JOURNAL OF BACTERIOLOGY, Mar. 1971, p. 1039-1046

Vol. 105, No. 3
Printed in U.S.A.

Transport of Aromatic Amino Acids by *Pseudomonas aeruginosa*

W. W. KAY[1] AND AUDREY F. GRONLUND

Department of Microbiology, University of British Columbia, Vancouver 8, British Columbia, Canada

Received for publication 29 December 1970

Kinetic studies of the transport of aromatic amino acids by *Pseudomonas aeruginosa* revealed the existence of two high-affinity transport systems which recognized the three aromatic amino acids. From competition data and studies on the exchange of preformed aromatic amino acid pools, the first transport system was found to be functional with phenylalanine, tyrosine, and tryptophan (in order of decreasing activity), whereas the second system was active with tryptophan, phenylalanine, and tyrosine. The two systems also transported a number of aromatic amino acid analogues but not other amino acids. Mutants defective in each of the two and in both transport systems were isolated and described. When the amino acids were added at low external concentrations to cells growing logarithmically in glucose minimal medium, the tryptophan pool very quickly became saturated. Under identical conditions, phenylalanine and tyrosine each accumulated in the intracellular pool of *P. aeruginosa* at a concentration which was 10 times greater than that of tryptophan.

Several properties of amino acid transport systems in *Pseudomonas aeruginosa* were described previously (7, 8, 10). These amino acid transport systems have high affinities for their respective substrates, are strongly stereospecific, dependent upon metabolic energy, and subject to metabolic regulation, and can be lost via mutagenesis (7-10). Preliminary results have indicated that the various amino acids are transported by a number of distinct nonoverlapping transport systems such as have been reported for *Escherichia coli* (4-6, 11).

From kinetic considerations, the aromatic amino acids are transported into *E. coli* by what appears, at first, to be a single transport system with essentially no overlap with other neutral amino acids (11). In *Salmonella typhimurium*, however, aromatic amino acids and histidine are transported by general and by specific transport systems (1) each of which has been elucidated, not only by kinetic analyses, but also by the isolation and characterization of specific transport mutants (2, 13).

The work presented here embodies a kinetic and a genetic analysis which defines a third bacterial system of aromatic amino acid transport in which two high-affinity aromatic amino acid transport systems operate with a definite overlap but do not operate with other neutral amino acids.

[1] Present address: Department of Surgery, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

MATERIALS AND METHODS

Microorganisms. *P. aeruginosa* ATCC 9027 and the transport-negative derivatives TA3, TC10, and 5FT3 were the strains used throughout this investigation. Maintenance of the cultures, methods of cultivation, and composition of the minimal medium were described previously (7).

Mutagenesis and selection. Wild-type, logarithmic-phase, glucose-grown cells were treated with 100 μg of *N*-methyl-*N*-nitro-*N'*-nitrosoguanidine per ml as described (7). Cultures were washed and allowed to grow out overnight in glucose minimal medium. Since *P. aeruginosa* is resistant to low concentrations of most toxic amino acid analogues (9), cells resistant to high concentrations were selected as follows. Approximately 10^9 cells of the above mutagenized culture were spread on glucose minimal agar, 5 to 10 mg of either 5-fluorotryptophan or DL-*p*-fluorophenylalanine were placed in a center well, and the agar plug was replaced. Resistant colonies which arose within the wide zone of inhibition were picked and purified by repeated single-colony isolation and tested for the ability to transport the respective radioactive amino acid.

Mutants unable to utilize tyrosine as a carbon source were isolated as previously described (9).

Uptake studies. The procedures for filtration of cell suspensions and assay for radioactivity have been described (1). Time course studies on the uptake and fate of the intracellular ^{14}C-amino acid pools were carried out at 30 C, with 0.2 mg (dry weight) of cells per ml, and the ^{14}C-amino acid was present at 10^{-6} to 10^{-7} M. Cell suspensions were maintained at 30 C on a magnetic stirrer.

To determine the kinetics of amino acid uptake as well as those of competitive inhibition, reactions were carried out in 4-ml volumes in culture vials with mechanical agitation. Cells from a heavy cell suspension were added to the otherwise complete reaction mixture to initiate uptake. Rates of amino acid uptake were calculated from 15-sec ^{14}C-amino acid incorporation data. Total uptake was linear over this period.

Studies of inhibition of amino acid uptake by structurally related amino acids and analogues were carried out by using 0.1 mg (dry weight of cells) per ml and ^{14}C-amino acid concentrations of 2×10^{-7} M. Amino acids and analogues, added as competitors, were present either at 10^{-4} or 10^{-5} M. The rates of transport for ^{14}C-tryptophan were calculated after subtracting the amount of ^{14}C-tryptophan bound to boiled cells of *P. aeruginosa*.

Exchange studies on the maintenance of intracellular pools were carried out at 10 C. Pools were formed at this temperature from 10^{-6} M ^{14}C-amino acid, and the exchange reactions were initiated by the addition of the ^{12}C-amino acid to a final concentration of 10^{-4} M.

