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Amino Acid Transport in *Pseudomonas aeruginosa*

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Properties of the transport systems for amino acids in *Pseudomonas aeruginosa* were investigated. Exogenous ^{14}C-labeled amino acids were shown to equilibrate with the internal native amino acid pool prior to incorporation into protein. When added at low external concentrations, the majority of the amino acids examined entered the protein of the cell unaltered. The rates of amino acid transport, established at low concentrations with 18 commonly occurring amino acids, varied as much as 40-fold. The transport process became saturated at high external amino acid concentrations, was temperature-sensitive, and was inhibited by sodium azide and iodoacetamide. Intracellular to extracellular amino acid ratios of 100- to 300-fold were maintained during exponential growth of the population in a glucose minimal medium. When the medium became depleted of glucose, neither extracellular nor intracellular amino acids could be detected.

The transport and accumulation of amino acids by microorganisms has received much attention in recent years (1–2, 6–9, 12, 13, 19); however, most studies have been restricted to a specific amino acid or to families of amino acids. There has been little or no attempt to establish the relationships among various amino acid transport systems or to determine the significance of amino acid transport in relation to other physiological parameters of the cell.

Members of the genus *Pseudomonas* characteristically have very simple nutritional requirements and catabolize a wide variety of substrates (18). The transport of metabolites, such as amino acids, which can serve as both carbon and nitrogen sources as well as sources of immediate precursors for the synthesis of proteins or other structural macromolecules, would be an important cellular function in determining the range of compounds metabolized and their rate of utilization.

The purpose of this study was to establish the general properties of the constitutive transport systems for naturally occurring amino acids in the type species *P. aeruginosa* and to attempt to correlate variations in transport rates with various physiological parameters of cell growth and composition.

MATERIALS AND METHODS

Organisms and media. *P. aeruginosa* (ATCC 9027) was used throughout this study. Stock cultures were maintained at 6 C on ammonium salts-minimal agar slants with glucose as the sole carbon source.

Cells required for experimental procedures were grown, from a 20-hr inoculum, in Roux flasks at 30 C in a medium (*p*H 7.4) containing 0.3% $NH_4H_2PO_4$, 0.2% K_2HPO_4, and iron as $FeSO_4 \cdot 7H_2O$ (0.5 μg/g). Glucose and $MgSO_4 \cdot 7H_2O$ were added separately, after sterilization, from 10% stock solutions to give final concentrations of 0.2% and 0.05%, respectively (minimal medium).

Preparation of cell suspensions. Cells from late exponential phase were harvested by centrifugation at room temperature and were quickly suspended to the desired concentration in minimal medium. The concentration of cells was established by measuring the optical density at 650 nm with a model B spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.). The cell suspensions were stirred on a submergeable magnetic stirrer (Henry Troemner Inc., Philadelphia, Pa.) for 10 min prior to the addition of the radioactive amino acid. This procedure was found to give reproducible incorporation data for any one amino acid.

Uptake of labeled amino acids. The incorporation of ^{14}C-amino acids into cells and cellular constituents was studied by two procedures, the choice of which depended upon the nature of the experiment.

In the first procedure, the incorporation of ^{14}C-amino acids into whole cells, trichloroacetic acid-insoluble material, and pools was determined by the filtration method of Britten and McClure (1). The trichloroacetic acid-insoluble material contains, in addition to protein, ribonucleic acid, deoxyribonucleic acid, lipid, and cell wall material. During transport experiments where low external concentrations of ^{14}C-amino acids and short incubation periods were employed, the majority of the radioactivity in the trichloroacetic acid-precipitable mate-

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rial was usually incorporated into protein. This fraction, therefore, has been referred to as "protein." Cells were filtered on an 0.45-μm pore size filter (Millipore Corp., Bedford, Mass.) in an E8B precipitation apparatus (Tracerlab, Waltham, Mass.) and immediately washed with 2 ml of minimal medium. The temperature of the wash medium was the same as the reaction mixture containing cells and amino acids. This procedure did not remove pool amino acids. Dried filters were placed in vials containing 10 ml of scintillation fluid (Liquifluor; New England Nuclear Corp., Boston, Mass.), and the vials were assayed for radioactivity in a model 725 liquid scintillation spectrometer (Nuclear Chicago Corp., Des Plaines, Ill.).

