A MODIFICATION OF THE 30 S RIBOSOMAL SUBPARTICLE IS RESPONSIBLE FOR STIMULATION OF "NON-ENZYMATIC" TRANSLOCATION BY *p*-CHLOROMERCURIBENZOATE

L.P. GAVRILOVA and A.S. SPIRIN
Institute of Protein Research, Academy of Sciences of the USSR, Poustchino, Moscow Region, USSR

Received 5 February 1972

1. Introduction

In a previous communication [1] we have shown that *p*-chloromercuribenzoate (PCMB) stimulates "non-enzymatic" translocation in a polyU-directed system of polyphenylalanine synthesis with purified *Escherichia coli* ribosomes without transfer factors and GTP. Data are presented here that the stimulation is induced by a modification of the 30 S ribosomal subparticle by PCMB.

2. Materials and methods

Ribosomal 30 S and 50 S subparticles were prepared from *E. coli* strain MRE-600 by sucrose gradient centrifugation in the presence of 0.5 M NH_4Cl with 0.001 M $MgCl_2$ [2]. Treatment of ribosomal subparticles with PCMB or dithiothreitol (DTT) was performed in a buffer containing 10 mM Tris-HCl–100 mM KCl–13 mM $MgCl_2$, $pH_{25°}$ 7.1, for 1–2 hr at 25°; the concentration of ribosomal particles was 4–6 mg/ml; the PCMB or DTT concentration was 10^{-4} M or 10^{-3} M, respectively. After such treatment excess PCMB or DTT was removed by gel-filtration through a G-50 Sephadex column (0.9 × 11 cm).

In the experiments on "non-enzymatic" translation the reaction mixture was prepared in a buffer with 10 mM Tris-HCl–100 mM KCl–13 mM $MgCl_2$, $pH_{25°}$ 7.1; 0.05 ml contained 13 μg 30 S subparticles, 26 μg 50 S subparticles, 20 μg polyU (K^+-salt) and 80 μg of ^{14}C-phe-tRNA (150,000 cpm per mg of total tRNA). Incubation was done at 25° for 6 hr. The radioactivity of ^{14}C-polyphenylalanine, insoluble in hot trichloroacetic acid, was determined as described previously [1], every hour during incubation.

3. Results

It is seen in fig. 1 that when both the 30 S and the 50 S subparticles are pre-treated with PCMB, active polymerization of ^{14}C-phenylalanine residues takes place, and that when both subparticles are pre-treated with DTT polymerization is much slower. If only the 30 S subparticle is treated with PCMB and the 50 S subparticle is treated with DTT, then polymerization is practically as active as in the case of PCMB treatment of both subparticles. On the contrary, treatment of the 50 S subparticle with PCMB and the 30 S subparticle with DTT gives a low activity of the system. It follows that activation of the system of "non-enzymatic" translation by PCMB [1] is found to depend on a modification (blocking of SH-groups) only in the small, 30 S subparticle, and not in the 50 S subparticle of the ribosome.

4. Discussion

The data presented provide evidence that the blocking of some SH-group(s) of the 30 S subparticle by PCMB discloses the potential capability of the ribosome to carry out translocation without the G-factor

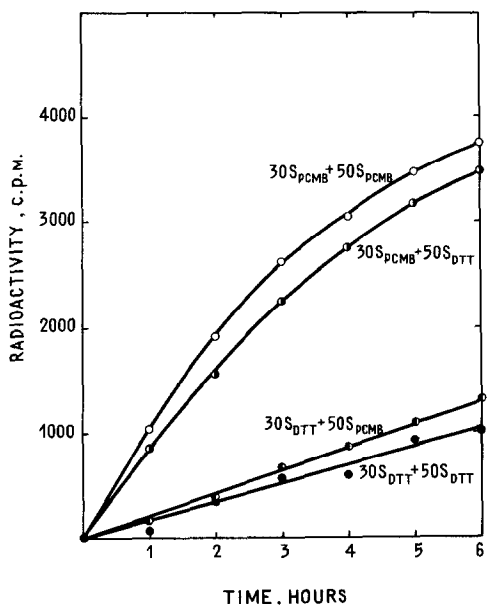

Fig. 1. Effect of preliminary treatment of ribosomal subparticles with PCMB on the polyU-directed synthesis of polyphenylalanine in the "non-enzymatic" cell-free system. The amount of hot trichloroacetic acid insoluble ^{14}C-polyphenylalanine is plotted vs incubation time.(○—○—○): Both 30 S and 50 S subparticles pre-treated with PCMB; (●—●—●): both 30 S and 50 S subparticles pre-treated with DTT; (◑—◑—◑): the 30 S subparticle pre-treated with PCMB, and the 50 S subparticle pre-treated with DTT; (◐—◐—◐): the 30 S subparticle pre-treated with DTT, and the 50 S subparticle pre-treated with PCMB.

and GTP, i.e., "non-enzymatically". Treatment of the 50 S subparticle with PCMB does not affect the "non-enzymatic" translation. At the same time recent investigations of the mechanism of translocation have focused attention on the 50 S subparticle, in particular, as being responsible for the binding of the G-factor [3]. The activation of the capability of the ribosome to carry out "non-enzymatic" translocation as a result of blocking of SH-group(s) in only the 30 S subparticle must suggest the participation of both ribosomal subparticles in the formation of the translocational mechanism of the ribosome.

Acknowledgements

We wish to thank V.E. Koteliansky for help in carrying out the experiments and A.G. Raiher for translating the manuscript into English.

References

[1] L.P. Gavrilova and A.S. Spirin, FEBS Letters 17 (1971) 324.
[2] N.V. Belitsina and A.S. Spirin, J. Mol. Biol. 52 (1970) 45.
[3] Y.W. Bodley and L. Lin, Nature 227 (1970) 60.