STIMULATION OF "NON-ENZYMIC" TRANSLOCATION IN RIBOSOMES BY *p*-CHLOROMERCURIBENZOATE

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1. Introduction

It was shown by Pestka [1] and supported in this laboratory [2] that purified *Escherichia coli* ribosomes are capable of accomplishing polyU-directed synthesis of oligo- and polyphenylalanine in the absence of transfer factors (T and G) and guanosine-5′-triphosphate (GTP). This evidently means that in the system studied there is a capability of repetition not only of peptide bond formation but also of translocation without the participation of transfer factors and GTP energy. In contrast to the usual "enzymic" (G-dependent) translocation, this system of "non-enzymic" translocation is not inhibited by guanylyl-5′-methylene diphosphonate and *p*-chloromercuribenzylsulphonate or *p*-chloromercuribenzoate [1, 2].

In this communication it is shown that *p*-chloromercuribenzoate (PCMB) stimulates the "non-enzymic" translocation in ribosomes. In the presence of SH-compounds required for the functioning of a complete protein-synthesizing system the capability of ribosomes for the "non-enzymic" translocation is found to be suppressed.

2. Materials and methods

Ribosomes were isolated from *Escherichia coli* MRE-600 and washed four or six times with 1 M NH_4Cl [1–3].

In experiments on the "non-enzymic" translocation the reaction mixture was prepared in 20 mM $MgCl_2$, 100 mM KCl and 10 mM Tris-HCl $pH_{25°}$ 7.1; 0.05 ml contained 40–60 μg of ribosomes, 20 μg of polyU and 80–100 μg of ^{14}C-phe-tRNA (130,000–170,000 counts/min per mg). The concentration of *p*-chloromercuribenzoate (PCMB) used was from 10^{-4} to 6×10^{-4} M, that of dithiothreitol (DTT) from 10^{-4} to 10^{-3} M and that of mercaptoethanol (ME) 2×10^{-3} M. Incubation was carried out at 25° from 30 min to several hours in different experiments. The reaction was stopped by adding 3 ml of 5% trichloroacetic acid (TCA). Each sample was hydrolyzed for 15 min at 90° and cooled; the precipitate was deposited on a nitrocellulose filter and washed with 5% TCA; the filter was dried at 90° and its radioactivity counted in the standard toluene–PPO–POPOP mixture.

3. Results

Fig. 1 shows that the system consisting of ribosomes, polyU and ^{14}C-phe-tRNA performs the synthesis of TCA-insoluble ^{14}C-polyphenylalanine in the presence of PCMB and practically does not do so in the presence of DTT.

Paper chromatography of alkali-hydrolyzed samples after a relatively short period of incubation of the active system (30 min at 25°) indicates the presence of ^{14}C-oligophenylalanines [2]. This proves that the observed incorporation of ^{14}C-phenylalanine actually represents polymerization of phenylalanine residues. An increase of the incubation time leads to the accumulation of insoluble ^{14}C-phenylalanine-containing products.

Polymerization of phenylalanine residues in the active system is polyU-dependent: in the absence of polyU no incorporation of ^{14}C-phenylalanine into the TCA-insoluble products is observed (fig. 1).

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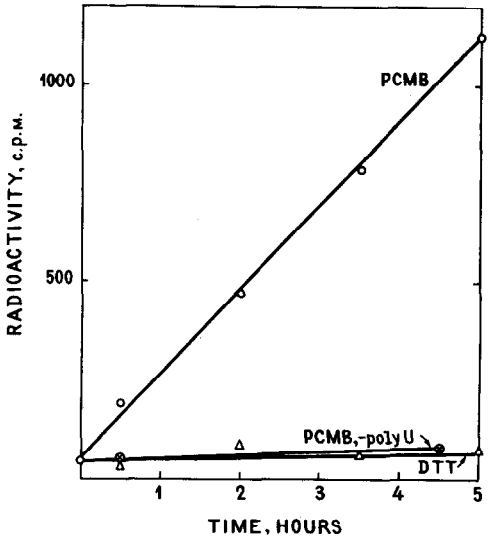

Fig. 1. Effect of PCMB and DTT on the polyU-directed synthesis of polyphenylalanine in the "non-enzymic" (without transfer factors and GTP) cell-free system. PCMB was present in a 10^{-4} M concentration; DTT in a 10^{-3} M concentration. The experiment was carried out at 25°. The amount of TCA-precipitated ^{14}C-phe-phenylalanine is plotted versus incubation time. ○ – with PCMB; △ – with DTT; ⊗ – with PCMB without polyU.

