Observations on Methionine Transport in Pseudomonas fluorescens UK1

By P. MÄNTSÄLÄ, S. LAAKSO AND V. NURMIKKO
Department of Biochemistry, University of Turku, 20500 Turku 50, Finland

(Received 5 December 1973; revised 10 May 1974)

SUMMARY

Pseudomonas fluorescens UK1 absorbed DL-methionine with a $K_m$ value of 25 $\mu$M, optimum temperature 42 to 45 °C. DL-Ethionine, S-methyl-L-cysteine, S-ethyl-L-cysteine and $\alpha$-methyl-DL-methionine inhibited methionine transport competitively, with $K_i$ values of 0.10, 0.35, 0.40 and 0.20 $\mu$M respectively.

Using lysozyme, EDTA and trypsin, membrane vesicles were prepared from cells grown with methionine. Methionine accumulation in the vesicles was enhanced both by ascorbate-phenazine methosulphate and by several physiological electron donors; the most effective were FAD$^+$ malate, malate, succinate or 2-oxoglutarate. Oxidation of succinate, but not of malate, was inhibited by malonate; oxidation of malate was inhibited by azide; FAD did not reverse azide inhibition.

Tracer methionine was recovered from the membranes both as the unchanged amino acid and methionine sulfoxide.

INTRODUCTION

A specific uptake system for methionine has been demonstrated in Escherichia coli (Cohen & Monod, 1957; Piperno & Oxender, 1968; Kadner, 1974), in Salmonella typhimurium (Ayling & Bridgeland, 1972) and in Saccharomyces cerevisiae (Gits & Grenson, 1967). Methionine transport is competitively inhibited by some analogues in S. typhimurium (Ayling & Bridgeland, 1972). Little is known, however, about the mechanism of methionine transport and its relationship to amino acid degradation, especially when methionine is the only source of carbon, nitrogen and sulphur. Kay & Gronlund (1969a, b) found that, although Pseudomonas aeruginosa transports methionine, the amino acid is a source of nitrogen only. Kaback & Milner (1970) reported that methionine is converted to an unidentified metabolite by the isolated membranes from E. coli ML308–225. When nitrogen-starved mycelium of Penicillium chrysogenum is incubated with high concentrations of labelled methionine, influx is followed by efflux of the corresponding labelled $\alpha$-keto acid (Hunter & Segel, 1973). However, some aerobic bacteria can utilize methionine as the only source of carbon, nitrogen and sulphur (Segal & Starkey, 1969); methionine is first deaminated and then demethiolated.

This paper presents our studies on methionine transport into whole cells and into isolated membrane vesicles of Ps. fluorescens UK1.

METHODS

Media. The basal medium in which methionine was the only source of carbon and nitrogen contained (g/l): $\text{KH}_2\text{PO}_4$, 1.35; $\text{MgSO}_4\cdot\text{7H}_2\text{O}$, 0.26; $\text{FeSO}_4\cdot\text{7H}_2\text{O}$, 0.003; methionine, 1.49. The solution was adjusted to pH 7.0 with KOH; 2% (w/v) agar was added as required.
Bacteria. The strain UKI of *Pseudomonas fluorescens* was isolated from heavily polluted sea water, and had been exposed for 2 h at 30 °C to a solution (100 μg/ml) of *N*-methyl-*N'*-nitro-*N*-nitrosoaniline in the basal medium. The bacteria were then collected by centrifugation at 5000 g for 15 min, washed with 0.9 % (w/v) NaCl and cultured overnight in the basal medium containing nutrient broth (0.5 %, w/v). They were next cultured for 3 days on the methionine agar medium, then several further times in the basal medium. The stock cultures produced in that way were stored on methionine agar slants at 4 °C.

