A SOLUBLE ENZYME FROM ESCHERICHIA COLI WHICH CATALYZES THE TRANSFER OF LEUCINE AND PHENYLALANINE FROM tRNA TO ACCEPTOR PROTEINS

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ABSTRACT - A soluble enzyme which catalyzes the transfer of leucine and phenylalanine from tRNA to protein has been partially purified from Escherichia coli. No activity is observed with other amino acyl tRNAs. The transfer reaction does not require magnesium ions but does require a monovalent cation and an acceptor protein such as bovine serum albumin.

Kaji and his coworkers (Kaji, Kaji and Novelli, 1965 a and b; Momose and Kaji, 1966) have described the incorporation of leucine and phenylalanine into protein by soluble fractions from E. coli. They have shown that amino acyl tRNA is an intermediate in the reaction and that the incorporated amino acid maintains a free alpha amino group. We have recently demonstrated that a comparable type of incorporation of arginine by soluble mammalian extracts is due to a specific enzyme, the arginyl tRNA-protein transferase, which catalyzes the transfer of arginine from tRNA to peptide linkage with the amino terminal residue of certain acceptor proteins (Soffer, 1968; Soffer and Horinishi, 1969). It seemed likely to us that the results of Kaji et al. could be attributed to a similar type of transfer enzyme in E. coli.

The assay which we have employed for this enzyme is the incorporation of radioactivity from leucyl-^{3}H tRNA and phenylalanyl-^{14}C tRNA into hot trichloroacetic acid-insoluble material. Magnesium ions are omitted from the reaction mixture so that incorporation which might occur as a result of ribosomal contamination is eliminated. The reaction is carried out in the presence of bovine serum albumin, which is the standard acceptor protein for the arginyl tRNA-protein transferase and appears to function similarly in the leucine-phenylalanine transfer reaction.

Our results indicate that there is a soluble enzyme(s) in E. coli which transfers these amino acids from their respective tRNAs to acceptor proteins.

Thus, although their function is not yet clear, specific amino acyl tRNA-protein transferases appear to occur in widely differing organisms.

MATERIALS AND METHODS

Cells of E. coli, Strain B, harvested in mid-log growth were purchased from Miles Laboratories, Elkhart, Indiana. Salmonella typhimurium LT-2, Strain G-30 was provided by Dr. R. Bevill. Stripped E. coli B tRNA was a product of General Biochemicals Corp., Chagrin Falls, Ohio. Uniformly labeled L-phenylalanine-^{14}C, 409 μc/μmole, was obtained from the New England Nuclear Corp., Boston, Mass., and L-leucine-4,5-^{3}H, 5500 μc/μmole, from Tracerlab, Waltham, Mass. Amino acyl tRNAs were prepared as described elsewhere (Soffer and Fernbach, 1967), using a modification of the technique of Von Ehrenstein and Lipmann (1961), and contained 2.16 mμmoles of L-leucine-^{3}H and 0.412 mμmoles of L-phenylalanine-^{14}C per mg of tRNA.

A mixture of 15 highly purified uniformly labeled L-amino acids-^{14}C was obtained from the New England Nuclear Corp. This mixture was used to prepare an amino acyl-^{14}C tRNA mixture (1.11 x 10^{6} cpm/mg tRNA) using the method described above. Similar preparations were made with the reaction mixtures containing 0.01 M leucine-^{12}C and phenylalanine-^{12}C, or arginine-^{12}C, representing an excess of at least 200-fold over the labeled amino acids. These preparations resulted in amino acyl tRNA mixtures containing 0.744 x 10^{6} cpm/mg tRNA (leucine and phenylalanine radioactivity diluted) and 1.10 x 10^{6} cpm/mg tRNA (arginine radioactivity diluted).

Proteins were estimated by the method of Lowry et al. (1951).