Chromatographic methods. Intracellular pools were extracted into 5% trichloroacetic acid at 0 C for 10 min from washed, filtered cells. The trichloroacetic acid was extracted with cold ethyl ether, and the pool extract was dried to a small volume. Analyses by chromatography and radioautography were performed by thin-layer chromatography as previously described (7).

Materials. Amino acids used either as carriers, competitors, or growth supplements were products of Nutritional Biochemicals Corp., Cleveland, Ohio. L-Tryptophan (^{14}C-methylene) was obtained from Nuclear-Chicago Corp. L-^{14}C-tyrosine and L-^{14}C-phenylalanine were obtained from Schwarz BioResearch Inc., Orangeburg, N.Y. All radioactive amino acids were checked for purity by chromatography and radioautography. D-Tyrosine, DL-β-methyltryptophan, kynurenine, and L-formylkynurenine were obtained from Calbiochem, Los Angeles, Calif.; *o*-hydroxyphenylacetic acid, *p*-hydroxyphenylacetic acid, *m*-hydroxyphenylacetic acid, and phenylpyruvic acid were from K & K Laboratories Inc., Plainview, N.Y.; *m*-fluoro-DL-phenylalanine, 5-fluorotryptophan, 6-fluorotryptophan, dibromotyrosine, diiodotyrosine, and kynurenic acid were from Nutritional Biochemicals Corp.; and DL-*o*-tyrosine, DL-*m*-tyrosine, L-mimosine, and 3,4-dihydroxyphenylalanine were from Sigma Chemical Co., St. Louis, Mo. DL-*o*-Tolylalanine, *m*-fluorotyrosine, 3-amino-L-tyrosine, and 4-amino-DL-phenylalanine were the generous gifts of G. Grant, Salk Institute, LaJolla, Calif.

RESULTS

Uptake of phenylalanine and tryptophan. The time course of phenylalanine and of tryptophan uptake by growing cells of *P. aeruginosa* are shown in Fig. 1A and B, respectively. These amino acids were rapidly incorporated and concentrated into the amino acid pool, and the radioactive amino acid pool was subsequently depleted during the course of protein synthesis. The amino acids did not undergo a chemical change during

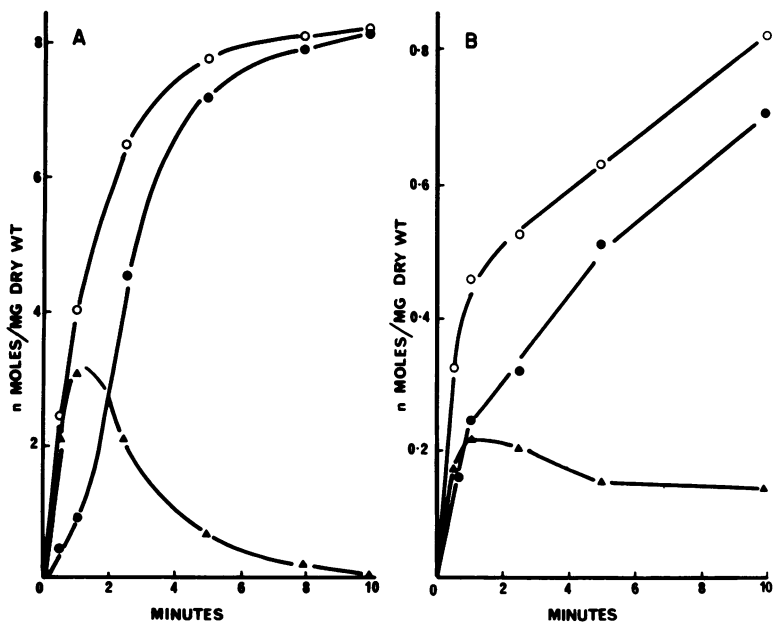

FIG. 1. *Time course of phenylalanine (A) and tryptophan (B) uptake by growing cells of Pseudomonas aeruginosa. The ^{14}C-L-amino acid was added at zero time to cells (0.2 mg dry weight/ml) in glucose minimal medium at 30 C. The radioactivity in the cells* (○) *and the 5% trichloroacetic acid precipitate* (●) *were assayed in a scintillation spectrometer, and the pool radioactivity* (▲) *was determined subtractively. Note the change in scale in the ordinate in B.*

the incorporation process, since the pools extracted from these cells during uptake experiments described in Fig. 1 contained essentially no other radioactive compounds when analyzed by radioautography of thin-layer chromatograms.

This transient accumulation of amino acid added at low external concentration is typical of other bacterial systems. Tryptophan did not accumulate to the degree exhibited by phenylalanine, and long time-course experiments showed that once the intracellular amino acid pool was saturated, entry from the external environment was dependent upon the rate of removal for protein synthesis.

