Methylammonium (ammonium) uptake in a glutamine auxotroph of the cyanobacterium *Anabaena cycadeae*

D.T. Singh, A.N. Rai+ and H.N. Singh*

School of Life Sciences, University of Hyderabad, Hyderabad 500134, and +*Department of Biochemistry, North-Eastern Hill University, Shillong 793014, India*

Received 22 April 1985

Methylammonium uptake was studied in a glutamine auxotroph of the cyanobacterium *Anabaena cycadeae* lacking glutamine synthetase activity. The uptake pattern was found to be biphasic, consisting of an initial fast phase lasting up to 60 s followed by a slower second phase. When cells were preincubated with L-methionine-DL-sulphoximine, an irreversible inhibitor of glutamine synthetase activity, the second uptake phase was abolished although the first phase was unaffected. Since the glutamine auxotroph did not have any glutamine synthetase activity the inhibition of the second phase by L-methionine-DL-sulphoximine could not have been due to the inhibition of glutamine synthetase activity. Thus, it is suggested that the two uptake phases may represent two different ammonium transport systems, the second one being sensitive to L-methionine-DL-sulphoximine.

Ammonium transport Anabaena cycadeae *Cyanobacteria*

1. INTRODUCTION

Ammonia is one of the commonest combined nitrogen sources on earth available for utilization by various organisms [1]. Ammonium transport systems are important for the uptake of exogenous ammonium and for the retention of ammonium produced during N_2 fixation inside the cells. Although ammonium transport systems have been studied extensively in many N_2-fixing bacteria [1–8] the information regarding ammonium transport systems in cyanobacteria is rather limited because research on such systems has commenced only recently. Since the first characterization of an ammonium transport system in the N_2-fixing cyanobacteria *Anabaena variabilis* and *A. azollae* by Rai et al. [9] ammonium transport systems have been characterized in the N_2-fixing cyanobacteria *Nostoc muscorum* [10] and *A. flos-aquae* [11] and non-nitrogen-fixing cyanobacteria *Anacystis nidulans* R-2 [12], *Synechococcus leopoliensis* UTEX 625 and *Synechococcus* sp. [13]. In all the cases reported so far, the uptake has been found to be biphasic, consisting of a fast first phase followed by a slower second phase [9,11,12]. The first phase has been shown to represent intracellular accumulation and the second slower phase to be linked with the GS activity [9,12,14]. Using a GS mutant we provide evidence here to show that the second phase may not necessarily be linked to GS activity in the nitrogen-fixing cyanobacterium *A. cycadeae* but it may represent a different ammonium transport system.

* To whom correspondence should be addressed

Abbreviations: GS, glutamine synthetase; MSX, L-methionine-DL-sulphoximine

2. MATERIALS AND METHODS

Axenic cultures of *A. cycadeae* Reinke and its glutamine auxotroph were grown in Chu-10 medium [15] as described in [16].

Chlorophyll *a* was estimated using the method of MacKinney [17]. For measurement of methyl-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

ammonium uptake, exponentially growing cells were centrifuged, washed and resuspended in 10 mmol·dm^{-3} Hepes-NaOH buffer, pH 7.0, and equilibrated for 20 min at 25°C and at a photon fluence rate of 50 μmol·m^{-2}·s^{-1}. ^{14}C-labelled methylammonium was then added to a final concentration of 50 μmol·dm^{-3} (spec. act. 7.5 kBq·cm^{-3}) and at time intervals samples were taken and cells separated from their bathing medium using oil-microcentrifugation [18] as in [9]. ^{14}C-incorporation in the cells was determined using a Beckman LS 1800 liquid scintillation spectrometer. Non-specific binding of methylammonium was determined by measuring its incorporation into toluene-treated cells as in [9].

3. RESULTS AND DISCUSSION

The ammonium analogue, [^{14}C]methylammonium, was used as probe for studying the transport system of *A. cycadeae* wild-type strain and its glutamine auxotroph (figs 1 and 2, respectively). The choice of [^{14}C]methylammonium as a probe was based on the observations that the presence of ammonium in the incubation mixture prevented [^{14}C]methylammonium uptake and that release of the free intracellular [^{14}C]methylammonium pool was observed when ammonium was added subsequent to the [^{14}C]methylammonium uptake (not shown). Fig.1 presents data on [^{14}C]methylammonium uptake by *A. cycadeae* wild-type cells. The uptake pattern was found to be biphasic which consisted of a fast initial phase

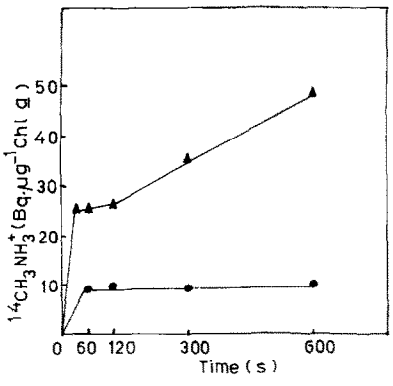

Fig.1. Methylammonium uptake by *A. cycadeae* wild-type strain. (▲) Normal cells, (●) cells pretreated with 1% (v/v) toluene for 15 min.

