INTERACTION OF SH-REAGENTS WITH THE RIBOSOMAL 30 S SUBPARTICLE AND 'NON-ENZYMATIC' TRANSLOCATION

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1. Introduction

It has been shown earlier that *p*-chloromercuribenzoate (PCMB) stimulates the ability of *E. coli* ribosomes to perform polyU-directed synthesis of polyphenylalanine in the absence of transfer factors (EF-T and EF-G) and GTP, i.e., to carry out 'non-enzymatic' translocation [1, 2]. This effect proved to be due to a modification of the 30 S ribosomal subparticle by PCMB [2, 3].

It is shown in this communication that the 30 S subparticle SH-groups, the blocking of which by PCMB is responsible for stimulating the 'non-enzymatic' translocation, are inaccessible to alkylation by iodoacetamide (IAAm) and *N*-ethylmaleimide (NEM). It is further shown that the only protein of the 30 S subparticle practically inaccessible to these alkylating SH-reagents and at the same time accessible to PCMB is S12. The results suggest that the blocking of the protein S12 SH-groups is responsible for stimulating the 'non-enzymatic' translocation.

2. Materials and methods

Ribosomal 30 S and 50 S subparticles were prepared from *E. coli* MRE-600 by sucrose gradient zonal centrifugation in the presence of 0.5 M NH_4Cl with 1 mM $MgCl_2$ [3, 4].

IAAm treatment of 30 S subparticles was done with 10 mM IAAm for 13–14 hr in the cold in a buffer containing 10 mM Tris–HCl–100 mM KCl–13 mM $MgCl_2$, pH 8.0; the concentration of 30 S subparticles was 2–3 mg/ml. After this the suspension of 30 S subparticles was dialyzed against the same buffer but with 0.1 mM dithiothreitol (DTT) and then against the buffer without DTT for a few hours.

Treatment of 30 S subparticles with NEM was performed at a 1 mM concentration of the reagent in the same buffer and under the same conditions as in the experiments with IAAm. The excess reagent was also removed with DTT and by dialysis.

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; 20 μl contained 5 μg of 30 S subparticles (original or pre-treated with IAAm or NEM), 10 μg of 50 S subparticles, 8–10 μg of polyU (K^+-salt, Calbiochem) and 20 μg of total tRNA containing 11–12 pmoles of [^{14}C]phenylalanyl-tRNA (the initial [^{14}C]phenylalanine was from Amersham, England, 513 mCi/mmole); the reaction mixture contained 0.1 mM PCMB (stimulated system) or 1 mM DTT (control system). Incubation was done at 25°C for 5 hr. The radioactivity of hot TCA-precipitated [^{14}C]polyphenylalanine was estimated as described earlier [1, 2] every hour during incubation.

For identification of the reacting proteins [^{14}C]-IAAm (52 mCi/mmole, Amersham, England), [^{14}C]-NEM (2.4 mCi/mmole, Amersham, England) and [^{14}C]PCMB (10.4 mCi/mmole, Schwarz/Mann, USA) were used. [^{14}C]IAAm was first diluted 10-fold with unlabeled IAAm. Treatment of 30 S subparticles with [^{14}C]IAAm and [^{14}C]NEM was performed as described above for the unlabeled reagents. Treatment of 30 S subparticles with [^{14}C]PCMB was done in the same buffer, 10 mM Tris–HCl–100 mM KCl-13 mM $MgCl_2$, $pH_{25°}$ 7.4 at a 0.1 mM concentration of the reagent for 2 hr at 25°C with a following removal of excess PCMB by Sephadex G-50 gel-filtration [3] or

by dialysis in the cold. The proteins were extracted from the 30 S subparticles by 67% acetic acid in the presence of 0.2 M $MgCl_2$ [5, 6].

The proteins were separated by two-dimensional polyacrylamide gel electrophoresis according to Kaltschmidt and Wittmann [7]. The stained regions of the gel corresponding to all the individual proteins of the 30 S subparticle were cut out, dried and then dissolved in 30% H_2O_2 with NH_4OH [8]; the radioactivity of each protein was counted in a Packard scintillation spectrometer in a toluene–PPO–POPOP–Triton X-100 (2:1) mixture [9].

