-induced cells (△). The algae at 10 μl of packed cells per ml were incubated with 1 mM L-arginine (specific activity, 150 nCi/μmol). The cells had been pretreated for 3 hr, with 15 mM glucose or 6-deoxyglucose or without sugar. Samples (100 μl) were filtered at intervals.

Is There a Sugar Effect for all Amino Acids? The rates of uptake for all 19 L-amino acids and glycine were determined before and after treatment of the cells with glucose (Table 1). According to the degree their uptake is affected by glucose, the amino acids can be divided into three groups. In the first group are the two basic amino acids and four neutral ones (L-alanine, glycine, L-proline, and L-serine). For this group, the rate of uptake increased 25- to 150-fold due to the sugar pretreatment. Another group of amino acids, including aromatic and acidic ones, were not at all affected by the preincubation of the cells with glucose. A third group (L-histidine, L-glutamine, L-methionine, and L-threonine) showed lesser degree of increase in rate of uptake after glucose pretreatment (approximately 10-fold); the absolute rate of uptake, however, of these amino acids amounted to only about 10% of the uptake rates of the amino acids of the first group. It cannot be excluded, therefore, that the amino acids of the third group might be taken up via a side activity of the transport systems responsible for the transport of the amino acids of group 1.

Because the four neutral amino acids of group 1 as well as the two basic ones showed clear competitive behavior among each other, whereas no competition was observed between the neutral and the basic amino acids, the uptake of the amino acids of group 1 is obviously catalyzed by at least two different transport systems. Neither the neutral nor the basic amino acids, however, are taken up via the hexose transport system because no mutual competitive inhibitory effects between sugars and amino acids were observed. The amino acids, on the other hand, did not induce their own uptake.

Induction Versus Activation of Amino Acid Transport Systems. From the results reported so far, it is obvious that, in *Chlorella*, at least two amino acid transport systems are drast-

Table 1. Effect of glucose preincubation on rate of amino acid uptake

Amino acid	Rate of uptake, μmol/hr/ml packed cells*	
	No glucose	With glucose
L-Alanine	0.52	78
Glycine	0.75	79
L-Proline	0.45	66
L-Serine	0.74	90
L-Arginine	0.83	46
L-Lysine	1.22	29
L-Asparagine	0.42	0.42
L-Aspartic acid	0.41	0.42
L-Glutamic acid	0.39	0.39
L-Cysteine	0.45	0.50
L-Phenylalanine	0.16	0.16
L-Tyrosine	0.16	0.16
L-Tryptophan	<0.01	<0.01
L-Isoleucine	0.16	0.16
L-Valine	0.17	0.20
L-Leucine	<0.01	<0.01
L-Histidine	<0.05	3.2
L-Glutamine	0.68	5.1
L-Methionine	0.07	0.6
L-Threonine	0.25	2.1

* The amino acid uptake rates were measured at 80 μl of packed cells per ml and a saturating amino acid concentration (0.1–1 mM).

Table 2. K_m values for amino acid uptake

Amino acid	K_m, μM	
	No glucose	With glucose
L-Alanine	28	48
L-Proline	40	85
L-Arginine	1.4	2.2

ically increased in their activity when the cells are pretreated with glucose. The question arises as to whether glucose (and 6-deoxyglucose) induces the synthesis of amino acid transport proteins or whether the sugars act as positive effectors of already available proteins. The K_m values for amino acids did not change significantly due to glucose pretreatment of the cells (Table 2). This does not exclude the possibility, however, that the sugars induce the synthesis of the same kind of transport proteins that are already present in small amounts constitutively.

The question of induction versus activation, however, cannot be easily decided by using protein synthesis inhibitors in this case because the induction of the hexose transport system—which may be required for the glucose effect on amino acid uptake—is also prevented by such inhibitors (e.g., cycloheximide). When *Chlorella* cells were incubated in the presence of a high concentration of 6-deoxyglucose (0.1 M) plus cycloheximide, it was possible to achieve an inside concentration of the sugar analogue of 33 mM. Under this condition the uptake of arginine did not increase at all (Table 3). Because with cells able to take up arginine at a high rate a 3-hr incubation in cycloheximide does not inhibit arginine uptake significantly (Table 3), these results together can be taken as evidence that sugars induce the synthesis of amino acid transport systems.

Intracellular Accumulation of Arginine and Proline. A possible objection to the above interpretation could be that sugars do not induce amino acid transport systems but instead induce some reactions responsible for further metabolism of the amino acids in question. This possible metabolic reaction cannot be protein synthesis, of course, because only a few amino acids are affected by sugars. That the rate-determining uptake reaction indeed is measured with glucose-pretreated *Chlorella* when disappearance of amino acids from the medium is followed is shown by the data of Table 4. Even after 60 min, 52% (arginine) and 76% (proline) of the total radioactivity incorporated still was present as free amino acid in the cell. The data also demonstrate that *Chlorella* cells actively transport proline and arginine; these amino acids accumulated several hundredfold. [The membrane potential, −135 mV, of *Chlorella* (10) cannot account for the arginine accumulation observed, and an overshoot by exchange transport has also been excluded.]

