Arginine Transport and Metabolism in Osmotically Shocked and Unshocked Cells of Escherichia coli W*

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ONSLOW H. WILSON‡ AND JOSEPH T. HOLDEN

From the Division of Neuroscience, City of Hope Medical Center, Duarte, California 91010

SUMMARY

Exogenously derived arginine is extensively metabolized by early stationary phase cells of Escherichia coli W. This metabolism is substantial even during exposure to the substrate for only 15 sec at room temperature; at 300 sec only about 15% of the intracellular pool radioactivity is found in arginine. The major route of exogenous arginine metabolism is via an amineoxyacetic acid-inhibitable arginine decarboxylase which leads to the production and accumulation of large amounts of agmatine and putrescine. Much smaller amounts of ornithine, citrulline, proline, and glutamate also accumulate intracellularly.

Cold osmotic shock treatment of the cells decreases the relative extent of arginine metabolism via the decarboxylase and increases metabolism via the other pathway (pathways). The net result is that proportionately more arginine is found in shocked cell pools than in the corresponding control cells. In the presence of 4 x 10^-3 M amineoxyacetic acid the bulk of the pool radioactivity is found in arginine. Thus, under these conditions the pool radioactivity is a measure of arginine transport.

Cold osmotic shock treatment reduces the amount of arginine-derived radioactivity associated with the cell in the presence and absence of 4 x 10^-3 M amineoxyacetic acid. The shock treatment also reduces the capacity of the cell to transport exogenous lysine and leucine but not proline.

In the presence of amineoxyacetic acid, L-lysine is a poor inhibitor of arginine transport, while L-canavanine, which itself is not well accumulated by these cells, is a modest inhibitor. L-Arginine is a good inhibitor of lysine transport while L-canavanine is a modest inhibitor. These data support the thesis that arginine and lysine are not transported by identical systems in this organism. Since lysine previously has been found to inhibit arginine uptake, these observations further support the conclusion that arginine transport cannot be reliably studied in E. coli W without utilizing inhibitors such as amineoxyacetic acid which greatly attenuate the extensive metabolism of this amino acid.

While studying the accumulation of radioactivity from 14C-amino acids within the intracellular pool of Escherichia coli W, it was observed that the time course for L-arginine uptake did not show signs of equilibration, as did L-lysine, L-proline, and L-leucine uptake. A similar observation can be found in a study reported by Mauls (1), although the unusual nature of this uptake pattern was not investigated.

In the early 1950's, Roberts et al. (2) showed that upon exposure to 14C-arginine a growing culture of E. coli B produced 14C-proline and 14C-gluatamate. It was implied that L-ornithine was the precursor of these two metabolites. Morris and Pardee (3, 4) have shown the formation, in E. coli B, of putrescine from L-ornithine and L-arginine. These authors found that cells grown in a minimal medium convert L-ornithine to putrescine via a Vitamin B6-dependent, constitutive ornithine decarboxylase (3), while L-arginine is first converted to agmatine by a similar decarboxylase. Agmatine is then converted to putrescine and urea by an agmatine ureohydrolase (4). It appeared, therefore, that exogenous arginine may be extensively metabolized by these organisms during transport experiments.

With these considerations in mind, we embarked on a study to determine the possible involvement of arginine metabolism in the apparent unusual time course pattern of arginine uptake by E. coli W. In the course of these studies the effects of osmotic shock on arginine transport and metabolism also were examined.

EXPERIMENTAL PROCEDURE

Materials

E. coli W (ATCC 9637) was used throughout these studies. Uniformly labeled 14C-L-arginine (226 mCi per mmole), 14C-L-leucine (275 mCi per mmole), 14C-L-lysine (237 mCi per mmole), and 14C-L-proline (220 mCi per mmole) were obtained from New England Nuclear. 14C-Guanidino-L-canavanine (31.6 mCi per mmole) was purchased from Schwarz BioResearch. Dowex AG 50W-X8 was obtained from BioRad. Aminooxyacetic acid (hemihydrochloride) was supplied by Upjohn. Chlorampheni
col was a gift from Parke Davis. All other compounds were of reagent grade.

