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A general amino-acid permease is inducible in *Chlorella vulgaris*

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Abstract. Glucose or non-metabolizable glucose analogues induce two systems of amino-acid transport in *Chlorella vulgaris*: an arginine-lysine system and a proline system. An additional third system of amino-acid transport is induced when glucose and an inorganic nitrogen source are present during glucose induction. The transport rates in glucose-NH_4^+-treated cells are 10 to 80 times higher than in untreated cells. The transport system shows a rather broad specificity and catalyses the transport of at least ten neutral and acidic amino acids. Three of these amino acids (L-alanine, L-serine and glycine) are transported by the proline system as well. The system is specific for L-amino acids and has a pH optimum between 5 and 6. Transport by this system seems to be active, since amino acids are accumulated inside the cells.

Key words: Amino acid transport – *Chlorella* – Transport induction (amino acid).

Introduction

Cells of lower and higher plants are able to use inorganic nitrogen in the form of nitrate or ammonia as their sole nitrogen source, and they grow perfectly well in the absence of external amino acids, amines or other organic N compounds. In all these plants, however, amino-acid transporting activities can be measured and in many cases these activities have been shown to be the result of transport systems specifically catalysing this uptake (Cho et al. 1981; Harrington and Henke 1981; Komor et al. 1981; Richards and Thurston 1980). In bacteria or yeast cells, most systems of amino-acid transport are constitutive or derepressible during nitrogen starvation (Eddy 1982; Grenson et al. 1970; Oxender 1972); the amino-acid transport systems in plant cells are usually also constitutive (Felle 1981; Komor et al. 1981; Richards and Thurston 1980; Van Sumere and Dedonder 1971; Pedersen and Knutsen 1974). In *Chlorella vulgaris*, surprisingly, glucose and glucose analogues can induce two amino-acid transport systems (Cho et al. 1981). One of these systems is specific for the basic amino acids L-arginine and L-lysine (the arginine system) and the other for neutral amino acids with small side chains i.e. L-alanine, glycine, L-serine and L-proline (the proline system). In contrast to some amino-acid permeases from yeast and bacteria, which are repressed in the presence of ammonia (Grenson et al. 1970; Higgins and Ferro-Luzzi Ames 1982; Schwenke and Magana-Schwenke 1969), high concentrations of ammonia or nitrate do not inhibit these transport systems (Sauer 1982). On the other hand, both the arginine system and the proline system are induced even in the absence of glucose when the cells are depleted of their nitrogen source (Sauer et al. 1983). This latter behaviour is very similar to the derepression mechanism in yeast and bacteria mentioned above. In this paper, a third amino-acid transport system with a broad specificity for ten amino acids will be described. This system is induced only under unusual conditions, when D-glucose plus relatively high concentrations of inorganic nitrogen are present in the incubation medium. Neither glucose nor ammonia (or nitrate) ions alone can induce this transport system. Some characteristics of this system have been studied.

Material and methods

Plant material. The strain of *Chlorella vulgaris* was that used by Tanner and Kandler (1967). The cells were grown under the conditions described by Sauer and Tanner (1984). Nitrogen-starved cells were prepared as described previously (Sauer et al. 1983).

Chemicals. L-[U-^{14}C]Arginine (specific activity $1.263 \cdot 10^{13}$ Bq mol^{-1}), L-[U-^{14}C]glutamine (specific activity $1.059 \cdot 10^{13}$ Bq mol^{-1}), L-[U-^{14}C]proline (specific activity $1.093 \cdot 10^{13}$ Bq mol^{-1}) and a L-[U-^{14}C]amino acid kit (specific activity $3.77 \cdot 10^{11}$ Bq mol^{-1} each) were purchased from New England Nuclear (Boston, Mass., USA). L-2-Amino-[1-^{14}C]isobutyric acid (specific activity $0.219 \cdot 10^{13}$ Bq mol^{-1}), L-[U-^{14}C]cysteine hydrochloride (specific activity $0.219 \cdot 10^{13}$ Bq mol^{-1}) and L-[methylene-^{14}C]tryptophan (specific activity $(0.291 \cdot 10^{13}$ Bq mol^{-1}) were obtained from Amersham Buchler (Braunschweig, FRG). D-Glutamine and D-methionine were from Sigma (Munich, FRG).

