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EVIDENCE FOR A ROLE OF SPECIFIC ISOACCEPTOR SPECIES OF tRNA IN AMINO ACID TRANSPORT

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Summary

The transport of phenylalanine by the general aromatic amino acid system and of threonine by a separate system in spheroplasts of Escherichia coli 9723 is stimulated by tRNA (E. coli B) and inhibited competitively by the respective aminoacylated tRNA. tRNA appears to increase the affinity of the transport system for the amino acid. The stimulatory activity of tRNA was found to reside in fractions containing minor isoacceptor species obtained by fractionation of the respective aminoacylated tRNA and deacylation.

Introduction

The transport of phenylalanine and of tyrosine by the general aromatic amino acid transport system in spheroplasts of Escherichia coli 9723 has been found to be stimulated by $tRNA^{Phe}$ and $tRNA^{Tyr}$, respectively, with a high degree of specificity (1). Some preliminary data suggested that minor isoacceptor species of $tRNA^{Phe}$ rather than the major species were involved. In the present investigation, the direct stimulation of amino acid transport has been extended to threonine, and significant specificity of minor isoacceptor species of $tRNA^{Phe}$ and $tRNA^{Thr}$ in stimulating the transport of phenylalanine and of threonine has been demonstrated.

The relative amounts of tRNA isoacceptors undergo changes in different developmental and functional stages of organisms (2,3). Also, a preferential utilization of particular isoaccepting species of tRNA in translation of mRNA for specific proteins has been observed and proposed as a basis for control of specific protein synthesis at different stages of development and in different organs (4-7). There has been a considerable interest in the possibility that there may be specific biological functions of isoaccepting species other than their preferential utilization in translation of specific codons. The present work indicates that one specific role, at least in some cases, is directly concerned with the transport of amino acids.

Materials and Methods

Preparation of penicillin-induced spheroplasts and the assay for amino acid uptake have been previously described (1). Unfractionated E. coli B tRNA was purchased from Schwarz/Mann, deacylated and dialyzed before use to make cer-

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tain that all tRNAs are free from amino acids. The deacylated tRNA was aminoacylated with either [^{14}C] L-phenylalanine or [^{14}C] L-threonine using the crude aminoacyl tRNA synthetase as previously described (8,9) except that aminoacylation with threonine was carried out at pH 8.2 instead of pH 7.5 (Tris·HCl). For separation of isoaccepting species, benzoylated DEAE-cellulose obtained from Boehringer-Mannheim was washed three times with 90% ethanol and then washed three times with 2 M NaCl. The resin was packed into a 1 cm x 50 cm glass column and washed with a solution of 7 M urea and 1 M NaCl in 20% ethanol, pH 3.0, until the A_{260} absorbance was negligible. Approximately 600-800 absorbancy units (A_{260}) of labeled, aminoacylated tRNA were loaded onto the column. The tRNA was eluted with a linear gradient of 0.22 M - 0.70 M NaCl (400 ml each) for tRNA acylated with phenylalanine and 0.20 M - 0.40 M NaCl (400 ml each) for tRNA acylated with threonine, in 7 M urea adjusted to pH 3.0 with HCl. The elutions were carried out at 24°C with a flow rate of about 15 ml/h, and 1.6 ml fractions were collected. The radioactivity in the eluate was determined in 0.1 ml aliquots in 5 ml of scintillation fluor prepared by mixing 2 parts of methoxy ethanol with 3 parts of toluene containing 0.5% (w/v) 2,5-diphenyl-1,3-oxazole. The combined fractions of eluate containing specific isoacceptor species were treated with cold ethanol and heated for 2 min at 60°C. The precipitated aminoacyl-tRNA was recovered by centrifugation, deacylated and dialyzed (10) to remove the labeled amino acids. The resulting $tRNA^{Phe}$ and $tRNA^{Thr}$ isoacceptors, free from phenylalanine and threonine, respectively, were used for determining their activity in amino acid transport.

