TRANSFER OF METHIONYL RESIDUES BY LEUCYL,PHENYLALANYL-tRNA-PROTEIN TRANSFERASE

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SUMMARY: Leucyl,phenylalanyl-tRNA-protein transferase also catalyzes transfer of methionyl residues as indicated by (i) copurification over a 1000-fold range of transfer activities for all three amino acids and (ii) loss of methionyl transfer activity in a mutant of E. coli lacking the transferase and reappearance of this activity in a transferase revertant. The purified enzyme was found to use Met-$tRNA_m^{Met}$ in preference to Met-$tRNA_f^{Met}$ as donor substrate. Peptides containing a basic amino acid at the NH_2-terminus functioned as acceptors for the transfer of methionyl residues.

Extracts from E. coli can incorporate leucine and phenylalanine into protein in the absence of ribosomes (1). This reaction is due to the presence of a soluble enzyme, leucyl,phenylalanyl-tRNA-protein transferase (EC 2.3.2.6) (2) which catalyzes transfer of these amino acids from tRNA into peptide linkage with basic NH_2-terminal aminoacyl residues of protein (3) or peptide (4) acceptors. The physiological role of the enzyme is not well understood. However, analysis of a mutant in which it is lacking (5) has suggested that one of its functions may involve regulation of proline catabolism at the level of proline oxidase (6). Horinishi et al. recently reported (7) that a crude soluble extract of E. coli B also catalyzed transfer of methionine from tRNA to acceptor proteins having a basic amino acid at their NH_2-terminus. This communication provides biochemical and genetic evidence that such methionyl transfer activity is associated with leucyl,phenylalanyl-tRNA-protein transferase.

MATERIALS AND METHODS

Partially purified $tRNA_f^{Met}$ and $tRNA_m^{Met}$ free from contamination with each other (8) were a gift from Dr. LaDonne Schulman. L-[U-^{14}C] leucine (250 μCi per μmole) and L-[U-^{14}C] phenylalanine (480 μCi per μmole) were from New England Nuclear. L-[U-^{14}C] methionine (260 μCi per μmole) was a product of

Schwarz/Mann. Stripped E. coli B tRNA from General Biochemicals was used to prepare (2) [^{14}C] leucyl-tRNA (0.9 nmoles per mg) and [^{14}C] phenylalanyl-tRNA (0.4 nmoles per mg). The partially purified isoaccepting species were used to obtain [^{14}C] methionyl-tRNA$^{Met}_{f}$ (5.5 nmoles per mg) and [^{14}C] methionyl-tRNA$^{Met}_{m}$ (2.2 nmoles per mg). Alpha casein was purchased from Nutritional Biochemicals and synthetic peptides were from Cyclo Chemicals or Bachem. Reaction mixtures containing these peptides in the place of α-casein were examined for the presence of radioactive products by paper electrophoresis at pH 6.5 and by paper chromatography in 1-butanol:acetic acid:water (120:30:50) (4).

W4977 is the E. coli K12 strain from which MS845, a mutant defective in leucyl,phenylalanyl-tRNA-protein transferase, was originally isolated (5). R18 is a spontaneous revertant for the enzyme (5). Soluble extracts from these cells and from E. coli B were prepared as before (2).

Protein concentrations were estimated by the method of Lowry et al. (9) using bovine serum albumin as the standard.

RESULTS

Activity in E. coli B for the transfer of methionine from tRNA to protein

Table I

Copurification from E. coli B of Transfer Activities

for Phenylalanine, Leucine and Methionine

Fraction	Amino acid			Ratio	
	Phenylalanine	Leucine	Methionine (nmoles/min/mg)	Phe/Leu	Phe/Met
105,000 x g extract	0.030	0.032	0.010	0.9	3.0
1st Phosphocellulose eluate	1.0	0.89	0.30	1.1	3.3
Ammonium sulfate fraction	6.8	6.8	2.3	1.0	3.0
Sephadex G-100	10	9.5	3.3	1.1	3.0
2nd Phosphocellulose eluate	30	25	10	1.2	3.0

Enzyme fractions were obtained by the purification procedure for leucyl,-phenylalanyl-tRNA-protein transferase (4). Assay reaction mixtures (50 µl) contained 50 mM Tris-HCl (pH 8.2), 50 mM 2-mercaptoethanol, 0.15 M KCl, 2 µM (with respect to amino acid) [^{14}C] aminoacyl-tRNA, 150 µg chloramphenicol per ml and 1.0 mg α-casein per ml. The reaction was initiated by addition of enzyme. Incorporation of radioactivity into material insoluble in hot 5% trichloroacetic acid was determined on 40 µl aliquots by the filter paper disc technique (10) after incubation for 5 min at 37°.

