Uptake of methionine sulfoximine by some N_2 fixing bacteria, and its effect on ammonium transport

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The N_2 fixing bacteria *Klebsiella pneumoniae, Azospirillum brasilense, Rhodopseudomonas sphaeroides* and *Rhodospirillum rubrum*, but not *Azotobacter vinelandii* accumulate the glutamine analogue methionine sulfoximine in the cell. In the accumulating cells methionine sulfoximine inhibits ammonium transport. Accumulation and inhibition are prevented by glutamine.

Methionine sulfoximine transport *Ammonium transport* Klebsiella pneumoniae
Azospirillum brasilense Rhodospirillum rubrum Rhodopseudomonas sphaeroides

1. INTRODUCTION

Ammonium transport in *Klebsiella pneumoniae* [1] and *Azospirillum brasilense* [2] is strongly inhibited by glutamine analogues, especially methionine sulfoximine (MSX). This inhibition could be prevented by glutamine, if added to the cells prior to MSX. If the cells were first incubated with MSX, addition of glutamine was without effect [1]. This and other results led to the proposal that glutamine prevents entry of MSX via a common carrier, and that therefore the MSX binding site is inside the cell [1].

We here support these conclusions by showing:

(a) that the inhibition of ammonium transport by MSX is found in several N_2 fixing species which accumulate MSX, and
(b) that both MSX accumulation and inhibition of ammonium transport are prevented by glutamine.

2. MATERIALS AND METHODS

K. pneumoniae M5a 1 (a gift from Professor R.H. Burris, University of Wisconsin) was grown aerobically with 20 mM histidine as the nitrogen source, washed and resuspended in 80 mM sodium phosphate and 0.5% glucose (pH 7.0). *Azs. brasilense* ATCC 29710 was grown in the semisolid nitrogen-free medium described in [4]. Before the transport assays the agar was removed by filtration, the bacteria were washed and resuspended in agar-free minimal medium [4]. *Rhodospirillum rubrum* ATCC 11170 and *Rhodopseudomonas sphaeroides* DSM 158 were grown aerobically in the dark with 10 mM glutamate as the nitrogen source as in [5]. Cells from the logarithmic phase were washed and resuspended in 20 mM potassium phosphate and 0.2% sodium malate (pH 7.0). *Azotobacter vinelandii* OP (a gift from Professor R.H. Burris) was grown in the nitrogen-free medium described in [6], washed and resuspended in 20 mM potassium phosphate (pH 7.0) with 0.5% glucose. Methylammonium uptake (an indicator of ammonium uptake) and transport of [^{14}C]MSX were measured by filtration as in [3]. Estimation of intracellular spaces and calculations of intracellular MSX concentrations were done using the values in [7–9].

Preparation of [^{14}C]MSX [10,11]: 0.66 mg [*methyl*-^{14}C]methionine (New England Nuclear) with an activity of 250 μCi, and 3.9 mg unlabeled methionine were dissolved in a mixture of 30 μl concentrated H_2SO_4 and 100 μl chloroform.

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9.5 mg NaN_3 were slowly added under shaking, and the mixture was incubated for 1 h at 40°C under shaking in a test tube. The content was then transferred into 30 ml H_2O + 0.1 ml HCl. This solution was put on a column of Dowex 50 in the protonated form (7.5 × 1.5 cm). The effluent contained about 1% of the label. The column was then washed with 50 ml H_2O, which removed a further 0.2% of the label. Then, MSX was eluted from the column with a 1/10 concentrated NH_4OH solution. 80% of the label was removed with the first 5 ml, and dried in an N_2 stream. The compound was subjected to chromatography on polyethylene imine cellulose as in [3]. Scanning the chromatograms with a Berthold LB 2723 scanner (Berthold, Karlsruhe) showed the labeled substance in one spot which co-chromatographed with unlabeled MSX, but not with methionine, methionine sulfone, or methionine sulfoxide.

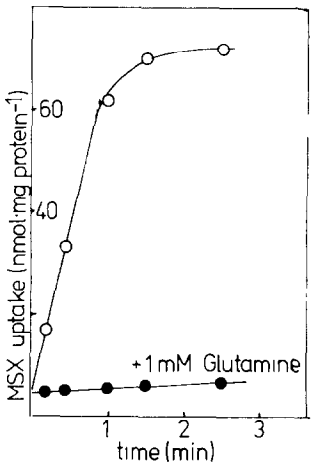

Fig.1. Uptake of MSX by *K. pneumoniae* in the presence (●) and absence (○) of 1 mM glutamine. Temperature 30°C, pH 7.0. Glutamine was added 1 min before the start by addition of 38 μM MSX.

3. RESULTS

Table 1 summarizes the results on MSX uptake, and on inhibition of methylammonium uptake by MSX in various N_2 fixing bacteria. All strains with the exception of *A. vinelandii* showed rapid accumulation of [^{14}C]MSX, which was to a large extent prevented by glutamine. As an example, the uptake kinetics by *K. pneumoniae* are given in fig.1. About 50% of the accumulated label could be chased from the cells by addition of 1 mM cold MSX.

Also, with the exception of *A. vinelandii*, small concentrations of MSX inhibited ammonium (methylammonium) transport. Furthermore, as already described for *K. pneumoniae* [1], this inhibition could be prevented by glutamine, if added to the cells prior to MSX, but not after MSX addition (not shown). In *R. rubrum* 1 mM glutamine inhibited ammonium transport to more than 90% so that these protection experiments could not be carried out with this strain.

Table 1

Uptake rates and accumulation of MSX by various N_2 fixing bacteria, and its effect on ammonium (methylammonium) transport

Organism	Uptake of MSX (nmol·min^{-1}·mg protein^{-1})		Final intracellular MSX concentration (mM)[a]	Concentration (μM) at which 50% inhibition of ammonium transport occurs
	No addition	+1 mM glutamine		
K. pneumoniae	64.0	0.05	2.8	40
Azs. brasilense	3.6	0.05	3.1	10
R. rubrum	3.4	0.20	1.0	5
R. sphaeroides	3.5	0.35	0.8	2
A. vinelandii	0.05	0.05	n.d.	>500

[a] At a final extracellular concentration of 10 μM
n.d., not determined

4. DISCUSSION

MSX has been widely employed as an inhibitor of glutamine synthetase (e.g., [12,13]), and has recently been shown to inhibit ammonium transport [1,2]. Our proposal, that MSX has to be transported into the cells before being inhibitory [1], is supported by the demonstration of rapid MSX uptake by various strains, resulting in an intracellular accumulation of about 1 mM MSX. Both the uptake of MSX and its effect on ammonium transport are prevented by glutamine, indicating entry via a common carrier. A similar competitive situation was reported for the transport of glutamine and methionine sulfoxide by *Salmonella typhimurium* [14]. The remarkable exception of *A. vinelandii* is in accord with our conclusion, since these organisms take up MSX only very slowly, and conversely very high concentrations of this compound are required to inhibit ammonium transport.

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