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Uptake of amino acids by the cyanobacterium *Anabaena* ATCC 27893

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SUMMARY

Active uptake of phenylalanine has been characterized in a species of cyanobacterium, *Anabaena* sp. ATCC 27893. Phenylalanine was apparently taken up by only one transport system over the concentration range 2–50 μM. Inhibition of phenylalanine uptake by glutamate and a range of neutral amino acids suggested that these were internalized by the same system. At least one other system appeared to be present, transporting the basic amino acids arginine and lysine. The K_m and V_{max} of phenylalanine uptake were comparable to those of phenylalanine uptake in eukaryotic unicellular freshwater algae. Uptake was energy dependent and internalized phenylalanine was rapidly incorporated into trichloracetic acid-insoluble material.

Key words: Amino-acid transport, cyanobacteria, *Anabaena* sp. ATCC 27893.

INTRODUCTION

The number and specificity of amino-acid transport systems varies considerably between different organisms. In *Escherichia coli*, there are as many as 30 amino-acid transport systems, most of which are very specific (Oxender, 1972; Anraku, 1982; Hama *et al.*, 1988). In the yeast *Saccharomyces cerevisiae*, a general amino acid permease and several narrowly specific amino-acid transport systems have been reported (Cooper, 1982). Conversely, there are only two or three broadly specific systems in higher plant cells (Kinraide & Etherton, 1980; Kinraide, 1981; Robinson & Beevers, 1981; Wyse & Komor, 1984; Vaisanen & Sopanen, 1986). Decreased number and non-specificity of amino-acid transport systems may be a consequence of integration of higher plant cells into tissue systems in which the extracellular environment is homeostatically maintained. Thus, the unicellular green alga *Chlorella*, subject to environmental fluctuations in availability of amino acids, not only possesses uptake systems of broad specificity, but also at least three that take up only a single amino acid, methionine, threonine and glutamine respectively (Sauer, Komor & Tanner, 1983; Cho & Komor, 1985).

Cyanobacteria are ideal organisms for experimental investigation of whether the narrow specificity and multiplicity of amino acid transport systems seen in prokaryotic heterotrophs and yeasts is a consequence of adaptation to a changeable environment, or to the absence of light dependent carbon fixation. Cyanobacteria are typical prokaryotes, but also possess a fully functional oxygen-evolving photosynthetic system similar to that of higher plants. Although they are autotrophs, and many strains are also capable of nitrogen fixation, many cultured cyanobacteria can use amino acids as nitrogen or carbon sources (Neilson & Larsson, 1980; Vaishampayan, 1982; Rawson, 1985; Spence & Stewart, 1986). However, there are only a few reports on the kinetics and specificity of amino-acid transport systems in these organisms (Faust, Orcutt & Prazma, 1974; Lee-Kaden & Simonis, 1982; Chapman & Meeks, 1983; Strasser & Falkner, 1986). In the work discussed in this paper, the energy dependent uptake of L-phenylalanine is used to characterize amino-acid transport systems in the filamentous, heterocyst-forming *Anabaena* sp. ATCC 27893.

MATERIAL AND METHODS

Organisms and chemicals

Anabaena sp. ATCC 27893 was the kind gift of Professor G. A. Codd and Dr G. C. Machray, De-

partment of Biological Sciences, University of Dundee. This strain contains an antibiotic resistant plasmid, pRL10 (McFarlane, Machray & Stewart, 1987; Schmetterer & Wolk, 1988), and stock cultures were grown on agar containing chloramphenicol to prevent bacterial contamination. Cultures were grown in BG11 medium (Stanier *et al.*, 1971) in air at 25 °C in a shaking incubator, and illuminated continuously by cold fluorescence tubes at 54 μmol m^{-2} s^{-1}.

[U-^{14}C]amino acids were obtained from Amersham International plc; unlabelled L-amino acids and carbonylcyanide *m*-chlorophenylhydrazine (CCCP) were from Sigma Chemical Co.

