Basis of Transport Discrimination of Arginine from Other Basic Amino Acids in Salmonella typhimurium*

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SUMMARY

Salmonella typhimurium, like Escherichia coli, takes up arginine both by an arginine-specific transport system and by a system accepting lysine and ornithine as well as arginine. The kinetic parameters for the two routes of arginine transport were obtained first by several direct methods of kinetic analysis under the mass-action law. The total uptake of arginine was then formally shown to be indeed the sum of the two assumed components, by eliminating first one component (the high affinity, arginine-specific component) through specific inhibition by homoserine, and then the other component (the nonspecific component) by specific inhibition with lysine, ornithine, or histidine. The kinetic parameters obtained for arginine uptake by these approaches were in reasonable agreement with those obtained directly. The action of homoserine as an inhibitor of the arginine-specific system is based on a limited recognition of the hydroxyamino acid, i.e., a recognition not leading to its transport. Not only is the position of the hydroxyl group critical to this partial recognition, whether on carbon atoms 3, 4, or 5 of the hydroxyamino acid; but also its orientation in space is decisive. trans-Hydroxyproline being an effective inhibitor; cis-hydroxyproline, ineffective. By analogy and from the circumstance that replacement of the ε-nitrogen hydrogen atom of homoaarginine with a methyl group eliminates transport reactivity, we conclude that the group >NH at the secondary nitrogen atom represents one essential point of recognition of the guanidino amino acids, and the cationic structure of the other nitrogen atoms of the guanidinium group a second essential point.

The results of studies of basic amino acid transport by gram-negative bacteria in general indicate the presence of an arginine-specific transport system (1-4), associated with the action of arginine-specific binding proteins (1, 5), and of a transport system that serves for lysine, arginine, and ornithine (1, 2, 4, 5), associated with the presence of a lysine, arginine, and ornithine (LAO)-binding protein (1). This report contains a description of basic amino acid transport in Salmonella typhimurium strain 1/T2. We conclude that the uptake of arginine, lysine, and ornithine by S. typhimurium occurs by a general transport system for basic amino acids, analogous to the LAO system described for Escherichia coli (1), and that in addition, arginine is transported by a system specific to this basic amino acid.

The amino acid analogues that served to confirm the separateness of these two components of arginine uptake also provided us with the clue to the way in which the specific system recognizes arginine but rejects ornithine and lysine. A two-component subsite is proposed that recognizes separately the cationic structure and the secondary nitrogen atom of the guanidinium group on the amino acid side chain.
pentacyanoaquoferrate color reaction characteristic for asymmetric dialkyl derivatives of guanidine.

The structure of the product was confirmed by NMR spectroscopy, using a Varian T-60 spectrometer. The spectrum of a 5% solution of the reaction product in D$_2$O with tetramethylsilane as external reference was recorded at 27°C. Chemical shifts were observed at $\tau$ 7.6 to 8.6 multiple (6H), 6.53 singlet (3H), 6.27 triplet (1H), and 6.24 triplet (2H). These values were assigned to the C1-C5 methylene groups, N$^{-}$methyl, C5-H, and the C4-methylene group, respectively. These assignments were supported by examination of the NMR spectra of L-homoarginine.

All other chemicals were obtained from commercial sources. Optically active amino acids were in the L form unless specified otherwise.

Transport Assay—Overnight cultures in late exponential phase of growth were collected by centrifugation and washed twice with 0.01 M choline phosphate buffer, pH 7.0. They were resuspended in the choline buffer and incubated at 37°C for 5 min. The reaction was started by adding the bacterial suspension to a solution of the radioactive amino acids and any other additions as described in the text. The time course of uptake was measured by removing 1-ml samples at appropriate intervals, filtering these through a 25-mm Millipore filter (0.45 μm pore size) and washing immediately with 5 ml of choline phosphate buffer at 37°C. Radioactivity was counted in a Packard liquid scintillation spectrometer with a standard scintillator in ethanol-toluene solution (8). The rate of uptake was linearly proportional to the dry weight of cells in the range used and remained constant during the time each experiment required. The quantity of cells was estimated from the absorbance at 000 nm, employing a previously determined standard curve relating absorbance and dry weight.