Kinetics of amino acid uptake. The effect of substrate concentration on the rate of phenylalanine and tyrosine uptake is shown in Fig. 2. The uptake system was saturable and displayed high affinities for both phenylalanine and tyrosine. The apparent Michaelis constants had values of 4.4×10^{-7} M and 5.4×10^{-7} M for phenylalanine and tyrosine, respectively, and velocity maximum [V_{max}, nanomoles per minute per milligram (dry weight of cells)] of 4.44 for phenylalanine and 7.70 for tyrosine. The apparent Michaelis constant for tryptophan was also relatively low, 1.4×10^{-6} M, and the V_{max} value was 4.8 (Fig. 3).

Thus all three aromatic amino acids were transported at similar initial velocities and, collectively, the transport proteins exhibited similar affinities for the three substrates.

Competition for aromatic amino acid transport. The effect of the presence of various amino acids on ^{14}C-phenylalanine and on ^{14}C-tryptophan transport was determined. When 18 nonaromatic

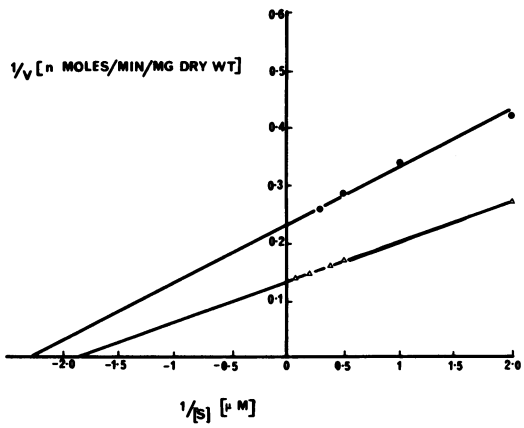

FIG. 2. *Double reciprocal plots of the rate of labeled phenylalanine and tyrosine uptake as a function of amino acid concentration. Symbols: ●, phenylalanine; △, tyrosine. Cells were added to glucose minimal medium containing the radioactive amino acid and cells were filtered at 15-sec intervals to determine initial uptake rates.*

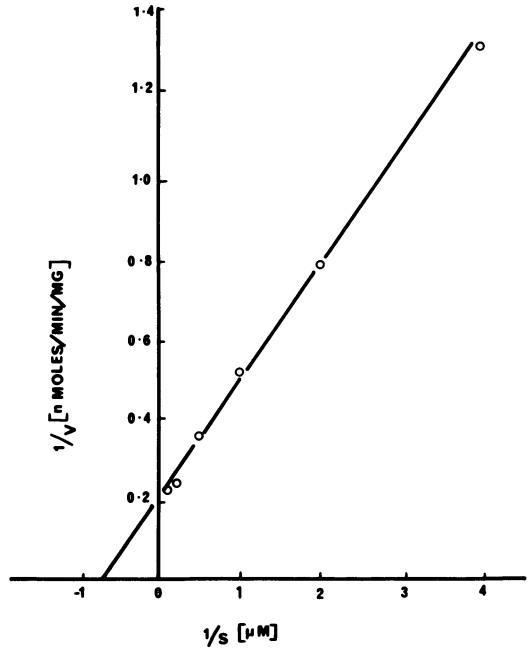

FIG. 3. *Double reciprocal plot of the rate of tryptophan uptake as a function of external concentration. The procedure was identical to that described in Fig. 2.*

amino acids were present, each at 100 times the concentration of ^{14}C-phenylalanine or ^{14}C-tryptophan, essentially no inhibition of uptake was observed. However, a very slight inhibition was observed with the amino acids isoleucine, leucine, and valine when each was present at 500 times the concentration of the ^{14}C-amino acid, and significant inhibition could be achieved only at very high aliphatic amino acid concentrations (10^{-3} to 10^{-2} M).

Each aromatic amino acid inhibited the uptake of the other two aromatic amino acids (Table 1), but to different degrees. Phenylalanine was more competitive for tyrosine and phenylalanine uptake than was tyrosine. This suggested the existence of an aromatic amino acid transport system functional for these two amino acids, with phenylalanine being most strongly recognized. These two amino acids were not as competitive for ^{14}C-tryptophan uptake as was ^{12}C-tryptophan, thereby suggesting the possible existence of a second aromatic amino acid transport system which primarily recognized tryptophan. ^{12}C-tryptophan also inhibited the uptake of ^{14}C-phenylalanine and ^{14}C-tyrosine but not to the degree exhibited by ^{12}C-phenylalanine or ^{12}C-tyrosine. However, tryptophan was more competitive for tyrosine uptake than phenylalanine uptake. These observations are commensurate with the existence of an aromatic permease which recognizes

TABLE 1. *Competitive inhibition of aromatic amino acid uptake in Pseudomonas aeruginosa*

^{12}C-competitor[a]	Per cent decrease in incorporation rate of		
	^{14}C-phenylalanine[b]	^{14}C-tyrosine	^{14}C-tryptophan
Phenylalanine	94.2	98.2	53.9
Tyrosine	88.4	97.0	52.0
Tryptophan	73.8	94.4	88.2

[a] Unlabeled L-amino acids were added to a final concentration of 10^{-4} M.

