Methylamine and Ammonium Transport Systems in 
*Rhizobium leguminosarum* MNF3841

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As the sole source of nitrogen, methylamine supported the growth of a range of species of *Rhizobium*. The methylamine assimilatory system was inducible in *R. leguminosarum* MNF3841, whereas the capacity to utilize NH$_4^+$ as a nitrogen source was constitutive. An uptake system for [14C]methylamine (methylamine permease) was induced by growth of MNF3841 on methylamine or ethylamine. The uptake was sensitive to 2,4-dinitrophenol, azide and carbonyl cyanide m-chlorophenylhydrazone. The methylamine permease had a $K_m$ of 0.035 mM, a $V_{max}$ of 2.2 nmol min$^{-1}$ (mg protein)$^{-1}$ and a $K_i$ for ammonium of 1.5 mM. Most of the [14C]methylamine accumulated by cells was rapidly incorporated into TCA-insoluble materials. An NH$_4^+$-sensitive methylamine-accumulating system distinct from the methylamine permease was demonstrated in ammonia-limited cells grown in continuous culture. This system, the ammonium permease, had a $K_m$ of 0.11 mM (for methylamine), a $K_i$ for NH$_4^+$ of 0.007 mM and a $V_{max}$ of 2.5 nmol min$^{-1}$ (mg protein)$^{-1}$. Methylamine was accumulated by chemostat-grown, N-limited cells and could exchange with unlabelled methylamine. Treatment with carbonyl cyanide m-chlorophenylhydrazone caused efflux of the accumulated methylamine, whereas high concentrations of NH$_4^+$ did not. Thus *R. leguminosarum* possesses a specific methylamine permease which is quite distinct from the ammonium permease.

**INTRODUCTION**

The mechanism of ammonia transport is of obvious importance to understanding the physiology of nitrogen fixation by *Rhizobium*, since both isolated bacteroids (Bergersen & Turner, 1967) and cells induced for nitrogenase in the laboratory (O'Gara & Shanmugam, 1976; Tubb, 1976; Bergersen & Turner, 1978) liberate into the medium as $^{15}$NH$_3$ most of the $^{15}$N$_2$ they fix.

Studies of ammonia transport mechanisms in bacteria have been approached in two major ways: the measurement of intracellular and extracellular ammonia concentrations (e.g. Dilworth & Glenn, 1982), and the use of [14C]methylamine as an analogue for ammonium (Stevenson & Silver, 1977).

In *Azotobacter vinelandii*, measurements of intra- and extracellular ammonia indicated that a 100-fold higher concentration existed inside the cells (Kleiner, 1975), but since cell suspensions were apparently toluenized prior to the measurements of intracellular ammonia, these data must be treated with caution. Similar concentration differences have been reported for *Clostridium pasteurianum* and *Klebsiella pneumoniae* (Kleiner & Fitzke, 1979; Kleiner, 1982). By contrast, in *Rhizobium leguminosarum* grown on either histidine or NH$_4^+$, no evidence was found for a gradient in ammonia concentration across the cell membrane (Dilworth & Glenn, 1982).

While uptake of methylamine by eukaryotic cells appears to parallel uptake of NH$_4^+$ (Kleiner, 1981), the situation in prokaryotes is not as definitive. Since methylamine uptake on an ammonium carrier would be expected to be less efficient than ammonium uptake, the inhibition constant ($K_i$) for ammonium competition with methylamine would be expected to be lower than

**Abbreviation:** CCCP, carbonyl cyanide m-chlorophenylhydrazone.
the Michaelis constant \((K_m)\) for methylamine uptake (Kleiner, 1981). Data which conform to this prediction have been reported for \(A.\ vinelandii\) (Barnes & Zimniak, 1981), \(C.\ pasteurianum\) (Kleiner & Fitzke, 1981), \(K.\ pneumoniae\) (Kleiner, 1982), \(Azospirillum\) species (Hartmann & Kleiner, 1982) and \(Rhodospirillum\ rubrum\) (Alef & Kleiner, 1982). While the ideal situation for measuring methylamine uptake would require that no further metabolism of methylamine occurs, it is clear that for \(A.\ vinelandii\) (Barnes & Zimniak, 1981), \(C.\ pasteurianum\) (Kleiner & Fitzke, 1981) and \(K.\ pneumoniae\) (Kleiner, 1982) methylamine is extensively metabolized to a less polar compound, probably \(N^5\)-methylglutamine \((\gamma\text{-glutamylmethylamide})\). However, methylamine does not serve as a nitrogen source for the growth of any of these bacteria.