In the second procedure, the rate of incorporation of amino acids into whole cells was determined essentially by the method of FerroLuzzi-Ames (5) which was modified as follows: 1 ml of a vigorously stirred heavy cell suspension was added to a test tube containing 3 ml of minimal medium and the ^{14}C-amino acid, at the desired specific activity. The reaction was stirred with a Troemner magnetic stirrer and miniature magnetic stirring bars. Samples were removed for filtration at 15-sec time intervals. Filters were dried and assayed for radioactivity, and rates of amino acid transport were calculated from the earliest linear incorporation data. This method was used for the demonstration of saturation kinetics, since it permitted a series of reactions to be completed within a short period of time and thereby eliminated variation in rates of transport due to cell proliferation.

It should be stressed at this time that, at least with *P. aeruginosa*, slight variations in the techniques employed in amino acid transport studies, such as rate of stirring, ratio of surface area to depth of reaction mixture, and so on, may result in slight differences in the overall pattern of amino acid incorporation. Therefore, in all instances where comparisons of a single aspect of transport or pool formation were made between different amino acids, either the procedure of Britten and McClure (1) or the modified procedure of FerroLuzzi-Ames (5) was utilized.

Experiments designed to determine the rates of amino acid uptake as a function of incubation temperature were performed in water-jacketed reaction vessels maintained at the desired temperature with a Lauda K-2/R temperature-controlled, circulating water pump (Brinkmann Instruments, Inc., Westbury, N.Y.). Cell suspensions were allowed to equilibrate at the desired temperature for 15 min prior to the initiation of the transport experiment by the addition of the ^{14}C-amino acid. The reaction mixtures were continuously agitated with a magnetic stirrer.

Measurement of amino acids in the cell pool and in the culture medium. A sensitive quantitative measurement of pool amino acids and amino acids excreted into the growth medium was devised as follows. Cells were grown in 10 ml of minimal medium containing 111 μmoles of uniformly labeled ^{14}C-glucose with a specific activity of 2 mc/mmole; exponential- or stationary-phase cultures were harvested by centrifugation at 10,000 $\times$ *g* at room temperature. The supernatant fluid was carefully decanted, and the tube was wiped free from adhering liquid. The resulting packed cells were extracted with 5 ml of 10% trichloroacetic acid, and the residual cellular material was removed by centrifugation at 10,000 $\times$ *g* for 10 min at 6 C. This procedure was repeated twice and the trichloroacetic acid extracts were combined. The growth medium was deproteinated with cold 5% trichloroacetic acid in a similar manner. The trichloroacetic acid was removed from the fractions by extracting five times with cold ethyl ether. The resulting aqueous sample was evaporated to dryness in an air stream. Each dried sample was dissolved in distilled water and applied to a column (1.2 $\times$ 10 cm) of Dowex 50 (H^+ form). The column was washed with distilled water until no further radioactivity was eluted, and the absorbed compounds were then eluted with 100 ml of 4 M ammonium hydroxide. The eluate was evaporated to dryness, and the residue was dissolved in 0.1 ml of distilled water. Samples were quantitatively applied to cellulose thin-layer plates (cellulose powder MN 300), and the radioactive compounds were separated two-dimensionally by the method of Jones and Heathcote (10). The resulting chromatograms were exposed for 1 week to medical X-ray film (Eastman Kodak Co., Rochester, N.Y.). The films were developed, and the radioactive areas detected by this method were scraped loose from the plates and were drawn, by vacuum, into scintillation vials which were subsequently filled with a toluene scintillation fluid and assayed for radioactivity in a liquid scintillation spectrometer. The addition of from 5 to 60 mg of cellulose caused no increase in quenching under these conditions. It was found by this method that amino acids could easily be detected on radioautograms in amounts as low as 2 $\times$ 10^{-6} μmoles.

Confirmatory qualitative amino acid analyses were carried out by two-dimensional chromatography on thin-layer plates and on paper. Thin-layer plates were coated with Silica gel G, spotted with the radioactive sample, and developed in a solvent system of chloroform-methanol-ammonia (2:2:1, v/v; reference 14) and in the second dimension in phenol-water (75:25, v/v). Paper chromatograms, spotted with the radioactive samples, were developed in the first dimension in a solvent system of *n*-butyl alcohol–acetic acid–water (120:30:50, v/v) and in the second dimension in phenol-ammonia (200:1, v/v; reference 17). Chromatograms from both these methods were analyzed by radioautography, and amino acids were identified by the coincidence of mobility of the radioactive compounds with the ninhydrin detection of the appropriate carrier ^{12}C-amino acids.

