INACTIVATION AND REACTIVATION OF THE ACCEPTOR BINDING SITE OF RIBOSOMAL PEPTIDYL TRANSFERASE

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1. Introduction

The ribosomal 50 S subunit may assume two conformations differing in biological activity [1, 2]. In one conformation of the 50 S subunit peptidyl transferase (which is a component of this subunit) catalyzes the transfer of the peptide chain from peptidyl-tRNA to puromycin. In the second conformation the enzyme is inactive. The enzymically active conformation is converted to the inactive conformation by removal of NH_4^+ ions from the medium. The biologically inactive conformation can be converted back to the active one upon incubation with ammonium ions at a higher temperature (40°) [1, 2]. The reactivation is markedly temperature-dependent so that practically no reactivation takes place at 0° even in the presence of ammonium ions. A similar temperature dependence was observed by Nomura and Erdmann for the reconstitution of the 50 S subunit from its components [3].

The inactivation of peptidyl transferase which takes place during the change of ribosome conformation may be caused by a decrease of affinity for the corresponding substrate at the donor site, the acceptor site or at both simultaneously. In addition the change in conformation of the 50 S subunit may result in a distortion of the spatial arrangement of the binding sites so that no peptide transfer can take place even if both binding sites are occupied by the proper substrates. In this paper the inactivation mechanisms were studied.

2. Methods

Ribosomes were prepared from *Escherichia coli* B in the logarithmic phase by as previously described [4].

Inactivation of ribosomes was performed according to Miskin et al. [1]: ribosomes (about 20 mg) were dialyzed against 2 × 2 liters 10 mM tris-succinate pH 8.0, 10 mM $MgCl_2$ and 2 mM β-mercaptoethanol at 0° for 20 hr.

Activation of ribosomes was performed according to Miskin et al. [1]: dialyzed ribosomes were incubated with 0.1 volume of 50 mM tris-succinate of pH 8.0, 50 mM $MgCl_2$ and 500 mM NH_4Cl at 40° for 5 min.

CACCA-Ac-^{3}H-Phe and CACCA-^{3}H-Phe fragments were prepared according to Monro et al. [5] by digestion of Ac-^{3}H-Phe-tRNA and ^{3}H-Phe-tRNA by RNase T_1 and were isolated electrophoretically.

The fragment reaction was determined by a modification of Monro's method [5].

The binding of the Phe-pentanucleotide to the ribosomes was determined according to Pestka [6]: The interaction was measured at 0° to prevent the transition of the inactive to active conformation of the 50 S ribosomal subunit, which takes place in the presence of ammonium ions at 24°.

Binding of the AcPhe-pentanucleotide to ribosomes was examined according to Celma et al. [7], using 70 S *E. coli* ribosomes instead of 50 S subunits.

3. Results and discussion

Using inactivated ribosomes, NH_4^+-reactivated ribosomes and ribosomes prepared by a standard technique, we investigated (1) the activity of peptidyl transferase, (2) binding of the 3′-terminal fragment of Phe-tRNA(CACCA-Phe) to the acceptor site, (3) binding of the 3′-terminal fragment of AcPhe-tRNA (CACCA-AcPhe) to the donor site.

Fig. 1 shows the transfer of the AcPhe-residue from CACCA-AcPhe to puromycin, catalyzed by ribosomal peptidyl transferase. The highest activity was found with peptidyl transferase in ribosomes reactivated by incubation with ammonium ions at 40°; the lowest activity was in inactivated ribosomes; the activity of standard ribosomes was intermediate. Our results are practically identical with that of Miskin et al. on the reaction of formylmethionyl-hexanucleotide with puromycin and *E. coli* ribosomes [1].

The binding of the acceptor substrate (CACCA-Phe) to the acceptor site (fig. 2) is affected by the conformation change of the ribosomes similarly to the activity of peptidyl transferase. The greatest amount of the acceptor substrate is bound to reactivated ribosomes, the least amount to inactivated ribosomes, ribosomes prepared by the standard technique showing intermediate values. The relatively higher residual binding of the CACCA-Phe fragment to inactivated ribosomes as compared with the residual activity of peptidyl transferase in inactivated ribosomes is apparently due to partial binding of CACCA-Phe to the donor site of peptidyl transferase [8].

The binding of the donor substrate CACCA-AcPhe to the donor site of peptidyl transferase is shown in fig. 3. There is no fundamental difference between the binding of the pentanucleotide CACCA-AcPhe (and also CACCA-Ac Leu) to the donor site of peptidyl transferase in inactivated, reactivated and standard ribosomes.