In bacteria capable of using methylamine as a source of nitrogen for growth the situation appears to be different. In \(Pseudomonas\) species MA, growth with methylamine as a sole source of carbon and nitrogen results in the synthesis of a transport system of considerable capacity but high \(K_m\) \((1 \text{ to } 3 \text{ mM})\), which is insensitive to ammonium inhibition (Bellion et al., 1980). When the same strain is grown on succinate with methylamine as the sole nitrogen source, a second transport system is induced which has a low \(K_m\) \((0.016 \text{ mM})\) and relatively low capacity, and which shows weak competitive inhibition \((K_i = 4.8 \text{ mM})\) by \(NH_4^+\) (Bellion & Weyland, 1982).

In bacteroids of \(R.\ leguminosarum\) loaded anaerobically with radioactive methylamine, Laane et al. (1980) showed that oxygen was necessary for methylamine export, and inferred that this applied to \(NH_4^+\) movement also. Dilworth & Glenn (1982) found that \[^{14}\text{C}\]methylamine uptake into \(R.\ leguminosarum\) WU235 was an active process that was only slightly inhibited by a considerable molar excess of \(NH_4^+\), and suggested that uptake of methylamine was not necessarily a good index of ammonium uptake in this strain. In \(Rhzobium melliloti\) also, ammonium did not significantly inhibit methylamine uptake (Osburne, 1982), and efflux could not be demonstrated in response to ammonium additions (Wiegel & Kleiner, 1982). By contrast, experiments with cowpea \(Rhizobium\) 32H1 showed that \(NH_4^+\) did inhibit methylamine uptake at very low concentrations, and that counterflow of methylammonium and ammonium ions could occur (Gober & Kashket, 1983). Extensive conversion of methylamine to a compound like \(N^5\)-methylglutamine also occurred, but whether this strain could grow on methylamine was not apparent. Methylamine transport was not induced in aerobic cultures using glutamate as the nitrogen source, but was induced in the same medium under the microaerophilic growth conditions needed to induce nitrogenase (Gober & Kashket, 1983). It is unclear how cowpea \(Rhizobium\) strains grow on low concentrations of \(NH_4^+\) under aerobic conditions (Bergersen & Turner, 1976) if a vital ammonium transport system cannot be synthesized.

It thus appears that where an organism is able to use methylamine as a nitrogen source for growth, methylamine uptake is likely to be via a specific inducible transport system that is unconnected with \(NH_4^+\) transport.

In this paper we present evidence for the existence in \(R.\ leguminosarum\) MNF3841 of two permease systems which will transport methylamine. The first is induced only by growth on methylamine (or ethylamine) and we regard it as a methylamine permease. The second is derepressed only under nitrogen limitation (ammonia or nitrate), is very sensitive to inhibition by ammonium ions and appears to be an \(NH_4^+\) permease.

**METHODS**

*Organisms.* The rhizobia used in this study are shown in Table 1.

*Media.* Strains were tested for growth on a solid medium containing the mineral salts used by Brown & Dilworth (1975) with phosphate at 0.3 mM, mannitol \((10 \text{ mM})\) and nitrogen sources at 10 mM. \(Rhizobium\ leguminosarum\) MNF3841 was grown in batch culture in liquid medium of the same composition supplemented with 40 mM-HEPES \((pH \text{ 7.2})\).

*Nodulation and preparation of bacteroids.* Pea plants \((Pisum\ sativum\ L.\ cv.\ Greenfeast)\) were nodulated by \(R.\ leguminosarum\) MNF3841 in pots and bacteroids isolated as described by Glenn et al., 1980): they were used immediately after isolation.