Chemicals. [1-14C]DL-Methionine (specific activity 7.9 mCi/mmol) and [35S]L-methionine (specific activity 96 Ci/mmol) were purchased from New England Nuclear, Boston, Massachusetts, U.S.A. DL-Ethionine, lysozyme and DNase II were obtained from Sigma. S-methyl-L-cysteine and S-ethyl-L-cysteine were from California Corporation for Biochemical Research, Los Angeles, U.S.A.

Membrane preparations. The bacteria, cultured on methionine or methionine + nutrient broth (at 30 °C), were harvested in mid-exponential phase by centrifugation at 5000 g for 15 min. One portion of the cells was washed with 0.05 M-potassium phosphate buffer (pH 6.6) and assayed for transport activity. The remaining portion, having been washed twice with 10 mM-tris-HCl buffer (pH 8.0), was converted to membrane vesicles; 8 mg washed cells, 0.5 mg lysozyme, 3 mg trypsin and 4 mg DNase/ml of suspension were used for this purpose (Kaback, 1971). The isolated vesicles were suspended (1.2 to 1.6 mg protein/ml) in 0.1 M-potassium phosphate buffer (pH 6.6).

Measurement of methionine transport. The reaction mixtures (Mäntsälä, 1974), of 0.45, 0.9 or 1.8 ml, contained 0.05 M-potassium phosphate buffer (pH 6.6), 1 mM-MgSO4, 0.01 mM-[14C]methionine (specific activity 15-6 mCi/mmol) and either 0.4 mg dry wt bacteria/ml, or membrane vesicles equivalent to 0.35 mg protein/ml. The solutions were usually 20 mM with respect to the electron donors.

Enzyme and protein assays. Succinate dehydrogenase (EC. 1.3.99.1) was measured spectrophotometrically (Veeger, der Vartanian & Zeylemaker, 1969), as was L-malate dehydrogenase (EC. 1.1.2.4) (Arrigoni & Singer, 1962). Protein was estimated by the method of Lowry, Rosebrough, Farr & Randall (1951).

Identification of radioactive compounds taken up by whole cells and membrane vesicles. Whole cells or isolated membrane vesicles (0.8 mg protein) were exposed to 60 μM-[14C]-methionine (in 0.5 ml) and to 10-5 μM-[35S]methionine in the presence of chloramphenicol. At 5 min intervals, 0.1 ml portions were filtered (Millipore, 0.45 μm pore-size). The filters were washed with 10 ml of 0.1 M-NaCl, extracted with 6 ml water at 80 °C and the extracts lyophilized. The residues that dissolved in 50 μl water were chromatographed on Whatman No. 1 paper with n-butanol-acetic acid–water (25:4:10, by vol.). The papers were autoradiographed and the radioactive spots assayed in a scintillation spectrometer (Wallac, Decem-NTL314).

Results

Effect of growth substrates and some metabolites of methionine degradation on methionine accumulation

Figure 1 demonstrates that the uptake of [14C]methionine continued linearly for 2 min in cells adapted to utilize methionine. Because 2-oxobutyrate and propionate, two of the end products of methionine degradation, hindered methionine uptake, we suggest that this is controlled by end-product inhibition. Succinate, on the other hand, only slightly decreased methionine accumulation. Bacteria grown with Casamino acids, nutrient broth or glucose and then incubated for 2 h under carbon starvation accumulated much less [14C]methionine.
Methionine transport in *Ps. fluorescens*

Fig. 1. Time course of L-methionine transport in the whole cells of *Ps. fluorescens* UK1. The reaction mixture contained, in 1.8 ml of 1 mM-MgSO₄, 0.01 mM-[¹⁴C]methionine (0.79 mCi/mmol) and 0.8 mg dry wt of bacteria. The bacteria were grown on the basal medium containing methionine (O), 0.5% (w/v) nutrient broth (△), 0.5% (w/v) Casamino acids (●), or 10 mM-glucose (▲). They were then starved for 2 h in the presence of 0.05 M-phosphate buffer (pH 6.6), 1 mM-MgSO₄ and 2 mM-(NH₄)₂SO₄, Citrate (□), 2-oxoglutarate (□), succinate (■), 2-oxobutyrate (●) and proprionate (◇) were added at 2 min (see arrow); final concentration 10 mM.