Treatment of the cells to induce transport activity. For induction of transport activity, the cells were harvested, washed with distilled water and resuspended to a density of 50 µl packed cells ml^{-1} in 25 mM sodium-phosphate buffer, pH 6.0. These cells were shaken for 3 h at 27° C in the presence of 13 mM D-glucose or in the presence of 13 mM D-glucose plus 10 mM NH_4Cl (or $NaNO_3$). Control cells (=non-induced cells) were shaken for the same time without the addition of sugar or a nitrogen source.

Uptake experiments. Uptake experiments were carried out as described previously (Sauer et al. 1983).

Determination of pH-dependence of uptake. For the determination of the pH-dependence, uptake experiments were performed with cells at a density of 30 µl packed cells ml^{-1}. For measurements at pH 5.0 or below, 25 mM citrate buffer was used, and measurements at pH 5.0 and above were made in 25 mM sodium-phosphate buffer. Uptake rates were determined in both buffer systems at pH 5.0, and the rates in citrate buffer were corrected for the difference in uptake rates obtained in the two buffer systems at pH 5.0.

Determination of apparent K_m values. Uptake experiments were performed as above. Cell densities varied from 5 µl packed cells ml^{-1} at 0.1 mM L-glutamine (specific activity $6.28 \cdot 10^{11}$ Bq mol^{-1}) to 0.1 µl packed cells ml^{-1} at 3.1 µM L-glutamine (specific activity $2.03 \cdot 10^{12}$ Bq ml^{-1}).

Determination of the accumulation factor. Intracellular and extracellular concentrations of free amino acids were determined as described by Cho et al. (1981).

Results

Induction of the general amino-acid transport system. D-Glucose and non-metabolizable glucose analogues induce two amino-acid transport systems in *Chlorella vulgaris* (Cho et al. 1981). The same two transport systems are induced after nitrogen starvation of the cells (Sauer et al. 1983). After *Chlorella* cells were incubated in the presence of 13 mM D-glucose and 10 mM NH_4Cl (or 10 mM $NaNO_3$) for 3 h, a further seven amino acids (L-glutamine, L-asparagine, L-cysteine, L-histidine, L-methionine, L-leucine and L-glutamic acid) are transported with distinctly increased uptake rates (Table 1). The uptake rates for these amino acids increase up to 80-fold and these rates are similar to those obtained with the two amino-acid transport systems after glucose induction or after nitrogen starvation (see also Table 3). In Fig. 1 it is shown that these increased transport rates cannot be explained by an effect of ammonium ions on the algae; the uptake rates determined after incubation of *Chlorella* cells in glucose or NH_4Cl alone differ only slightly from the uptake rates in untreated control cells. The high transport activities are observed only in cells treated with both substances at once. Glucose cannot be replaced by its non-metabolizable analogue 6-deoxyglucose. In Fig. 2 it is demonstrated that pretreatment of the cells with 13 mM D-glucose plus 10 mM $NaNO_3$ (glucose/NO_3^-) instead of 13 mM D-glucose plus 10 mM NH_4Cl (glucose/NH_4^+) has almost the same effect on the transport acitivty of the algae. These inorganic nitrogen sources could not be substituted with organic nitrogen compounds, not even with the amino acids transported after glucose-NH_4^+ incubation (data not shown).

How many transport systems are responsible for the increased amino-acid transport? First it had to be clarified whether one or more transport systems

Table 1. Effect of glucose-NH_4Cl preincubation on amino-acid transport in *Chlorella vulgaris*. The uptake rates of pretreated cells were measured at 10 µl packed cells ml^{-1}, the rates of untreated cells at 30–50 µl packed cells ml^{-1}. The final substrate concentration was 1 mM, rates were determined during the first 4 min after amino-acid addition