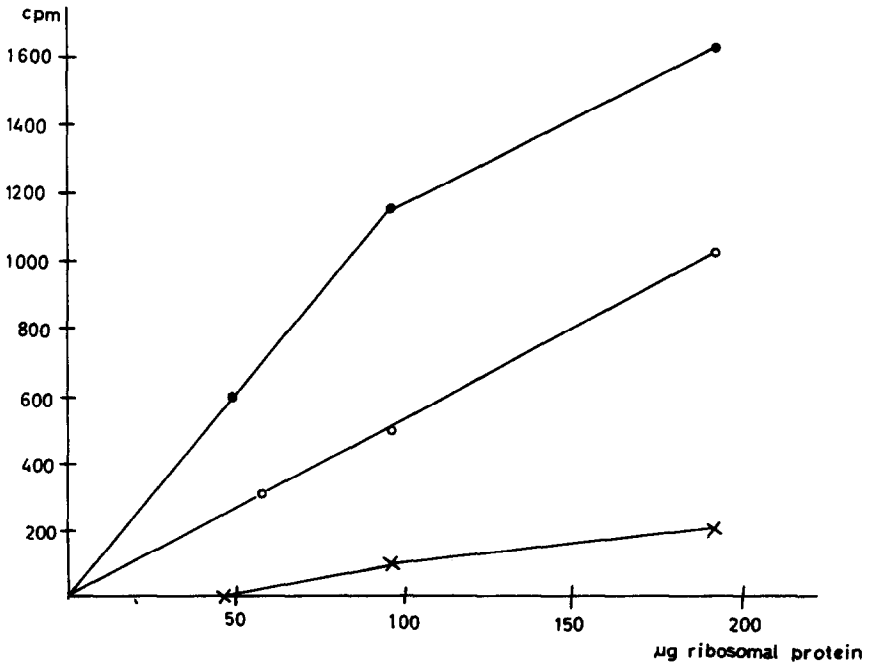

Fig. 1. The fragment reaction of CACCA-Ac-^{3}H-Phe with puromycin and ribosomes prepared by the standard technique, inactivated, and reactivated ones. The reaction mixture contained (before adding methanol) 50 mM tris-HCl of pH 7.4, 400 mM KCl, 20 mM $Mg(OAc)_2$, ribosomes (50–200 μg protein), CACCA-Ac-^{3}H-Phe (1150 cpm, specific activity 21 Ci/mmole) and 250 μM puromycin in 100 μl. The reaction was started by adding 50 μl methanol and the incubation proceeded for 40 min at 0°. It was stopped by adding 100 μl 200 mM NaOAc at pH 5.5 saturated with $MgSO_4$ and the AcPhe-puromycin was extracted with 1.5 ml ethyl acetate. The ethyl acetate phase (1.2 ml) was transferred to a planchet, dried and radioactivity was assayed in a flow counter. o——o ribosomes, x——x inactivated ribosomes, •——• reactivated ribosomes.