Fig. 2. The effect of sodium azide on the time course of methionine transport. Experimental conditions were as in Fig. 1. The arrow indicates when azide was added. Control (O); 2 mM-azole (●); 5 mM-azole (◇); 10 mM-azole (●); 10 mM-azole from time zero (△).
Fig. 3. Conversion of methionine to other metabolites in the whole cells and in the membrane vesicles of *Ps. fluorescens* U.K.I. (a) The bacteria (2.2 mg dry wt) and (b) the membrane vesicles (0.8 mg protein) were put with 0.06 mM-[14C]methionine in 0.5 ml of 0.03 M-phosphate buffer (pH 6.6) and chloramphenicol (100 μg). Ascorbate (20 mM) + PMS (0.1 mM) was the electron donor system in (b). In (c) the reaction mixture was chromatographed without washing. In (d) [35S]methionine was used. In (e) [14C]methionine was incubated with 20 mM-ascorbate and 0.1 mM-PMS for 10 min in the absence of the vesicles. In (f) membrane vesicles were subsequently added to such a mixture.

**Effect of azide on methionine transport**

The presence of NaN₃ inhibited methionine transport (Fig. 2). Some concentrative accumulation appeared to occur even in the presence of 10 mM-azide, which also caused the efflux of the accumulated compounds.

**Identification of the accumulated materials**

Methionine was partly converted to the corresponding α-keto acid, to methionine sulphoxide and to several other compounds when the bacteria were used (Fig. 3). However, some methionine sulphoxide formed spontaneously during the assays (Fig. 3c). Methionine was recovered from the membrane vesicles both as methionine itself and as the sulphoxide (Fig. 3b). Methionine sulphoxide was apparently partly converted to methionine again after addition of membrane vesicles (Fig. 3f).
Methionine transport in *Ps. fluorescens*

**Fig. 4.** The reciprocal of the initial velocity (v) of methionine uptake as a function of the reciprocal of the methionine concentration. The amount of DL-methionine absorbed in 2 min was assayed (see Fig. 1). The methionine concentration varied up to 200 μM.

**Table 1.** The effect of some methionine analogues, sulphur compounds and amino acids on methionine uptake by the bacteria

The concentration of DL-methionine was 20 μM; other experimental conditions were as in Fig. 1.

<table>
<thead>
<tr>
<th>Analogue (2 mM)</th>
<th>Methionine absorbed (nmol/min/mg dry wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No additions</td>
<td>2.10</td>
</tr>
<tr>
<td>DL-Ethionine</td>
<td>0.80</td>
</tr>
<tr>
<td>α-Methyl methionine</td>
<td>1.15</td>
</tr>
<tr>
<td>S-methyl-L-cysteine</td>
<td>1.25</td>
</tr>
<tr>
<td>S-ethyl-L-cysteine</td>
<td>1.40</td>
</tr>
<tr>
<td>No additions</td>
<td>2.60</td>
</tr>
<tr>
<td>N-formyl-L-methionine</td>
<td>1.70</td>
</tr>
<tr>
<td>L-Methionine sulfoxide</td>
<td>1.75</td>
</tr>
<tr>
<td>DL-Homocysteine</td>
<td>1.60</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>1.80</td>
</tr>
<tr>
<td>N-acetyl-DL-methionine</td>
<td>2.65</td>
</tr>
<tr>
<td>DL-Allocystationine</td>
<td>2.40</td>
</tr>
<tr>
<td>L-Cysteic acid</td>
<td>2.75</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>2.55</td>
</tr>
<tr>
<td>L-Glutathione</td>
<td>2.30</td>
</tr>
<tr>
<td>DL-Homocysteic acid</td>
<td>2.20</td>
</tr>
<tr>
<td>DL-Homocysteine thiolactone</td>
<td>2.60</td>
</tr>
<tr>
<td>3-Mercapto propionic acid</td>
<td>2.25</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>2.40</td>
</tr>
<tr>
<td>L-Aspartic acid</td>
<td>2.60</td>
</tr>
</tbody>
</table>
Fig. 5. Accumulation of methionine in isolated membrane vesicles from *Ps. fluorescens* UKI. Concentration of the added electron donors was 20 mM (PMS was 0·1 mM and FAD was 0·01 mM). The reaction mixture (see Fig. 1) contained 0·32 mg of membrane protein. (a) Ascorbate-PMS (○), ascorbate (●), no added electron donor (○). (b) Malate + FAD (△), malate (▲). (c) Succinate (□), DL-lactate (■). (d) 2-Oxoglutarate (■), fumarate (■).