Amino acid	Rates of uptake [µmol h^{-1} ml packed cells^{-1}]		
	Cells not pretreated	Cells pretreated with glucose-NH_4^+	
L-Arginine	1.2	152.0	Arginine system
L-Lysine	3.2	140.7	
L-Proline	1.0	58.0	Proline system
L-Serine	<0.5	75.4	
L-Alanine	1.7	96.1	
Glycine	2.4	108.4	
L-Asparagine	1.9	18.2	General amino-acid transport system
L-Glutamine	0.9	78.8	
L-Glutamic acid	2.6	34.3	
L-Cysteine	0.4	46.0	
L-Histidine	1.2	38.6	
L-Methionine	1.1	24.3	
L-Leucine	1.0	25.1	
L-Aspartic acid	1.1	3.5	
L-Threonine	0.5	6.4	
L-Phenylalanine	1.5	3.5	
L-Tyrosine	0.5	5.8	
L-Tryptophan	1.0	0.7	
L-Isoleucine	<0.5	2.1	
L-Valine	<0.5	7.9	

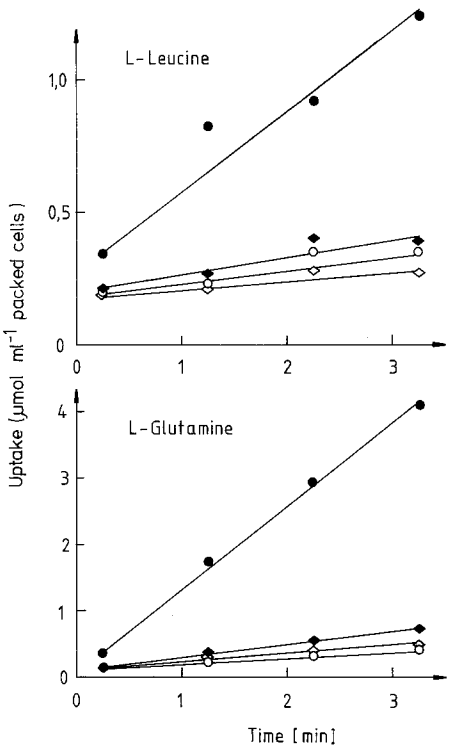

Fig. 1. Transport of L-leucine and L-glutamine in untreated (= control) cells (o—o), in Glucose-treated cells (◆—◆), in NH_4Cl-treated cells (◇—◇) and in cells treated with glucose plus NH_4Cl (●—●). Incubations were performed as described in Material and methods

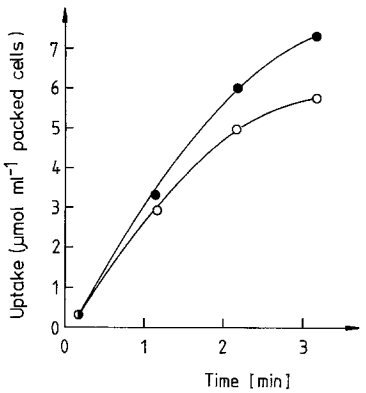

Fig. 2. Comparison of glucose-NH_4^+ treatment and glucose-NO_3^- treatment. Cells were induced as described in Material and methods in the presence of 13 mM D-glucose plus 10 mM NH_4Cl (●—●) or plus 10 mM $NaNO_3$ (o—o). After 3 h the uptake rates for L-glutamine were determined. The same results were obtained for the other amino acids transported after glucose-NH_4^+ treatment

were responsible for the high transport rates of these amino acids. This was done by competition experiments. The uptake rates for the seven amino acids were determined with and without an excess of L-glutamine and the percentage of inhibition was calculated. Owing to its high rate of transport (Table 1), L-glutamine seemed to be a suitable substrate and a good inhibitor of the uptake of those amino acids transported by the same system as L-glutamine. The data are summarized in Table 2. The transport of all these amino acids is drastically inhibited in the presence of L-glutamine. The inverse, inhibition of L-glutamine transport by L-histidine and L-methionine, is also shown. The uptake of L-proline and L-arginine, two amino acids that are transported by the systems mentioned above, is almost not influenced by L-glutamine (Table 3). The transport of the amino acids of the last group in Table 1, which show increased, but comparatively low transport rates after glucose-NH_4^+ incubation (for instance L-tyrosine, L-valine or L-aspartic acid), is inhibited to some extent in the presence of L-glutamine (Table 2). The absolute decrease in the uptake rates for these amino acids, however, is relatively small compared with the other amino acids.