*Continuous culture.* The medium for continuous culture was similar to that for batch cultures, but contained 20 mM-HEPES, \(pH\ 7.2\). The carbon source was fructose or sucrose \((10 \text{ mM})\). Cells were cultured under \(NH_4^+\)-limitation \((0.5 \text{ mM-}NH_4^+:D = 0.04\text{ or }0.12\text{ h}^{-1})\), methylamine limitation \((0.5 \text{ mM-methylamine};D = 0.04\text{ h}^{-1})\),
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Table 1. Rhizobium strains used

<table>
<thead>
<tr>
<th>Fast-growing rhizobia</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>R. leguminosarum WU235</td>
<td>Institute of Agriculture, University of Western Australia, Australia</td>
</tr>
<tr>
<td>R. leguminosarum MNF3841</td>
<td>Str derivative of strain 300 of Johnston &amp; Beringer (1975)</td>
</tr>
<tr>
<td>R. trifolii TA1</td>
<td>Dr D. L. Chatel, W. Australian Department of Agriculture, Australia</td>
</tr>
<tr>
<td>Cowpea Rhizobium NGR234</td>
<td>Dr M. J. Trinick, CSIRO, Canberra, Australia</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Slow-growing rhizobia</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>Cowpea Rhizobium 32H1</td>
<td>Dr J. Burton, Nitragin Co., USA</td>
</tr>
<tr>
<td>Cowpea Rhizobium CB756</td>
<td>Dr R. A. Date, CSIRO, Brisbane, Australia</td>
</tr>
<tr>
<td>Rhizobium japonicum WU40</td>
<td>Institute of Agriculture, University of Western Australia, Australia</td>
</tr>
<tr>
<td>Rhizobium lupini WU8</td>
<td></td>
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</tbody>
</table>

phosphate limitation (0.05 mM; \( D = 0.12 \text{ h}^{-1} \)) or under \( O_2 \) limitation (\( D = 0.092 \text{ h}^{-1} \)). The chemostats had working volumes of 75 ml and were operated at 28°C.

**Uptake experiments.** Cells were prepared for uptake measurements as described by Dilworth & Glenn (1982) except that they were centrifuged and washed rather than Millipore-filtered. Competitors or inhibitors were added 60 s prior to the addition of 0.1 mM[^14]C methylamine, and samples were taken at intervals (up to 10 min), filtered and washed with 10 ml minimal salts solution; radioactivity on the filter was measured by liquid scintillation counting (Hudman & Glenn, 1980).[^14]C Methylamine hydrochloride (specific activity 43.3 mCi mmol\(^{-1} \); 1.6 GBq mmol\(^{-1} \)) was from New England Nuclear.

**Incorporation experiments.** Cells of MNF3841 were grown at 28°C in mannitol–methylamine to a cell density of approximately 0.23 mg dry wt ml\(^{-1} \), centrifuged, washed twice and resuspended at a cell density of approximately 0.175 mg dry wt ml\(^{-1} \) in minimal salts–mannitol containing 0.4 mM[^14]C methylamine (0.2 µCi). Samples (0.5 ml) were taken at intervals into cold 5% (w/v) TCA containing 0.05% methylamine, stored for 30 min, filtered and washed twice with the same solution.

**Growth experiments.** Cells of strain MNF3841 were grown on minimal salts medium containing mannitol (10 mM) plus either methylamine or \( NH_4^+ \) or nitrate (10 mM) to a cell density of approximately 0.31 mg dry wt ml\(^{-1} \), centrifuged, washed with minimal salts solution, and resuspended in a similar medium containing the appropriate nitrogen source at 10 mM.

**Analytical methods.** Ammonia was measured by the phenol–hypochlorite method of Fawcett & Scott (1960) and methylamine by a modification of the ninhydrin method of Spies (1957), with a heating time of 20 min at 100°C for colour development. Bacterial protein was measured by the Lowry method with bovine serum albumin as the standard.

**RESULTS**

**Growth of rhizobial strains on methylamine.** All fast-growing strains of *Rhizobium* (*R. leguminosarum* WU235 and MNF3841, *Rhizobium trifolii* TA1 and cowpea *Rhizobium* NGR 234) grew on solid media with methylamine as the sole source of nitrogen. Slow-growing strains (*Rhizobium lupini* WU8, *Rhizobium japonicum* WU40 and cowpea *Rhizobium* CB756 and 32H1) also grew, *R. lupini* being the slowest.