Methods

Preparation of Cells—Cells were grown in the minimal medium of Davis and Mingioli (5) with vigorous aeration at 37°C. They were harvested either in the midexponential or in the early stationary phases and washed twice with ice-cold 0.01 M Tris-HCl (pH 7.2) containing NaCl at 0.03 M. The washed cells were then suspended at room temperature in 0.033 M Tris-HCl (pH 7.2). An aliquot was removed and kept in the cold as “control cells.” In most experiments, the remainder of the cells was subjected to the cold osmotic shock treatment described by Nossal and Heppel (6) which entails suspension of cells in a solution (room temperature) containing 20% sucrose, 0.03 mM Tris, and 0.1 mM EDTA, followed by resuspension in ice-cold 0.5 mM MgCl₂. The “shocked cells” were resuspended in 0.033 M Tris-HCl and kept in the cold. An aliquot was diluted (as were control cells) into growth (minimal) medium containing 1.0 mM Mg++ and 0.1 mM Ca++ (7) and plated on Difco nutrient agar to determine viability after the shock treatments. Viabilities for control cells were determined before and after storage (~90 min) in cold Tris-HCl. No significant change in viable cell count was observed after storage.

Uptake of [¹⁴C]-Amino Acids—Cells at 1 mg (wet weight) per ml were previously incubated in minimal medium containing glucose (0.2%) and chloramphenicol (80 µg per ml) for 30 min at room temperature (24-25°C). After preliminary incubation, radioactive amino acid was added to a final concentration of 5 µg per ml (0.02 µCi per ml) and uptake was monitored by Millipore filtration as described by Kessel and Lubin (8). The cells were washed on the filters with 1 ml of room temperature uptake medium (minimal with all supplements described above). The washed filters were cemented to aluminum planchets and dried, and radioactivity was assessed by gas flow counting. Under these conditions, less than 1% of the total cell isotope is incorporated into nonextractable material, consequently, direct counting of the filtered cells can be used to measure entry of amino acids into the intracellular pool.

Analysis of Radioactivity Accumulated from [¹⁴C]-Arginine—After exposing cells to [¹⁴C]-arginine under standard conditions for 15 or 300 sec (plus 5 and 10 sec for subsequent removal of medium from control and shocked cells, respectively), uptake was stopped by pouring the cells onto 47-mm diameter Millipore filters which were immediately placed into 2.5% trichloroacetic acid (w/v) at room temperature. Cells were extracted in trichloroacetic acid for 30 min at room temperature. The cell residues were removed by centrifugation at 15,000 x g and the clear supernatant was evaporated twice with 2 volumes of anhydrous ether to reduce the level of trichloroacetate. An aliquot of the remaining water phase was put onto a Dowex AG 50-X8 (H⁺ form) column (0.8 x 15 cm) which was eluted with a linear gradient of HCl (0 to 2.5 N) according to Morris and Pardee (4). Five-milliliter fractions were collected and 0.1-ml aliquots were assayed for radioactivity by scintillation counting.

Identification of Radioactive Peaks from Dowex Column—Individual radioactive fractions were evaporated to dryness and taken up into a small volume (approximately 1 ml) of H₂O. Metabolites in these column fractions were identified by paper chromatography (after addition of carrier compounds). Paper strips were developed in butanol-1-acetic acid-H₂O (2:1:1) (9), and the fraction constituents were located by radioactive scanning and ninhydrin staining.

Inhibition of Arginine Decarboxylase Activity in Intact Cells—

![Fig. 1. Time course of (a) [¹⁴C]-lysine (LYS), (b) [¹⁴C]-l-proline (PRO), (c) [¹⁴C]-leucine (LEU), and (d) [¹⁴C]-arginine (ARG) uptake by shocked and unshocked cells of E. coli W, under standard conditions.](http://www.jbc.org/)