These data indicate that only one transport system is responsible for the increased transport rates after glucose-NH_4^+ treatment of the algae. It cannot be said with certainty whether the amino acids with relatively low transport rates after glucose-NH_4^+ incubation are also taken up by this system or not.

Effect of glucose-NH_4^+ pretreatment on the arginine and proline systems. The six amino acids that are transported by the arginine and the proline systems after glucose induction are also taken up with high rates after glucose-NH_4^+ preincubation (Table 1). In order to determine whether this uptake is caused by these two systems alone, or whether there is at least some overlap with the glucose-NH_4^+-induced transport system described above, the transport rates for these six amino acids were measured after glucose treatment alone and after simultaneous incubation with glucose and ammonia in parallel experiments. The transport rates of both L-arginine and L-lysine (arginine system) are higher after glucose-NH_4^+ treatment than after glucose treatment alone (Table 3, group A). It seems unlikely, however, that this additional activity is caused by the transport system that also catalyses the uptake of the other amino acids after glucose-NH_4^+ incubation, because only a very small inhibition of L-arginine uptake by L-glutamine occurred. Further, it is demonstrated in Table 4 that the increase in transport activity for L-arginine and L-lysine in glucose-NH_4^+-treated cells is the result of an effect of ammonium ions alone. When *Chlorella* cells were incubated in 10 mM NH_4Cl for 3 h, the

Table 2. Inhibition of amino-acid uptake in glucose-NH_4^+-treated cells by other amino acids. The concentration of the amino acids tested was 1 mM. The uptake rates measured for L-glutamine without addition reflect the variation between different experiments

Amino-acid tested	Addition	Uptake rate without addition	Uptake rate with addition	Inhibition (%)
L-Leucine	10 mM L-Glutamine	25.1	7.5	70
L-Asparagine	10 mM L-Glutamine	18.1	1.0	95
L-Cysteine	10 mM L-Glutamine	46.0	9.0	80
L-Glutamic acid	10 mM L-Glutamine	34.3	1.0	95
L-Histidine	10 mM L-Glutamine	38.6	15.4	60
L-Methionine	10 mM L-Glutamine	24.3	6.6	72
L-Tyrosine	10 mM L-Glutamine	5.8	5.0	14
L-Valine	10 mM L-Glutamine	7.9	6.1	23
L-Aspartic acid	10 mM L-Glutamine	3.5	2.0	43
L-Isoleucine	10 mM L-Glutamine	2.1	1.7	19
L-Glutamine	10 mM L-Glutamic acid	42.2	5.0	88
L-Glutamine	2 mM L-Methionine	37.3	9.9	76
L-Glutamine	10 mM L-Methionine	42.2	3.0	93
L-Glutamine	5 mM L-Histidine	59.5	45.9	23
L-Glutamine	10 mM L-Histidine	59.5	38.3	36
L-Glutamine	10 mM L-Proline	35.2	34.6	2
D-Amino acids:				
L-Glutamine	2 mM D-Methionine	37.3	37.6	0
L-Glutamine	2 mM D-Glutamine	37.3	36.9	0

Table 3. Transport rates in the presence and in the absence of 10 mM L-glutamine (μmol h^{-1} ml packed $cells^{-1}$)

Amino-acid transported	Glucose-treated cells	Glucose-NH_4^+-treated cells	
			+L-glutamine
A			
L-Arginine	122.8	152.0	142.9
L-Lysine	86.4	140.7	149.7
B			
L-Proline	51.0	58.0	54.1
L-Serine	42.8	75.4	51.9
L-Alanine	39.3	96.1	46.4
Glycine	51.6	108.4	66.7

Table 4. Transport rates in cells after different pretreatments (μmol·h^{-1}·ml packed $cells^{-1}$)

Amino acid	No pretreatment	Glucose pretreatment	Glucose-NH_4^+ pretreatment	NH_4^+ pretreatment
L-Arginine	1.2	80.7	113.1	44.6
L-Lysine	3.2	86.4	140.7	30.2
L-Proline	1.0	51.0	58.0	4.5
L-Glutamine	0.9	4.0	45.2	4.0
L-Leucine	1.1	3.6	19.2	0.8

transport rates for L-arginine and L-lysine increased to values similar to the difference in rates between glucose- and glucose-NH_4^+-treated cells. In contrast, the uptake of the other amino acids did not increase after NH_4Cl incubation (Table 4).

