The Transport of L-Glutamate by *Rhizobium leguminosarum* Involves a Common Amino Acid Carrier

By P. S. POOLE,* M. FRANKLIN, A. R. GLENN AND M. J. DILWORTH

*Nitrogen Fixation Research Group, School of Environmental and Life Sciences, Murdoch University, Murdoch, Western Australia 6150*

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*Rhizobium leguminosarum* MNF3841 grown on glucose/NH₄Cl constitutively transported L-asparagine, L-aspartate, L-glutamate, L-glutamine, glycine, L-leucine, L-methionine and L-phenylalanine. Transport rates were increased 1.5-4-fold by growth on glucose/L-glutamate. Uptake of L-glutamate, L-glutamine, L-asparagine and L-leucine was inhibited to varying extents by a broad range of L-amino acids. Analogue of L-glutamate in which the amino group or α-hydrogen was methylated inhibited L-glutamate transport much less effectively. Also while 2- and 3-amino acids interfered with L-glutamate uptake, D-glutamate did not. Inhibition by 2,4-dinitrophenol, carbonyl cyanide m-chlorophenylhydrazone and cyanide indicated that amino acid transport was active. The ratio of the intracellular to extracellular concentration of L-leucine after 5 min accumulation was 768. Cells loaded with L-[14C]leucine exhibited exchange not only with external L-leucine but also with L-glutamate. The apparent *Kₘ* for L-glutamate transport was 0.081 μM. Both L-aspartate and L-alanine were competitive inhibitors of L-glutamate uptake with apparent *Kₘ* values of 0.164 μM and 2.3 μM, respectively. These results suggest that there is an extremely high affinity carrier for L-glutamate that is not only very sensitive to inhibition by L-aspartate but also capable of being inhibited by a broad range of amino acids at an order of magnitude higher concentration.

**INTRODUCTION**

Legume root nodules contain a complex mixture of compounds including sugars, organic acids and amino acids. A central problem in understanding nodule metabolism is to know which of these compounds are transported into and out of the plant cytosol and by what mechanism(s). The transport of sugars (Hudman & Glenn, 1980; Glenn & Dilworth, 1981; de Vries et al., 1982) and C₂-dicarboxylic acids (Glenn et al., 1980; Finan et al., 1981; McAllister & Lepo, 1983) has been studied, largely in an attempt to understand the nature of the carbon source(s) used by nitrogen-fixing bacteroids.

These studies on the transport of carbon compounds have been complemented by experiments on movements of nitrogen sources. At high concentrations, ammonia appears to move by diffusion (Dilworth & Glenn, 1982), while at low concentrations, ammonia is actively transported by a specific permease (Glenn & Dilworth, 1984; O’Hara et al., 1985). The lack of this ammonium permease in isolated pea bacteroids suggests that ammonia fixed from N₂ moves into the plant cytosol by diffusion (O’Hara et al., 1985). Methylamine, which in rhizobia can serve as a sole source of nitrogen but not generally of both carbon and nitrogen, is taken up via an active methylamine permease (Glenn & Dilworth, 1984).

Amino acids, which can be used as a source of both carbon and nitrogen by rhizobia (Poole et al., 1984) and which are present in high concentrations in the nodule (Pate, 1977), have so far been largely overlooked. The lack of ammonia-assimilating enzymes in the bacteroids (Brown &

*Abbreviation*: CCCP, carbonyl cyanide m-chlorophenylhydrazone.
Dilworth, 1975; Kurz et al., 1975) supports the suggestion (O’Gara & Shanmugam, 1976) that the bacteroid may need to be supplied with amino acids by the plant. Some amino acids must presumably move from the plant to the bacteroid since amino acid auxotrophs of *Rhizobium meliloti* (Scherrer & Dénarié, 1971) and *R. leguminosarum* (Pain, 1979) form effective nodules. If protein synthesis is to occur in such auxotrophs then transport of amino acids from the plant to the bacteroid must occur.

In this study we have determined the general properties of amino acid transport in free-living cells of *R. leguminosarum* MNF3841.

**METHODS**

**Organism.** *Rhizobium leguminosarum* MNF3841 is a *Str* derivative of strain 300 (Johnston & Beringer, 1975) and has been described before (Glenn et al., 1980).