[b] Labeled amino acids were added to a final concentration of 10^{-6} M.

phenylalanine, tyrosine, and tryptophan, respectively (transport system I), and a second aromatic permease (transport system II) which must recognize tryptophan, phenylalanine, and tyrosine, respectively.

The effect of phenylalanine and tryptophan on the uptake of tyrosine in *P. aeruginosa* (Fig. 4) demonstrates that these two amino acids do, in fact, behave as competitive inhibitors of tyrosine uptake. Similar experiments have demonstrated that all three aromatic amino acids inhibit the uptake of one another in a strictly competitive manner.

Pool formation and pool exchange. When *P. aeruginosa* was preincubated with chloramphenicol, labeled tyrosine and tryptophan accumulated in the intracellular pool against a concentration gradient (Fig. 5). Pool formation was rapid and reached a steady state within 2 min after the addition of tryptophan and 10 min after the addition of tyrosine. A considerable difference in the ability of the organism to accumulate these amino acids was observed as both tyrosine and phenylalanine accumulated to a concentration approximately 10 times greater than that found for tryptophan. These pools remained chromatographically homogenous for the duration of the experiment and behaved like other amino acid pools studied in this microorganism; that is, they were lost to the external environment at low temperatures and on the addition of inhibitors of energy metabolism. The accumulated radioactive amino acids could be exchanged with unlabeled exogenous amino acids, and the relative order of exchange (Table 2) agreed fairly well with the competitive inhibition data (Table 1); however, a perfect correlation between exchange and uptake inhibition was not observed.

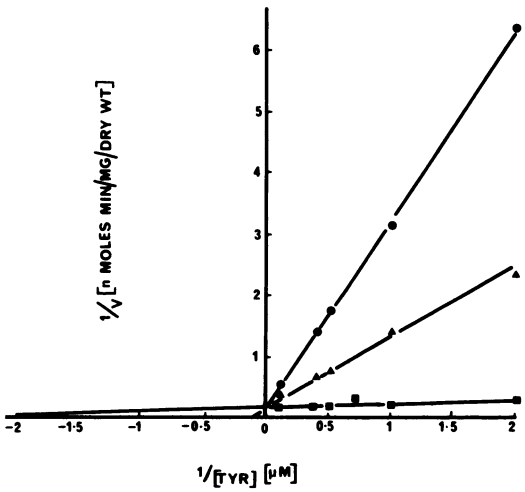

FIG. 4. *Kinetics of competitive inhibition of ^{14}C-tyrosine uptake by ^{12}C-phenylalanine and ^{12}C-tryptophan. Symbols: ■, tyrosine uptake in the absence of competitors; ●, tyrosine uptake in the presence of phenylalanine; ▲, tyrosine uptake in the presence of tryptophan. The uptake procedure was identcal to that described in Fig. 2. ^{12}C-amino acid concentration was 10^{-4} M.*

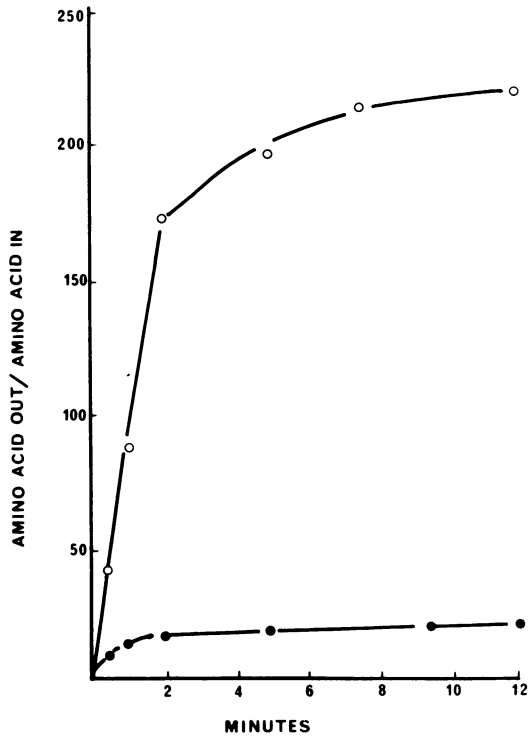

FIG. 5. *Time course of pool formation of ^{14}C-L-tyrosine and ^{14}C-L-tryptophan. Symbols: ○, tyrosine; ●, tryptophan. The amino acids were added to a cell suspension of Pseudomonas aeruginosa in glucose minimal medium which had previously been incubated with 200 μg of chloramphenicol per ml for 30 min at 30 C. The labeled amino acids were added to a final concentration of 10^{-6} M and the 5% trichloroacetic acid-soluble pool was determined at various time intervals. The pool levels are expressed as a ratio between the exogenous amino acid the level calculated for the intracellular pool.*

TABLE 2. *Exchange of aromatic amino acid pools*

^{14}C-pool amino acid[a]	Per cent of pool exchanged in 10 min with		
	^{12}C-phenylalanine[b]	^{12}C-tyrosine	^{12}C-tryptophan
Phenylalanine	84.2	51.3	31.1
Tyrosine	78.3	77.9	26.7
Tryptophan	4.6	5.2	72.7

[a] Cells (0.2 mg dry weight/ml) were pretreated with chloramphenicol (200 μg/ml) at 10 C, and the pool was allowed to reach the maximum level from the ^{14}C-L-amino acid added to a final concentration of 10^{-6} M.