Amino acid composition of cellular protein. Cells from a culture in exponential growth were harvested by centrifugation and fractionated according to the procedure of Roberts et al. (16). The protein residue remaining after extraction with hot trichloroacetic acid was further extracted twice with ethyl ether to remove residual trichloroacetic acid, and the samples were air-dried. The protein was hydrolyzed with 6 N HCl for 18 hr at 100 C in sealed glass vials. After hydrolysis, the acid was removed by flash evaporation, and the water-washed residue was dried and dissolved in 0.1 M citrate buffer (*p*H 5.5). Amino acid analyses were

carried out by using a Beckman model 120C amino acid analyzer.

Qualitative ^{14}C-amino acid analyses of the protein of cells which were grown in the presence of low concentrations of radioactive amino acids were carried out by paper chromatography as described above.

Chemical fractionation of whole cells. The chemical fractionation procedure used was essentially that of Roberts et al. (16), with the modification of Clifton and Sobek (3). The hot trichloroacetic acid-soluble fraction was prepared by heating the sample in 5% trichloroacetic acid for 20 min at 90 C, rather than at 100 C for 30 min. Samples of the cell fractions were plated in duplicate onto stainless steel planchets, dried under an infrared lamp, and counted at infinite thinness with a thin end-window Geiger tube attached to a Nuclear-Chicago model 181A scaler equipped with an automatic gas-flow counter. Corrections were made for background. To reduce statistical deviation, at least 1,000 counts were recorded when possible.

Chemicals. Amino acids used as carriers for ^{14}C-amino acids were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. L-Tryptophan (^{14}C-methylene) was obtained from Nuclear Chicago Corp. The other radioactive amino acids and ^{14}C-glucose were purchased from Schwarz Bio Research Inc., Orangeburg, N.Y., and were obtained as the uniformly labeled ^{14}C product, with the exception of L-hydroxyproline-^{3}H. All radioactive amino acids were checked for purity by thin-layer chromatography (10) and radioautography, and those preparations containing less than 5% of the radioactivity as impurities were considered adequate and within the limits of accuracy of the transport experiments. When necessary, the presence of impurities was corrected for in experiments where pool components were identified.

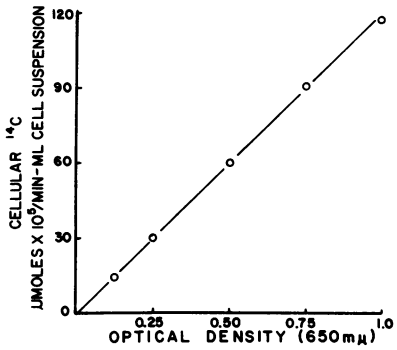

FIG. 1. *Increase in the rate of proline uptake with increasing cell mass. External proline concentration was 10^{-6} M; specific activity, 25 μc/μmole [optical density$_{650}$ of 0.50 equals 0.2 mg (dry weight)/ml]. Procedure: Britten and McClure (1).*

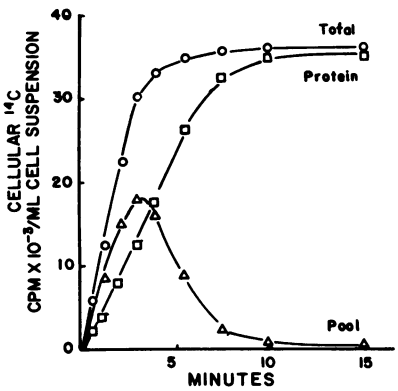

FIG. 2. *Time course of tyrosine uptake and incorporation into protein by a growing suspension of P. aeruginosa (0.2 mg (dry weight)/ml). External tyrosine concentration was 10^{-6} M; specific activity, 12.5 μc/μmole. Procedure: Britten and McClure (1).*

RESULTS

Uptake of amino acids. The initial rate of incorporation of amino acids into whole cells during exponential growth was first order with respect to cell mass and was reproducible under the experimental conditions used (Fig. 1).

The time course of amino acid uptake and distribution within the cells was studied. Figure 2 shows the results of an experiment measuring the uptake of ^{14}C-tyrosine. Tyrosine was taken into the cell immediately and rapidly until the supply in the medium became exhausted. The entry of labeled tyrosine into trichloroacetic acid-precipitable material very quickly became linear, and after 2 to 3 min continued at the expense of the amino acid pool. At 3 min, the tyrosine concentration in the pool was 13,350 times greater than in the medium. This pattern of incorporation corresponds to the patterns of amino acid uptake into *Echerichia coli* (1).

Precursor-pool relationships. The incorporation of tyrosine (Fig. 2) was typical of many of the amino acids studied. The negligible lag in the entry of amino acid into the protein fraction suggested either that passage of the amino acid through the pool was not obligatory for entry into protein, or possibly, that the amino acid concentration in the native pool of growing cells was very low and thus had a negligible influence on the specific activity of the labeled amino acid transported into the cells.