Results and Discussion

Analagous to the stimulation of phenylalanine transport by tRNA previously reported, a similar enhancement of threonine transport by tRNA was observed. That each amino acid transport is stimulated by its specific tRNA is indicated by not only the loss of stimulation but an inhibitory effect of tRNA loaded with the respective amino acid. As shown in the double-reciprocal plots of Fig. 1A and 1B, tRNA (*E. coli* B) stimulates the transport of the corresponding amino acid by enhancing the affinity of the transport system for the amino acid. tRNA aminoacylated with phenylalanine and threonine competitively inhibit phenylalanine and threonine transport, respectively. Similar results are obtained even though the aminoacylation of tRNA is carried out with amino acid of the same specific activity as the amino acid used in the transport, which rules out the possibility of any significant effects of hydrolysis of the aminoacyl-tRNA to free amino acid.

In order to determine the specificity of the stimulatory effect of tRNA, tRNA from *E. coli* which had been loaded with phenylalanine was fractionated into [^{14}C] phenylalanyl-tRNA isoacceptors as indicated in Fig. 2 by chromatography on benzoylated DEAE-cellulose. The early eluting fractions contained phenylalanyl derivatives of two minor isoaccepting species ($tRNA_I^{Phe}$ and $tRNA_{II}^{Phe}$) which were not distinctly separated, and subsequent fractions contained the major component ($tRNA_{III}^{Phe}$). Fractions containing the specific isoacceptor species of Phe-$tRNA^{Phe}$ were combined and deacylated, and the stimulatory effect on phenylalanine transport of the preparations of each of free $tRNA^{Phe}$ was determined (Table 1). The combinations of fractions containing the major species of $tRNA^{Phe}$ ($tRNA_{III}^{Phe}$) did

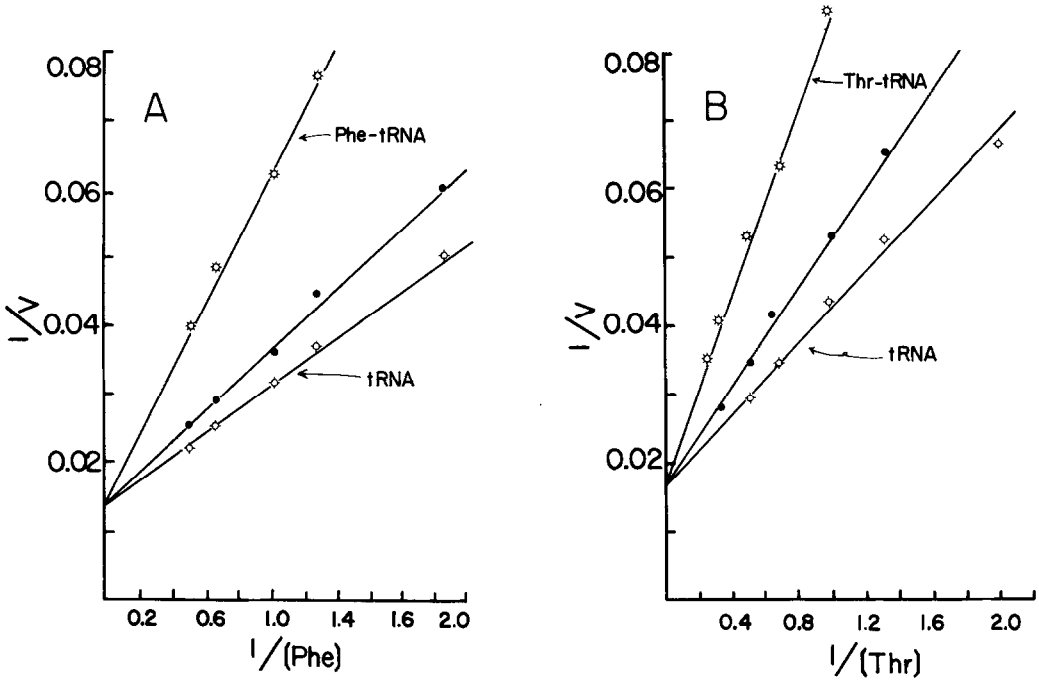

Fig. 1. Double-reciprocal plots of amino acid concentration versus amino acid uptake in spheroplasts. The amino acid uptakes without (●●) and with supplements of either free tRNA (☼ ☼) or aminoacylated tRNA (☼ ☼) are expressed as pmoles of amino acid uptaken/min/mg dry wt spheroplasts versus amino acid concentrations in μM.