Table 3 (group B) shows that L-glutamine has no influence on the L-proline transport in cells pretreated with glucose-NH_4^+. Thus L-proline is trans-

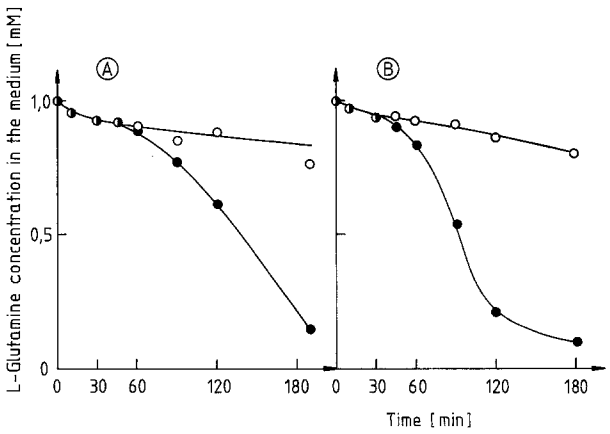

Fig. 3A, B. Disappearance of L-glutamine from the medium. In **A** the algae were incubated at a cell density of 50 μl packed cells ml^{-1} in buffer containing 13 mM D-glucose, 10 mM NH_4Cl and 1 mM L-glutamine (specific activity $6.26 \cdot 10^9$ Bq ml^{-1}) in the absence (●——●) or presence (○——○) of 15 μM cycloheximide. **B** shows the same experiment, but the cells were preincubated in 13 mM D-glucose for 3 h before the start of the experiment. The radioactivity in 50 μl of supernatant was determined

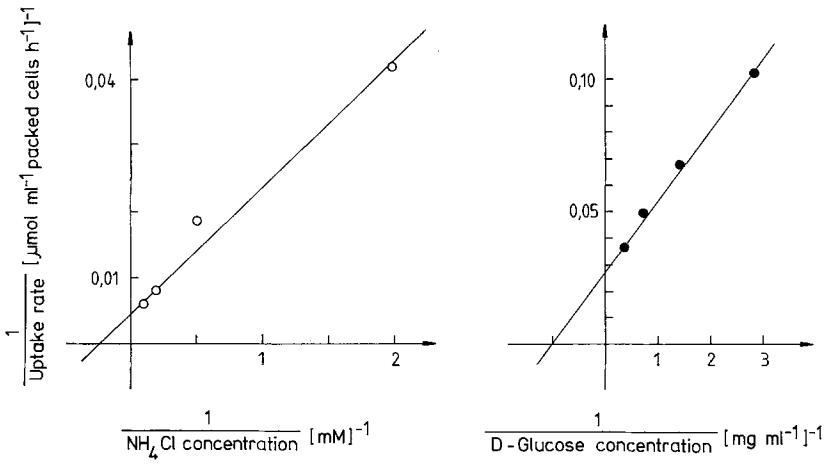

Fig. 4. Determination of the concentrations of D-glucose and NH_4Cl necessary for the induction of 50% transport activity of the general permease. To test the glucose concentration the algae were induced with a constant NH_4Cl concentration (10 mM). The amount of glucose varied from 0.35 mg ml^{-1} to 2.8 mg ml^{-1}. To determine the NH_4Cl concentration, the concentration of glucose was kept at 13 mM and NH_4Cl varied from 0.5 mM to 10 mM. After 3 h of incubation the uptake rates for L-glutamine were determined. The data are presented as double-reciprocal plots

ported only by the proline system and can be used as a specific substrate for determining the activity of this system. It is clear from the data in Table 3, however, that L-serine, L-alanine and glycine are transported much better after glucose-NH_4^+ treatment than after glucose treatment alone. This additional transport can be inhibited by L-glutamine to 70–80% of its original value. This means that besides the seven amino acids mentioned above, three amino acids transported by the proline system are also transported by the newly described permease. Since this permease catalyses the transport of at least ten different amino acids (and maybe even more, although with very low uptake rates) it can be designated the "general amino-acid transport system" of *Chlorella vulgaris*.

