PEPTIDYL TRANSFERASE ACTIVITY OF Escherichia coli RIBOSOMES HAVING AN ALTERED PROTEIN COMPONENT IN THE 50S SUBUNIT

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SUMMARY

Ribosomes from the mutants of Escherichia coli having an altered specific 50S ribosomal protein component showed much the same ability to bind N-acetylphenylalanyl-tRNA in the presence of polyuridylic acid but were found to have distinctly lower peptidyl transferase activity than those from parent Q13 cells. The ribosomes "less active" in peptidyl transferase activity have been shown to be convertible to the "active" state by changing the environmental K^+ or NH_4^+ concentration.

We have previously found that ribosomes from most erythromycin resistant mutants of E. coli have a much reduced erythromycin binding ability and a much lower polypeptide synthesizing activity than those from parent Q13 cells at low concentrations of K^+ or NH_4^+ (1, 2). The chromatographic analysis of protein components of such erythromycin resistant ribosomes revealed that in these ribosomes a specific protein component of the 50S subunit, the 50-8 protein component, was mutationally changed, and that the altered component was eluted earlier from a CM-cellulose column than was its counterpart from the Q13 ribosomes. The 201 ribosomes, which have a distinctly lower peptide synthesizing activity, were found to contain a 50-8 component, which eluted much earlier than that found in other mutant ribosomes (2). These facts may suggest that the structural alteration of this protein component leads the 50S ribosomal subunit to the "less active" state for peptide synthesis. We could also show that these ribosomes were convertible into the "active" state favorable to protein synthesis by increasing the K^+ or NH_4^+ concentration (2).

Peptidyl transferase, the enzyme responsible for peptide bond formation, appears to

be located on the ribosomes (3-5) as a component of the 50S subunit (6, 7). Further investigation revealed that this enzyme activity was reversibly altered by changing the environmental K^+ or NH_4^+ concentration (8).

In this paper the peptidyl transferase activity of the ribosomes having an altered ribosomal protein component was examined at various concentration of K^+ (and NH_4^+).

MATERIALS AND METHODS

The bacterial strains used for this study were E. coli Q13 and erythromycin resistant mutants (QE005 and QE201) isolated from Q13 strain by treatment with mutagens (2). Ribosomes prepared from each strain were washed with an NH_4Cl solution, according to Nishizuka and Lipmann (9). N-acetyl-^{14}C-phenylalanyl-tRNA (N-acetyl-^{14}C-Phe-tRNA) was prepared by the method of Lapidot et al. (10). As a model of the peptidyl transfer reaction, we used the reaction between N-acetyl-^{14}C-Phe-tRNA and puromycin, the activity being determined by measuring the release of N-acetyl-^{14}C-phenylalanine from tRNA by puromycin (11). The reaction mixture contained the following components in a final volume of 125 µl; 50 mM Tris-HCl (pH 7.8), 16 mM magnesium acetate, 10 mM 2-mercaptoethanol, 30 µg of N-acetyl-^{14}C-Phe-tRNA (13,000 cpm), 50 µg of polyuridylic acid, 5.0 A_{260} units of ribosomes, 0.3 mM puromycin, and a specified concentration of K^+ or NH_4^+. Incubation was carried out at 37°C for 8 min. After incubation, 50 µl aliquots were applied to filter paper discs and unreacted N-acetyl-^{14}C-Phe-tRNA was assayed as ordinary aminoacyl-tRNA by cold 5% trichloroacetic acid precipitation (12). In the absence of puromycin, no significant release of N-acetylphenylalanine from tRNA was seen during incubation. The paper chromatographic analysis of the reaction mixture, using n-butanol-pyridine-H_2O (46 : 31 : 23, v/v) as the solvent, confirmed the formation of N-acetyl-^{14}C-phenylalanyl-puromycin. Binding of N-acetyl-^{14}C-Phe-tRNA to ribosomes was determined by adsorption of the ribosomes onto nitrocellulose filters (13). The reaction mixture used for this binding was the same as that described for the

peptidyl transfer reaction, except that puromycin was not added.

^{14}C-Phenylalanine (355 μC/μmole) was obtained from Schwarz BioResearch Inc. Orangeburg, N.Y. (U.S.A.).

RESULTS AND DISCUSSION

As shown in Fig. 1, with the ribosomes from E. coli Q13 and from its mutants, no significant difference was observed in the ability to form the ternary complex of polyuridylic acid, ribosome, and N-acetyl-^{14}C-Phe-tRNA. It is also evident that the ternary complex formation with these ribosome preparations is not significantly affected by the environmental K^+ concentration in the range tested in this experiment. Considered together with the previous findings described above, these facts may indicate that the alteration of 50-8 protein component of ribosomes has little effect on the binding of N-acetyl-^{14}C-Phe-tRNA to E. coli ribosomes.