Concentrations of tRNA and tRNA acylated with the indicated amino acid were 3.7 mg/ml and 2.7 mg/ml respectively. A: Phenylalanine, B: Threonine.

not have significant activity in stimulating phenylalanine transport in the spheroplasts. However, the combinations of fractions of the two minor components, $tRNA_I^{Phe}$ and $tRNA_{II}^{Phe}$, were about equally active. Phenylalanine transport by the general aromatic amino acid system was stimulated 32.4% and 32.7% in the presence of 0.17 mg/ml of tRNA fractions containing $tRNA_I^{Phe}$ and $tRNA_{II}^{Phe}$, respectively. It is apparent that the activity for stimulating phenylalanine transport resides in the minor isoacceptor species, but the overlapping of these fractions makes the determination of relative activity difficult.

Fractionation of E. coli B tRNA acylated with threonine resulted in three peaks (Fig. 3). The major band ($tRNA_I^{Thr}$) was the first threonine derivative eluted from the column and was followed by two bands ($tRNA_{II}^{Thr}$ and $tRNA_{III}^{Thr}$) with successive decreases in relative amounts. Fractions containing the specific isoacceptor species were combined, deacylated, and assayed. The combination of fractions containing the two most abundant forms of $tRNA^{Thr}$ does not have any significant activity in stimulating the transport of threonine in E. coli sphero-

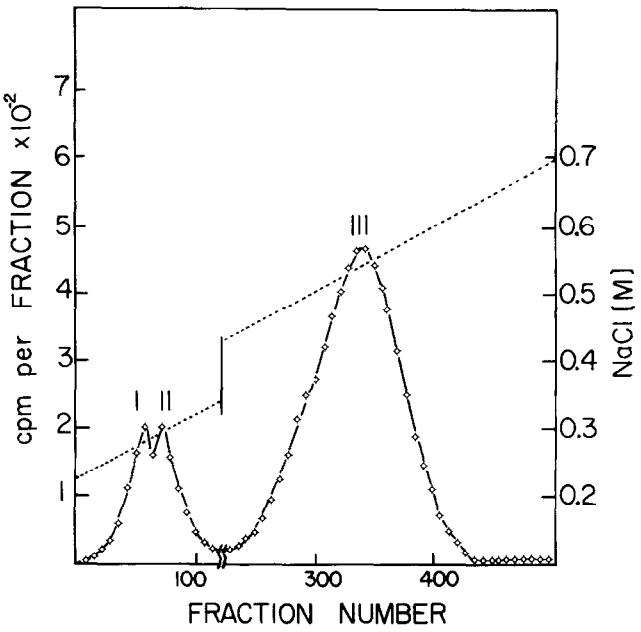

Fig. 2. Separation of tRNA (E. coli B) loaded with [^{14}C]phenylalanine into isoacceptors on BD-cellulose in 7 M urea, pH 3.0. NaCl gradient of 0.22 M - 0.70 M was used.

Table 1

Stimulation of phenylalanine uptake by $tRNA^{Phe}$ isoacceptors in E. coli spheroplasts

Supplement[a]	Phenylalanine uptake by general aromatic transport system[b]	% Stimulation by tRNA supplement
None	35.8	0
$tRNA_I^{Phe}$	47.4	32.4
$tRNA_{II}^{Phe}$	47.5	32.7
$tRNA_{III}^{Phe}$	37.0	3.3

[a] The tRNA of the combined fractions containing each isoaccepting species was added at a level of 0.17 mg/ml.