Valine was selected as the amino acid to test these possibilities. When added at 10^{-6} M, the amino acid was completely removed from the external medium by 90 to 120 sec, precisely the time at which the pool level was maximal (Fig. 3, inset). Also in this experiment, a 90-sec preincubation with ^{12}C-valine at 10^{-6} M was followed at zero-time by a pulse of ^{14}C-valine at the same concentration. The prolonged lag of ^{14}C-valine incorporation into trichloroacetic acid-insoluble

material (Fig. 3) was due, therefore, to the equilibration of the radioactive amino acid with the ^{12}C-valine pool.

These results suggested that exogenously supplied amino acids entered the cell and accumulated in an intracellular metabolic pool, and that entry into this pool was necessary for incorporation into protein. To account for the negligible lag of ^{14}C-amino acid entry into cellular protein, concentrations of de novo-synthesized amino acids in the native pool of growing cells must, therefore, be relatively low.

When the time course of ^{14}C-amino acid uptake was determined for all the commonly occurring amino acids, it was found that only the incorporation of glycine, serine, and glutamate exhibited a lag prior to entry into protein. Figure 4 illustrates the incorporation of ^{14}C-glutamate into whole cells and into the trichloroacetic acid-insoluble material of *P. aeruginosa*. With most amino acids tested, ^{14}C entry into protein was essentially linear from zero-time.

These results suggested that most amino acids were present in the pool of growing cells at low levels, but that a few, such as glutamate, were present at relatively high concentrations. Glutamate has also been reported to be present in relatively high concentrations in the pool of *E. coli* (1).

Comparative rates of transport. From the results of rate studies of amino acid uptake, it became obvious that a large variation in rates of transport for different amino acids existed. Experiments to determine ^{14}C-amino acid rates were performed for all the common amino acids by rapid sampling to obtain accurate, comparative data. Under these conditions of low external amino acid concentrations (10^{-6} M), there was approximately a 40-fold variation in the rates of transport; from a low of 1.92×10^{-4} μmoles $\times$ $min^{-1} \times mg^{-1}$ (dry weight) of cells for cysteine to 80×10^{-4} μmoles $\times$ $min^{-1} \times mg^{-1}$ (dry weight) for leucine (Table 1).

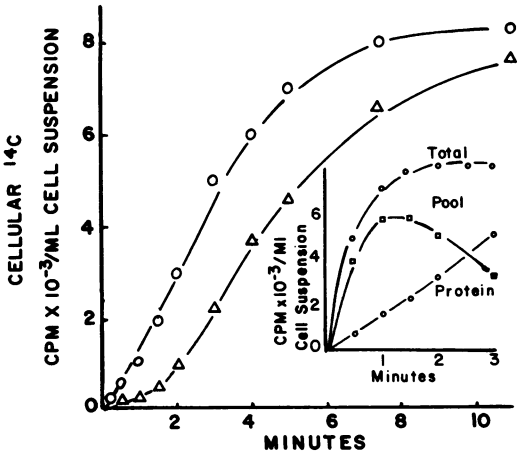

FIG. 3. *Incorporation of ^{14}C-valine into the protein of P. aeruginosa. Symbols:* O, *10^{-6} M ^{14}C-valine;* △, *10^{-6} M ^{14}C-valine added 90 sec after preloading the cells with 10^{-6} M ^{12}C-valine. Inset: Incorporation of 10^{-6} M ^{14}C-valine into whole cells, protein and pool. Procedure: Britten and McClure (1).*

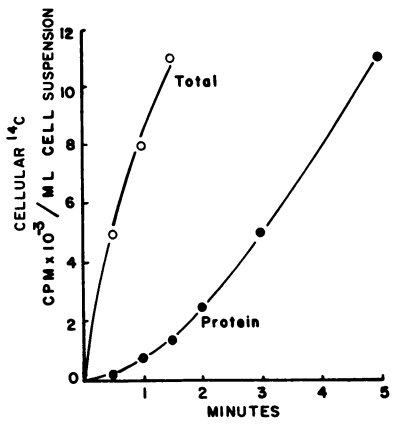

FIG. 4. *Total uptake of ^{14}C-glutamate and incorporation into protein of cell suspensions (0.1 mg, dry weight, of cells/ml). External glutamate concentration 10^{-6} M; specific activity, 12.5 μc/μmole. Procedure: Britten and McClure (1).*

The only amino acid tested to which these cells were impermeable was hydroxy-L-proline. Experiments to detect uptake of this amino acid showed that no radioactivity was incorporated either into the amino acid pool or into the protein.