Enzyme assays were performed using reaction mixtures which contained 0.05 M Tris-HCl, pH 8.6; 0.16 M KCl; and 5 mM 2-mercaptoethanol. Amino acyl tRNA, bovine serum albumin, and enzyme were added as indicated. Aliquots were removed after various time intervals at 37° and incorporation of radioactivity into hot TCA-insoluble material was determined on filter paper discs (Mans and Novelli, 1960) which were processed as described elsewhere (Soffer and Mendelsohn, 1966). A control omitting enzyme was always included.

RESULTS

A) Enzyme Purification. The procedure employed will be described elsewhere in greater detail. All buffers contained 5 mM 2-mercaptoethanol. A 105,000 x g supernatant fraction was prepared from an alumina extract in

Table I

Enzyme Purification

Fraction	Total Protein (mg)	Total Activity (units)	Specific Activity (units/mg)	Purification	Recovery (%)
S105	5880	12.8/3.02	0.0022/0.00052	1/1	100/100
Protamine	225	6.5/1.39	0.029 / 0.0062	13.2/12.0	50.8/46.0
Sephadex G-100	45.5	6.6/1.37	0.146 / 0.030	66.7/58.5	51.6/45.3

Enzyme assays (0.1 ml) were performed as described in MATERIALS AND METHODS in the presence of 5 mg/ml bovine serum albumin; in separate reactions leucyl-^{3}H and phenylalanyl-^{14}C tRNA concentrations were 1.27 and 10.3 mg/ml, respectively. Aliquots of 50 µl were tested for hot TCA-insoluble radioactivity after 6 minutes. The data are expressed as leucine/phenylalanine incorporation. One enzyme unit corresponds to the incorporation of 1 mµmole of amino acid per minute.

the presence of DNAase by conventional techniques. The dialyzed extract at a concentration of 10 mg/ml in 20 mM Tris-HCl, pH 6.5 was treated with 0.3 volumes of 0.5% protamine sulfate in the same buffer. The resulting precipitate was washed with 0.2 M NaH_2PO_4, pH 6.5 and with 50 mM NaH_2PO_4 pH 6.5, 0.1 M $(NH_4)_2SO_4$. The enzyme was then extracted with 50 mM NaH_2PO_4, 0.5 M $(NH_4)_2SO_4$ (extracting buffer). The protamine eluate was then subjected to gel filtration on Sephadex G-100 equilibrated in extracting buffer. The active fractions, which were in the retained volume under these conditions, were dialyzed against saturated $(NH_4)_2SO_4$. The precipitate was taken up in a small volume of extracting buffer and dialyzed against this buffer (Sephadex G-100 fraction).

Results of a typical preparation are presented in Table I. Both transfer activities were purified to similar extents. This was found to be the case in three separate preparations which yielded overall purifications of

Table II

Specificity of Amino Acyl tRNA-Protein Transferases

Source of Enzyme	Incorporation (cpm/ml)		
	Amino Acyl-^{14}C tRNA Mixture	Mixture Including Leucyl-^{12}C and Phenylalanyl-^{12}C tRNA	Mixture Including Arginyl-^{12}C tRNA
E. coli, S105 2.0 mg/ml	7440	840	9360
S. typhimurium, S105 1.4 mg/ml	7400	480	7000
E. coli, purified enzyme 0.16 mg/ml	7720	520	7560
Rabbit liver, S105 1.4 mg/ml	20560	26560	280

Reactions were run as described in MATERIALS AND METHODS in the presence of bovine serum albumin (10 mg/ml) and the indicated amino acyl-^{14}C tRNA mixture (10^6 cpm/ml). After 30 minutes incubation hot TCA-insoluble radioactivity was determined on 50 µl aliquots.

60-200-fold. The partially purified preparation was free of leucyl and phenylalanyl tRNA synthetase activities, which were not precipitated in the protamine step. The enzyme preparation was stable for one month at 0° but was rapidly inactivated by desalting procedures such as dialysis or gel filtration at low ionic strength.