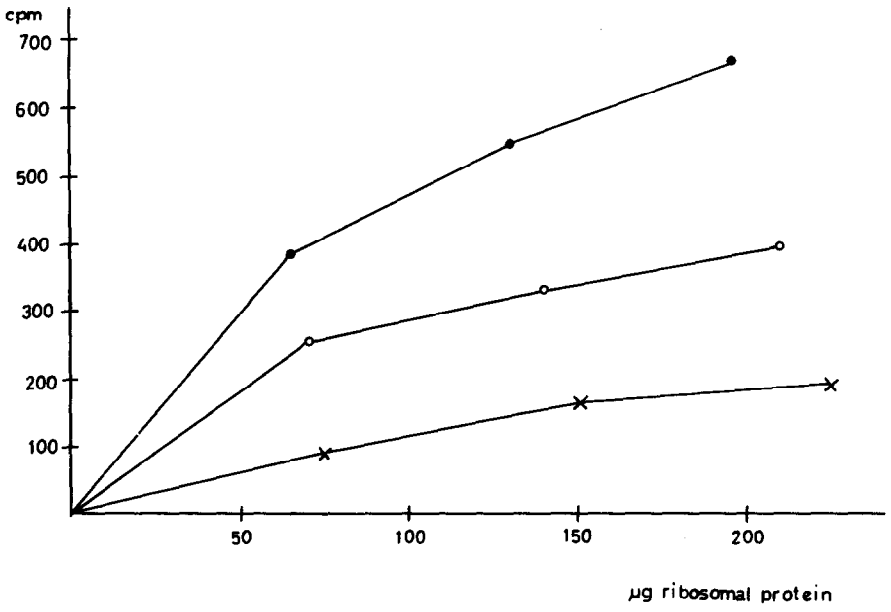

Fig. 2. Binding of CACCA-^{3}H-Phe to ribosomes prepared by the standard technique, inactivated, and reactivated. The reaction mixture contained in 100 μl 50 mM tris-acetate pH 7.2, 100 mM NH_4Cl, 40 mM $MgCl_2$, 400 mM KCl, ribosomes (50–200 μg protein), CACCA-^{3}H-Phe (9500 cpm, specific activity 21 Ci/mmole) and 20 μl ethanol. The medium was incubated for 60 min at 0°, filtered through a Millipore filter, the filter washed with the buffer used in the reaction mixture, dried and the radioactivity assayed in a toluene scintillation liquid. o—o ribosomes, x—x inactivated ribosomes, •—• reactivated ribosomes.

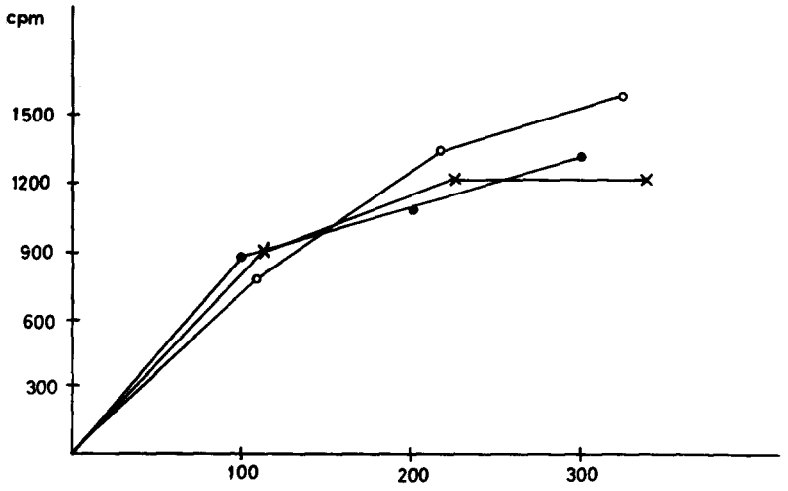

Fig. 3. Binding of CACCA-Ac-^{3}H-Phe to ribosomes prepared by the standard technique, inactivated, and reactivated. The incubation mixture (150 μl) contained 40 mM tris-HCl at pH 7.4, 13 mM $Mg(OAc)_2$, 270 mM KCl, ribosomes (100–300 μg protein), CACCA-Ac-^{3}H-Phe (5200 cpm, specific activity 21 Ci/mmole) and 50% (v/v) ethanol. The medium was incubated for 40 min at 0°, ribosomes centrifuged at 4°, 100 μl supernatant mixed with Bray's scintillation solution and radioactivity assayed. o—o ribosomes, x—x inactivated ribosomes, •—• reactivated ribosomes.

The results indicate that the inactivation of peptidyl transferase observed during the change of conformation of the 50 S subunit in the absence of NH_4^+ ions is caused by the change of conformation of the acceptor site which results in decreased affinity of the acceptor site for acceptor substrates. Under conditions preventing the transition of inactive to active conformation, NH_4^+ ions alone show no effect on the binding of acceptor substrates. The results suggest that the primary effect of the ammonium ions, which are essential for the activity of peptidyl transferase, is to maintain the proper conformation of the acceptor site.

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References

[1] R. Miskin, A. Zamir and D. Elson, Biochem. Biophys. Res. Commun. 33 (1968) 551.
[2] R. Miskin, A. Zamir and D. Elson, J. Mol. Biol. 54 (1970) 355.
[3] M. Nomura and V.A. Erdmann, Nature 228 (1970) 744.
[4] I. Rychlík, J. Černá, S. Chládek, J. Žemlička and Z. Haladová, J. Mol. Biol. 43 (1969) 13.
[5] R.E. Monro, J. Černá and K.A. Marcker, Proc. Natl. Acad. Sci. U.S. 61 (1968) 1042.
[6] S. Pestka, in: Methods in Enzymology, Vol. 12, Part C (1970).
[7] M.L. Celma, R.E. Monro and D. Vazquez, FEBS Letters 6 (1970) 273.
[8] K. Igarashi and A. Kaji, European J. Biochem. 14 (1970) 41.