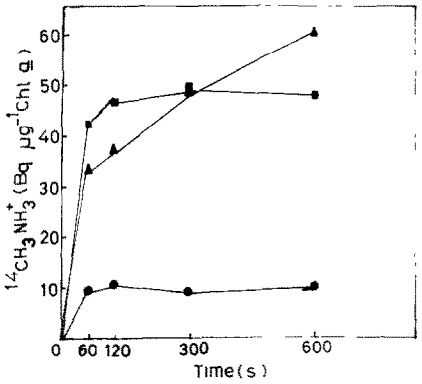

Fig.2. Methylammonium uptake by GS-lacking glutamine auxotroph of *A. cycadeae*. (▲) Normal cells, (■) cells pretreated with MSX (100 μmol·dm^{-3}) for 3 h, (●) cells pretreated with 1% (v/v) toluene for 15 min.

equilibrating within 60 s, followed by a slower second phase. To ensure that the first phase of uptake was not merely due to non-specific adsorption, [^{14}C]methylammonium incorporation into toluene-treated cells was measured and found to be less than 30% of that in normal cells after 60 s. This together with the fact that the [^{14}C]methylammonium in toluene-treated cells remained constant over a period of time suggested that the non-specific binding of [^{14}C]methylammonium was small and the biphasic pattern was a true reflection of the actual uptake. Similar results have been reported in *A. variabilis* [9] and *Ac. nidulans* [12] and it has been shown that the first phase of the uptake corresponded with [^{14}C]methylammonium accumulation in the cells while the second phase was linked with the GS activity [9,12,14]. To determine the role of GS during the second phase of [^{14}C]methylammonium uptake in such systems it would be ideal to have a GS-lacking mutant. Such a mutant of *A. cycadeae* has been isolated by Singh et al. [16] and used in various other studies [16,19,20]. We have used the same strain here to monitor [^{14}C]methylammonium uptake (fig.2). Despite the lack of GS activity in the glutamine auxotroph, the [^{14}C]methylammonium uptake pattern was found to be similar to that in the wild-type strain containing normal GS activity, i.e., biphasic. Apparently, GS activity did not seem to be involved in the second phase of [^{14}C]methylammonium uptake. When cells were preincubated with MSX, an analogue of glutamate and irrevers-

ible inhibitor of GS activity, prior to the [^{14}C]methylammonium uptake experiments, only the first phase of uptake was observed. That is, MSX abolished the second phase of [^{14}C]methylammonium uptake in the GS-lacking glutamine auxotroph. This effect of MSX could not be explained by the inhibitory effect of MSX on GS activity, because the organism had no GS activity in the first place. Thus, it is clear from the above observations that the biphasic pattern of [^{14}C]methylammonium uptake in the glutamine auxotroph of *A. cycadeae* was not due to [^{14}C]methylammonium assimilation by GS. The two phases may represent two different ammonium transport systems. The first one responsible for the first phase of the uptake is fast and MSX-insensitive while the second one responsible for the second phase of the uptake is slower and MSX-sensitive.

ACKNOWLEDGEMENTS

Financial support from UGC and INSA to H.N.S. and A.N.R., respectively, is thankfully acknowledged.

REFERENCES

[1] Kleiner, D. (1981) Biochim. Biophys. Acta 639, 41–52.
[2] Kleiner, D. (1982) Biochim. Biophys. Acta 688, 702–708.
[3] Kleiner, D. and Castorph, H. (1982) FEBS Lett. 146, 201–203.
[4] Alef, K. and Kleiner, D. (1982) Arch. Microbiol. 132, 79–81.
[5] Hartmann, A. and Kleiner, D. (1982) FEMS Microbiol. Lett. 15, 65–67.
[6] Wiegel, J. and Kleiner, D. (1982) FEMS Microbiol. Lett. 15, 61–63.
[7] Gordon, J.K. and Moore, R.A. (1981) J. Bacteriol. 148, 435–442.
[8] Gober, J.W. and Kashket, E.R. (1983) J. Bacteriol. 153, 1196–1201.
[9] Rai, A.N., Rowell, P. and Stewart, W.D.P. (1984) Arch. Microbiol. 137, 241–246.
[10] Kashyap, A.K. and Johar, G. (1984) J. Gen. Appl. Microbiol. 30, 267–275.
[11] Turpin, D.H., Edie, S.A. and Canvin, D.T. (1984) Plant Physiol. 74, 701–704.
[12] Boussiba, S., Dilling, W. and Gibson, J. (1984) J. Bacteriol. 160, 204–210.
[13] Boussiba, S., Resch, C. and Gibson, J. (1984) Arch. Microbiol. 138, 286–292.
[14] Boussiba, S. and Gibson, J. (1985) FEBS Lett. 180, 13–16.
[15] Gerloff, G.C., Fitzgerald, G.P. and Skoog, F. (1950) Am. J. Bot. 37, 216–218.
[16] Singh, H.N., Rai, U.N., Rao, V.V. and Bagchi, S.N. (1983) Biochem. Biophys. Res. Commun. 111, 180–187.
[17] MacKinney, G. (1941) J. Biol. Chem. 140, 315–322.
[18] Scott, I.D. and Nicholls, D.G. (1980) Biochem. J. 186, 21–33.
[19] Bagchi, S.N., Rai, U.N., Rai, A.N. and Singh, H.N. (1985) Physiol. Plant. 63, 322–326.
[20] Singh, H.N., Rai, A.N. and Bagchi, S.N. (1985) Mol. Gen. Genet., in press.