Evidence for a Direct Role of tRNA in an Amino Acid Transport System

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SUMMARY

The transport of phenylalanine by the general aromatic transport system in spheroplasts of Escherichia coli 9723 has been found to be stimulated by oxenogenous tRNA. Neither periodate-treated tRNA nor phenylalanine-charged tRNA stimulated, and the latter inhibited, phenylalanine uptake. Among preparations of specific tRNAs, tRNA<sup>phenyl</sup> and tRNA<sup>tyr</sup> were effective in stimulating the uptake of phenylalanine and tyrosine, respectively, and tRNA<sup>thi</sup> and tRNA<sup>val</sup> gave no detectable stimulation of phenylalanine or tyrosine transport. The preparation of tRNA<sup>tyr</sup> was ten times as active as unfractionated tRNA and gave as much as 167% stimulation of tyrosine transport. Correspondingly, the preparation of tRNA<sup>phenyl</sup> was at least 3.5 times as active as the unfractionated tRNA and 2.5 times as active as the preparation of tRNA<sup>tyr</sup> in stimulation of phenylalanine transport. Preliminary results in fractionation of the active component of tRNA for stimulating phenylalanine uptake show that the major activity resides in minor isoacceptor(s) tRNA<sup>thi</sup> rather than the major component tRNA<sup>phenyl</sup>, and the slight activity of preparations of tRNA<sup>tyr</sup> is probably due to a contamination of the active tRNA<sup>phenyl</sup>. Other preliminary results indicate that this type of stimulation occurs with uptake of other amino acids and their tRNA.

Escherichia coli is known to have at least four aromatic amino acid transport systems: a general aromatic amino acid transport system that utilizes phenylalanine, tyrosine, and tryptophan which compete for a common receptor, and three systems, each of which transport specifically one of the three amino acids (1, 2). An inducible system transporting tyrosine or tryptophan has been reported (3, 4) in addition to the above four systems.

On the basis of studies of mutants of E. coli, repression of branched chain amino acid transport has been proposed to involve the interaction of leucine with its aminoacyl-tRNA synthetase and its cognate leucyl-tRNA (<sup>5-8</sup>). A significant enhancement of the initial rate of uptake of leucine and other branched chain amino acid transport has been proposed to involve the interaction of leucine with its aminoacyl-tRNA (<sup>5-8</sup>). A significant enhancement in the initial rate of uptake of leucine and other amino acids was observed in Chinese hamster ovary cell lines when the temperature-sensitive leucyl-tRNA synthetase and its cognate leucyl-tRNA (<sup>5-8</sup>) were obtained from Calbiochem. Phenylalanine, tRNA<sup>phenyl</sup>, tRNA<sup>tyr</sup>, tRNA<sup>thi</sup>, and tRNA<sup>val</sup> were purchased from the Sigma Chemical Co. Millipore filters (13 mm, 0.8 µm) and Sephadex G-25 were obtained from Millipore Corp. and Pharmacia Fine Chemicals, Inc., respectively.

Preparation of Penicillin Spheroplasts—Penicillin-induced spheroplasts of E. coli 9723 were prepared as described by Lederberg (16) with minor modifications. Growth medium (130 ml) containing 0.53 µmol sucrose, 15 mM MgSO<sub>4</sub>, and 1.75 µmol (w/v) Bacto-Tryptone was sterilized for 15 min in the autoclave and inoculated with 4.5 ml of a 50-ml, 14-h culture grown at 37°C in the same medium without MgSO<sub>4</sub>, and sucrose. The growth flask was incubated in a New Brunswick gyraatory shaking water bath (model G76) at gyratory speed 5. After the cell density doubled, 0.11 µg of penicillin (1.8 × 10<sup>6</sup> units) was added to the growing culture. Spheroplast conversion was followed by phase contrast microscopy, and upon complete conversion, the spheroplasts were harvested at 20°C by centrifugation at 12,000 × g for 3 min and washed twice with a buffer containing 10 mM potassium phosphate, pH 7.0, 400 mM ultrapure sucrose, 30 mM MgSO<sub>4</sub>, and 1 mM MnCl<sub>2</sub>. For uptake studies, the spheroplasts were resuspended in the same buffer to a final absorbance (A<sub>500</sub>) of 1.3 to 1.6. A phase contrast microscope was used to confirm the integrity of the spheroplasts at each step. Only samples that contained primarily whole spheroplasts were used for transport studies. The cells were used within about 40 min, since the activity declined with storage time. Used within this time limit, the spheroplasts on a weight basis were approximately 40% as active as intact cells in the uptake of phenylalanine or tyrosine by the general aromatic transport system under the conditions described below.

Amino Acid Uptake—The total uptake of amino acids (phenylalanine, tyrosine) was determined by a technique adapted from that of Weiner and Heppel (17). The uptake medium contained 1 mg of glucose, 2.2 nmol of L-[<sup>14</sup>C]phenylalanine, or L-[<sup>14</sup>C]tyrosine and appropriate amounts of tRNA in 0.1 ml. The spheroplast suspension and the substrates were kept at room temperature and 0°C to 4°C, respectively, until used. Just prior to the assay, the tubes containing assay substrates were incubated for 2 min at 23°C. The uptake was initiated by addition of 0.1 ml of the spheroplast suspension to the assay tubes. After 2 min at 27°C, the uptake was terminated by the addition of 2 ml of the phosphate buffer described above, and followed by rapid filtration of the reaction mixture on a 25-mm, 0.8-µm pore size, Millipore filter. The filters were dried and placed in vials containing 10 ml of toluene with 0.5% (w/v) 2,5-diphenyl-1,3-oxazole for counting.