**Growth of MNF3841 on methylamine as a nitrogen source.** Cells grown in mannitol–methylamine to a cell density of approximately 0.23 mg dry wt ml\(^{-1} \) and resuspended in the same medium grew immediately with a mean generation time of 10-3 h. Cells grown and resuspended in mannitol–\( NH_4^+ \) grew immediately with a generation time of 3.5 h. Methylamine-grown cells transferred to mannitol–\( NH_4^+ \) also grew immediately with a low generation time (3.5 h).

Cells grown in mannitol–\( NH_4^+ \) and transferred to mannitol–methylamine showed a limited increase in turbidity for 2 h after which growth ceased. After 5 h the cells began to grow with a mean generation time of 9-9 h, a rate virtually identical to that observed previously for cells growing on methylamine as a nitrogen source.

Cells of strain MNF3841 grown on a mannitol–minimal salts medium containing nitrate (10 mM) as the nitrogen source grew with a mean generation time of 4-5 h when resuspended in a similar medium. Nitrate-grown cells resuspended in mannitol–\( NH_4^+ \) media grew immediately with a generation time of 3.5 h, providing additional evidence that cells grown on nitrogen sources other than \( NH_4^+ \) are able to grow immediately when transferred to it.
Substrate consumption rates. During growth experiments similar to those described, samples of supernatant were taken to measure the utilization rates for NH\(_4^+\) and methylamine. Ammonium- or methylamine-grown cells of MNF3841 consumed NH\(_4^+\) at 2.1 and 1.8 pmol h\(^{-1}\) (mg dry wt\(^{-1}\)), respectively, and there was no change in the rate of utilization with time. Methylamine-grown cells when resuspended in fresh methylamine medium utilized methylamine at 0.5 pmol h\(^{-1}\) (mg dry wt\(^{-1}\)). When ammonium-grown cells were resuspended in methylamine-containing medium they did not utilize any methylamine for 5 h and thereafter consumed it at a rate of 0.6 pmol h\(^{-1}\) (mg dry wt\(^{-1}\)).

These growth data provided clear evidence that the methylamine utilization system in *R. leguminosarum* MNF3841 was inducible and that it was clearly different from the NH\(_4^+\)-consuming system.

The methylamine uptake system. Cells of *R. leguminosarum* MNF3841 were grown on a variety of nitrogen sources, and their rate of \(^{14}C\)methylamine uptake was examined after they had been centrifuged and washed. Cells grown on methylamine and ethylamine as the sole nitrogen source were able to take up \(^{14}C\)methylamine [1.5 and 1.09 nmol min\(^{-1}\) (mg protein\(^{-1}\)], respectively] but cells grown on nitrate, glutamate or NH\(_4^+\) had only low levels of uptake [0.01, 0.03 and 0.08 nmol min\(^{-1}\) (mg protein\(^{-1}\)], respectively]. These data were consistent with the induction of the methylamine permease before methylamine could be used as a nitrogen source of rhizobial growth.

The addition of carbonyl cyanide m-chlorophenylhydrazone (CCCP; 0.025 mM) or 2,4-dinitrophenol (0.5 mM) or azide (1 mM) inhibited uptake of \(^{14}C\)methylamine by more than 95%. The process thus appeared to be active and dependent on an energized membrane, though these conclusions are limited by the possible effects of further metabolism of methylamine.

Kinetic data. The apparent \(K_m\) for the methylamine permease was determined on five occasions over the concentration range 0.01 to 0.15 mM and the following values were obtained: Lineweaver–Burk plot, 0.035 mM (SD, 0.010); Hans–Woolf, 0.050 mM (SD, 0.012); and non-linear regression (Duggleby, 1981), 0.03 mM. The \(V_{max}\) was 2.2 nmol min\(^{-1}\) (mg protein\(^{-1}\)).

The effect of ammonium and amines on methylamine transport. The capacity of cells grown on methylamine as sole N source to accumulate \(^{14}C\)methylamine in the presence of NH\(_4^+\) and amines was investigated. The accumulation of methylamine by adapted cells of MNF3841 was found to be relatively insensitive to NH\(_4^+\). Ammonium was a poor competitive inhibitor with a \(K_i\) of 1.5 mM. Ethylamine was a more potent inhibitor of methylamine transport with a \(K_i\) of 0.5 mM. Propylamine was also a poor inhibitor of methylamine transport with a \(K_i\) of 2.6 mM. Glycine appeared to have no significant effect on methylamine uptake even when present in a 100-fold excess. In the presence of 1 mM-NH\(_4^+\) the apparent \(K_m\) for methylamine uptake was 0.118 mM.