Distribution of radioactive amino acids in the cell. To establish whether the exogenously supplied ^{14}C-amino acids which were incorporated into the trichloroacetic acid-insoluble material remained unchanged during passage through the intracellular pool, cultures were allowed to incorporate specific ^{14}C-amino acids added at low concentrations, and the distribution of radioactivity within the cells was determined by chemical fractionation. For ^{14}C-histidine, ^{14}C-isoleucine, ^{14}C-phenylalanine, and ^{14}C-proline, approximately 90% of the radioactivity was incorporated into the protein fraction, whereas, with ^{14}C-arginine, ^{14}C-glycine, and ^{14}C-serine, only 58.3, 50.7, and 56.7%, respectively, was found in the hot trichloroacetic-insoluble fraction (Table 2). A significant percentage of the label found in the cold trichloroacetic acid-soluble fraction for the ^{14}C-arginine experiment accounted for this deviation. With ^{14}C-glycine, a large percentage of the added radioactivity was found, understandably,

TABLE 1. *Rates of transport of amino acids into whole cells of P. aeruginosa*[a]

Amino acid (10^{-6} M)	Rate of transport[b]	Relative rate
Hydroxyproline...	0	0
Cysteine.....	1.92×10^{-4}	1.00×10^{-4}
Threonine.....	3.54×10^{-4}	1.85×10^{-4}
Aspartate.....	4.11×10^{-4}	2.14×10^{-4}
Serine.....	4.20×10^{-4}	2.19×10^{-4}
Glutamate.....	4.67×10^{-4}	2.43×10^{-4}
Proline.....	5.75×10^{-4}	2.99×10^{-4}
Glycine.....	5.90×10^{-4}	3.08×10^{-4}
Tyrosine.....	1.40×10^{-3}	7.50×10^{-4}
Methionine.....	1.99×10^{-3}	9.90×10^{-4}
Valine.....	2.36×10^{-3}	12.30×10^{-4}
Histidine.....	2.74×10^{-3}	14.30×10^{-4}
Alanine.....	2.76×10^{-3}	14.40×10^{-4}
Phenylalanine....	3.23×10^{-3}	16.80×10^{-4}
Lysine.....	3.36×10^{-3}	17.50×10^{-4}
Isoleucine.....	3.92×10^{-3}	20.42×10^{-4}
Tryptophan.....	5.20×10^{-3}	27.10×10^{-4}
Arginine.....	6.75×10^{-3}	35.19×10^{-4}
Leucine.....	8.00×10^{-3}	41.60×10^{-4}

[a] External amino acid concentration was 10^{-6} M.
[b] Expressed as micromoles $\times$ min^{-1} $\times$ mg^{-1} (dry weight).

in the nucleic acid fraction. Also, the nucleic acid fraction accounted for a large percentage of the label of cells grown in the presence of ^{14}C-serine. This was attributed to the presence of the transhydroxymethylase. The relatively low amounts of label found in the acid-ethyl alcohol-soluble fractions from the majority of the ^{14}C-amino acids were assumed to be present as alcohol-soluble protein; however, the higher proportion of label in this fraction from cells labeled with ^{14}C-serine was attributed to the synthesis of lipids. When the hot trichloroacetic acid residue was acid-hydrolyzed and subjected to paper chromatography, the identity of the recovered amino acids was found, for the most part, to be the same as the added amino acid. However, the label from ^{14}C-serine was present as serine and, to a lesser extent, glycine in the protein fraction, again suggesting the presence of serine transhydroxymethylase.

Energy requirement for amino acid incorporation. Most metabolite transport systems in microorganisms are sensitive to poisoning by metabolic inhibitors such as dinitrophenol, azide, or other uncouplers of energy metabolism. Cell suspensions of *P. aeruginosa* were tested for their ability to transport amino acids when treated with such inhibitors. When cells were preincubated for 30 min at 30 C in the presence of 30 mM sodium azide or 30 mM sodium azide plus 1 mM iodoacetamide, a marked reduction in amino acid transport ability was observed as measured by ^{14}C-proline incorporation into whole cells. Preincubation of the cell suspensions for 30 min with sodium azide inhibited the rate of incorporation of this amino acid by 97% and by 99% when both sodium azide and iodoacetamide were added to the preincubation mixture. This second, relatively minor, increase in inhibition of proline uptake with iodoacetamide was presumably due to the termination of substrate level phosphorylation. The conclusion, that the incorporation of amino acids was energy-dependent, was substantiated by the observation that cell suspensions of *P. aeruginosa* did not incorporate ^{14}C-proline at 0 C.