Determination of amino-acid uptake

Cells were harvested from exponentially growing cultures by centrifugation. All but 0·5 ml of the medium was taken off from the pellet, and 0·5 ml of 4 mM sodium phosphate buffer (pH 7·6) was added. The resuspended cyanobacteria were then incubated for 30 min under temperature and light conditions similar to those of growth. To start the experiments, 0·5 ml of the cyanobacterial suspension was added to 0·5 ml of 2 mM sodium phosphate buffer containing radioactively labelled amino acid. At intervals of 5 or 10 min, samples were filtered under low vacuum pressure through prewetted Whatman GF/C 2·5 cm diameter filter discs and washed with 10 ml distilled water. Discs were dried in air and their radioactivity measured by scintillation counting. The amount of amino acid taken up was determined as described by McAuley (1986). Uptake rates were determined by linear regression; affinity constants (K_m) and maximum uptake rates (V_{max}) were calculated from Woolf plots (S *vs.* S/v).

Partition of L-phenylalanine between trichloracetic acid soluble (pool) and insoluble (protein) fractions was determined from the amount of radioactivity in the cells before and after extraction for 2 h with ice-cold 5% (w/v) trichloroacetic acid (Pedersen & Knutsen, 1974; McAuley, 1987). Competition between different amino acids for the same transport system was determined by measuring uptake of radioactive amino acid (0·7–10·0 μM) in the presence or absence of an unlabelled amino acid (50 μM).

Chlorophyll determination

Cells were centrifuged and resuspended in 80% acetone. After extraction for 24 h at 4 °C, chlorophyll was determined by the method of MacKinney (1941).

RESULTS

Transport systems

Anabaena sequestered a wide variety of amino acids (Fig. 1). At the low concentrations used to test for the presence of amino-acid transport systems, uptake of all amino acids was linear. Uptake of arginine was measured at a much greater concentration (20 μM) because the affinity of *Anabaena* for this amino acid appeared to be very high.

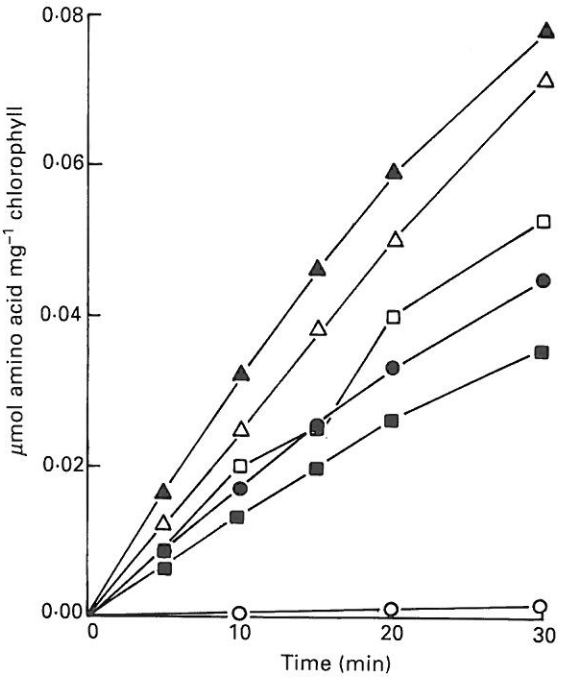

Figure 1. Uptake of [U-^{14}C]amino acids by *Anabaena* ATCC 27893. Cells isolated from culture were allowed to equilibrate in experimental conditions for 30 min before addition of [U-^{14}]amino acid [3·7 kBq ml^{-1}, except for phenylalanine (7·4 kBq ml^{-1}) and arginine (111·0 kBq ml^{-1})]. At intervals, samples were removed and filtered, after which retained radioactivity was measured as described in Materials and Methods. ●——●, Phe (0·9 μM); ○——○, Asp (1·0 μM); ■——■, Asn (1·0 μM); □——□, Arg (20·0 μM); ▲——▲, Ala (1·3 μM); △——△, Lys (0·7 μM).

Uptake of phenylalanine

The rate of uptake of phenylalanine was dependent upon substrate concentration, showing Michaelis–Menten kinetics, with K_m and V_{max} of 6·7 μM and 15·0 nmol min^{-1} mg chl^{-1} respectively, over the concentration range 2–50 μM. The low value for K_m (indicating high affinity for substrate) was similar to those of 7–10 μM (Van Sumere & Dedonder, 1971) and 28 μM (Cho & Komor, 1985) for phenylalanine uptake in *Chlorella vulgaris* and 5 μM for phenylalanine uptake by *Chlorella fusca* (Pedersen & Knutsen, 1974). Uptake of phenylalanine by the cyanobacterium *Microcystis* PCC 7280 also showed a low K_m, 20·2 μM (Xu & McAuley, unpublished results). Sequestered phenylalanine was rapidly incorporated into protein, although a proportion of radioactivity remained in the TCA-soluble pool (Fig. 2).