[b] Unlabeled amino acid was added to a final concentration of 10^{-4} M.

The phenylalanine and tyrosine pools exchanged more readily with the respective exogenous ^{12}C-amino acid than did the tryptophan pool, and a very small percentage of the tryptophan pool exchanged with either exogenous phenylalanine or tyrosine. The amount of displacement of preformed phenylalanine and tyrosine pools by tryptophan was also relatively small. These results support the allocation of two systems for aromatic amino acid transport in *P. aeruginosa*.

Amino acid analogues. A number of amino acid analogues inhibited the uptake of tyrosine (Table 3). Of these analogues, the fluorinated derivatives were the most strongly inhibitory. The aromatic transport system was not specific for the L-isomers since both D-phenylalanine and D-tyrosine were competitive for L-tyrosine uptake. The fact that D-phenylalanine was a stronger competitor than D-tyrosine emphasizes that transport system 1 has a higher affinity for phenylalanine than tyrosine. The comparative degree of inhibition by *m*-fluorophenylalanine and *m*-fluorotyrosine (Table 3) again strengthens this conclusion. The recognition of tyrosine by this system would seem to be permitted by the parahydroxyl group, but hydroxyl groups at the meta and ortho positions are apparently less acceptable. The requirement for an α-amino group is essential since phenylpyruvic acid was not at all competitive. Whereas β-2-thienylalanine was a strong competitor, thiazolylalanine was not, thus indicating that the replacement of a carbon by nitrogen in the aromatic ring detracts from the aromatic character of the molecule.

Large substitutions in the aromatic ring preclude any detectable recognition by the transport system, thus dibromotyrosine and diiodotyrosine were not competitive inhibitors of tyrosine uptake.

It was also of interest to note that the fluoro analogues of tryptophan which competitively inhibited the uptake of labeled tryptophan (Table 4) had virtually no effect on tyrosine uptake; this

TABLE 3. *Inhibition of ^{14}C-tyrosine uptake by amino acid structural analogues*

Analogue added[a]	Per cent inhibition of uptake at analogue concn of	
	10^{-5} M	10^{-4} M
L-Tyrosine	93.0	—[b]
p-Fluorophenylalanine	89.7	—
m-Fluorophenylalanine	79.1	—
m-Fluorotyrosine	69.9	—
DL-β-Thienylalanine	58.3	87.3
D-Phenylalanine	43.0	68.8
DL-*o*-Tolyalanine	42.6	85.6
3-Amino-L-tyrosine	32.1	67.4
m-Tyrosine	27.7	63.1
4-Amino-L-phenylalanine	20.0	53.0
D-Tyrosine	14.8	17.6
3,4-Dihydroxyphenylalanine	13.8	69.4
o-Tyrosine	10.1	—

[a] Unlabeled analogue and labeled tyrosine (2×10^{-7} M) were added together at time zero. Cells were removed over a period of 2 min and filtered at 15-sec intervals. The following analogues caused no inhibition of uptake when added to 10^{-5} M: phenylpyruvic acid, phenylacetic acid, *o*, *m*, or *p*-hydroxyphenylacetic acids, thiazolyl-DL-alanine, L-mimosine, dibromotyrosine, diiodotyrosine, 5-methyltryptophan, 5-fluorotryptophan, and 6-fluorotryptophan.

[b] Not tested.

TABLE 4. *Inhibition of ^{14}C-tryptophan uptake by amino acid analogues*[a]

Analogue added	Per cent inhibition of uptake
L-Tryptophan	88.6
6-Fluorotryptophan	79.8
5-Fluorotryptophan	77.2
Kynurenic acid	11.8
Kynurenine	9.3
N-Formylkynurenine	9.1
5-Methyltryptophan	0

[a] Unlabeled analogues (final concentration of 10^{-4} M) and labeled tryptophan (final concentration 5×10^{-7} M) were added together at time zero.

further emphasizes the difference between the two aromatic transport systems. The competitive inhibition of tryptophan uptake by 5-fluorotryptophan and 6-fluorotryptophan is shown in Fig. 6. 6-Fluorotryptophan was not inhibitory to growth of the organism; however, 5-fluorotryptophan was inhibitory at high external concentrations. 5-Methyltryptophan had no effect on uptake of tryptophan. The degradation products of tryptophan metabolism, kynurenine, formylkynurenine, and kynurenic acid had only slight effects on this uptake process.