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\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Time course of (a) [¹⁴C]-lysine (LYS), (b) [¹⁴C]-l-proline (PRO), (c) [¹⁴C]-leucine (LEU), and (d) [¹⁴C]-arginine (ARG) uptake by shocked and unshocked cells of E. coli W, under standard conditions.}
\end{figure}
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Control cells, under standard uptake conditions, were used in these studies. Cells were incubated in minimal medium containing all supplements in the apparatus described by Roberts and Simonsen (10) which allows diffusion of 14CO₂ from a reaction vessel into 1 ml of hydroxide of Hyamine contained in a scintillation counting vial. The reaction was started by injecting a desired amount of substrate (5 μg per ml; 0.02 μCi per ml) through a rubber stopper into the reaction vessel. Aminoxyacetic acid, at varying concentrations, was present during a 30-min preliminary incubation period. The reaction was allowed to continue for 30 min, at the end of which time HCl was added to a final concentration of 1 N. The flasks were shaken for an additional 90 min to permit the 14CO₂ to diffuse and be trapped in the Hyamine. Scintillation fluid was then added to the counting vials and radioactivity was measured.

Assay of Pool Radioactivity after Exposure to 14C-Arginine in Presence of Aminoxyacetic Acid—Control and shocked cells were allowed to take up radioactivity from 14C-arginine under standard conditions in the presence and absence of aminoxyacetic acid. The cells were extracted with trichloroacetic acid and the distribution and identity of radioactivity were determined chromatographically as described previously.

**RESULTS**

**Relationship between Culture Age and Survival after Osmotic Shock**—In our hands, cells harvested during the exponential phase of growth did not survive the shock procedure very well. Viabilities varied from 30 to 50%, a level not acceptable for studies on the effects of osmotic shock on substrate accumulation.

**Fig. 2.** Dowex 50 elution pattern of trichloroacetic acid-extracted pool radioactivity from E. coli W after exposure to 14C-arginine for 5 min under standard conditions. The following substances were detected and identified as described under “Methods”: urea-glutamic acid (UREA-GLUT.), proline (PRO), citrulline (CIT.), ornithine (ORN.), putrescine (PUTRES.), arginine (ARG.), and agmatine (AGMAT.).

**Table 1**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control cells</th>
<th>Shocked cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15-20 sec</td>
<td>300 sec</td>
</tr>
<tr>
<td>Arginine</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Urea + glutamate</td>
<td>12.0</td>
<td>14.1</td>
</tr>
<tr>
<td>Proline</td>
<td>7.4</td>
<td>59.5</td>
</tr>
<tr>
<td>Arginine</td>
<td>19.0</td>
<td>21.8</td>
</tr>
<tr>
<td>Urea + glutamate</td>
<td>1.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Proline</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>Citrulline</td>
<td>1.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Ornithine</td>
<td>0.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Recovery</td>
<td>90.0</td>
<td>99.2</td>
</tr>
</tbody>
</table>

* Per cent of total radioactivity applied to the column. The total counts per min per 0.1 ml of cell extract were 8,340 and 41,280 for control cells at 15 and 300 sec, respectively, and 6,400 and 36,430 for shocked cells at 15 and 300 sec, respectively.

In the meantime, with early stationary cells, however, consistently good viabilities (95 to 103%) were obtained with the use of the shock procedure described under “Methods.” Piperno (11) and Piperno and Oxender (12) have made similar observations with E. coli K12. Nossal and Heppel (6) supplemented their growth medium with various divalent cations and maintained a high level of viability in E. coli harvested at the exponential phase. To avoid complications related to the supplementation of a citrate-containing medium with divalent cations, all experiments were carried out instead with early stationary cells.

**Time Course of 14C-Arginine Uptake**—Fig. 1 shows the time course of uptake for 14C-arginine by control and shocked cells. A steady state level of cellular radioactivity was reached between 1 and 3 min with L-lysine, L-proline, and L-leucine (Fig. 1, a to c) under these conditions. For L-arginine, however (Fig. 1d), after 30 sec, uptake continued at a linear rate for at least 5 min. It can also be seen that shock had little effect on L-proline accumulation (Fig. 1b), while it affected both the initial rate and steady state level of lysine uptake (Fig. 1a). The initial (15 sec) rates of L-leucine and L-arginine uptake were not altered appreciably (Fig. 1, a to c).