The rate of phenylalanine uptake by *Anabaena*

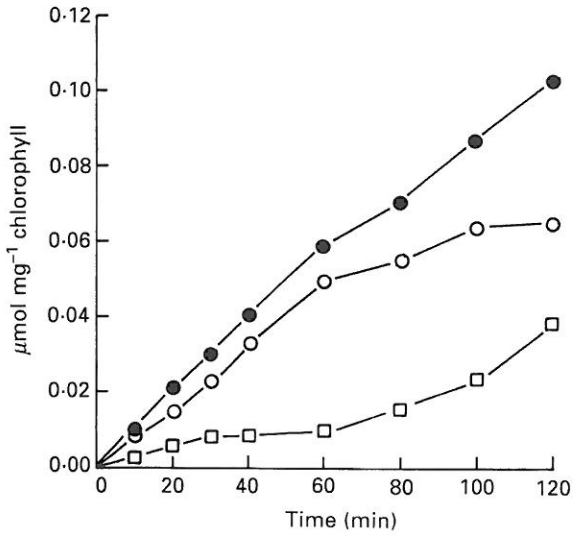

Figure 2. Metabolism of sequestered [U-^{14}C]phenylalanine by *Anabaena* ATCC 27893. Cells were incubated in the presence of 0·9 μM [U-^{14}C]phenylalanine (7·4 kBq ml^{-1}) after which total [U-^{14}C]phenylalanine assimilated by the cells (●——●), and [U-^{14}C]phenylalanine in TCA-soluble (○——○) and TCA-insoluble (□——□) pools, were determined as described in Materials and Methods.

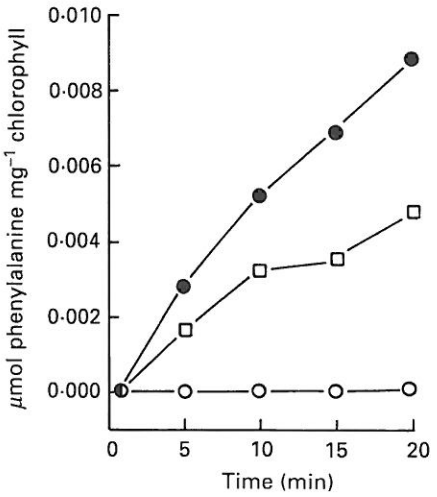

Figure 3. The effect of metabolic inhibitors on uptake of [U-^{14}C]phenylalanine by *Anabaena* ATCC 27893. Cells were incubated for 30 min in experimental conditions in unsupplemented medium (●——●), or medium containing 20 μM CCCP (○——○) or 5 mM KCN (□——□). [U-^{14}C]phenylalanine was then added to give a final concentration of 1 μM (8·3 kBq ml^{-1}), after which uptake was determined as described in Materials and Methods.

grown at 25 °C was determined over a range of temperatures to determine the Q10 of this process. As would be expected of an energy requiring process, uptake rose over the temperature range 20–30 °C, although between 30–45 °C there was no change in uptake rate. The Q10 between 20 and 30 °C was 3·4, about twice that of the phenylalanine uptake system of *Chlorella fusca* (Pedersen & Knutsen, 1974). The presence of the respiratory chain inhibitor potassium cyanide or the H^+ transport uncoupler CCCP strongly inhibited phenylalanine uptake by *Anabaena*, again suggesting that internalization was energy dependent (Fig. 3).

Competition between amino acids for uptake by cyanobacteria

Phenylalanine uptake was inhibited by neutral amino acids and glutamate, but not by the basic amino acid arginine, or by the acidic amino-acid aspartate and its derivative, asparagine (Table 1). Other experiments suggested the presence of at least one other amino-acid carrier, transporting arginine and lysine (Table 2). This may be equivalent to the basic amino-acid carrier found in microalgae and higher plants. It appeared to have a very high specificity for arginine, since uptake of this amino acid was not affected by lysine, but arginine did inhibit uptake of lysine. Results of inhibition experiments using histidine either as substrate or as inhibitor were ambiguous. Histidine inhibited neither arginine nor lysine uptake, and uptake of histidine was only poorly inhibited by basic and neutral amino acids.