The influence of some of the amino acid analogues on the uptake of tyrosine is illustrated in Fig. 7. These results demonstrate that the ana-

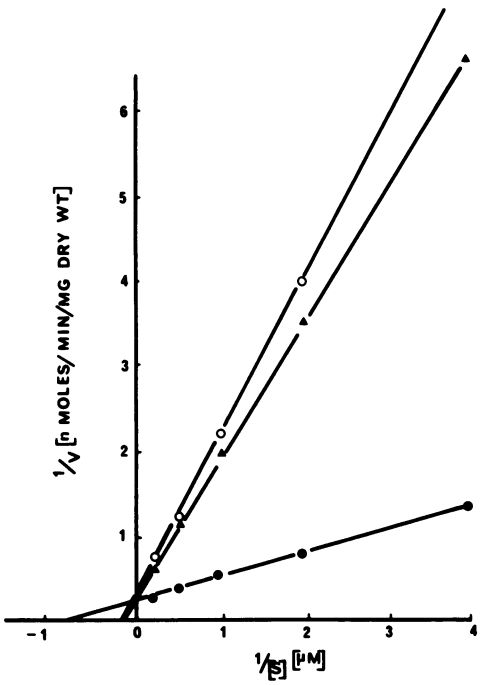

FIG. 6. *Kinetics of competitive inhibition of* L-*tryptophan uptake by 6-fluorotryptophan and 5-fluorotryptophan. Symbols:* ●, *tryptophan uptake in the absence of inhibitors;* ○, *in presence of* 10^{-4} M *6-fluorotryptophan;* ▲, *in the presence of* 10^{-4} M *5-fluorotryptophan.*

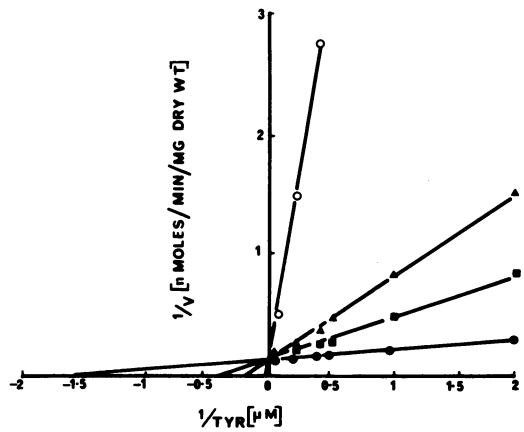

FIG. 7. *Kinetics of competitive inhibition of* L-*tyrosine uptake by amino acid analogues. Symbols:* ●, *tyrosine uptake in the absence of inhibitors;* ○, *uptake in the presence of* D-*phenylalanine;* ▲, *uptake in the presence of* DL-*β-2-thienylalanine;* ■, *uptake in the presence of* DL-*o-tolylalanine. Analogue concentration was* 10^{-4} M.

logues D-phenylalanine, DL-α-2-thienylalanine, and DL-*o*-tolylalanine behave as competitive inhibitors of tyrosine uptake; this was also true for the other amino acid analogues tested. The three above-mentioned analogues were not inhibitory to the growth of *P. aeruginosa*.

Transport-negative mutants. Since kinetic analysis had strongly indicated that there were two aromatic transport systems operating in *P. aeruginosa*, we attempted to isolate mutants defective in each and in both transport systems. Three such mutants were isolated and characterized as to their ability to transport aromatic amino acids. Mutants TC10 and TA3 were isolated as strains which could only slowly utilize tyrosine as a sole carbon source after mutagenesis with nitrosoguanidine. Mutant 5FT3 is a nitrosoguanidine-induced, 5-fluorotryptophan-resistant mutant. Of 30 DL-*p*-fluorophenylalanine-resistant mutants isolated, none was defective for aromatic amino acid transport, yet all were unable to use tyrosine readily as a sole carbon source.

The ability of the above mutants to transport tyrosine and tryptophan is illustrated in Fig. 8 and Fig. 9. Strain TC10 took up tryptophan (Fig. 8) at a rate similar to the parent strain (90% of the wild type rate) but was partially defective in

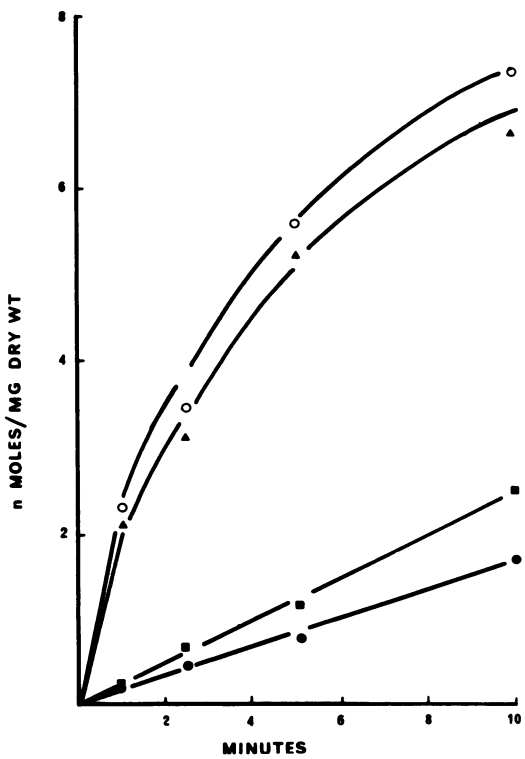

FIG. 8. *Tryptophan transport activity of wild-type and mutant strains of Pseudomonas aeruginosa. Symbols:* ○, *wild type;* ▲, *mutant TC10;* ■, *mutant 5FT3;* ●, *mutant TA3.* ^{14}C-L-*tryptophan was added to a final concentration of* 5×10^{-7} M *to cell suspensions (0.2 mg dry weight of cells/ml) in glucose minimal medium, and the rate of total uptake was determined at various intervals.*