Effect of temperature on the rate of amino acid transport. The uptake of amino acids into whole cells of *P. aeruginosa* is temperature-dependent. The rate of ^{14}C-valine incorporation into whole cells at various temperatures is shown in Fig. 5. Below 10 C, the rate of ^{14}C-valine incorporation was negligible, but it increased rapidly with increasing temperature to the maximum rate exhibited at 45 C. At temperatures exceeding 45 C, the rate of ^{14}C-valine uptake declined sharply, a characteristic common to many enzyme systems. Incubation of whole cells for 30 min at 55 C was found to inactivate permanently the valine transport system. The fact that transport of valine could be dissociated from growth was evident from the observation that the rate of valine transport was high at 45 C, whereas growth of the organism does not occur at this temperature. The Q_{10} for valine uptake was found to be approximately 2.75.

Dependence of the rate of transport on substrate concentration. The rate of uptake, for most of the amino acids studied, increased concomitantly with the exogenous amino acid concentration. The

TABLE 2. *Distribution of radioactivity in the cell fractions of P. aeruginosa grown in the presence of ^{14}C-amino acids (10^{-6} M)*

^{14}C-Amino acid	Fraction[a]			
	Cold trichloroacetic acid soluble	Acid ethyl alcohol-soluble	Hot trichloroacetic acid-soluble	Residual protein
Arginine.....	35.5	3.8	2.4	58.3
Glycine.....	4.1	4.8	40.4	50.7
Histidine.....	2.5	3.7	2.7	91.1
Isoleucine....	0.7	4.3	4.7	90.3
Phenylalanine.....	1.1	4.6	4.2	90.1
Proline.....	2.0	6.5	3.7	87.8
Serine.....	6.3	11.9	25.1	56.7

[a] Per cent of total radioactivity incorporated into cellular material.

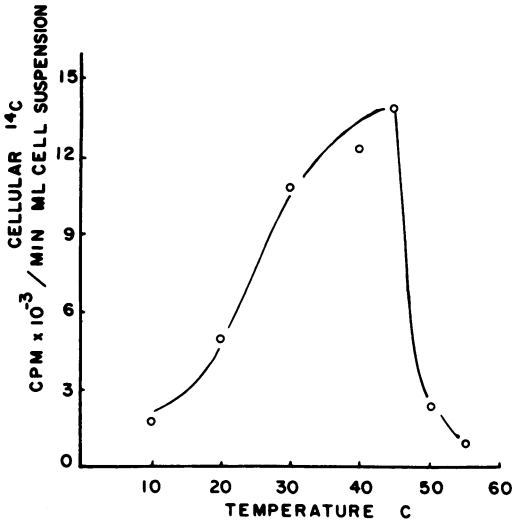

FIG. 5. *Rate of 10^{-6} M ^{14}C-valine incorporation into whole cells of P. aeruginosa as a function of temperature. Cells were equilibrated for 10 min at the appropriate temperature prior to the addition of label. Samples were removed at 30-sec intervals to obtain initial uptake velocities. Procedure: Britten and McClure (1).*

uptake system, however, tended to become saturated at high external amino acid concentrations. The kinetics of incorporation of ^{14}C-phenylalanine are shown in Fig. 6. Michaelis constants were of the order of 10^{-7} to 10^{-6} M for most of the amino acids studied. The adherence of the uptake process to Michaelis-Menton kinetics, the dependence upon energy metabolism, temperature sensitivity, and the loss of this uptake process by mutation (Kay and Gronlund, *unpublished data*) strongly suggest that the amino acid incorporation process is mediated by a protein, presumably a permease.

Concentration of amino acids in the intracellular pool. From the kinetics of amino acid incorporation into the protein fractions, it was assumed that possibly a wide variation in concentrations of the different amino acids existed within the native amino acid pool. Direct measurement of these amino acids was carried out to determine whether a correlation existed between native pool composition and the variation in rates of transport. The amino acid composition of the growth medium was also determined in order to establish the concentration gradients maintained between the intracellular and extracellular amino acids during growth. The amino acids present in the growth medium were released by the cells, and the concentration of these amino acids was directly proportional to cell numbers during exponential growth.

The composition of the amino acid pool of cells from populations growing exponentially was found to be extremely heterogeneous. Of 35 positively charged compounds present in the pool of *P. aeruginosa*, only 11 were identified as amino acids. The quantitative amino acid compositions of the pool, the culture medium, and the concentration ratios are listed in Table 3.