The system is specific for L-amino acids, because neither D-methionine nor D-glutamine have any influence on the transport of L-glutamine (Table 2). α-Aminoisobutyric acid is not transported by any of the three amino-acid transport systems in *Chlorella* described so far.

Is the general amino-acid permease activated or newly synthesized? Most of the experiments to characterize further this new permease were performed with L-glutamine, since its transport is exclusive to this system and since it has the highest transport rate of all amino acids carried by this system.

Is this increased rate of uptake caused by protein activation or by the de-novo synthesis of an amino-acid transport system? From the results shown in Fig. 3, one can see that a lag-period of 30–45 min passes after the addition of glucose and NH_4Cl to the cells before a difference in the transport behaviour can be detected. This period is as long as the lag-phase for induction of the glucose transport system in *Chlorella* (Haaß and Tanner 1974). Further, in the presence of 15 µM cycloheximide, no induction takes place (Fig. 3). This is evidence against protein activation. It could be argued that in the presence of cycloheximide the induction of the glucose transport system is also abolished, preventing enough of the molecules important for induction from entering the cells. This problem can be overcome by using glucose-pretreated cells, in which the glucose transport system is already induced. In such cells, both glucose and ammonia can enter the cells from the outset, but the same lag-period and the same inhibition with cycloheximide is observed (Fig. 3 B).

Which concentrations of D-*glucose and* NH_4Cl *are necessary for induction of the general amino-acid transport system?* The concentrations of D-glucose or NH_4Cl at which half-maximal induction takes place were calculated from the plots in Fig. 4. For D-glucose a value of about 5 mM and for NH_4Cl a concentration of about 3 mM were found to give half the transport activity of fully induced cells after 3 h incubation. For this reason 13 mM D-glucose and 10 mM NH_4Cl were chosen for induction to get maximal transport rates. There is no indication that NH_4Cl is inhibitory or poisonous to the cells at this concentration. Figure 2 shows that glucose plus 10 mM $NaNO_3$ (which can be used instead of NH_4Cl during pre-treatment) in-

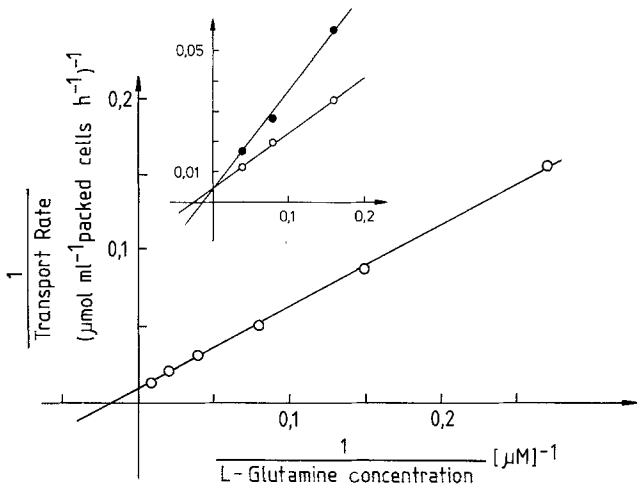

Fig. 5. Determination of the apparent K_m value of the unspecific permease for L-glutamine (o—o) (Lineweaver-Burk plot). The *inset* shows the determination of the apparent K_m value in the presence (●—●) and in the absence (o—o) of 12.5 µM L-methionine. The experiments were performed as described in Material and methods

duces about 80% of the transport activity obtained after preincubation with glucose-NH_4^+.

Apparent affinity of the general amino-acid transport system for glutamine. The uptake experiments described so far for the general amino-acid transport system were performed at pH 6.0 with a substrate concentration of 1 mM. Glutamine is saturating at this concentration and the apparent K_m (derived from two experiments, and presented in the plot in Fig. 5) is 50 µM. The inset in Fig. 5 shows that L-glutamine transport is competitively inhibited by L-methionine. The apparent K_m value for L-glutamine is comparable to the values determined for arginine and proline in the respective transport systems for their amino acids in *Chlorella* (Cho et al. 1981). The maximal uptake rates for L-glutamine are measured at pH values between 5 and 6 (Fig. 6). The arginine and the proline systems also show their highest activities at a pH value below pH 7 (Fig. 6).