Methylamine transport in NH\(_4^+\)-grown cells. Ammonium-grown cells of MNF3841 have a low, but measurable, rate of \(^{14}C\)methylamine transport [0.08 nmol min\(^{-1}\) (mg protein\(^{-1}\)]. To determine if this represented a basal level of the methylamine permease, or if transport involved another, separate uptake system, the \(K_i\) values for NH\(_4^+\), ethylamine and propylamine were determined. The \(K_i\) values were 0.55 mM for ethylamine, 1.2 mM for NH\(_4^+\) and 2.6 mM for propylamine, values which are essentially the same as those obtained for methylamine-grown cells. It appeared therefore that the low rate of methylamine transport observed in NH\(_4^+\)-grown cells was due to a basal level of the methylamine permease, not an NH\(_4^+\) permease.

Efflux experiments with the methylamine permease. Cells grown in batch culture on mannitol–methylamine were loaded with \(^{14}C\)methylamine and then treated with methylamine (25 mM) or NH\(_4^+\) (25 mM) or CCCP (0.02 mM) or azide (1 mM). None of these treatments resulted in a loss of radioactivity from the cells.

Incorporation into TCA-insoluble material was therefore measured as a fraction of the total accumulated label as described in Methods. Samples (0.1 ml) were also filtered to measure the rate of accumulation of label. About 85% of the radioactivity was incorporated into TCA-insoluble material, thus explaining the lack of efflux described earlier.
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Chemostat culture studies. Cells from chemostat cultures of MNF3841 grown under a variety of limitations were examined for their capacity to transport \(^{14}\text{C}\)methylamine. Methylamine-limited cells transported methylamine via a system which was relatively insensitive to NH\(_{4}\)\(^+\) (Table 2). The \(K_c\) (for NH\(_{4}\)\(^+\)) for this system was 1.25 mM and the \(K_m\) 0.050 mM (Hans-Woolf), indicative of the methylamine permease described for batch cultures. Phosphate-limited cells and oxygen-limited cells showed only a basal activity of the methylamine permease (Table 2).

Cells growing on 0.5 mM-NH\(_{4}\)\(^+\) were ammonia-limited; the effluent NH\(_{4}\)\(^+\) concentration was 0.016 mM. Increasing the NH\(_{4}\)\(^+\) concentration to 1 mM resulted in a doubling of the cell density from 0.19 to 0.38 mg dry wt ml\(^{-1}\). Such cells showed a \(^{14}\text{C}\)methylamine uptake activity which was clearly sensitive to NH\(_{4}\)\(^+\) inhibition (Table 2). Cells from nitrate-limited chemostats also showed NH\(_{4}\)\(^+\)-sensitive \(^{14}\text{C}\)methylamine uptake.

Kinetics of the ammonium permease. \(^{14}\text{C}\)methylamine uptake in NH\(_{4}\)\(^+\)-limited cells of MNF3841 was investigated in more detail – particularly the effect of substrate concentration and the effect of NH\(_{4}\)\(^+\). The \(K_m\) values quoted are the means of four separate determinations and the \(s_d\)s were less than 0.015 mM. The \(K_m\) determined over the range 0.010 to 0.150 mM was 0.177 mM (Lineweaver–Burk) or 0.11 mM (Hans–Woolf) with a \(V_{\text{max}}\) of 2.5 nmol min\(^{-1}\) (mg protein\(^{-1}\)). Over the range 0.005–0.15 mM, NH\(_{4}\)\(^+\) was a powerful competitive inhibitor (\(K_i\) 0.007 mM; Fig. 1). When the kinetic characteristics of this system were compared with those for the methylamine permease, it was clear that the apparent \(K_m\) values for methylamine varied only two- to threefold, while the NH\(_{4}\)\(^+\) sensitivity of the two systems was vastly different. Because the NH\(_{4}\)\(^+\)-sensitive system found in cells growing under inorganic N limitation has the characteristics predicted for NH\(_{4}\)\(^+\) transport we are terming it a rhizobial ammonium permease.