RESULTS

The uptake of the basic amino acids by Salmonella typhimurium strain LT2 was characterized at 37°C. The rate of uptake of arginine, lysine, or ornithine at 20 μM concentrations was linear for at least 3 min and reached a steady state in 5 to 7 min. Aminoxyacetic acid has previously been used with E. coli during studies of arginine uptake to prevent the action of arginine decarboxylase (3). Neither aminoxyacetic acid (8 mM) nor choloramphenicol (80 mg per liter) affected the initial rate of uptake of arginine although they decreased the intracellular accumulation at the steady state by 15 and 35%, respectively. These reagents would be expected to affect the initial rate of uptake only in the special case where the transport process is not the rate-limiting step in arginine uptake and subsequent metabolism. The effects of exogenous energy sources and of two inhibitors of energy transfer on the uptake of arginine and lysine were examined. Adding glucose or glycerol stimulated the initial rate of uptake. Use of cyanide or 2,4-dinitrophenol to interfere with energy conservation diminished transport to less than 50% of control rates. These initial experiments led us to use washed cells without added chloramphenicol or aminoxyacetic acid for these studies and to approximate initial rates of uptake by taking 15- and 30-s samples.

Kinetics—The kinetic constants for basic amino acid transport were determined by several methods. The saturation of the transport of arginine, lysine, and ornithine as a function of concentration was determined and was plotted according to Lineweaver and Burk (Fig. 1). The data for lysine and ornithine (Fig. 1B) are those anticipated for a single saturable uptake process, although the results for arginine (Fig. 1A) show more complexity. If we assume provisionally that arginine is taken up by two simultaneous saturable processes, we can obtain by iteration (9) two sets of $K_m$ and $V_{max}$ values (see Table III, "Arginine-specific" and "Lysine-arginine-ornithine (LAO) Transport System").

The repression of arginine transport was demonstrated by growth on 20 mM arginine (Fig. 1A). The extent of repression was 90% and 78%, respectively, for the L. monocyctogenes strain and for the E. coli (2), was observed. The $K_m$ and $V_{max}$ for this component was 0.03 μM and 0.03 mmole per kg of cells. Since this rate represents 1% of the total arginine transport capacity at saturating arginine levels, and less than 6% of the arginine specific capacity, we did not study this process further. No corrections of our data were made for this component.

The deviation from simple Michaelis-Menten kinetics observed for arginine transport has been interpreted as arising from the action of two independent transport systems. A hypothesis about arginine, lysine, and ornithine uptake in S. typhimurium consistent with basic amino acid transport in E. coli (2) and Pseudomonas putida (10) is that one of these arginine transport systems is shared with lysine and ornithine. We looked for confirmation of this view. Inui and Christensen (11) have described a graphical method for obtaining the kinetic parameters of a transport mechanism involving two saturable uptake systems, when only one is sensitive to a particular inhibitor. This method was used with arginine as substrate and lysine as inhibitor by varying the lysine concentration at a number of arginine concentrations (Fig. 2). Because the experiments were made with cells containing unmodified pools of cellular amino acids, any trans effects from that source could be regarded as constant. One can easily see in the figure that the extent of lysine inhibition is greater at higher arginine concentrations. If we subtract the rate of arginine uptake at a given lysine concentration ($v_{Lys}$) from the rate in the absence of lysine ($v_0$) we can calculate the portion of arginine uptake inhibited by lysine. One could not, however, have obtained this portion by simple inspection.

According to Inui and Christensen (11), a plot of $(1/(v_0 - v_{Lys})$ against $1/[Lys]$ for each arginine level in Fig. 2 should yield a series of straight lines whose intercepts measure that part of arginine uptake subject to lysine inhibition (Fig. 3, left). A plot of the intercept of these lines against $1/[Arg]$ should yield the $K_m$ and $V_{max}$ for this process (Fig. 3, right). A $K_m$ value of 45 μM and a $V_{max}$ of 1.5 mmole per kg of dried cells/min
were obtained by this method, values similar to the parameters of the low affinity process observed in the initial Lineweaver-Burk analysis (Table III).