**Media.** Bacteria were routinely grown in batch culture at 28 °C in the liquid minimal medium of Brown & Dilworth (1975) with glucose (10 mM) as the carbon source, NH₄Cl or amino acid (10 mM) as the nitrogen source, phosphate (0.4 mM) and with the pH maintained at 7.0 with 40 mM-HEPES. From here on all media will be referred to by the nitrogen source used. Amino acids were filter-sterilized and added aseptically to autoclaved minimal salts medium.

**Chemostat culture.** For experiments on transport kinetics bacteria were grown at 28 °C in ammonia-limited (0-75 mM) chemostat culture (dilution rate 0.09 h⁻¹) with 10 mM-sucrose as the carbon source and HEPES decreased to 20 mM.

**Uptake assays.** Cells were prepared for uptake assays as described by Hudman & Glenn (1980). Assays were conducted at 28 °C in 1 ml minimal salts medium at pH 7.0 with 0.1 ml cell suspension (0.084-16 mg protein ml⁻¹) being sampled at 30 s or 1 min intervals for 3 to 5 min. Inhibitors were added 10 s prior to addition of the labelled amino acid (0.05 mM). After Millipore filtration under vacuum, cells were washed twice with minimal salts medium at 28 °C and added to 3 ml of the scintillant described by Glenn et al. (1984). For the measurement of transport kinetics, assays were conducted in a 10 ml volume with 3 ml cell suspension (0.004 mg protein ml⁻¹) being sampled at 5, 15 and 25 s.

**Incorporation experiments.** During an uptake assay over 5 min, 0.1 ml cell suspension was added at each sampling time to 2 ml ice-cold 10% (w/v) TCA containing unlabelled carrier amino acid (10 mM). Samples were kept on ice for 30 min and filtered. The filters were washed twice with minimal salts medium and then added to scintillation fluid for counting.

**Cell volumes.** Cell volumes were calculated from a value of 1.45 ml (g dry wt)⁻¹ (Glenn et al., 1984) for this strain.

**Paper chromatography.** Cell suspension (3 ml) incubated with L-[¹⁴C]leucine for 3 min was centrifuged, washed twice and resuspended in minimal salts medium before addition of L-glutamate (10 mM). After 11 min the extracellular fluid was collected by Millipore filtration and its components were separated by descending chromatography on 25 cm sheets of Whatman no. 3 chromatography paper. Two solvents were used for separation: A, phenol (500 g phenol in 125 ml water/ethanol/water (15:4:1, by vol.) and B, tert-butanol/methylketone/water/NH₄OH (4:3:2:1, by vol.). Carrier L-leucine (20 nmol) was added to all filtrate samples while 500 nmol of L-glutamate were carried over from the exchange experiment. After running the chromatogram the internal standards of L-leucine and L-glutamate were located by spraying with ninhydrin (0.1%, w/v, in ethanol). The chromatograms were then cut into 1 cm sections and placed in scintillation fluid for counting.

**Radiochemicals.** L-[¹⁴C]Glutamate (10-9 GBq mmol⁻¹) was obtained from New England Nuclear. L-Asparagine (4.4 GBq mmol⁻¹), L-leucine (12.2 GBq mmol⁻¹), L-aspartate (8.1 GBq mmol⁻¹), L-phenylalanine (16-6 GBq mmol⁻¹), L-glutamate (1-8 GBq mmol⁻¹) were all [¹⁴C] and were obtained from Amersham, as were [²⁻¹⁴C]glycine (2.1 GBq mmol⁻¹), L-[methyl-¹⁴C]methionine (2.2 GBq mmol⁻¹) and D-[¹⁴C]glucose (10.8 GBq mmol⁻¹). Immediately before use L-glutamate and L-asparagine were purified using Dowex-1 resin by the technique of Prusiner & Miler (1970).

**Protein determination.** Protein was estimated by the Lowry method with bovine serum albumin as the standard.

**RESULTS**

**Constitutivity of transport.** *R. leguminosarum* MNF3841 was able to transport a wide range of amino acids after growth on NH₄Cl (Table 1). Uptake rates for the five compounds tested all increased 1.5-4-fold when the cells were grown on L-glutamate.