Table 2. Rates of \(^{14}\text{C}\)methylamine uptake in the presence and absence of 1 mM-NH\(_{4}\)\(^+\) by 
*R. leguminosarum* MNF3841 grown under various limitations

<table>
<thead>
<tr>
<th>Limitation</th>
<th>Dilution rate (h(^{-1}))</th>
<th>Control rate [nmol min(^{-1}) (mg protein(^{-1}))]</th>
<th>Rate in presence of 1 mM-NH(_{4})(^+) [nmol min(^{-1}) (mg protein(^{-1}))]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylamine</td>
<td>0.04</td>
<td>1.87</td>
<td>1.13</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.04</td>
<td>0.73</td>
<td>0.052</td>
</tr>
<tr>
<td>Ammonia</td>
<td>0.04</td>
<td>0.76</td>
<td>0.078</td>
</tr>
<tr>
<td>Oxygen</td>
<td>0.12</td>
<td>0.02</td>
<td>—</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.09</td>
<td>0.02</td>
<td>—</td>
</tr>
</tbody>
</table>

Fig. 1. Dixon plot showing the effect of NH\(_{4}\)\(^+\) on the uptake of \(^{14}\text{C}\)methylamine by NH\(_{4}\)\(^+\)-limited chemostat cells of *R. leguminosarum* MNF3841. Methylamine concentration was 0.02 mM (○) or 0.1 mM (●).
Fig. 2. The effect of methylamine, CCCP or NH₃⁺ on the retention of accumulated [¹⁴C]methylamine by NH₃⁺-limited chemostat-grown cells of *R. leguminosarum* MNF3841. Cells were allowed to take up [¹⁴C]methylamine for 5 min, after which time (a) 25 mM-methylamine, (b) 0.02 mM-CCCP, or (c) 25 mM-NH₃Cl was added to the cells.

**Characteristics of the rhizobial ammonium permease.** Cells from ammonia-limited chemostats were unable to accumulate [¹⁴C]methylamine when treated with CCCP (0.02 mM), azide (1 mM) or 2,4-dinitrophenol (1 mM). Like the methylamine permease this system was probably active and dependent on an energized membrane.

**Efflux of radioactive [¹⁴C]methylamine from NH₃⁺-limited cells.** The efflux of [¹⁴C]methylamine from NH₃⁺-limited cells was followed after they were allowed to accumulate label for 5 min and then treated with 25 mM (final concentration) methylamine. Samples taken over the next 25 min showed clear evidence for efflux of label when there was a large external concentration of methylamine (Fig. 2a). The half-time for the exchange was about 25 min. This was clearly different from the methylamine-grown cells which rapidly incorporated most of the radioactivity into TCA-precipitable material. When a similar experiment was carried out on cells accumulating [¹⁴C]methylamine by treating them with CCCP (0.02 mM), a clear loss of label from the cells occurred (Fig. 2b), a result again quite distinct from that with methylamine-grown cells.

It might be considered that NH₃⁺ itself should induce an efflux of [¹⁴C]methylamine from cells of *R. leguminosarum* MNF3841. However, there was no NH₃⁺-induced efflux of intracellular [¹⁴C]methylamine (Fig. 2c). This result is in accord with our previous work (Dilworth & Glenn, 1982), which showed that relatively high concentrations of ammonia (at least greater than 2.5 mM) rapidly equilibrate across the rhizobial membrane. Under these conditions, there will be a high concentration (25 mM) of ammonia inside the cells within 1.5 min which will effectively prevent label from leaving.

The data presented provide clear evidence that in laboratory-grown cultures of *R. leguminosarum* there are two separate and distinct permeases capable of transporting methylamine. One of these is the true methylamine permease induced by growth on methylamine; the other has the characteristics of an NH₃⁺ permease and is produced only when the cells are grown under inorganic nitrogen limitation.