Inui and Christensen (11) have demonstrated that a plot of the ratio slope-intercept of the lines in Fig. 3, left, against [Arg] will be linear only when the inhibition under description is competitive and of Dixon's type Ia (12); i.e., when the mediator-lysine complex is totally inactive in producing transport of arginine. Such a plot is seen in Fig. 3, left, indicating the application of the kinetic parameters for arginine uptake by the LAO system. The solid line represents a saturable process with a $K_m = 45 \mu M$ and a $V_{max}$ of 1.5 mmol per kg of dried cells-min.

In Fig. 2, the portion of arginine uptake subject to competitive inhibition by lysine was determined from the data of Fig. 4, right, namely $K_m$ of 3.2 $\mu M$ and $V_{max}$ of 0.5 mmol per kg of dried cells-min, a value well with the parameters of the high affinity arginine uptake process (Table III). This agreement supports the hypothesis that the high $K_m$ arginine transport system is inhibited by lysine and that the low $K_m$ arginine uptake system is sensitive to lysine.

To see if this hypothesis can be extended to include ornithine transport, the inhibition of ornithine uptake by arginine was studied to determine the inhibition constant ($K_i$) for arginine by the Dixon method (12). A $K_i$ of 90 $\mu M$ was obtained for arginine (plot not shown) which agrees with the value of 95 $\mu M$ obtained for the low affinity system by simple Lineweaver-Burk analysis of arginine uptake and is similar to the value of 45 $\mu M$ determined by the Inui-Christensen method.

By these analyses we come to the conclusion, stated qualitatively, that this organism takes up arginine by a high affinity, arginine-specific system, and also by a system (LAO) shared with lysine and ornithine, for which the affinity for arginine is relatively low.

Specificity—To investigate further the specificities of these uptake systems, the natural amino acids at 2 mM were tested as inhibitors of 40 $\mu M$ arginine, a concentration at which about 60% of its uptake occurs by the LAO system. Only arginine, histidine, and lysine caused inhibition greater than 13%. Under the same conditions, these 3 amino acids caused 95%, 82%, and 42% inhibition, respectively. In testing the effect of $\omega$-hydroxyamino acids on arginine uptake our attention was drawn to the unexpected inhibition by homoserine not shown by serine, 2-amino-5-hydroxyvalerate, or 2-amino-6-hydroxycaproate. Under conditions such that homoserine decreased arginine uptake by 58% (1 $\mu M$ arginine with 5 mM inhibitors) the other $\omega$-hydroxyamino acids displayed less than 8% inhibition. To determine which system or systems for arginine transport histidine and homoserine were inhibiting, and to confirm the foregoing results with lysine and ornithine, an experiment was made in which each of these amino acids at 1 mM was tested as inhibitors of 40 $\mu M$ arginine uptake by 58% (1 $\mu M$ arginine with 5 mM inhibitors) the other $\omega$-hydroxyamino acids displayed less than 8% inhibition. The presence of large excess should yield a Lineweaver-Burk plot of arginine uptake by a single system; the $K_m$ and $V_{max}$ observed being the values for the residual, uninhibited system. In other words, their presence should convert the nonlinear plot of Fig.
uptake of leucine, arginine, alanine, and lysine, among the amino acids. Table I shows that at 100 μM the number of amino acids was examined in the presence of homoserine (A), lysine (O), or histidine (Δ).