Phenylalanine-specific transport was determined as described above except that supplements of 125 nmol each of t-tyrosine and t-tryptophan were added. Higher levels of tyrosine and tryptophan did not further reduce the uptake of phenylalanine in spheroplasts, and the remaining activity is presumed to result primarily from the phenyl-
alanine-specific transport system. Phenylalanine uptake by the GAT system was calculated by the difference in the specific and total uptake. Tyrosine-specific transport was similarly determined in the presence of 125 nmol of L-phenylalanine and L-tryptophan, and the tyrosine uptake by the GAT system was calculated by the difference between total and specific tyrosine uptake. The uptake of amino acids was linear under these conditions for more than 10 min, and the uptake, for experiments in the absence of chloramphenicol, represents amino acid accumulated in the amino acid pool and incorporated into protein.

Also, the kinetics of transport of phenylalanine in spheroplasts of E. coli is similar to that in intact cells. The $K_m$ of the general aromatic transport determined by this assay method is 1.26 $\mu$m which compares with 0.27 $\mu$m observed with intact E. coli cells and the V_max is 40 pmol/min/mg dry weight of spheroplasts and is comparable to that observed in intact cells. For the uptake of phenylalanine not inhibited by tyrosine and tryptophan and acylated to phenylalnine-specific transport, kinetic data indicate a $K_m$ of 0.3 $\mu$m for the above spheroplast technique compared with 3.1 $\mu$m for intact cells.

Preparation of tRNA—E. coli B, unfraccionated tRNA was deacylated, dialyzed, and stored according to usual procedures (18). Periodate-treated tRNA was prepared according to the methods of Chousterman and Chapeville (19) and Prieis et al. (20). Periodate treatment destroyed at least 99% of the aminoacylation capacity of the tRNA as measured in a phenylalanyl-tRNA synthetase assay. [$^{14}$C]Phenylalanyl-tRNA was prepared by the method of Ravel (21). Upon dissolution, the tRNA solution was aspirated for 30 min at room temperature in order to remove traces of ethanol.

RESULTS AND DISCUSSION

In a study of the transport of phenylalanine in spheroplasts of E. coli, a slight stimulation by tRNA on the total transport was noted. The observed stimulation of total transport was found to be primarily associated with the GAT system (Table I). The stimulation did not occur with periodate-treated tRNA, and a significant inhibition of uptake rather than stimulation was found to occur with tRNA acylated with phenylalnine (data not shown). These results indicated the possibility of specific tRNA effects on the general aromatic amino acid transport system. Accordingly, the effects of different tRNA species on the transport of phenylalanine by the GAT system were determined. The uptake of [$^{14}$C]phenylalanine into E. coli spheroplasts from a concentration of 11 $\mu$m amounted to 57.2 pmol/min/mg dry weight of spheroplast in the 2-min incubation period, and the stimulatory effects of preparations of various species of tRNA were determined as shown in Fig. 1. Of the various preparations of amino acid-specific tRNAs, tRNA$^{\phi\psi}$ and tRNA$^{\phi\psi}$ showed no detectable activity, while the preparation of tRNA$^{\phi\psi}$ was at least 3.5 times as active as the mixture of tRNAs (E. coli B tRNA).

Since the preparation of tRNA$^{\phi\psi}$ used in this experiment contained 9.7% tRNA$^{\phi\psi}$, the slightly greater activity of the tRNA$^{\phi\psi}$ preparation than that of the unfractionated tRNA is probably the result of the impurity rather than the major species, tRNA$^{\phi\psi}$. Since the activity of the preparation of tRNA$^{\phi\psi}$ does not account for the total activity of E. coli B tRNA, the possibility of isooacceptor species accounting for the activity appeared likely. Preliminary work of the fractionation of tRNA acylated with [$^{14}$C]phenylalanine into minor and major isooacceptors followed by hydrolysis of the phenylalanine group has provided samples which show activity for only the minor isooacceptor(s). Thus, it appears likely that the slight activity of tRNA$^{\phi\psi}$ over that of the crude tRNA represents a contamination with the active tRNA$^{\phi\psi}$ isoacceptor(s).

In order to investigate further the specificity of tRNA species for stimulation of uptake by the general aromatic amino acid transport system, the effect of tRNA species on

1 The abbreviation used is: tRNA, general aromatic amino acid transport.
2 M. L. Pratt (1977) Ph.D. dissertation, The University of Texas at Austin, Austin, Tex.

![Figure 1](image-url)
Preliminary investigation of glutamic acid and valine uptake have shown tRNA stimulations which appear to have specificity. Preparations of tRNA\(^{\text{Glu}}\) are more active than crude tRNA, and the tRNA\(^{\text{Phe}}\) preparation is inactive in stimulating glutamic acid uptake.

Whether the exposed cell membrane in the absence of the cell wall allows tRNAs to act externally on the membrane receptors or to penetrate into the spheroplasts requires further investigation. However, it is apparent that the spheroplast technique offers an unusual system for further investigation of roles of tRNA, particularly of isoacceptor species.

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