The linear amino acid incorporation kinetics into the protein fraction for most of the amino acids tested suggested that the concentration of some amino acids in the native pool was low. Glycine, serine, alanine, and glutamate were present in concentrations approximately 7- to 30-fold higher than the other amino acids found in the native pool, and the incorporation data for glycine, serine, and glutamate (Fig. 4) demonstrated "precursor-pool" kinetics. However, alanine deviated from this trend (Fig. 7). This suggested either that alanine was the only amino acid which did not first equilibrate with all of the native alanine pool prior to entry into protein, or that, in fact, a considerable proportion of the alanine pool consisted of D-alanine.

From the amino acid concentration ratios (Table 3), it can be seen that concentration gradients of approximately 100- to 300-fold are maintained within the cell during exponential growth of the population.

An identical experiment, with cells that had been in the stationary growth phase for 3 hr, demonstrated that all intracellular and extracellular amino acids had been utilized, with the exception of methionine, which was found in high con-

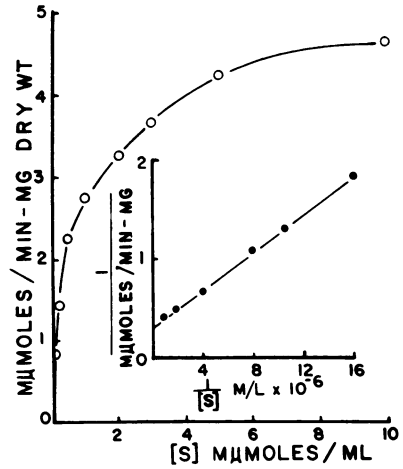

FIG. 6. *Saturation kinetics of phenylalanine incorporation by P. aeruginosa. Cells, in minimal medium, were incubated for 30 sec at 30 C in the presence of increasing concentrations of ^{14}C-phenylalanine. Inset: Lineweaver-Burk plot. Procedure: FerroLuzzi-Ames (5).*

TABLE 3. *Composition of the amino acid pool and culture supernatant fluid of P. aeruginosa during logarithmic growth*[a]

Amino acid	Intracellular water	Extracellular medium	Cell, water/medlum
	μmoles/ml	*μmoles/ml*	*μmoles/ml*
Leucine	6.64×10^{-2}	3.74×10^{-4}	178
Isoleucine	6.67×10^{-2}	3.54×10^{-4}	188
Phenylalanine	7.23×10^{-2}	3.44×10^{-4}	210
Valine	13.70×10^{-2}	10.70×10^{-4}	128
Tyrosine	14.70×10^{-2}	0.0×10^{-4}	0
Threonine	21.4×10^{2}	15.2×10^{-4}	140
Glycine	44.8×10^{2}	42.4×10^{-4}	105
Serine	93.6×10^{2}	28.9×10^{-4}	322
Alanine	95.3×10^{2}	53.1×10^{-4}	179
Glutamate	173.0×10^{-2}	58.8×10^{-4}	295
Glutamine	22.6×10^{-2}	0.0	0
Glucosamine	0.0	138.0×10^{-4}	0

[a] Cells were grown in the presence of ^{14}C-glucose.

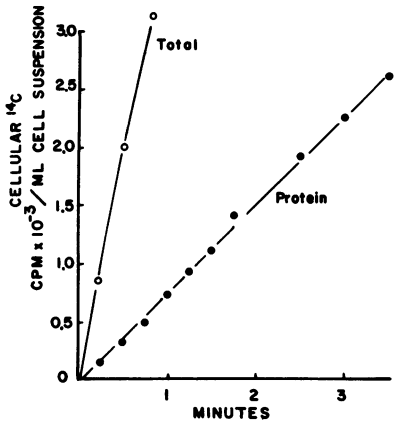

FIG. 7. *Early time course of 10^{-6} M ^{14}C-alanine uptake by a growing suspension of P. aeruginosa (0.1 mg, dry weight of cells/ml). Procedure: Britten and McClure (1).*

TABLE 4. *Composition of the cellular protein of P. aeruginosa*

Amino acid	Relative concn
Methionine	1.00
Histidine	1.38
Lysine	1.85
Tyrosine	2.27
Phenylalanine	3.09
Isoleucine	3.76
Proline	3.95
Serine	4.28
Arginine	4.46
Threonine	4.54
Glutamic acid	5.48
Valine	6.52
Glycine	8.40
Leucine	8.51
Aspartic acid	8.94
Alanine	11.95

centration in the medium of the stationary-phase culture.