**pH profile.** L-Glutamate transport had a pH optimum between 6.0 and 7.0 when measured in a mixture of 2.5 mM-MES and 2.5 mM-HEPES, pH 5.0 to pH 8.0.
Amino acid transport in Rhizobium

Table 1. Rates of amino acid transport in Rhizobium leguminosarum MNF3841

Cells of *R. leguminosarum* MNF3841 grown on glucose/NH₄Cl or glucose/L-glutamate were washed and resuspended in minimal salts medium at pH 7.0 and 28 °C in the presence of 14C-labelled amino acids (0.05 mM). Samples were removed and the radioactivity in cells was estimated as described in Methods. Rates of uptake are expressed as nmol min⁻¹ (mg protein)⁻¹. All values are the mean ± SEM of at least three experiments, except the value for L-phenylalanine, which is the result from a single experiment.

<table>
<thead>
<tr>
<th>Amino acid transported</th>
<th>Nitrogen source</th>
<th>NH₄Cl</th>
<th>L-Glutamate</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Asparagine</td>
<td>4.9 ± 0.9</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>L-Aspartate</td>
<td>4.9 ± 1.2</td>
<td>12.2 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>L-Glutamate</td>
<td>9.0 ± 1.1</td>
<td>37.0 ± 3.9</td>
<td></td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>5.5 ± 0.8</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>14.4 ± 1.9</td>
<td>20.8 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>L-Leucine</td>
<td>4.1 ± 0.6</td>
<td>16.2 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>L-Methionine</td>
<td>3.1 ± 0.2</td>
<td>9.0 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>2.4</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

ND, Not determined.

Table 2. Inhibition of L-glutamate transport in R. leguminosarum MNF3841

Cells were grown on glucose/NH₄Cl and prepared for the measurement of transport as described in Table 1. The appropriate inhibitor was added to give a 20-fold excess 10 s before the addition of either 0.05 or 0.1 mM-L-glutamate. All values are the mean of at least two replicates.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Percentage inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>3-Aminopropionate</td>
<td>67</td>
</tr>
<tr>
<td>4-Aminobutyrate</td>
<td>49</td>
</tr>
<tr>
<td>D-Glutamate</td>
<td>5</td>
</tr>
<tr>
<td>Methylamine</td>
<td>12</td>
</tr>
<tr>
<td>Propylamine</td>
<td>6</td>
</tr>
<tr>
<td>2-Methyl-DL-glutamate</td>
<td>18</td>
</tr>
<tr>
<td>N-Methyl-L-glutamate</td>
<td>34</td>
</tr>
<tr>
<td>L-Glutamate 5-methyl ester</td>
<td>81</td>
</tr>
<tr>
<td>L-Glutamate dimethyl ester</td>
<td>81</td>
</tr>
<tr>
<td>L-Histidinol</td>
<td>4</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>2</td>
</tr>
<tr>
<td>2-Oxoglutarate</td>
<td>11</td>
</tr>
</tbody>
</table>

Nature of uptake system. Potassium cyanide (1 mM), carbonyl cyanide *m*-chlorophenylhydrazone (CCCP; 0.05 mM) and 2,4-dinitrophenol (DNP; 1 mM) caused >90% inhibition of L-glutamate uptake. Sodium azide (1 mM) and *N*,*N*-dicyclohexylcarbodiimide (DCCD; 0.1 mM) caused 69% and 51% inhibition respectively, while sodium fluoride (5 mM) and sodium arsenate (5 mM) caused <25% inhibition.

Simple amines, or 2-oxoglutarate, did not significantly inhibit L-glutamate uptake, but 3-amino propionate and 4-amino butyrate did (Table 2). D-Glutamate was a very poor inhibitor, demonstrating that the system had a high specificity for the L-isomer. Use of substrate analogues of L-glutamate showed that free carboxyl groups were not required since both the 5-methyl and dimethyl esters of L-glutamate caused substantial inhibition of uptake. An unsubstituted α-hydrogen and amino group were necessary as shown by the lack of inhibition by 2-methylglutamate and *N*-methylglutamate. This pattern of inhibition suggests the presence of a transport system with broad specificity for L-amino acids. If this interpretation is correct, other L-amino acids should also have a very broad pattern of cross inhibition, although this may be complicated by the presence of multiple transport systems.