**Table I**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control rate (μmol/hr/g dried cells)</th>
<th>Inhibition by 100 μM homoserine (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8 μM leucine</td>
<td>0.16</td>
<td>80</td>
</tr>
<tr>
<td>3 μM arginine</td>
<td>1.12</td>
<td>40</td>
</tr>
<tr>
<td>1.5 μM alanine</td>
<td>3.17</td>
<td>26</td>
</tr>
<tr>
<td>1 μM lysine</td>
<td>0.25</td>
<td>18</td>
</tr>
<tr>
<td>2 μM histidine</td>
<td>1.5</td>
<td>5</td>
</tr>
<tr>
<td>1.5 μM proline</td>
<td>1.05</td>
<td>3</td>
</tr>
<tr>
<td>6 μM tryptophan</td>
<td>1.3</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

The inhibition by homoserine was an unexpected phenomenon, and focused our attention on the structural requirements for the so-called arginine-specific system. To test whether this inhibitory action might be a nonspecific one, the uptake of a number of amino acids was examined in the presence of homoserine. Table I shows that at 100 μM, homoserine inhibits the uptake of leucine, arginine, alanine, and lysine, among the amino acids tested. Among these, only the action on lysine uptake may be seen as paradoxical. Because the action was weak and not increased by raising the homoserine level to 1 mM, it was not pursued further. The uptake of homoserine itself and the effect of various amino acids on that uptake were examined next. Table II shows that of the amino acids tested, leucine, valine, and alanine give the best inhibition, whereas arginine and lysine are without effect. This behavior is consistent with a prior conclusion that homoserine is transported in *E. coli* by the leucine-iso-leucine-valine system (15).

**Table II**

<table>
<thead>
<tr>
<th>Inhibitor (100 μM)</th>
<th>Inhibition of homoserine uptake (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td>87</td>
</tr>
<tr>
<td>Homoserine</td>
<td>85</td>
</tr>
<tr>
<td>Valine</td>
<td>82</td>
</tr>
<tr>
<td>Alanine</td>
<td>80</td>
</tr>
<tr>
<td>Proline</td>
<td>5</td>
</tr>
<tr>
<td>Lysine</td>
<td>3</td>
</tr>
<tr>
<td>Arginine</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

The inhibition by homoserine was an unexpected phenomenon, and focused our attention on the structural requirements for the so-called arginine-specific system. To test whether this inhibitory action might be a nonspecific one, the uptake of a number of amino acids was examined in the presence of homoserine. Table I shows that at 100 μM, homoserine inhibits the uptake of leucine, arginine, alanine, and lysine, among the amino acids tested. Among these, only the action on lysine uptake may be seen as paradoxical. Because the action was weak and not increased by raising the homoserine level to 1 mM, it was not pursued further. The uptake of homoserine itself and the effect of various amino acids on that uptake were examined next. Table II shows that of the amino acids tested, leucine, valine, and alanine give the best inhibition, whereas arginine and lysine are without effect. This behavior is consistent with a prior conclusion that homoserine is transported in *E. coli* by the leucine-iso-leucine-valine system (15).

1A, to a straight line pertaining to the uninhibited transport process. Such an experiment is presented in Fig. 5. The *Km* and *Vmax* values for arginine uptake observed in the presence of histidine, lysine, or ornithine are close to those given for the high affinity system for arginine (cf. Table III). That is, the low affinity system for arginine is identified as the one shared independently of the presence of alkali metal ions. The results from this experiment show that inhibition of arginine transport by homoserine occurs independently of the presence of alkali metal ions. The inhibition by homoserine was an unexpected phenomenon, and focused our attention on the structural requirements for the so-called arginine-specific system. To test whether this inhibitory action might be a nonspecific one, the uptake of a number of amino acids was examined in the presence of homoserine. Table I shows that at 100 μM, homoserine inhibits the uptake of leucine, arginine, alanine, and lysine, among the amino acids tested. Among these, only the action on lysine uptake may be seen as paradoxical. Because the action was weak and not increased by raising the homoserine level to 1 mM, it was not pursued further. The uptake of homoserine itself and the effect of various amino acids on that uptake were examined next. Table II shows that of the amino acids tested, leucine, valine, and alanine give the best inhibition, whereas arginine and lysine are without effect. This behavior is consistent with a prior conclusion that homoserine is transported in *E. coli* by the leucine-iso-leucine-valine system (15).