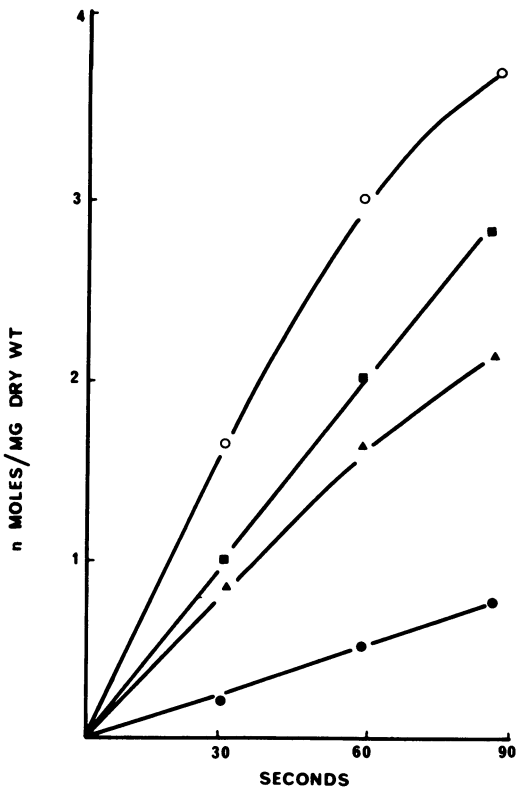

FIG. 9. *Tyrosine transport activity of wild-type and mutant strains of Pseudomonas aeruginosa. Symbols:* ○, *wild type;* ■, *mutant 5FT3;* ▲, *mutant TC10;* ●, *mutant TA3.* ^{14}*C-L-tyrosine was added at a final concentration of* 5×10^{-7} *M to cell suspensions [0.2 mg (dry weight of cells)/ml] in glucose minimal medium, and the rate of uptake was determined at 30-sec intervals.*

the incorporation of tyrosine (47% of the wild type rate), and it was also defective in the uptake of phenylalanine. This suggested that this mutant harbored a defect in the aromatic transport system I (phenylalanine, tyrosine, and tryptophan). On the other hand, strain 5FT3 was very defective in the uptake of tryptophan (15% of the wild type rate) but only partially defective in tyrosine uptake (66% of the wild type rate) and hence could be tentatively classified as an aromatic transport system II mutant (tryptophan, phenylalanine, and tyrosine). The isolation of these two mutants confirmed the existence of two aromatic amino acid transport systems and the overlap in amino acid transport between these systems. Mutant TA3 was highly defective in both amino acid transport systems; that is, both tyrosine and tryptophan were transported at 10% of the wild type rate. The nature of the mutation has not as yet been resolved and may be a double mutation, although this is unlikely. Because the rates of tyrosine and tryptophan transport by this mutant (TA3) were both reduced to 10% of the rates normally observed in the parent strain, this suggests that the mutation may exist at a step which is common to both of the aromatic amino acid systems described. The data regarding the decreased rates of transport in the three mutants indicate that the transport defect in mutant TA3 does not represent a third system for the ransport of aromatic amino acids in *P. aeruginosa*. Revertants of these classes of mutants have not as yet been isolated.

DISCUSSION

On the basis of the experimental data reported here, we have concluded that there are two amino acid permeases for aromatic amino acids in *P. aeruginosa*, both of which demonstrate high affinity for their substrates. These permeases are responsible for the concentrative uptake of phenylalanine, tyrosine, tryptophan, and a number of aromatic amino acid analogues. The two permeases have varying orders of affinity for the three amino acids; however, their combined activities yield similar V_{max} values for the three amino acids.

Direct measurements of the initial velocity of transport of phenylalanine, tyrosine, and tryptophan showed that nonaromatic amino acids did not inhibit the uptake of these amino acids by the cell. Only the aromatic amino acids themselves inhibited uptake, and in a competitive manner. The various degrees of competitive inhibition of the uptake of one aromatic amino acid by high concentrations of another permitted the postulation that two aromatic transport systems exist in *P. aeruginosa*. The data cannot be explained in terms of a single transport system mediating the uptake of all three aromatic amino acids. Also, the relative rates of aromatic amino acid pool displacement by the aromatic amino acids further substantiate this postulation. The existence of these two permeases were verified by the isolation of transport-negative strains defective in either one or both of the aromatic amino acid transport systems. The fact that mutants defective in a single aromatic transport system showed somewhat reduced uptake in the second transport system suggests that the aromatic amino acids normally enter *P. aeruginosa* by both systems, to give the maximal uptake rates demonstrated by the parent strain. The kinetic parameters of the two systems are sufficiently similar that an inflection in reciprocal plots could not be detected.