Table 1. *Inhibition by other amino acids of uptake of phenylalanine by* Anabaena *ATCC 27893*

Unlabelled amino acid added to [U-^{14}C]phenylalanine	Uptake (% control)
——	100·0
Phenylalanine	12·7
Alanine	17·6
Arginine	99·2
Aspartate	131·3
Leucine	10·2
Asparagine	93·6
Phenylalanine	12·7
Glutamate	11·5
Methionine	94·9
Phenylalanine	13·2
Glycine	6·0
Threonine	17·5
Serine	11·2
Valine	11·2
Phenylalanine	3·9
Glutamine	36·3
Histidine	63·3
Isoleucine	14·9
Proline	91·7

The initial rate of uptake of 1 μM [U-^{14}C]phenylalanine (8·3 kBq ml^{-1}) in the absence or presence of 50 μM unlabelled amino acid was determined from 0·2 ml samples taken 1, 5 and 10 min after addition of amino acid to a suspension of *Anabaena* cells. The relative rates of uptake are expressed as a percentage of uptake in the presence of [U-^{14}C]phenylalanine alone. Each block of results represents a single experiment.

Table 2. *Inhibition by other amino acids of uptake of arginine, lysine and histidine by* Anabaena *ATCC 27893*

Labelled Amino acid	Additional Unlabelled Amino acid	Uptake (% control)
Arginine	——	100·0
	Arginine	3·8
	Phenylalanine	80·9
	Glutamate	106·6
	Methionine	90·8
	Aspartate	89·2
	Histidine	95·1
	Lysine	62·2
Lysine	——	100·0
	Lysine	3·9
	Arginine	6·8
	Histidine	94·3
	Phenylalanine	105·7
	Methionine	104·3
Histidine	——	100·0
	Histidine	26·1
	Arginine	74·3
	Lysine	62·9
	Phenylalanine	59·1
	Methionine	49·7

Uptake of 10 μM [U-^{14}C]arginine (55·5 kBq ml^{-1}), or 1·3 μM [U-^{14}C]lysine or [U-^{14}C]histidine (7·4 kBq ml^{-1}), was determined in the absence or presence of unlabelled amino acids as described in Table 1.

DISCUSSION

Cyanobacteria are photoautotrophs capable of synthesizing all their amino acids from ammonium and photosynthetically fixed carbon. However, the strain of *Anabaena* studied here exhibited a non-specific energy-requiring transport system capable of sequestering phenylalanine against a concentration gradient. Inhibition of transport by the proton translocator CCCP suggested that uptake of phenylalanine by *Anabaena* may be dependent, directly or indirectly, upon an H^+ gradient. Lee-Kaden & Simonis (1982) concluded that α-aminoisobutyric-acid uptake in *Anacystis nidulans* is driven by H^+ co-transport down an H^+ ATPase-generated gradient.

There are probably at least two amino-acid carriers present in *Anabaena*. Phenylalanine appeared to be taken up by only one transport system, which also transported neutral amino acids (alanine, glycine, serine, threonine), neutral, branched-chain amino acids (leucine, isoleucine and valine), and glutamate, but not basic amino acids or aspartate and asparagine. In addition, *Anabaena* may also possess a basic amino-acid carrier, transporting arginine and lysine. *Synechococcus cedrorum* possesses a single, common transport system for various neutral, basic and dicarboxylic amino acids (Faust *et al.*, 1974). In contrast, 'high' and 'low' affinity systems specific for glutamine/glutamate have been identified in *Anabaena varabilis* (Chapman & Meeks, 1983), and specific leucine–isoleucine–valine and D-alanine-α-aminoisobutyric acid–glycine carriers in *Anacystis nidulans* (Lee-Kaden & Simonis, 1982).

The presence of both general and specific carriers for amino acids in cyanobacteria parallels the situation in the green alga *Chlorella*, which possesses a general transport system for most neutral and acidic amino acids but also specific systems for glutamine, methionine and threonine (Cho & Komor, 1985). Therefore, it would seem that evolution of photoautotrophy was not the step at which multiple, often highly specific, amino-acid transport systems were lost. However, whether cyanobacteria possess the extreme diversity of bacterial uptake systems, in which one amino acid may be taken up via several different but highly specific systems (Anraku, 1982; Hama *et al.*, 1988), remains to be investigated.

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