Table 2. The effect of various compounds on methionine transport by *Ps. fluorescens* UKI membrane vesicles

Experimental conditions were as in Fig. 6.

<table>
<thead>
<tr>
<th>Addition (20 mM)</th>
<th>Methionine absorbed (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0·065</td>
</tr>
<tr>
<td>NADH</td>
<td>0·105</td>
</tr>
<tr>
<td>ATP</td>
<td>0·080</td>
</tr>
<tr>
<td>Glucose-6-P</td>
<td>0·060</td>
</tr>
<tr>
<td>Glucose-1-P</td>
<td>0·080</td>
</tr>
<tr>
<td>Glucose</td>
<td>0·070</td>
</tr>
<tr>
<td>Acetate</td>
<td>0·030</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0·065</td>
</tr>
<tr>
<td>Citrate</td>
<td>0·075</td>
</tr>
<tr>
<td>2-Oxobutyrate</td>
<td>0·100</td>
</tr>
<tr>
<td>Choline</td>
<td>0·205</td>
</tr>
<tr>
<td>Ethanolamine</td>
<td>0·150</td>
</tr>
</tbody>
</table>

**Kinetics of methionine transport**

A Lineweaver–Burk plot of methionine transport is shown in Fig. 4. One process exhibits saturation kinetics at methionine concentrations below 20 μM. A second system may represent simple diffusion, since (i) the extrapolated *v*ₙₐₓ. (Fig. 4, inset) appears to be very large.
Methionine transport in *Ps. fluorescens*

Fig. 6. Effect of malonate and azide on methionine uptake when succinate and malate were used as electron donors. Membrane vesicles were incubated (see Fig. 1) in the presence of: 0.01 mM-[14C]methionine, 20 mM-succinate and 20 mM-malonate (○); 0.01 mM-[14C]methionine, 10 mM-succinate, 10 mM-malate, 20 mM-malonate and 10 mM-azide (□); and 0.01 mM-[14C]methionine, 20 mM-malate, 10 mM-azide and 10 μM-FAD (△). The arrows show when the compounds were added.

Table 3. The effect of malonate and azide on the activities of succinate, L-malate and D-lactate dehydrogenases in membrane vesicles prepared from *Ps. fluorescens* UK1 grown with methionine

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Succinate dehydrogenase (nmol/min/mg protein)</th>
<th>L-Malate dehydrogenase (nmol/min/mg protein)</th>
<th>D-Lactate dehydrogenase (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.092</td>
<td>0.122</td>
<td>0.016</td>
</tr>
<tr>
<td>Malonate (20 mM)</td>
<td>0.034</td>
<td>0.116</td>
<td>—</td>
</tr>
<tr>
<td>Azide (10 mM)</td>
<td>0.068</td>
<td>0.102</td>
<td>—</td>
</tr>
</tbody>
</table>

and (ii) the addition of azide (Fig. 2) and cyanide (10 mM) failed to stop it. Among the compounds listed in Table 1, DL-ethionine, α-methyl-DL-methionine, S-methyl-L-cysteine and S-ethyl-L-cysteine were the strongest inhibitors. Their effects were competitive; apparent \( K_i \) values were 0.10, 0.20, 0.35 and 0.40 mM, respectively, when the methionine concentration was below 20 μM.