Is the transport by the general amino-acid transport system active? Finally the question was addressed, whether the transport of amino acids by the general permease is active or not. This was investigated by determining the internal concentrations of several amino acids after a 1-h uptake experiment. In Table 5 it is shown that after this time, 71% of the incroporated L-leucine and 14% of L-glutamine are still present as free amino acids inside the cells, and their accumulation factors were 15.9-fold and 2.4-fold, respectively. Since both of these amino acids are "zwitterions" at pH 6.0, their uptake will not be influenced by the membrane potential; an active transport has to be postulated, therefore, to explain these accumulation ratios.

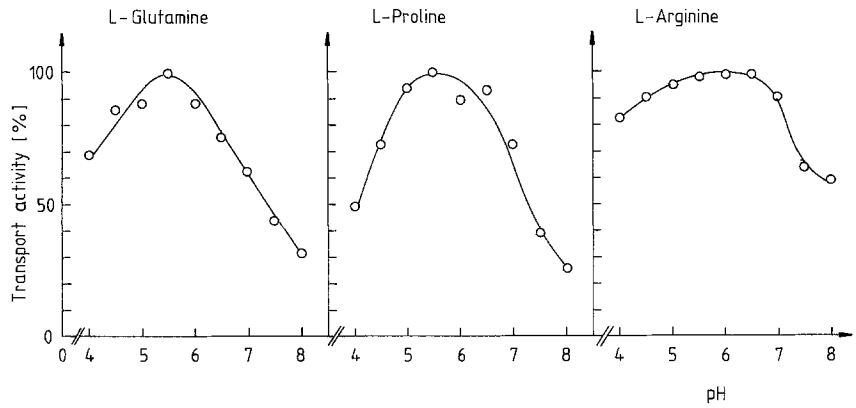

Fig. 6. pH-Dependence of the proline system, the arginine system and the general permease. The dependence of the general permease was determined with L-glutamine as described in Material and methods

Table 5. Accumulation of amino acids by algae pretreated with glucose-NH_4^+. The cell density during amino-acid uptake was 30 µl packed cells ml^{-1}. The specific activity of both amino acids was $6.41 \cdot 10^9$ Bq mol^{-1}. Concentrations of free amino acids in the cells before incubation were determined as described by Sauer et al. (1983)

Amino acid	Free amino acid before incubation [mM]		Free amino acid after incubation [mM]		Accumulation factor	Free amino acids (% of total taken up)
	In medium	In cells	In medium	In cells		
L-Glutamine	2.0	0.17	1.70	4.0	2.4	14
L-Leucine	1.0	0.35	0.75	11.9	15.9	71

Discussion

Chlorella vulgaris, a unicellular, non-flagellate green alga is able to induce a sugar transport system when certain hexoses are present in the culture medium. Two amino-acid transport systems with rather narrow specificities are synthesized by the cells at the same time. In this paper it is shown that the simultaneous presence of glucose and NH_4Cl (or $NaNO_3$) enables the cells to induce a third uptake system for amino acids, which has a rather broad specificity. This system is newly synthesized, since no induction occurred in the presence of cycloheximide. With this permease, the cells are able to make use of amino acids as different as L-glutamic acid and L-methionine. It is demonstrated by competition experiments that at least one acidic and nine neutral amino acids share this transport system. It cannot be ruled out that some amino acids with low transport rates are also recognized by this system. Basic amino acids, however, most probably are not substrates for this general permease. As has been shown for the general amino-acid permease in yeast (Grenson et al. 1970), L-proline is also not transported by the general amino-acid transport system in *Chlorella*. From the data in Table 3 it is clear that the other three amino acids taken up by the proline system, are transported as well or even better by the general system. From these data, it follows that the amino configuration is necessary to allow transport by the general amino-acid transport system.

The general amino-acid transport system can translocate its substrates against a concentration gradient. This uptake is not influenced by the membrane potential, since the transported amino acids are neutral or even negatively charged. An H^+-amino acid cotransport might be responsible for the accumulation ratios obtained. The importance to *Chlorella* of this transport system, which is synthesized only when the supply of carbon and nitrogen in the medium is plentiful is unclear at the moment.

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