3. Results and discussion

3.1. *Pre-treatment of the 30 S subparticles with IAAm and NEM does not affect the 'non-enzymatic' translocation*

Figs. 1–3 show that pre-treatment of the 30 S subparticles with IAAm (fig. 1) or NEM (fig. 2) or IAAm

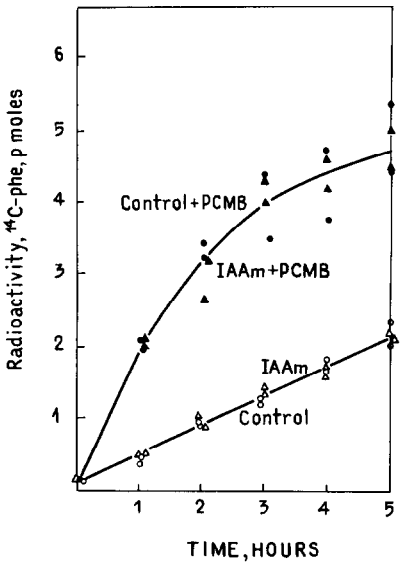

Fig. 1. Effect of pre-treatment of ribosomal 30 S subparticles with IAAm on polyphenylalanine synthesis in the 'non-enzymatic' system. The amount of hot TCA-precipitated [^{14}C]-polyphenylalanine is plotted versus incubation time (25°C). The incubation mixture included: ○ – Not pre-treated (control) 30 S subparticles; ● – Not pre-treated (control) 30 S subparticles plus PCMB; △ – IAAm pre-treated 30 S subparticles; ▲ – IAAm pre-treated 30 S subparticles plus PCMB.

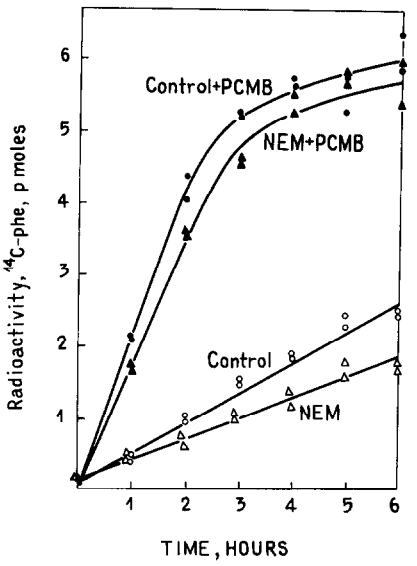

Fig. 2. Effect of pre-treatment of ribosomal 30 S subparticles with NEM on polyphenylalanine synthesis in the 'non-enzymatic' system. Designations are the same as in fig. 1 with the exception that NEM was used instead of IAAm.

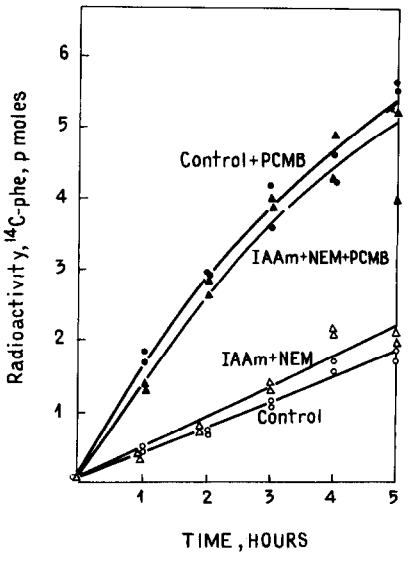

Fig. 3. Effect of pre-treatment of ribosomal 30 S subparticles with IAAm and NEM successively on polyphenylalanine synthesis in the 'non-enzymatic' system. Designations are the same as in fig. 1 with the exception that instead of just IAAm, both IAAm and NEM were applied.

and NEM successively (fig. 3) does not stimulate poly U-directed synthesis of polyphenylalanine in the absence of transfer factors and GTP, i.e., contrary to the effect of PCMB [1–3], it does not activate 'non-enzymatic' translocation.

It is interesting to note that IAAm and NEM pre-treatment of 30 S subparticles in our conditions had no effect either on the poly U-directed binding of phenylalanyl-tRNA with the 30 S subparticle or on the poly U-directed polyphenylalanine synthesis in the complete ('enzymatic') cell-free system [10]. (In contrast, inactivation of some fraction of the 30 S particles by NEM as tested in the complete cell-free system was observed by Traut and Haenni [11] and by Moore [12].)

Figs. 1–3 also show that PCMB treatment of the 30 S subparticle pre-treated with IAAm and NEM gives the same stimulation of 'non-enzymatic' translocation as in the case of 30 S subparticles not pre-treated with IAAm or NEM. Hence, IAAm and NEM do not prevent the consequent stimulating effect of PCMB.

Thus, the SH-groups the blocking of which by PCMB is responsible for stimulating the 'non-enzymatic' translocation are not alkylated by IAAm and NEM within the 30 S subparticle.