was copurified with leucyl,phenylalanyl-tRNA protein transferase over approximately a 1000-fold range (Table I), thus providing biochemical evidence that it is due to the same enzyme. Activity of the purified enzyme was completely dependent upon the presence of α-casein. Acceptor specificity for methionyl transfer was examined by testing dipeptides containing various NH_2-terminal residues linked to alanine for their ability to (i) inhibit α-casein-dependent transfer of [^{14}C] methionine from tRNA into hot acid-insoluble material and (ii) give rise to enzymatically generated radioactive products when substituted for α-casein as acceptor (4). The dipeptides were tested at 25 mM and included Lys-Ala, Arg-Ala, Trp-Ala, Asp-Ala, Ser-Ala, Pro-Ala, Gly-Ala, Met-Ala, Cystinyl-bis-Ala, Ile-Ala, Leu-Ala and Phe-Ala. Only Arg-Ala and Lys-Ala fulfilled both criteria for acceptance. Phe-Ala, Asp-Ala and Trp-Ala were also inhibitory. However, no peptide-dependent, enzymatically generated product could be demonstrated in reaction mixtures containing them under conditions where the presence of Lys-Ala or Arg-Ala resulted in transfer of 45% of the radioactivity from tRNA to a peptide-dependent derivative. The remaining dipeptides were not inhibitory.

Table II

Polyribonucleotide Donor Specificity for the Transfer of Methionine

tRNA	Incorporation (pmoles)
$Met\text{-}tRNA_m^{Met}$	28
$Met\text{-}tRNA_f^{Met}$	0.9
$Met\text{-}tRNA_f^{Met}$ + $Met\text{-}tRNA_m^{Met}$	29

Reaction mixtures were similar to those described in Table I and contained 0.2 μg of enzyme purified through the second phosphocellulose step. The concentration of each [^{14}C] methionyl-tRNA was 2 μM. Incorporation was determined using 40 μl aliquots after incubation for 60 min.

These results suggest that acceptor substrate specificity for methionine depends on a basic NH_2-terminal aminoacyl residue as has been established (4) for the transfer of leucine and phenylalanine.

Donor substrate specificity with respect to methionyl transfer by the purified enzyme was examined using the different isoaccepting species of tRNA found in E. coli. There was a marked preference for Met-$tRNA_m^{Met}$ as shown in Table II.

Confirmatory genetic evidence that methionyl transfer activity is due to leucyl,phenylalanyl-tRNA-protein transferase was obtained with a mutant previously shown to be defective in the enzyme (5). Activity for the transfer of methionine, like those for leucine and phenylalanine, was lost in the mutant and reacquired in the spontaneous revertant (Table III).

Table III

Transfer Activities for Leucine, Phenylalanine and Methionine in Soluble Extracts of K12 Strains

Strain	Amino acid		
	Phenylalanine	Leucine	Methionine
	(pmoles/min/mg)		
W4977	6.1	6.8	2.1
MS845	<0.03	<0.03	<0.03
R18	6.6	6.7	2.0

Activities were determined as described in Table I.

DISCUSSION

These data indicate that leucyl,phenylalanyl-tRNA-protein transferase is responsible for the soluble methionine incorporating activity discovered in E. coli by Horinishi et al. (7). This conclusion is not inconsistent with

our earlier studies on the enzyme, since methionine was not present in the mixture of 15 radioactive amino acids originally used (2) to characterize donor specificity. Of special interest is the fact that methionine is transferred from Met-$tRNA_m^{Met}$ but scarcely at all from Met-$tRNA_f^{Met}$. The enzyme has previously been shown to distinguish different aminoacyl residues esterified to identical polyribonucleotide chains (3). However, this is the first example of discrimination between different intact polyribonucleotide chains containing a donor aminoacyl residue.

Methionine is the major NH_2-terminal residue found in bulk E. coli protein (11). Its presence there is thought to derive from the participation of fMet-$tRNA_f^{Met}$ in the initiation of protein synthesis (12). Post-translational enzymatic transfer should be considered as an alternative explanation for those proteins containing NH_2-terminal methionine linked to a basic amino acid.

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