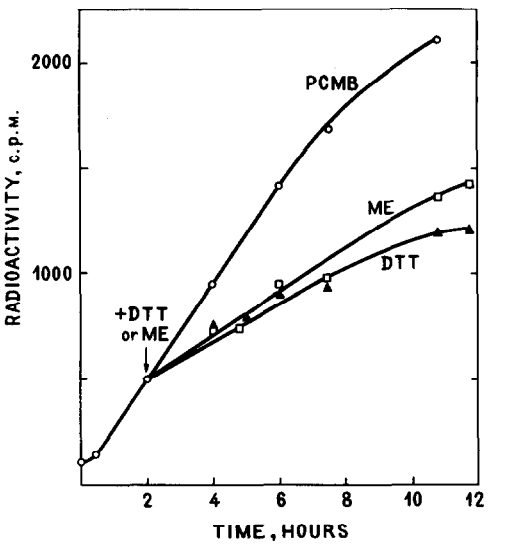

Fig. 2. Effect of adding DTT and ME to the PCMB-stimulated cell-free "non-enzymic" system of polyU-directed synthesis of polyphenylalanine. PCMB was initially present in a concentration of 10^{-4} M. After 2 hr incubation DTT was added up to 10^{-3} M or ME up to 2×10^{-3} M to the experimental sample, while the control sample was further incubated without additions (in the presence of PCMB). The experiment was carried out at 25°. The amount of TCA-precipitated ^{14}C-phenylalanine is plotted versus incubation time. ○ – with PCMB; ▲ – plus DTT; □ – plus ME.

Such antibiotics as chloramphenicol, tetracycline and thiostrepton known to be specific inhibitors of different functions of ribosomes were shown to inhibit the PCMB-activated "non-enzymic" system as well [4].

Thus in the system studied, in the presence of PCMB a real "non-enzymic" translation of the template polyU by ribosomes with the formation of polyphenylalanine is observed. It is natural that the principal distinction between the "non-enzymic" system and the complete "enzymic" cell-free system is the big difference in the rate of translation: the "non-enzymic" system is less efficient.

Fig. 2 shows that if DTT or ME is added to the already working PCMB-activated system, the rate of "non-enzymic" translation decreases (but is not completely suppressed).

Conversely, it is seen in fig. 3 that if the system has been incubated for a few hours with DTT and has not revealed any noticeable polymerizing activity, the addition of PCMB activates it, inducing an accumulation of TCA-insoluble ^{14}C-phenylalanine.

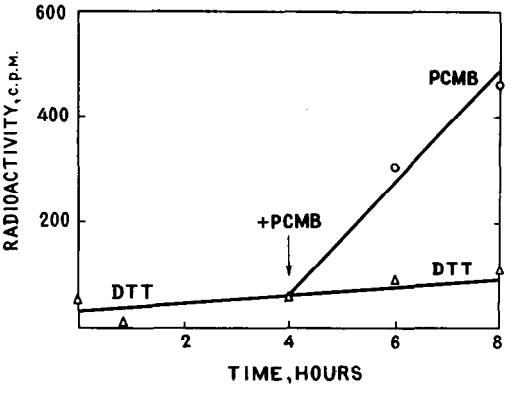

Fig. 3. Effect of adding PCMB to the DTT-inhibited cell-free "non-enzymic" system of polyU-directed synthesis of polyphenylalanine. DTT was initially present in a 10^{-4} M concentration. After 4 hr incubation PCMB was added up to 5×10^{-4} M to the experimental sample, while the control sample was further incubated without the addition (in the presence of DTT). The experiment was carried out at 25°. The amount of TCA-precipitated ^{14}C-phenylalanine is plotted versus incubation time. ○ – with DTT; △ – plus PCMB.

In special experiments we corroborated that DTT and ME do not inhibit either the "non-enzymic" binding of ^{14}C-phe-tRNA to ribosomes in the presence of polyU or the peptidyltransferase activity of ribosomes. Thus, the inhibiting action of DTT and ME and the activating effect of PCMB in the "non-enzymic" system studied are most probably applied to the capacity of ribosomes for spontaneous translocation: in the presence of SH-compounds it is suppressed, while it is activated as a result of oxidation or blocking of some SH-groups of the ribosome by PCMB.

4. Discussion

In a normal cell-free system with the participation of transfer factors and GTP the ribosome always works in the presence of SH-containing compounds such as 2-mercaptoethanol or dithiothreitol. The data presented in this communication indicate that the ribosome in the presence of SH-compounds, i.e., evidently in a normal atmosphere for its working, practically cannot perform "non-enzymic" translocation or does so to a low extent.

Ribosomes washed and incubated without SH-compounds [1, 2] and, as seen from the data presented, ribosomes in the presence of PCMB (fig. 1) are capable of accomplishing translocation without G-factor and GTP. This confirms the idea that in principle the capability for translocation is intrinsic to the structural organization of the ribosome itself and not to the protein transfer factors [2, 4]. The role of the protein transfer factors may be chiefly catalytical.

The main fact found in this study is that PCMB is not indifferent but stimulates "non-enzymic" translocation in ribosomes. It may be presumed that the functional effect of PCMB detected is connected with oxidation or blocking of some ribosomal SH-group (or groups) which is (are) reduced and free in normal conditions. It is probable that just this oxidation or blocking reveals the potential capability of the ribosome itself to carry out translocation. In normal conditions this capability is efficiently realized by G-factor with GTP.

Acknowledgements

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