FEMS Microbiology Letters 15 (1982) 65–67
Published by Elsevier Biomedical Press

Ammonium (methylammonium) transport by *Azospirillum* spp.

Anton Hartmann and Diethelm Kleiner

Lehrstuhl Genetik and Lehrstuhl Mikrobiologie, Universität, 858 Bayreuth, F.R.G.

Received 7 April 1982
Accepted 13 April 1982

1. INTRODUCTION

In recent years soil bacteria of the genus *Azospirillum* found increasing biological and ecological interest because of their ability to microaerobically fix N_2 and to live in close association with plant roots, stimulating growth of nitrogen-limited plants [1–5]. The physiology of N_2 fixation, especially its repression by NH_4^+, has been studied extensively [6–9]. The question, however, how NH_4^+ traverses the membrane of these organisms has not yet been studied. In a number of soil bacteria, NH_4^+ uptake is mediated by specific carriers [10]. The results presented here also indicate the existence of a specific NH_4^+ carrier in *Azospirillum*.

2. MATERIALS AND METHODS

Azospirillum brasilense ATCC29710 and ATCC29145 and *Azospirillum lipoferum* ATCC29708 were grown aerobically at 30°C in batch cultures with the minimal medium described [6], containing 1% malate and 10 mM NH_4^+ or aspartate as carbon and nitrogen sources. N_2 fixing cultures were grown with 0.5% O_2 in the gas phase.

$^{14}CH_3NH_3^+$ transport assays were carried out with cultures of a density (at 580 nm) of 0.5 to 1.0, corresponding to 1.0 to $2.0 \cdot 10^8$ cells/ml [11], which were kept on ice. Routinely the cells were pre-incubated for 15 min at 30°C aerobically, before the transport assay was started by the addition of 4 μM $^{14}CH_3NH_3^+$. 0.1 ml samples were removed at intervals and filtered through polycarbonate filters (Nuclepore, Pleasanton, CA). Radioactivities were determined by liquid scintillation counting. Extracellular NH_4^+ was determined with an ammonia electrode (Orion, Cambridge, MA). The intracellular volume of one cell was calculated to be $2.5 \cdot 10^{-12}$ ml, taking dimensions of 3×1 μm [2].

3. RESULTS AND DISCUSSION

When *Azospirillum* strains were cultured on nitrogen-free, semisolid agar, the cells were able to remove traces of NH_4^+ ($5 \cdot 10^{-5}$ M), in agreement with the report of Volpon et al. [9]. This suggests an energy-dependent uptake mechanism. When growing under nitrogen-limiting conditions, *A. brasilense* and *A. lipoferum* took up $CH_3NH_3^+$, forming up to 500-fold gradients across the membrane (Fig. 1). The accumulated $CH_3NH_3^+$ to a great part could be chased from the cells by adding 50 mM NH_4^+, indicating the existence of an internal pool of free $CH_3NH_3^+$.

$CH_3NH_3^+$ uptake followed saturation kinetics (K_m = 50–200 μM, V_{max} = 0.1–0.4 nmol/10^8 cells/min) and was competitively inhibited by NH_4^+ (K_i = 5–10 μM), indicating a common binding site at the carrier. The lower K_i for NH_4^+ as

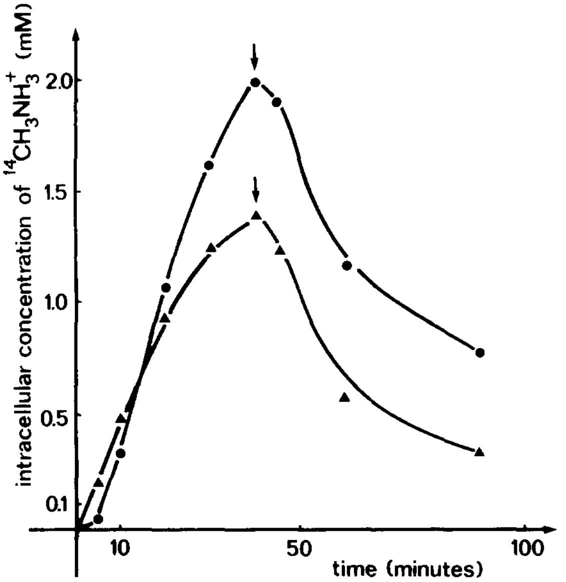

Fig. 1. Uptake and release of $^{14}CH_3NH_3^+$ by *A. brasilense* ATCC29710 (●) and *A. lipoferum* (▲). The cells were grown under N_2-fixing conditions to a density of 0.5. At the times indicated by arrows 50 mM CH_3COONH_4 was added.