The transport of tryptophan by *P. aeruginosa* differs considerably from that described for *P. acidovorans* (12) since, in *P. acidovorans*, the tryptophan transport system is highly specific—although 5-fluorotryptophan is inhibitory. Also,

tryptophan transport in *P. acidovorans* was strongly inhibited by L-formylkynurinine and L-kynurenine, whereas these compounds had little effect on *P. aeruginosa*.

Ames (1) found aromatic permeases in *S. typhimurium* for all three aromatic amino acids and a general aromatic amino acid permease which recognized histidine. A specific histidine permease also mediates the uptake of histidine into *S. typhimurium*. Mutants for both the specific histidine permease and the aromatic permease were isolated by resistance to D(+)-α-hydrazinoimidazolepropionic acid (13) and azaserine or phenylalanine phosphonate (1, 2). These aromatic permeases exhibited similar affinities for the aromatic amino acids as demonstrated here for *P. aeruginosa*.

E. coli has a high affinity aromatic amino acid transport system which seemingly recognizes phenylalanine, tyrosine, and tryptophan; however, no extensive kinetic nor genetic analyses have been performed as yet to reveal whether more than one aromatic permease exists in this organism (3, 11).

It is interesting that the kinetics of tyrosine and phenylalanine uptake are similar in *P. aeruginosa*, but tyrosine serves as a much better carbon or nitrogen source. It is likely that the transport of phenylalanine is not limiting during growth on this carbon source but that a catabolic step is growth limiting.

The metabolic role of high-affinity microbial amino acid transport systems is as yet open to question; however, it is likely that, at least in *P. aeruginosa*, they serve a scavenging function in times of nutrient deprivation or as recapture mechanisms for amino acids which may diffuse out of the cell (7). In enteric bacteria, aromatic transport systems do not function in a catabolic sense, but in pseudomonads which can utilize these amino acids as a carbon and nitrogen source (14) they undoubtedly do.

ACKNOWLEDGMENT

This investigation was supported by a grant from the National Research Council of Canada.

LITERATURE CITED

1. Ames, G. F. 1964. Uptake of amino acids by *Salmonella typhimurium*. Arch. Biochem. Biophys. **104**:1–18.
2. Ames, G. F., and J. R. Roth. 1969. Histidine and aromatic permeases of *Salmonella typhimurium*. J. Bacteriol. **96**: 1742–1749.
3. Boezi, J. A., and R. D. DeMoss. 1961. Properties of a tryptophan transport system in *Escherichia coli*. Biochim. Biophys. Acta **49**:471–484.
4. Britten, R. J., and F. T. McLure. 1962. The amino acid pool in *Escherichia coli*. Bacteriol. Rev. **26**:293–335.
5. Cohen, G. N., and H. V. Rickenberg. 1955. Existence d'accepteurs spécifiques pour les aminoacides chez *Escherichia coli*. C. R. Acad. Sci. **240**:2086–2088.
6. Cohen, G. N., and H. V. Rickenberg. 1956. Concentrations spécifique reversible des aminoacides chez *Escherichia coli*. Ann. Inst. Pasteur **91**:693–720.
7. Kay, W. W., and A. F. Gronlund. 1969. Amino acid transport in *Pseudomonas aeruginosa*. J. Bacteriol. **97**:273 281.
8. Kay, W. W., and A. F. Gronlund. 1969. Amino acid pool formation in *Pseudomonas aeruginosa*. J. Bacteriol. **97**: 282–291.
9. Kay, W. W., and A. F. Gronlund. 1969. Isolation of amino acid transport-negative mutants of *Pseudomonas aeruginosa* and cells with repressed transport activity. J. Bacteriol. **98**:116–123.
10. Kay, W. W., and A. F. Gronlund. 1969. Proline transport by *Pseudomonas aeruginosa*. Biochim. Biophys. Acta **193**:444–445.
11. Piperno, J. R., and D. L. Oxender. 1968. Amino acid transport systems in *Escherichia coli* K12. J. Biol. Chem. **243**: 5914–5920.
12. Rosenfeld, H., and P. Feigelson. 1969. Product induction in *Pseudomonas acidovorans* of a permease system which transports L-tryptophan. J. Bacteriol. **97**:705 714.
13. Shifrin, S., B. N. Ames, and G. F. Ames. 1966. Effect of the α-hydrazino analogue of histidine on histidine transport and arginine biosynthesis. J. Biol. Chem. **241**:3424 3429.
14. Stanier, R. Y., N. J. Palleroni, and M. Doudoroff. 1966. The aerobic pseudomonads: a taxonomic study. J. Gen. Microbiol. **43**:159–271.