A report from this laboratory in 1969 described the inhibition of basic amino acid transport in the Ehrlich cell by hydroxyamino acids and alkali metal ions, notably Na+, acting in concert (16). The finding that homoserine greatly inhibited arginine transport in *S. typhimurium* led us to test the possibility that this bacterial system might show similar properties. An experiment was made in which arginine uptake was examined in choline phosphate buffer, supplemented with 5 mM of the chloride salts of the alkali metal ions. The results from this experiment show that inhibition of arginine transport by homoserine occurs independently of the presence of alkali metal ions. These results indicate that homoserine is not transported by a pathway shared with arginine, but that features of its structure nevertheless cause it to bind at the arginine receptor site. We infer that the feature of homoserine recognized as arginine-like might well be the —NO₂ group, mimicking the —N— group present by the secondary nitrogen of arginine. If this is the case, only one of the homoserine analogues, 4-trans- and 4-cis-hydroxyproline, should serve as a potential effector of arginine transport. Fig. 6 shows that cis-hydroxyproline is without effect on lysine, arginine, or ornithine uptake. On the other hand, the trans isomer shows strong inhibition of arginine transport, although the uptake of lysine and ornithine is unaffected.
Difficulties in accommodating an $\omega$-quaternary nitrogenous group have also been observed for the broad specificity system $L^+$ serving for the transport of various basic amino acids in mammalian cells (17, 18). $\omega$-Arginine fully inhibited System LAO, whereas the arginine-specific system appears quite stereotype-specific, as we might logically expect from its complex mode of side-chain recognition.

Several synthetic derivatives of piperidine and cyclohexane have been used as basic amino acid analogues to study transport in mammalian cells (7). The arginine analogue, $N$-guanyl-4-amino-4-carboxypiperidine, and the ornithine analogue, 1,4-diamino-1-carboxycyclohexane, were tested as inhibitors of arginine and lysine transport, respectively. These compounds at 1 mM were poor inhibitors in both cases, showing less than 18% inhibition. These results are in contrast to the effects of these compounds in mammalian cells, where they show modest to good inhibition of basic amino acid transport (7).

One can postulate two ways in which the secondary nitrogen of arginine may be recognized. This group could act either as a donor or as an acceptor of a hydrogen bond with the transport receptor site. To test these alternatives, we synthesized $\epsilon$-$N$-methylhomoaarginine. This compound lacks a hydrogen on the $\epsilon$-nitrogen and thus should fail as a hydrogen donor. Fig. 7 shows that it is in fact only able to block arginine transport to the extent that arginine is transported by the LAO system, whereas it inhibits lysine and ornithine uptake nearly as well as does homoaarginine. These results support the hypothesis that the secondary nitrogen of arginine acts as a hydrogen donor during transport.

Incidentally, $N$-methylhomoaarginine proved not to be subject to the action of bovine liver arginase (Worthington), using 25 times the amount required to split arginine quantitatively (experiments by M. E. Handlogten). Homoaarginine is split slowly by arginase.

To investigate the recognition site of the LAO system, several analogues of lysine were tested. The effect on lysine uptake of methyl substitutions on the $\epsilon$-nitrogen group is reported in Fig. 8. Substitution by methyl groups of 2 or of 3 hydrogen atoms on the terminal amino group yields lysine analogues which are poor inhibitors, whereas it inhibits lysine and ornithine uptake nearly as well as does homoaarginine. These results support the hypothesis that the secondary nitrogen of arginine acts as a hydrogen donor during transport.

DISCUSSION

In this paper we make use of several kinetic and biochemical approaches for the discrimination of transport systems, and for demonstrating that the observed transport is the sum of two or more discrete parts. We may place these approaches in two groups.

1. Those based on an analysis under the mass-action law of the competition between two substrates for which heterogeneous transport interaction has been observed.

2. Those based on the identification or design of analogues that sharpen the discrimination between the two or more transport agencies underlying the observed heterogeneity.

It is the combination of these two approaches that we have previously recommended (19-21).