Turnover of the Induced Amino Acid Transport System. It has been shown before that *Chlorella* cells induced for hexose uptake lose this ability after several hours when the inducing sugar or sugar analogue is removed from the cells (12). This even happens when the cells are kept under nongrowth conditions. Because the appearance and disappearance of a membrane protein correlate with the sugar transport activity of the cells (13), the inducible protein required for sugar transport is obviously degraded once the inducer is removed. To determine whether evidence for a turnover of the amino acid transport systems could be obtained, the change in activity for amino acid uptake was followed after the inducing sugar had been removed from the algae. The uptake system for neutral amino acids rapidly lost its activity in nongrowing cultures (Fig. 3). Its half-life was about 7 hr, the same as that of the hexose transport system in these experiments. The turnover of the protein responsible for transport of the basic amino acids was considerably slower; it had a half-life of about 25 hr.

Table 3. Effect of cycloheximide on the increase in uptake rates

Pretreatment of algae	Uptake, μmol/hr/ml packed cells	
	6-Deoxyglucose	Arginine
None	2.46	2.04
15 mM 6-deoxyglucose, 3 hr	256	28.1
100 mM 6-deoxyglucose + 15 μM cycloheximide, 3 hr	2.46	2.24
15 mM 6-deoxyglucose, 3 hr; then 15 μM cycloheximide, 3 hr	222	23.4

The rate of uptake was determined in the presence of 1 mM L-[^{14}C]-arginine (140 nCi/μmol) or 1 mM 6-deoxy[^{14}C]glucose (110 nCi/μmol).

Table 4. Accumulation of amino acids by algae pretreated with glucose

Amino acid	Free amino acid after incubation, mM		Accumulation factor	Free amino acids, % of total taken up
	In medium	In cells		
Proline	0.15	33	220	76
Arginine	0.04	25	625	52

Chlorella at 20 μl of packed cells per ml was incubated for 60 min in the presence of 1 mM amino acid (specific activity, 170 nCi/μmol).

DISCUSSION

Glucose and glucose analogues are taken up by *Chlorella* cells more than 100 times faster when the cells are induced for uptake (12). The induction is brought about by a number of hexoses and nonmetabolizable hexose analogues; because they all act coinductively *for* each other and compete *with* each other during uptake, it has been concluded that the various hexoses are all taken up by the same transport system (8). Surprisingly, however, glucose as well as the nonmetabolizable 6-deoxyglucose also coinduce two amino acid transport systems. Because little is known about the molecular mechanism of induction in *Chlorella*—as is the case for most eukaryotic cells—one has to speculate on the basis of the terminology and ideas available for bacterial cells. Thus, it might be possible that various transport systems required for heterotrophic growth are aligned in one operon which is under the inductive control of hexoses. The

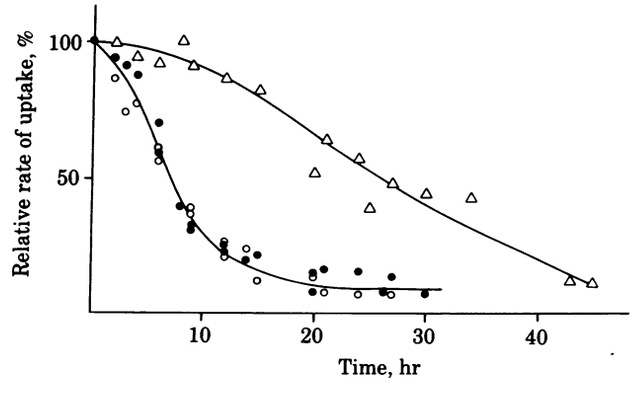

FIG. 3. Turnover of the glucose, L-proline, and L-arginine uptake systems. Cells (at 20 μl of packed cells per ml) were induced for sugar and amino acid uptake with glucose, washed free of external sugar at 0 min, and then incubated without sugar. At various times the uptake activity was tested with 1 mM 6-deoxyglucose (○), L-proline (●), or L-arginine (△).

observation that the rate of turnover of the basic amino acid transport system is considerably slower than that for hexoses and for neutral amino acids does not exclude a common operon because the rate of turnover might be mainly determined by the rate of breakdown of these transport proteins. The possible proteolytic hydrolysis would have to act more slowly on the protein catalyzing the transport for basic amino acids.

The occurrence of a new integral membrane protein with a molecular weight of 40,000 (14) after induction with glucose or 6-deoxyglucose has been described in double-labeling experiments (13). From the results reported here, it seems likely that at least three proteins are present in this 40,000 band; this will have to be checked by using two-dimensional gel separations.

The biological importance of the inducible hexose transport system of *Chlorella* is not understood. But whatever the natural circumstances which make autotrophically grown *Chlorella* switch to heterotrophic carbon metabolism, a supply of amino acids for complete or partial heterotrophic nitrogen metabolism might be available at the same time. This at least could be the teleonomic (15) explanation of the coinduction phenomena observed.

It cannot be ruled out that the phenomenon described remains a queer one restricted to *Chlorella*, but it might also be more common among heterotrophic organisms. Supply of carbon and organic nitrogen in nongreen parts of higher plants also could be coordinated as described here for *Chlorella*. Similarly, the proton cotransport feature of sugar uptake of Chlorella (9, 16) has helped to elucidate sucrose uptake, including phloem loading, in higher plants (17, 18).

Finally, it should be pointed out that, in induced *C. vulgaris*, the rates of uptake of the four neutral amino acids alanine, serine, proline, and glycine as well as of the two basic amino acids arginine and lysine are more than 5–10 times faster than the rate of amino acid uptake reported for any other plant cell. Thus, *C. vulgaris* induced for amino acid uptake should also be a good eukaryotic organism to use in studies of active transport of amino acids in detail.

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