3.2. *Different SH-reagents interact with somewhat different sets of proteins within the 30 S subparticle*

The data on two-dimensional gel-electrophoretical analysis of 30 S subparticle proteins pre-treated with [^{14}C]IAAm, [^{14}C]NEM or [^{14}C]PCMB are given in table 1. Table 1 is composed from the results of several independent analyses. The protein nomenclature is given according to Wittmann et al. [13]. Only the proteins containing SH-groups [14] are enumerated in table 1. In the rest of the proteins in the ^{14}C-label was not observed. More detailed results, including quantitative data on the incorporation of the labelled SH-reagents into all the proteins of the 30 S ribosomal subparticle, are published elsewhere [15].

It is seen from table 1 that IAAm alkylates proteins S1, S13, S18 and S21. This is in accordance with earlier data on the effect of IAAm [12] and iodoacetate [16] with one exception: instead of protein S13 Moore indicates protein 4d [12] which is not identified in the nomenclature of Wittmann et al. [13], and Craven and Gupta report protein 11 [16] which is identified as S11 [13]. As the two-dimensional gel electrophoretic protein map according to Wittmann et al., was directly used only in our experiments, we are inclined to think that this divergence is due to an inexact identification of the protein in previous communications [12, 16].

Table 1
Accessibility of ribosomal proteins to SH-reagents within the 30 S subparticle.

SH-containing proteins of the 30 S subparticle	[^{14}C]IAAm	[^{14}C]NEM	[^{14}C]PCMB
S1	++	+++	+++
S2	–	–	–
S4	–	++	+
S8	–	–	–
S11	±	++	+
S12	–	+	+++
S13	+++	++	+
S14	–	–	–
S15	–	+	–
S17	–	++	+
S18	+++	+++	+++
S19	–	–	–
S21	+++	+++	+++

Table 1 shows that NEM alkylates S1, S4, S11, S13, S17, S18, S21 and to a small degree S12 and S15. These data on the whole are in agreement with the results of Moore [12] where a good reactivity to NEM is reported for proteins 13 (S1), 4c (S11), 2b (S18) and 0 (S21), and a lesser reactivity for 3a (S17), 9 (S4) and 4d (S13?). (In contrast, Chang reported recently on reactivity towards NEM of proteins S4, S9, S1.1, S13, S14 and S15 [17]; we cannot understand the reasons of such a great divergence of his results from the data of Moore [12] and ourselves.)

It is seen from table 1 that PCMB reacts with proteins S1, S12, S18 and S21 and also to a lesser degree with S4, S11, S13 and S17. Consequently, the only protein poorly accessible to the alkylating SH-reagents and at the same time highly reactive with PCMB is S12.

The results suggest that it is the modification of protein S12 that is responsible for stimulation of the 'non-enzymatic' translocation by PCMB.

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References

[1] Gavrilova, L.P. and Spirin, A.S. (1971) FEBS Letters 17, 324.
[2] Gavrilova, L.P. and Spirin, A.S. (1972) Molekul. Biol. 6, 311.
[3] Gavrilova, L.P. and Spirin, A.S. (1972) FEBS Letters 22, 91.
[4] Belitsina, N.V. and Spirin, A.S. (1970) J. Mol. Biol. 52, 45.
[5] Hardy, S.J.S., Kurland, C.G., Voynow, P. and Mora, G. (1969) Biochemistry 8, 2897.
[6] Kaltschmidt, E. and Wittmann, H.G. (1972) Biochimie 54, 167.
[7] Kaltschmidt, E. and Wittmann, H.G. (1970) Anal. Biochem. 36, 401.
[8] Goodman, D. and Matzura, H. (1971) Anal. Biochem. 42, 481.
[9] Patterson, M.S. and Greene, R.C. (1965) Anal. Chem. 37, 854.
[10] Gavrilova, L.P., Kostiashkina, O.E. and Rachkus, J.A. Molekul. Biol. in press.
[11] Traut, R.R. and Haenni, A.L. (1967) Eur. J. Biochem. 2, 64.
[12] Moore, P.B. (1971) J. Mol. Biol. 60, 169.
[13] Wittmann, H.G., Stöffler, G., Hindennach, I., Kurland, C.G., Randall-Hazerbauer, L., Birge, E.A., Nomura, M., Kaltschmidt, E., Mizushima, S., Traut, R.R. and Bickle, T.A. (1971) Mol. Gen. Genet. 111, 327.
[14] Craven, G.R., Voynow, P., Hardy, S.J.S. and Kurland, C.G. (1969) Biochemistry 8, 2906.
[15] Gavrilova, L.P., Smolianinov, V.V. and Spirin, A.S., Dokl. Akad. Nauk SSSR, in press.
[16] Craven, G.R. and Gupta, V. (1970) Proc. Natl. Acad. Sci. U.S. 67, 1329.
[17] Chang, F.N. (1973) J. Mol. Biol. 78, 563.