In the present case, one could say on examination of Table III that our kinetic analysis of arginine and lysine uptake provided a complete description of the heterogeneity in arginine transport without the need of agency-specific analogues. The interpretation that the latter approach is unnecessary to most systems discrimination must, however, meet two objections.

1. Only in the special case where there exists a wide separation of the parameters for the two routes of uptake can direct analysis succeed. For the present case we see that the quotient, $K_a/V_{\text{max}}$ (which determines the slope of Lineweaver-Burk plots) for the arginine-specific system is about one-tenth as great as the same quotient for the LAO system. Graphically, this advantage shows in the clarity with which two lines may be discriminated in each plot in Fig. 1A.

2. If the curvature in such Lineweaver-Burk plots actually arises on a more complex basis than the simple additivity of uptake by two separate components, the parameters calculated by iteration would have no physical meaning. The cases where one can extract only with great uncertainty two or three straight lines from a plot by a linear transformation are furthermore also the cases that one might suspect most of deviating from the Michaelis-Menten equation on a more fundamental basis. In this study, the circumscribed inhibition of arginine uptake by homoserine and trans-hydroxyproline helped clarify the kinetic complexities observed for arginine uptake alone.

We began this research with the desire to test whether some model substrates designed for the cationic amino acid transport systems of mammalian cells would also serve in microorganisms. As the low inhibition by 4-amino-1-guanylpiperidine-4-carboxylic acid revealed, this arginine analogue has little transport reactivity in S. typhiium. Either of two factors might have accounted for its unacceptability: the width of the molecule at
**TABLE III**

**Summary of kinetic constants for basic amino acid transport in Salmonella typhimurium strain LT2**

Uptake was examined at 27°C in 0.01 M choline phosphate, pH 7.0, as described under **"Experimental Procedure."** Method of analysis refers to those forwarded by Lineweaver-Burk (22), Dixon (12), Inui-Christensen (11), or Winter-Christensen (9).

<table>
<thead>
<tr>
<th>Figures in this paper</th>
<th>Experimental conditions</th>
<th>Method of analysis</th>
<th>Transport systems</th>
<th>Arginine-specific</th>
<th>LAO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>K&lt;sup&gt;a&lt;/sup&gt;</td>
<td>V&lt;sub&gt;max&lt;/sub&gt;</td>
<td>K&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1A</td>
<td>Glucose grown cells — arginine uptake</td>
<td>Winter</td>
<td>2.8</td>
<td>0.54</td>
<td>95</td>
</tr>
<tr>
<td>1A</td>
<td>Glucose + arginine growth — arginine uptake</td>
<td>Winter</td>
<td>2.4</td>
<td>0.06</td>
<td>130</td>
</tr>
<tr>
<td>1B</td>
<td>Lysine uptake Ornithine uptake</td>
<td>Lineweaver</td>
<td>N.A.</td>
<td>N.A.</td>
<td>5.9</td>
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<tr>
<td>1B</td>
<td>Ornithine uptake</td>
<td>Lineweaver</td>
<td>N.A.</td>
<td>N.A.</td>
<td>28</td>
</tr>
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<td>2-4</td>
<td>Arginine uptake with lysine as inhibitor</td>
<td>Inui</td>
<td>3.9</td>
<td>0.5</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Ornithine uptake, arginine as inhibitor</td>
<td>Dixon</td>
<td>N.A.</td>
<td>N.A.</td>
<td>90</td>
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<td>5</td>
<td>Arginine uptake with excess lysine</td>
<td>Lineweaver</td>
<td>5.0</td>
<td>0.66</td>
<td>N.A.</td>
</tr>
<tr>
<td>5</td>
<td>Arginine uptake with excess ornithine</td>
<td>Lineweaver</td>
<td>0.1</td>
<td>0.76</td>
<td>N.A.</td>
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<tr>
<td>5</td>
<td>Arginine uptake with excess histidine</td>
<td>Lineweaver</td>
<td>2.1</td>
<td>0.57</td>
<td>N.A.</td>
</tr>
<tr>
<td>5</td>
<td>Arginine uptake with excess homoserine</td>
<td>Lineweaver</td>
<td>N.A.</td>
<td>N.A.</td>
<td>83</td>
</tr>
</tbody>
</table>

* K<sup>a</sup> refers to K<sub>i</sub>. values except in the inhibition study, analyzed by the Dixon method, which yields K<sub>i</sub> values.