**Intracellular Metabolites after Exposure to 14C-Arginine**—Fig. 2 shows the spectrum of 14C-metabolites resolved on a Dowex 50 column after a 5-min exposure of control cells to 14C-arginine under standard conditions. The first peak is a minor contaminant found in the original 14C-arginine and represents 0.3 to 0.5% of the total radioactivity applied to the column. There are at least seven metabolites. The identity of the constituents in each fraction was confirmed by paper chromatography as described under “Methods.” Table 1 shows the percentage distribution of isotope in these metabolites and the effects of incubation time and osmotic shock on this distribution. It is clear that arginine is actively degraded along a major pathway leading to putrescine and, considering the large amounts of agmatine which accumulate, that this may involve the constitutive arginine deiminase described by Morris and Pardee (4). Furthermore, the percentage of agmatine in the pool did not change appreciably between 20 and 300 sec while that of pu-
Osmotic shock also affected the distribution of radioactivity. The combined percentages of putrescine and agmatine in shocked cells were of the same order of magnitude as that of agmatine alone in control cells at 15 sec. At 300 sec they were of the same order of magnitude as putrescine alone. It is noteworthy also that ornithine accumulated only in small amounts, and then only in the 300-sec sample, although it is an apparent obligate intermediate in the pathway from arginine to proline (13).

Effect of Amineoxyacetic Acid on °CO₂ Evolution from °C-Arginine by Intact Control Cells—To determine whether arginine degradation occurs via a Vitamin B₆-dependent decarboxylase such as that described by Morris and Pardec (4), the effect of varying concentrations of amineoxyacetic acid on °CO₂ evolution from °C-arginine by intact cells was studied in a gas diffusion apparatus as described under "Methods." The inhibition of pyridoxal phosphate-dependent enzymes by amineoxyacetic acid has been described previously in several laboratories (10, 14). Fig. 3 shows that levels of amineoxyacetic acid on the order of 2 × 10⁻⁴ M are required to inhibit the reaction by 90 to 95%. Examination of the intracellular pool in cells which took up arginine in the presence of comparable levels of amineoxyacetic acid showed a corresponding increase in the amount of isotope remaining in arginine (see also Table II). These data and the experiments described above showing the presence of agmatine in the intracellular pool support the suggestion that an arginine decarboxylase is involved in the degradation of arginine by this organism.

Effect of Amineoxyacetic Acid on Time Course of °C-Arginine Uptake by Control Cells—Fig. 4 shows the effect of varying concentrations of amineoxyacetic acid on the time course of °C-arginine uptake by control cells. Between 8 × 10⁻³ M and 2 × 10⁻⁴ M amineoxyacetic acid, the apparent initial rates of entry were progressively enhanced, while the final amounts of isotope accumulated were progressively decreased. At 8 × 10⁻⁴ M amineoxyacetic acid, both the initial rates and the final
Effect of varying concentrations of (a) L-canavanine (CANAV.) and of (b) L-lysine (LYS) on the time course of \(^{14}\)C-arginine accumulation by unshocked cells of *E. coli* W in the presence of \(4 \times 10^{-4}\) M amineoxyacetic acid.

![Graph](http://example.com/graph1)

**Fig. 5.** Effect of 50 \(\mu\)g per ml of L-canavanine (CANAV.) and L-arginine (ARG.) on the time course of \(^{14}\)C-lysine accumulation by unshocked cells of *E. coli* W under standard conditions.

Levels were reduced. These concentrations of amineoxyacetic acid did not affect the time course of L-lysine uptake and had minimal effects on L-lysine uptake. In subsequent experiments, \(4 \times 10^{-3}\) M amineoxyacetic acid was used routinely to study arginine uptake.

**Effect of Aminooxyacetic Acid on Radioactivity Distribution during Exposure of Cells to \(^{14}\)C-Arginine**—Table II shows the percentage distribution of radioactivity in control and shocked cells after exposure to \(^{14}\)C-arginine in the presence of \(4 \times 10^{-4}\) and \(4 \times 10^{-3}\) M amineoxyacetic acid. The increase in the amount of arginine in the pool in relation to the decrease in the amounts of putrescine and agmatine (as well as the decrease in \(^{14}\)CO\(_2\) evolution under similar conditions (Fig. 3)) is evident. It is also clear that amineoxyacetic acid has little, if any, effect on the pathway (pathways) leading to proline and glutamate. Note, however, that the percentage of isotope found in ornithine was increased in its presence.