Transport specificity. In cells grown on L-glutamate a wide range of amino acids inhibited L-glutamate transport, even though their side chains were structurally unrelated (Table 3). Growing cells on NH₄Cl lowered the absolute rate of transport but did not change the pattern of inhibition significantly.
Table 3. Inhibition of amino acid transport in R. leguminosarum MNF3841

L-Glutamate, L-leucine and glycine transport was measured in cells grown on glucose/L-glutamate; L-asparagine transport was measured in cells grown on glucose/L-asparagine, and L-glutamine transport was measured in cells grown on glucose/L-glutamine. Cells were prepared for transport measurements as described in Table 1. The compound whose transport was being measured was added at a concentration of either 0.05 or 0.1 mM. Inhibitors were added 10 s prior to this to give a fivefold excess. All values are the mean of at least two replicates.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>L-Glutamate</th>
<th>L-Leucine</th>
<th>Glycine</th>
<th>L-Asparagine</th>
<th>L-Glutamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Alanine</td>
<td>87</td>
<td>84</td>
<td>52</td>
<td>82</td>
<td>78</td>
</tr>
<tr>
<td>L-Asparagine</td>
<td>89</td>
<td>54</td>
<td>19</td>
<td>–</td>
<td>46</td>
</tr>
<tr>
<td>L-Aspartate</td>
<td>78</td>
<td>ND</td>
<td>ND</td>
<td>46</td>
<td>22</td>
</tr>
<tr>
<td>L-Glutamate</td>
<td>–</td>
<td>35</td>
<td>25</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>L-Glutamine</td>
<td>95</td>
<td>84</td>
<td>39</td>
<td>92</td>
<td>–</td>
</tr>
<tr>
<td>Glycine</td>
<td>46</td>
<td>68</td>
<td>–</td>
<td>71</td>
<td>ND</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>75</td>
<td>–</td>
<td>42</td>
<td>73</td>
<td>58</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>43</td>
<td>27</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>L-Serine</td>
<td>77</td>
<td>84</td>
<td>ND</td>
<td>78</td>
<td>66</td>
</tr>
</tbody>
</table>

ND, Not determined.

If the inhibition of L-glutamate transport by other amino acids were caused by competition for a common carrier then other families of amino acids should show a similar pattern of inhibition of transport. The effect of various amino acids on the transport of L-glutamine, L-asparagine, L-leucine and glycine was therefore examined.

Amide transport. The transport of L-glutamine and L-asparagine was assayed in cells of R. leguminosarum MNF3841 grown on L-glutamine or L-asparagine, respectively, as the nitrogen source (Table 3). There was broad inhibition of both L-glutamine and L-asparagine transport by other amino acids. However, both were less sensitive to inhibition than L-glutamate transport. For example, while L-glutamate transport was inhibited 95% by a five-fold excess of glutamine, a five-fold excess of L-glutamate only caused a 46% inhibition of L-glutamate transport. Overall uptake of both L-glutamine and L-asparagine was less sensitive to inhibition by dicarboxylic amino acids than by neutral amino acids. Such a difference may be due to the presence of a specific amide transport system and/or a higher apparent affinity of a common system for L-glutamine than for L-glutamate.

Leucine and glycine transport. Cells grown on L-glutamate were assayed for transport of both L-leucine and glycine. L-Leucine transport was inhibited by a wide range of L-amino acids (Table 3), though inhibition was less severe than noted previously for L-glutamate transport. A fivefold excess of L-glutamate caused a 35% inhibition of L-leucine transport whereas a 1000-fold excess of L-glutamate still allowed 7% of the rate for L-leucine alone. These results suggest that a common system capable of transporting both L-leucine and L-glutamate may be present, together with a specific system for L-leucine. This was more clearly shown with glycine uptake, where a 200-fold excess of L-glutamate caused only a 37% inhibition of glycine uptake. The very high rate of constitutive glycine transport and its lower sensitivity to inhibition by L-glutamate suggest that there is also a transport system specific for glycine.

The intracellular concentration of L-leucine after 5 min incubation was 29.2 mM compared with 0.038 mM remaining outside the cells, a concentration ratio of 768.

Kinetics of L-glutamate transport. Cells of strain MNF3841 were grown in chemostat culture to ensure physiologically better adapted cell samples for the determination of transport kinetics. An apparent $K_m$ for L-glutamate of 0.081 ± 0.01 μM was determined by fitting the data from seven replicates (Fig. 1) to the Michaelis-Menten equation using nonlinear regression methods (Duggleby, 1981). L-Glutamate transport was competitively inhibited by both L-aspartate and L-
Amino acid transport in Rhizobium

Fig. 1. Substrate saturation curve for L-glutamate transport in R. leguminosarum MNF3841. The apparent $K_m$ was 0.081 μM. Bars indicate the SEM.