* N.A., not available by this experimental method.

The two β-carbons, which is recognized already as almost too great for transport into animal cells; or the absence of a hydrogen atom on the proximal nitrogen atom of the guanidine group.

The latter possibility is the one supported by our observations. The finding of an arginine-specific uptake system with minimal reactivity toward simple diamino acids implies that the acceptor site for this system must have sufficient complexity to recognize a guanidinium group placed at a given distance from the α-carboxyl group, but to reject a distal amino group in a similar position. Since both types of groups are largely in the cationic state at the pH values studied, this recognition of the ionicity. Even though 4-amino-1-methylpiperidine-4-carboxylic acid is reactive with this system, along with 4-amino-1-guanylylperidine-4-carboxylic acid, the 1-dimethylpiperidine amino acid is not (7). Even though the space seems adequate for the two methyl groups, the quaternization of the piperidine nitrogen, whereby it comes to be locked into a positively charged state, appears unacceptable to that system. The implication may be that a degree of proton mobility on the guanidinium and amino groups is necessary in that more weakly discriminating transport system.

For the present case, the strongest clue to a role of the secondary nitrogen atom of arginine in the recognition step lay in the inhibitory action of homoserine and 4-trans-hydroxyproline. These amino acids eliminate the component of arginine uptake minimally inhibited by lysine (Figs. 5 and 6). We were guided in our interpretation of the unexpected effectiveness of these hydroxyamino acids as inhibitors by a parallel earlier finding in this laboratory for the Ly<sup>+</sup> system of the Ehrlich cell (17, 18).

In that cell the results were related to the present findings, but with two differences.

1. The optimal position of the hydroxyl group lies on carbon 5 rather than on carbon 4; and 2. a strong inhibitory action of the neutral amino acid requires the presence of an alkali metal ion; Na<sup>+</sup> being the most effective.

In yeast, similar effects have been seen, although the selectivity among cations is inconspicuous (23). In the present case, the hydroxyamino acids are effective in the presence of either choline or the Tris cation. None of the alkali metal ions, Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, or Rb<sup>+</sup>, offers any enhancement over the effect obtained by the presence of choline. The simplest interpretation is that the monovalent cation present does not participate in the action.

Since no measurable component of the uptake of homoserine is subject to inhibition by arginine, we may conclude that this amino acid and the analogous trans-hydroxyproline do not provide all the structural features needed for recognition for transport, the deficiency presumably lying in the absence of the cationic structure represented by the two primary nitrogen atoms of the guanidinium group. Our finding of a difference in the requirements for binding and for subsequent transport has a number of precedents in our own experience and in that of others. The observation that the position of the hydroxyl group in hydroxyamino acids and the direction in which the γ-oxygen atom projects from hydroxyproline are both decisive to the inhibition indicates that this oxygen atom is a key to the degree of recognition shown for these amino acids. On the supposition that the hydroxyl group might be recognized through hydrogen bonding to a complementary subsite, we were led to test the effect of replacing the hydrogen atom on the analogous R<sub>2</sub>NH group of homoarginine with a methyl group. We Interpret the loss of reactivity on N-methylation as favoring the action of this group as a donor rather than an acceptor of a hydrogen bond, although other possible roles of the R<sub>2</sub>NH structure are not excluded.

The question may well be asked: has not the parallel problem already been met and solved in the enzymology of arginase? Note that simple chemical considerations tell us a priori why arginase fails to release urea from ornithine or homoserine, whereas we face two real problems in explaining why the arginine-specific transport system fails to produce its catalytic effect, transport, on ornithine and homoserine.

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Basis of Transport Discrimination of Arginine from Other Basic Amino Acids in 
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