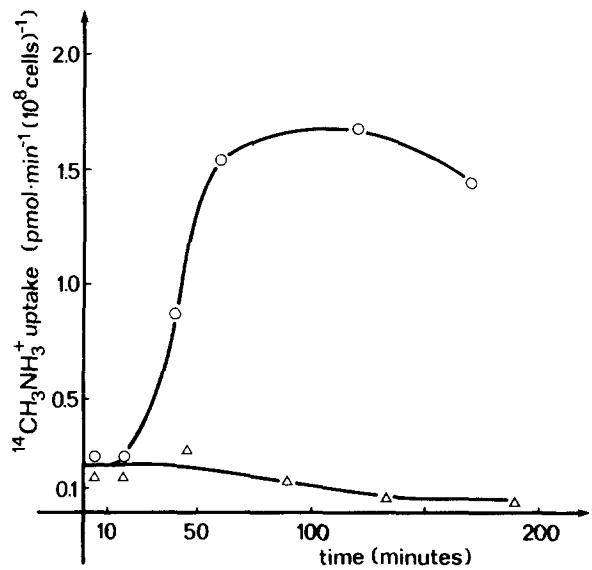

Fig. 2. Derepression of $CH_3NH_3^+$ uptake by *A. brasilense* ATCC29145. A culture grown to the exponential phase with 10 mM NH_4^+ was washed twice and resuspended in minimal medium containing 10 mM aspartate at 37°C to a density of 0.4 with (△) and without (○) chloramphenicol (50 mg/l).

Table 1

Effect of inhibitors on $^{14}CH_3NH_3^+$ uptake by *A. brasilense* (ATCC29145)

The culture was grown under aerobic conditions with 10 mM aspartate as nitrogen source. The inhibitors were added 5 min before the transport assay was started. Uptake rates were calculated from the amount of $^{14}CH_3NH_3^+$ taken up after 20 min at 30°C.

Inhibitor	Concentration (mM)	Residual uptake rate (%)
No addition	–	100
N_3^-	5	60
CN^-	1	0
Dinitrophenol	1	0
Carbonylcyanide-*m*-chlorphenylhydrazone (CCCP)	0.02	3
Iodoacetate	5	3
N-Ethylmaleimid	5	0

compared to the K_m for $CH_3NH_3^+$ indicates a higher affinity for NH_4^+. Uptake activity for *A. brasilense* was optimal at pH 7.5, while *A. lipoferum* showed a less distinct optimum. Uptake by *A. brasilense* was energy-dependent, since it was reduced to 5% in the absence of a carbon source, and since it was inhibited by various inhibitors of energy metabolism (Table 1). Similar results were obtained for *A. lipoferum*. These data argue for the existence of a specific carrier for $CH_3NH_3^+$ and NH_4^+. The findings that the affinity of the carrier is considerably higher for NH_4^+ than for $CH_3NH_3^+$, and that $CH_3NH_3^+$ is toxic for *Azospirillum* spp. and cannot serve as a carbon or nitrogen source (Hartmann, unpublished), indicate that NH_4^+ must be the natural substrate for the carrier [10].

Uptake was not detected by cells pre-grown with 10 mM NH_4^+. After transfer to NH_4^+-free media containing aspartate or glutamate, $CH_3NH_3^+$ transport became derepressed in *A.*

brasilense (Fig. 2). This derepression was prevented by chloramphenicol, indicating the necessity of de novo protein synthesis. Although similar derepression experiments were unsuccessful with *A. lipoferum*, $CH_3NH_3^+$ transport was repressed in cultures grown on NH_4^+, while N_2 fixing cultures were as active as *A. brasilense.* Thus in both strains the carrier is under genetic control. Regulations of the synthesis of NH_4^+ carriers have been found for *Klebsiella pneumoniae* [12] and *Rhodospirillum rubrum* (submitted), while the transport system of *Azotobacter vinelandii* seems to be constitutive [13].

In conclusion, we found ample evidence that *Azospirillum* spp. can absorb extracellular NH_4^+ via a repressible specific transport system with the expenditure of energy. Further investigations, however, are required to understand the role of the NH_4^+ carrier in the association between plant and *Azospirillum.*

ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft.

REFERENCES

[1] Von Bülow, J.F.W. and Döbereiner, J. (1975) Proc. Natl. Acad. Sci. USA 72, 2389–2393.
[2] Tarrand, J.J., Krieg, N.R. and Döbereiner, J. (1978) Can. J. Microbiol. 24, 967–980.
[3] Umali-Garcia, M., Hubbell, D.H., Gaskins, M.H. and Dazzo, F.B. (1980) Appl. Environ. Microbiol. 39, 219–226.
[4] Nur, I., Okon, Y. and Henis, Y. (1980) Can. J. Microbiol. 26, 482–485.
[5] Van Berkum, P. and Bohlool, B.B. (1980) Microbiol. Rev. 44, 491–517.
[6] Okon, Y., Albrecht, S.L. and Burris, R.H. (1976) J. Bacteriol. 127, 1248–1254.
[7] Okon, Y., Albrecht, S.L. and Burris, R.H. (1976) J. Bacteriol. 128, 592–597.
[8] Gauthier, D. and Elmerich, C. (1977) FEMS Microbiol. Lett. 2, 101–104.
[9] Volpon, A.G.T., De-Polli, H. and Döbereiner, J. (1981) Arch. Microbiol. 128, 371–375.
[10] Kleiner, D. (1981) Biochim. Biophys. Acta 639, 41–52.
[11] Okon, Y., Albrecht, S.L. and Burris, R.H. (1977) Appl. Environ. Microbiol. 33, 85–88.
[12] Kleiner, D. (1982) Biochim. Biophys. Acta, in press.
[13] Gordon, J.K. and Moore, R.A. (1981) J. Bacteriol. 148, 435–442.