Fig. 2. Inhibition of L-glutamate transport by L-aspartate in R. leguminosarum MNF3841. ○, No addition; ●, L-aspartate (0.2 μM); □, L-aspartate (0.4 μM); ■, L-aspartate (0.8 μM).

Fig. 3. Inhibition of L-glutamate transport by L-alanine in R. leguminosarum MNF3841. ○, No addition; ●, L-alanine (2 μM); □, L-alanine (6 μM); ■, L-alanine (10 μM).

alanine (Figs 2 and 3). Re-plotting $K_m/V_{max}$ against L-aspartate or L-alanine concentration gave apparent $K_i$ values of 0.16 μM and 2.3 μM respectively.

Amino acid exchange. While cross-inhibition is most easily interpreted as competition for a common carrier, similar results could be generated by intracellular metabolism, competition for a common cofactor or cross-inhibition of solutes that are transported by separate systems. To distinguish between these alternatives, cells of R. leguminosarum MNF3841 which had taken up a 14C-labelled amino acid were tested for the incorporation of label into TCA-insoluble material and for exchange with external amino acids.

Cells grown on L-glutamate incorporated 70% of the transported L-glutamate and 1.4% of the transported L-leucine into TCA-precipitable material in 5 min, while NH$_4$Cl-grown cells incorporated 38% of L-glutamate under the same conditions. Fig. 4 shows the effect of adding an excess (20 mM) of non-radioactive L-leucine or L-glutamate to cells grown on L-glutamate which had been allowed to accumulate L-[14C]leucine for 3 min. Efflux of L-[14C]leucine in response to external L-leucine was essentially complete within 23 min. Addition of the metabolic inhibitor CCCP (0.05 mM) also caused rapid efflux (Fig. 4), suggesting that the retention of a concentration gradient in the cells was energy dependent.

L-Glutamate initially exchanged rapidly with L-[14C]leucine, followed by a slow recovery of intracellular L-[14C]leucine. The immediate exit of L-[14C]leucine after L-glutamate addition is consistent with the presence of a common transport system, with further uptake expected when the chaser L-glutamate has saturated the common carrier on both sides of the membrane. At this
point, reached after 10 min, L-[14C]leucine was apparently unable to gain access to the common carrier and further leucine movement would occur via a specific system.

Addition of 20 mM-glucose or succinate instead of an amino acid had no effect on the intracellular concentration of L-[14C]leucine, while lowering the concentration of chaser amino acid to 1 mM did not alter the efflux pattern. Furthermore, glucose was still transported after addition of 20 mM-amino acid, implying that the efflux upon addition of external amino acid is not due to impaired membrane function.

The material that effluxed when L-glutamate was added to cells that had previously been allowed to accumulate L-[14C]leucine was separated by paper chromatography in two different solvent systems. In both solvents two major peaks were obtained, the first of which comprised 35% of the total radioactive material and co-chromatographed with L-leucine (Rf, 0.76 in solvent A and Rf, 0.65 in solvent B). The second peak (65% of the radioactive material) chromatographed (Rf, 0.60) behind L-leucine in solvent A and ahead of L-leucine in solvent B (Rf, 0.82). L-Glutamate had an Rf value of 0.14 in solvent A and 0.15 in solvent B. While a large proportion of the radioactivity had been converted to an unidentified compound with similar chromatographic properties to L-leucine, a significant fraction clearly remained as unaltered L-leucine. Though this might suggest that the minimum intracellular concentration of L-leucine was 10-2 mM, the time required for such a resuspension experiment (32 min) is much longer than for uptake experiments (5 min) and allows for extensive metabolism of L-leucine. In any event, the concentration factor between intra- and extra-cellular L-leucine was at least 269.

Unlabelled amino acids (20 mM) were added to cells grown on NH₄Cl and allowed to take up L-[14C]glutamate. Of the radioactivity not precipitable with TCA, 48% appeared in the medium when either L-glutamate or L-glutamine was added (Fig. 5). L-Leucine, glycine or L-asparagine addition resulted in an efflux of L-[14C]glutamate equivalent to that caused by L-glutamate.