However, distinct differences in ribosomal peptidyl transferase activity were

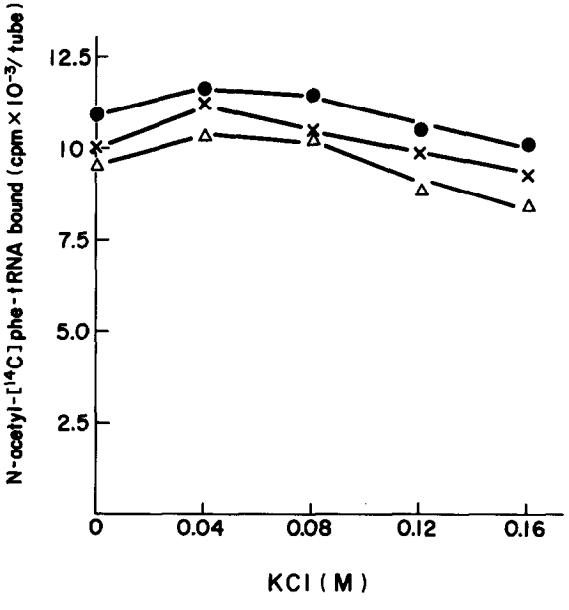

Fig. 1. Comparison of the binding ability of the ribosome preparations from Q13 and its mutants to N-acetyl-^{14}C-Phe-tRNA.
●——● Q13 ribosomes; Δ——Δ QE005 ribosomes; X——X QE201 ribosomes

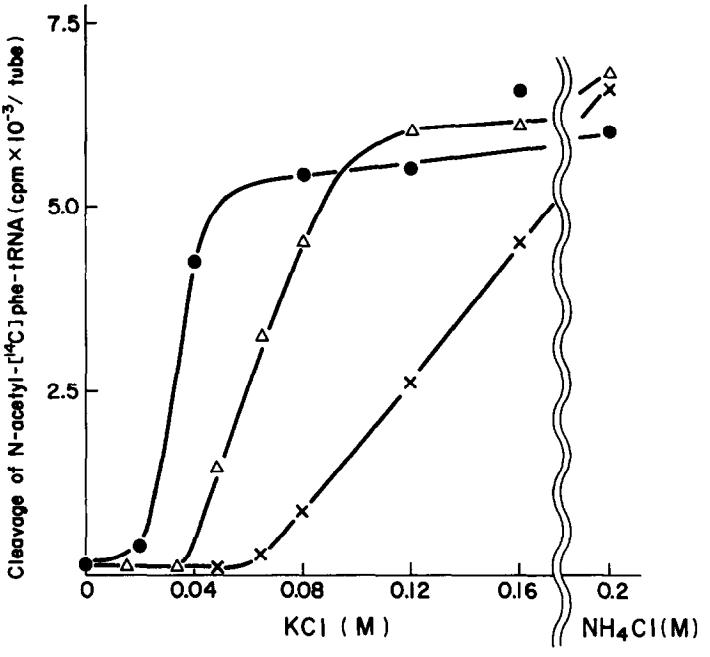

Fig. 2. Comparison of the peptidyl transferase activity of the ribosome preparations from Q13 and its mutants.
●—● Q13 ribosomes; Δ—Δ QE005 ribosomes; X—X QE201 ribosomes

observed among these three ribosome preparations. As shown in Fig. 2, ribosomes having an altered protein component were less active in the puromycin reaction than Q13 ribosomes. QE201 ribosomes especially have much lower activity than either Q13 ribosomes or QE005 ribosomes. The peptidyl transferase activity of these mutant ribosomes increased very sharply with increase in environmental K^+ concentration and reached the same maximum level as that reached by Q13 ribosomes on the addition of 0.2 M NH_4^+. This situation was similar to that found in the previous study of the peptide synthesizing activity of these ribosomes (2). Since the synthesis of peptides includes much more complicated processes and requires many more factors than the reaction of peptidyl transfer, direct comparison of the dependencies of these two reactions on environmental K^+ concentration may not be rational.

Two explanations are given for this finding, though other possibilities may not be

excluded. First, the simplest but less probable, it is suggested that the 50-8 protein component of ribosomes is peptidyl transferase itself and its structural alteration induces the reduction of the activity and alters the dependence on K^+ or NH_4^+ concentration. The second, and perhaps more probable explanation is that the alteration of the chemical structure of the 50-8 protein component resulted in a change in the ability of ribosomes to hold their ordered conformation. This plausible explanation would appear to involve the idea that at a low concentration of K^+ or NH_4^+, altered ribosomes may be no longer able to keep the ordered conformation necessary either to fix the N-acetyl-^{14}C-Phe-tRNA in a position where the puromycin reaction can be catalized by peptidyl transferase or to hold the peptidyl transferase molecule itself in active shape.

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