**Methylamine transport in isolated bacteroids.** Pea bacteroids accumulated [¹⁴C]methylamine only very slowly [0.024 nmol min⁻¹ (mg protein)⁻¹]. This uptake was relatively insensitive to inhibition by 1 mM-NH₃⁺ [0.017 nmol min⁻¹ (mg protein)⁻¹]. Control experiments showed that these bacteroids were able to accumulate [¹⁴C]succinate, but unable to take up fructose, a pattern described previously for this strain (Glenn *et al.*, 1980; Glenn *et al.*, 1984). The rates and ammonia insensitivity of the accumulation of [¹⁴C]methylamine by bacteroids suggest that this material was accumulated by a basal level of the methylamine permease and not via the ammonium permease.
DISCUSSION

The results presented suggest that *R. leguminosarum* MNF3841 resembles *Pseudomonas* species MA (Bellion et al., 1980; Bellion & Weyland, 1982) in its ability to synthesize a methylamine permease with methylamine as a nitrogen source for growth. Thus, the apparent $K_m$ values for methylamine and the degree of inhibition by NH$_3$ are low, and the permeases are induced by growth on alkylamines rather than by NH$_3$. Induction of the transport system apparently parallels induction of the necessary enzymes for methylamine metabolism, and leads to extensive incorporation of methylamine carbon into cells of MNF3841.

Cells of MNF3841 grown on high (10 mM) concentrations of nitrogen sources other than methylamine also transport methylamine to a limited extent, and for ammonia-grown cells the system responsible has the same properties as that induced by methylamine. Accordingly, under most conditions used for growth of *Rhizobium* in the laboratory, measurement of methylamine transport is likely to represent repressed levels of a methylamine permease relatively insensitive to inhibition by NH$_3$. Such appears to be the case for *R. meliloti* (Osborne, 1982). If the other enzymes of methylamine utilization are induced to roughly the same extent as the permease, efflux of radioactive methylamine would not be expected, a result noted by Wiegel & Kleiner (1982) for *R. meliloti*.

Since the low level of $[^{14}C]$methylamine uptake by pea bacteroids has the characteristics of the methylamine permease rather than the ammonium permease, it is probable that the bacteroid environment is non-limiting with respect to nitrogen. It is also probable that active methylamine export by bacteroids (Laane et al., 1980) will involve repressed levels of the methylamine permease and be unreliable as an index of ammonia export.

Growth of MNF3841 in a chemostat on limiting ammonia or nitrate results in a methylamine uptake activity with entirely different kinetic parameters. This system is inhibited by low concentrations of NH$_3$, as are the methylamine transport activities of *A. vinelandii* (Barnes & Zimniak, 1981), *C. pasteurianum* (Kleiner & Fitzke, 1981), *K. pneumoniae* (Kleiner, 1982), *Azospirillum* spp. (Hartmann & Kleiner, 1982) and *R. rubrum* (Alef & Kleiner, 1982), none of which is able to use methylamine as a source of nitrogen for growth. Unlike *R. leguminosarum*, these other organisms appear to be able to synthesize an NH$_3$ permease under conditions where nitrogen is not limiting. In *R. leguminosarum*, however, nitrogen-excess conditions appear to lead to repressed levels of a methylamine permease rather than an NH$_3$ permease.

The situation in the cowpea *Rhizobium* (*Bradyrhizobium*) 32H1 is somewhat confusing. Methylamine transport in this strain is mediated by a system with a low $K_m$ for methylamine and is inhibited by low concentrations of NH$_3$ (Gober & Kashket, 1983). Methylamine taken up can efflux, though not necessarily as methylamine, and the general picture would seem to be one of an organism unable to use methylamine for growth. However, we find that this strain grows on plates with methylamine as the sole source of nitrogen, albeit slowly. Another unusual finding is that only under microaerophilic conditions does strain 32H1 produce the methylamine-transporting (NH$_3$ permease) system (Gober & Kashket, 1983), yet it can grow under aerobic conditions with NH$_3$ as the nitrogen source (Bergersen & Turner, 1976). The apparent inconsistency may be resolved if 32H1 also produces the NH$_3$ permease system at low nitrogen concentrations, but relies on a diffusive mechanism for ammonia uptake at higher concentrations, as suggested for *R. leguminosarum* by Dilworth & Glenn (1982).

Further study of ammonium transport in fast-growing rhizobia such as *R. leguminosarum* will need to use ammonia- or nitrate-limited chemostats to separate the ammonium permease from the methylamine permease, as well as mutants completely lacking the methylamine permease system to allow the ammonium permease to be studied directly.

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REFERENCES