Accumulation of methionine in the membrane vesicles

As shown in Fig. 5, accumulation was enhanced by addition of 20 mM ascorbate with 0.1 mM phenazine methosulphate (PMS), the maximum levels being reached in 4 to 6 min. Several other compounds also served as electron donors. The most effective were FAD+, malate, malate, succinate and 2-oxoglutarate (Fig. 5), and NADH, choline and ethanolamine (Table 2). Figure 6 demonstrates that malonate (20 mM) inhibited methionine
accumulation when succinate was the electron donor, but was less effective when succinate and malate were used together. When azide (10 mM) was added to the reaction mixture, methionine accumulation was completely inhibited even in the presence of FAD. Succinate dehydrogenase was inhibited by malonate (20 mM) and both succinate and L-malate dehydrogenases were partly inhibited by azide (10 mM) (Table 3). D-Lactate dehydrogenase was almost absent from the membrane vesicles prepared from the bacteria grown in the basal medium.

**Effect of temperature on methionine transport in whole cells and membrane vesicles**

The initial rate of uptake of methionine was greatest at 42 to 45 °C in both cases. Using the vesicles, an activation energy of about 75 kJ/mol was estimated from the temperature-dependence of the rate.

**DISCUSSION**

Many transport systems for amino acids are constitutive (Cohen & Monod, 1957; Britten & McClure, 1962; Kepes & Cohen, 1962); however, especially in pseudomonads, some are inducible (Rosenfeld & Feigelson, 1969; Gryder & Adams, 1970; Miller & Rodwell, 1971a, b), as we found the strain UK1 to be with respect to methionine uptake. It seems relevant that Kay & Gronlund (1969a) found that, when cultures of *Ps. aeruginosa* were maintained in the stationary growth phase for 3 h in a glucose minimal medium, all the intracellular and extracellular amino acids were utilized, except for methionine which accumulated outside the cells.

Membrane vesicles from *E. coli* ML308–225 convert accumulated methionine to an unknown compound (Kaback & Milner, 1970), which may be either the corresponding α-keto acid or possibly methionine sulphone formed in the presence of ascorbate-PMS. In *Ps. fluorescens* UK1 methionine is obviously transported by the vesicles without deamination. If 2-oxomethionine had been demethylated, the 2-oxobutyrate formed would be labelled and at least three radioactive spots would be detected on the autoradiograms. These were not observed. Consequently demethylation may be a cytoplasmic process.

Ethionine is a competitive inhibitor of methionine in several micro-organisms (Gits & Grenson, 1967; Piperno & Oxender, 1968; Ayling & Bridgeland, 1972). In *Ps. fluorescens* UK1, too, ethionine inhibited methionine accumulation competitively. On the other hand, although methionine sulphone had no inhibitory effect in *Salmonella typhimurium* (Ayling & Bridgeland, 1972), it did in *Ps. fluorescens*.

For most organisms studied the artificial system, ascorbate-PMS, has been found to be the most effective electron donor. However, a wide variety of oxidizable compounds such as lactate (Kaback & Milner, 1970; Matin & Konings, 1973), succinate, di-α-hydroxybutyrate (Lombardi & Kaback, 1972), malate, pyruvate, oxalacetate, glucose (Stinnett, Guyman & Eagon, 1973), NADH (Sprott & MacLeod, 1972) and α-glycerol-P (Short, White & Kaback, 1972) act as electron donors. Succinate, malate and 2-oxoglutarate were evidently electron donors in *Ps. fluorescens* UK1. This depended on the way the vesicles were prepared; when ultrasonic disintegration or an X-press were used, only ascorbate-PMS accelerated methionine transport (unpublished observations).

**REFERENCES**


Methionine transport in *Ps. fluorescens* 27