It is apparent that, with the exceptions of alanine, amino acids present in the native intracellular pool at high concentrations are transported at a relatively low rate into *P. aeruginosa* (Tables 1 and 3).

Amino acid composition of the cellular protein. In order to determine whether the composition of the cellular protein reflected the efficacy of amino acid transport, protein was isolated from exponential-phase cells and submitted to amino acid analysis. The results (Table 4) were compared to the tabulated amino acid transport rates (Table 1).

Although the transport rates of some of the 18 amino acids tested (proline, valine, leucine, and alanine) compared favorably with their relative abundance in the protein of *P. aeruginosa*, the majority of the amino acids showed no strict correlation. However, when the data of Table 3 were compared with those of Table 4, it was observed that those amino acids present in relatively high concentrations in the native amino acid pool were essentially those present in highest concentrations in the protein. The amino acids present in the pool in relatively low concentrations, that is, leucine, isoleucine, valine, and phenylalanine, all had high rates of transport, whereas the amino acids present in relatively high concentrations were transported slowly.

DISCUSSION

The enzymatic nature of metabolite incorporation into cells, first demonstrated with carbohydrates by Rickenberg et al. (15), has been ex-

tended in recent years to encompass the amino acids, as well as other compounds (1, 11). The data presented here revealed that the amino acid transport systems of *P. aeruginosa* have all the properties ascribed to active transport systems: the transport process is seemingly energy-dependent, temperature-sensitive, and saturates at high substrate concentrations. Amino acid transport in *P. aeruginosa* is subject to loss due to mutagenesis and is also a rigid specific process (Kay and Gronlund, *unpublished data*).

Amino acids which are transported into *P. aeruginosa* equilibrate with amino acids synthesized de novo which reside in the intracellular pool, and also with amino acid pools which have been transiently preestablished from an exogenous source. This demonstrated that, in this organism, as in *E. coli* (1) and in *Neurospora crassa* (19), incorporated amino acids equilibrate with, and are subject to, the same metabolic fates as amino acids synthesized de novo. In *N. crassa* (13), some exogenous amino acids serve as substrates for reactions from which synthesized amino acids are excluded. Roberts et al. (16) suggested a similar fate for ^{14}C-lysine and ^{14}C-aspartic acid incorporated into *E. coli*.

Although some exogenously incorporated amino acids do undergo minor chemical changes during passage through the intracellular pool of *P. aeruginosa* (*unpublished data*), the amino acids incorporated into the protein fraction, for the most part, maintained their chemical integrity.

The total amino acid pool levels for *P. aeruginosa* during exponential growth are of the same order of magnitude as the levels reported for *E. coli* (7).

When the individual components of the intracellular pool were examined, some differences in the amino acid concentrations were observed. For instance, Ferroluzzi-Ames (5) reported intracellular concentrations for histidine of 0.06 μmoles/g (dry weight) in *Salmonella typhimurium*, yet no measurable amount of histidine was found in the native pool of *P. aeruginosa*. However, the glutamate concentration of 0.3 μmoles/g (dry weight) in *P. aeruginosa* compares favorably with the values of 0.5 to 2 μmoles/g (dry weight) in *E. coli*, as reported by Britten and McClure (1).

Pool concentrations achieved transiently during uptake experiments or those artificially prolonged are many times higher than those maintained during growth on glucose minimal medium. Britten and McClure (1) showed that, when cells deprived of a carbon source were pulsed with low levels of high specific activity ^{14}C-glucose, large concentration gradients for most amino acids were established. *P. aeruginosa* does not exhibit these high amino acid pool levels during exponential growth on glucose, undoubtedly owing to the functioning of biosynthetic control mechanisms. Therefore, the ability to maintain large concentration gradients of pool amino acids is expressed only in the presence of an added exogenous amino acid source.

Amino acids such as glutamate, alanine, glycine, and serine, which have multiple physiological roles not only as direct precursor material for protein, cell wall, and cell membrane synthesis, but also as precursors for other cellular metabolites, would be expected to be present in the pool in relatively higher concentrations than most other amino acids. One also would expect, on first analysis, that these amino acids would be transported more efficiently than those for which the cell has a lesser requirement. It is reasonable, however, that the low transport rates found for these amino acids are due to the fact that the cell does not rely on an exogenous supply of these amino acids which are readily synthesized from the carbon skeletons available from the metabolism of glucose. The only notable exception to these observations with *P. aeruginosa* was the absence of aspartic acid as a component of the native amino acid pool.

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