B) Specificity of the Transfer Reaction. As shown in Table II the purified enzyme and crude soluble fractions from E. coli and S. typhimurium catalyzed the transfer of radioactivity from a mixture of amino acyl-^{14}C tRNAs into protein. This transfer was reduced by 90% or more when the amino acyl tRNA mixture was prepared in the presence of excess leucine-^{12}C and phenylalanine-^{12}C. When the mixture was prepared with excess arginine-^{12}C there was no effect on the transfer. On the other hand with a crude supernatant fraction from rabbit liver dilution with arginyl-^{12}C tRNA completely eliminated the transfer of radioactivity, whereas dilution with leucyl-^{12}C and phenylalanyl-^{12}C tRNA's was without effect. The data demonstrate that in the bacteria tested soluble amino acyl tRNA-protein transferase activity is specific for leucine and phenylalanine whereas in rabbit liver it is specific for arginine.

As shown in Table III the transfer of both leucine and phenylalanine with the purified enzyme preparation was highly dependent upon the presence of bovine serum albumin. With the crude extracts no dependence was observed. The albumin retained much of its stimulatory effect even after boiling, whereas similar treatment of the enzyme completely abolished incorporation. When the reaction was run in the absence of albumin, and subsequently albumin was added together with ribonuclease to prevent further incorporation from amino acyl tRNA, no increase in hot TCA-insoluble radioactivity was observed. Albumin is thus acting as an acceptor and not simply as a carrier for an endogenously formed product. This interpretation was supported by the fact that incorporated leucine and phenylalanine co-electrophoresed with albumin.

Incorporation was also found to require a monovalent cation. Sodium, potassium, and ammonium were effective, whereas magnesium was not. The stimulation of leucine transfer by these cations was somewhat greater than that of phenylalanine.

Table III also demonstrates that ribonuclease abolished transfer of both amino acids, but a 500-fold excess of unlabeled amino acid did not sig-

Table III

Requirements and Inhibitors of Incorporation of Leucine and Phenylalanine into Hot TCA-Insoluble Material

	μμMoles of Amino Acid Transferred per ml	
	Leucine	Phenylalanine
Complete system	49	30
boiled (1 min) enzyme	<1	<1
boiled (1 min) bovine serum albumin	29	23
minus bovine serum albumin	10	5
plus pancreatic RNAase (0.1 mg/ml)	3	1
plus unlabeled amino acid (1 mM)	45	27
minus KCl	9	15
minus KCl plus K_2SO_4	37	29
minus KCl plus NaCl	55	30
minus KCl plus $(NH_4)_2SO_4$	50	26
minus KCl plus $MgSO_4$	11	3

The complete system was as described in MATERIALS AND METHODS, and included bovine serum albumin (10 mg/ml). Separate reactions (0.15 ml) were run to measure leucine and phenylalanine incorporation. Leucine incorporation experiments included purified enzyme protein (48 μg/ml) and leucyl-^{3}H tRNA (1.27 mg/ml); phenylalanine incorporation was assayed with purified enzyme protein (97 μg/ml) and phenylalanine-^{14}C tRNA (3.45 mg/ml). All cations were used at a concentration of 0.16 M. Hot TCA-insoluble radioactivity was determined on 50 μl aliquots after 30 minute incubations.

nificantly dilute incorporation. These data provide further evidence that the transfer of the amino acid is directly from tRNA to protein.

DISCUSSION

These results indicate that E. coli possesses a soluble enzyme(s) which specifically catalyzes the transfer of leucine and phenylalanine from tRNA to acceptor proteins. Magnesium ions are not required for this reaction which clearly differentiates it from the transfer of amino acids associated

with the synthesis of proteins de novo. This enzymatic activity is presumably responsible for the original observations made by Kaji and his coworkers.

The evidence thus far suggests that one enzyme mediates the transfer of both leucine and phenylalanine. Both activities were purified together and displayed similar requirements for a monovalent cation and a non-enzymatic protein acceptor. Moreover, using the partially purified enzyme we have found identical inactivation kinetics at 60°.

We have suggested that the amino acyl tRNA-protein transferases may provide a mechanism for regulating the function of acceptor proteins (Soffer and Horinishi, 1969). The use of purified enzymes as acceptor molecules will allow us to test this hypothesis.

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