[b] Phenylalanine uptake is expressed as pmoles/min/mg dry wt spheroplasts. Phenylalanine concentration in the media was 11 µM. General aromatic transport is determined by difference between total transport and specific phenylalanine transport. The latter amounted to 26.9 pmoles/min/mg dry wt spheroplasts.

plasts (Table 2). The activity resides in the combination of fractions of the least abundant species ($tRNA_{III}^{Thr}$) which shows 18% stimulation of transport at a

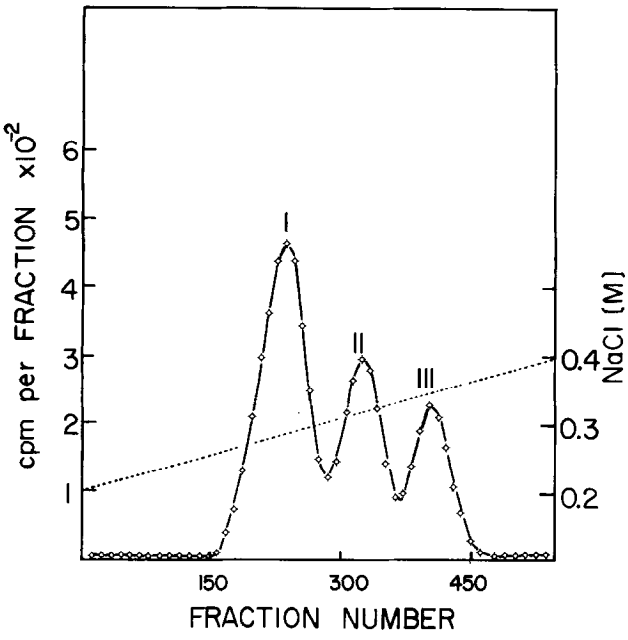

Fig. 3. Separation of tRNA (E. coli B) loaded with [^{14}C]threonine into isoacceptors on BD-cellulose in 7 M urea, pH 3.0. NaCl gradient of 0.20 M - 0.40 M was used.

Table 2

Stimulation of threonine uptake by $tRNA^{Thr}$ isoacceptors in E. coli spheroplasts

Supplement[a]	Threonine uptake[b]	% Stimulation by tRNA supplement
None	78.7	0
$tRNA_I^{Thr}$	81.0	3.0
$tRNA_{II}^{Thr}$	80.4	2.2
$tRNA_{III}^{Thr}$	92.8	18.0

[a]The tRNA of the combined fractions containing each isoacceptor was added at a level of 0.25 mg/ml.

[b]Threonine uptake is expressed as pmoles/min/mg of dry wt spheroplasts. Threonine concentration in the media was 3.4 µM. Procedure as previously described (1) except using threonine as substrate.

concentration of 0.25 mg/ml. Although the above procedure was effective in separating isoacceptor species, the single fractionation by the column does not provide pure tRNAs. Consequently, the concentrations of tRNA indicated in Tables 1 and 2 represent the total tRNA present including other contaminating tRNAs.

From these results, it is apparent that specific minor isoaccepting species of tRNA are involved in the transport of these two amino acids. Preliminary results with other amino acids suggest that there are other examples of minor isoaccepting species having a high degree of specificity in stimulating transport.

If similar effects of tRNA are exerted from within the cell upon the transport system, a role of tRNA in control of transport is indicated by the enhancement of transport by free tRNA and inhibition by aminoacyl-tRNA. The inhibitory effect of aminoacyl-tRNA on transport could account for the reported involvement of aminoacyl-tRNA synthetases in the control of amino acid transport (11).

The potential for isoacceptor species of tRNA to have a role in biological control mechanisms is indicated by changes which can be induced in the profiles of isoacceptor tRNAs. Leucine starvation of E. coli is known to result in the formation of new isoacceptor species of leucine-, histidine-, arginine-, valine- and phenylalanine-specific tRNA and changes in the profiles of isoacceptor tRNAs specific for serine, glycine and isoleucine (12). Threonine or isoleucine deficiency in E. coli causes changes in the relative amounts of the isoacceptor tRNAs specific for threonine or isoleucine, respectively (13). Such effects of amino acids upon isoacceptor species of tRNA are widespread (14-18). Therefore, it appears that the relative amounts of each isoaccepting species can vary depending on the intracellular amino acid concentration or on physiological demand of the organism (19,20). Not only could specific isoacceptor species of tRNA thus modulate the transport of amino acids by stimulating transport in the free form and inhibiting in the acylated form, but also the amino acid could regulate the level of the specific isoacceptor species involved in transport.

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