Competitive Interaction of *Escherichia coli* Leucyl-Transfer-Ribonucleic Acids with the Enzyme-Leucyladenylate Complex

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An aminoacyl-tRNA synthetase must undergo two successive reactions to esterify tRNA (Hoagland, Stephenson, Scott, Hecht & Zamecnik, 1958). The process involves first activation of the amino acid with ATP with the formation of the enzyme-aminoacyladenylate complex and secondly the transfer of the activated amino acid to cognate tRNA to form the charged tRNA. Though tRNA may be required for some amino acids in the first reaction (Mehler & Mitra, 1967), it is generally thought that interaction of tRNA with the aminoacyl-tRNA synthetase is in the second reaction. It is therefore desirable to eliminate the first reaction (amino acid activation) and to examine only the second reaction by using the enzyme-aminoacyladenylate complex isolated *in vitro* for the study of the interaction between tRNA species and the aminoacyl-tRNA synthetases (Norris & Berg, 1964; Kondo & Woese, 1969). Here I report that tRNA^{Leu}_I and tRNA^{Leu}_{II} fractions from a column of methylated albumin on kieselguhr can compete for the same enzyme-leucyladenylate complex *in vitro*. This result confirms several earlier observations that a single leucyl-tRNA synthetase can recognize different leucyl-tRNA fractions in *Escherichia coli* (Yamane & Sueoka, 1964; Bennett, 1969).

E. coli K 12 leucine tRNA was chromatographed on a column of methylated albumin on kieselguhr to give two fractions, tRNA^{Leu}_I and tRNA^{Leu}_{II} (Kondo, 1967). The tRNA^{Leu}_I fraction includes tRNA species recognizing codons CUA and CUG, and the tRNA^{Leu}_{II} fraction includes tRNA species recognizing CUU, CUC and UUG codons (Kan, Kano-Sueoka & Sueoka, 1968). The enzyme-leucyladenylate complex was prepared *in vitro* as described by Kondo & Woese (1969).

Fig. 1 illustrates the kinetics of [³H]leucyl-tRNA synthesis with enzyme-leucyladenylate and the tRNA fractions. The addition of a small amount of tRNA^{Leu}_I fraction substantially lowers the initial rate of leucine transfer with tRNA^{Leu}_{II} fraction (Fig. 1). This is not due to some non-diffusible inhibitor(s) in the tRNA^{Leu}_{II} fraction because the same fraction does not affect the rate of lysyl-tRNA synthesis and the inhibition of leucyl-tRNA syn-

thesis can be overcome after a longer period (2h) of incubation (Fig. 1).

The observed decrease in the initial rate of reaction indicates that some tRNA species in the tRNA^{Leu}_I fraction binds more strongly to the

Fig. 1. Effect of tRNA^{Leu}_I fraction on leucyl-tRNA synthesis with the enzyme-leucyladenylate complex and tRNA^{Leu}_{II} fraction. *E. coli* K 12 tRNA (20 mg) was chromatographed on a column of methylated albumin on kieselguhr as previously described (Yamane & Sueoka, 1963; Kondo, 1967). tRNA^{Leu}_I and tRNA^{Leu}_{II} fractions were pooled and dialysed overnight against 0.1M-tris-HCl buffer, pH 8.8; the leucine-acceptor activities of the pooled fractions were 146 and 72.2 pmol/0.35 *E*₂₆₀ unit respectively. Enzyme-[³H]leucyladenylate complex (2500 c.p.m./2.6 pmol; [³H]leucine, 5.05 mCi/μmol; New England Nuclear Corp., Boston, Mass., U.S.A.) was incubated at 0°C with tRNA^{Leu}_{II} fraction (8.4 pmol) in the reaction mixture (0.55 ml), which contained (final concentrations) 50 mM-sodium succinate, pH 6.0, 50 mM-KCl, 10 mM-2-mercaptoethanol and 1 mM-Na₂EDTA (curve 1). To the separate reaction mixtures described above was added tRNA^{Leu}_I fraction (0.84 pmol, curve 2; 1.7 pmol, curve 3; 3.4 pmol, curve 4; 5.1 pmol, curve 5). Then 0.1 ml portions were removed at 1, 10, 30, 60 and 120 min, and [³H]leucyl-tRNA formed was collected on nitrocellulose filters (Schleicher und Schüll B6) as cold-5%-trichloroacetic acid-insoluble material. The radioactivities of the dried filters were counted under toluene-based scintillation fluid.

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enzyme-leucyladenylate complex than do other leucine tRNA species, but the rate of leucine charging of this tRNA on the enzyme complex might be lower. Kinetic analysis of the reaction shows competitive interaction of leucine tRNA species for the enzyme-leucyladenylate complex. The affinity constant of this tRNA^{Leu} species is about an order of magnitude lower than that of other leucine tRNA species (M. Kondo, unpublished work). In addition, the results of Fig. 1 support the observation that a single leucyl-tRNA synthetase exists in *E. coli* (Yamane & Sueoka, 1964; Bennett, 1969). Further, experiments on the thermal inactivation of the leucyl-tRNA synthetase at 45°C revealed a first-order rate of inactivation with the same slope for both leucine tRNA fractions. This is also in accordance with the results of Yamane & Sueoka (1964). The multiple forms of leucyl-tRNA synthetase in *E. coli* reported by Yu (1966) might be explained by a possible aggregation of this synthetase.

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