**Effect of L-Lysine and L-Canavanine on L-Arginine Transport**—Control cells were previously incubated with \(4 \times 10^{-3}\) M amineoxyacetic acid and then allowed to take up arginine in the presence and absence of L-lysine and L-canavanine. Fig. 5a shows that L-canavanine is a modest inhibitor of L-arginine accumulation (17% inhibition at 50 \(\mu\)g per ml). L-Lysine, on the other hand, is much less effective as an inhibitor of L-arginine uptake. It gave only a 25% inhibition at 500 \(\mu\)g per ml, a molar concentration in excess of 100 times that of L-arginine (Fig. 5b).

**Effect of L-Arginine and L-Canavanine on L-Lysine Transport**—Fig. 6 shows that L-arginine and L-canavanine are modest inhibitors of L-lysine transport under standard conditions, giving 18% and 26% inhibition, respectively, at 50 \(\mu\)g per ml.

**DL-Canavanine-Guanidino-\(^{14}\)C-accumulation by Control Cells of *E. coli* W**—Cells were exposed to DL-14C-canavanine (made up to a final concentration of 5 \(\mu\)g per ml or 50 \(\mu\)g per ml with L-14C-canavanine) under standard conditions. It was observed that this structural analogue of arginine is not well accumulated by the cell although it is a moderate inhibitor of L-arginine transport. Fig. 7 shows the time course of its uptake under conditions permitting the rapid accumulation of L-14C-arginine. Aminoxyacetic acid, \(4 \times 10^{-3}\) M, was present in all cases.

**DISCUSSION**

The data presented indicate that exogenous L-arginine is rapidly and extensively metabolized by *E. coli* W during or shortly after its passage into the cell. The major route of this metabolism appears to involve the constitutive arginine de...
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The chromatographic evidence accumulated in this investigation suggests strongly that proline and glutamate are formed from arginine, a conversion which most likely involves the intermediary formation of ornithine. The reactions involved in this hypothetical pathway are not clear, but there are three readily apparent possibilities. (a) The conversion of arginine to citrulline via a decarboxylase activity of the cells possibly by release of this enzyme into the shock fluid. This possibility is considered further in the accompanying article (19). The failure of the shock procedure to reduce proline accumulation suggests that the components of this transport system are not readily detached from the cell. This observation is consistent with the findings of Kaback (20), who has shown that membrane fragments (obtained by sonic oscillation) of this organism retain the ability to accumulate proline at a fast rate.

The observations (a) that L-lysine has minimal effects on the accumulation of L-arginine (Fig. 5) and (b) that L-canavanine is not well accumulated by the cell (Fig. 7), although it is a moderately good inhibitor of L-arginine accumulation (Fig. 5), raise a number of questions. First, there appears to be a high degree of specificity in the arginine transport system, confirming earlier observations (21-23). However, this is in contrast with the findings of Schwartz, Maas, and Simon (19), who concluded on the basis of the simultaneous loss of several transport activities in a canavanine-resistant mutant that the arginine accumulating system in E. coli W has a much broader specificity. The apparent discrepancy in these findings may be related to the likelihood that the latter investigators studied arginine uptake under conditions which permitted extensive metabolism of this amino acid to occur. Second, if L-canavanine is not well accumulated by E. coli W, then its inhibitory effect on the growth of this organism (24) becomes more difficult to interpret. It is possible that the canavanine effect is restricted to the cell surface as has been suggested by Schachtele, Anderson, and Rogers (25). On the other hand, it is of interest to note that Kihara, Prescott, and Snell (26) reported that the growth of E. coli W was not sensitive to canavanine inhibition. This observation has been confirmed in this laboratory, which raises the possibility that the uptake of this analogue and its ability to inhibit growth of E. coli may be a variable phenomenon, subject to a specific set of growth and incubation conditions.

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