**DISCUSSION**

Although bacterial amino acid transport systems are usually highly specific for a single amino acid (Oxender, 1972) or for a family of amino acids (Ames, 1964), a general amino acid permease has been found in *Saccharomyces cerevisiae* (Grenson et al., 1970; Eddy, 1982) and *Streptomyces*...
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*Streptomyces hydrogenans* (Gross & Ring, 1971; Ring et al., 1977; Fritsch & Gross, 1983). In *Streptomyces hydrogenans* there are two separate transport systems for L-glutamate; one has a high affinity for L-glutamate and L-aspartate and operates optimally at pH 6.5, and a second has a low affinity for L-glutamate and transports its zwitterion, as well as a broad range of amino acids, optimally at pH 4.5. Amino acid transport in *R. leguminosarum* MNF3841 showed distinct differences from this pattern, since at pH 7.0 both broad cross-inhibition (Table 3) and a very high affinity for L-glutamate (Fig. 1) were evident.

If a general amino acid transport system were present in *R. leguminosarum* MNF3841 in addition to specific systems, several predictions might be made. Firstly, if growth on an amino acid increases its rate of transport it should also increase the rate of transport of other amino acids. Secondly, amino acid transport should be widely cross-inhibited, although the presence of specific systems would cause varying degrees of inhibition. The properties of transport in *R. leguminosarum* MNF3841 are compatible with these first two predictions (Tables 1 and 3). Possible explanations for a broad pattern of cross-inhibition include: (1) competition at a metabolic drag step, that is, inhibition of the metabolism of an amino acid leading to an increase in the intracellular concentration of the unaltered amino acid; (2) competition for a common cofactor; (3) formation of dead-end transport complexes between dissimilar amino acids and specific transport systems, and (4) trans-inhibition, that is, the inhibition of transport caused by the prior accumulation of a dissimilar compound. Since trans-inhibition in *Streptomyces hydrogenans* requires prolonged incubation with a neutral amino acid (Ring et al., 1970), this explanation is unlikely for *R. leguminosarum* MNF3841.

A third prediction, if confirmed, would make the other alternatives to cross-inhibition unlikely. This prediction is that if self-exchange occurs then cross-exchange between amino acids should also occur. L-Leucine is exchanged out of strain MNF3841 by L-glutamate, and even after 32 min, 35% of the exchanged material could be identified as L-leucine (Fig. 4). When taken in conjunction with the low incorporation rate of L-leucine into TCA-precipitable material, this suggests that over the 5 min period of most uptake experiments L-leucine forms a freely soluble pool inside the cell. Such a free pool seems unlikely to exhibit any substantial metabolic drag. The ability to rapidly cross-exchange amino acids is only easily explained by the presence of a general carrier and largely excludes the formation of extensive dead-end complexes.

If specific transport systems exist in conjunction with a common system they should be difficult to totally inhibit and should add a second kinetic component to any exchange experiment. L-Leucine and glycine transport showed these properties in *R. leguminosarum* MNF3841, with L-leucine being accumulated at 7% of the control rate in the presence of a 1000-fold excess of L-glutamate. The re-uptake of L-leucine seen in exchange experiments with L-glutamate (400-fold excess) occurs at a similar rate, a necessary result if this re-uptake is caused by a specific system.

A fourth requirement of a common system is that the inhibition between structurally unrelated amino acids should be competitive. Both L-aspartate and L-alanine caused competitive inhibition of L-glutamate transport although L-aspartate had an apparent *K*, an order of magnitude less than that of L-alanine (Figs 2 and 3). Thus high specificity was demonstrated towards L-glutamate and L-aspartate at concentrations around the apparent *Km* for L-glutamate. At concentrations which were 10-fold higher, but still in the micromolar concentration range, L-alanine and presumably other amino acids were inhibitory. Furthermore, L-glutamate transport failed to show any substantial deviation from Michaelis-Menten kinetics like that found for *Streptococcus faecalis* (Reid et al., 1970), *Streptomyces hydrogenans* (Gross & Ring, 1971) and *Mycobacterium smegmatis* (Yabu, 1971). This suggests that there need only be one kinetically important transport system for L-glutamate in strain MNF3841 under the growth conditions used.

It appears that *R. leguminosarum* MNF3841 has developed a very high affinity L-glutamate transport system that should enable it to scavenge traces of L-glutamate from its environment. If higher concentrations of amino acids are encountered the system may well transport other amino acids.
REFERENCES


