THE POSSIBLE ROLE OF SULFHYDRYL GROUPS IN THE DIMERIZATION OF 70S RIBOSOMES FROM ESCHERICHIA COLI[1]

Jerry Hsueh-Ching Wang[2] and A. T. Matheson

Division of Biosciences, National Research Council,

Ottawa, Canada

Received May 2, 1966

The amount of 100S (dimer) and 70S (monomer) ribosomes in extracts of Escherichia coli is dependent on the ionic environment; increased concentrations of either Mg^{+2} (Tissieres et al, 1959) or spermidine (Cohen and Lichtenstein, 1960) favor dimer formation while increased concentration of K^{+} favors monomer formation (Capecchi, 1964). However, under given ionic conditions, for example 10^{-2} M Mg^{+2}, the monomer-dimer relationship is also dependent on the physiological state of the cells. The 100S ribosomes predominate in extracts from cells from stationary phase cultures, while extracts from cells in early log phase cultures contain nearly equal amounts of 70S and 100S particles (McCarthy, 1960; Feiss and DeMoss, 1965). It has also been shown that on addition of glucose to cells in a culture medium depleted of glucose, there is a rapid transition from 100S ribosomes to 70S ribosomes as exponential growth commences (McCarthy, 1960). These results suggest that other factors besides the ionic environment may control the interconversion of 100S to 70S ribosomes in vivo.

---

[1] NRC No. 9045.

[2] National Research Council Postdoctorate Fellow 1965-66.

In this communication it is shown that the interaction of E. coli ribosomes with certain sulfhydryl reagents results in the conversion of the dimeric form of the ribosome into the monomeric form.

E. coli B (NRC No. 745) were grown in Medium C of Roberts et al (1955) and the ribosomes were isolated and washed repeatedly with TSM 10^{-2} buffer[3] as previously described (Matheson and Tsai, 1965).

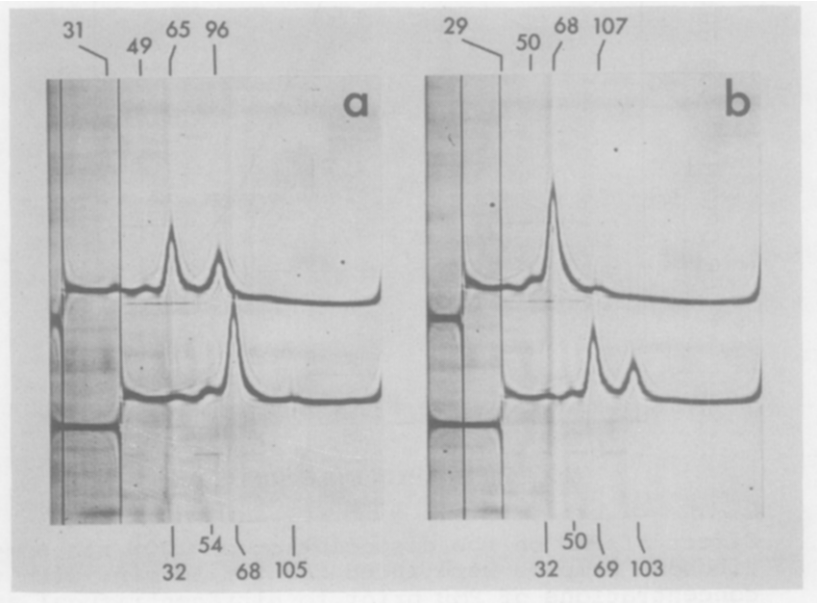

Fig. 1 Effect of p-mercuribenzoate (PMB) and β-mercaptoethanol on the sedimentation of E. coli ribosomes.
a) Upper - Ribosomes (in TSM 10^{-2} buffer) from cells in exponential growth
Lower - The above after incubation in 5 x 10^{-4} M PMB for 1 hour at 25°C.
b) Upper - PMB treated ribosomes (a), lower) after incubation for 1 hour in 2 x 10^{-2} M Mg^{+2}.
Lower - PMB treated ribosomes after incubation for 1 hour in 10^{-3} M β-mercaptoethanol.
Picture a) was taken 9 minutes and picture b) 11 minutes after centrifugation reached 35,600 r.p.m. Sedimentation constants, as indicated in the figure, are uncorrected. Sedimentation is from left to right.

[3] TSM 10^{-2} is 0.01M Tris-succinate pH 7.6 containing 10^{-2} M Mg^{+2}.

When the ribosomes were treated with 5×10^{-4} M p-mercuribenzoate (PMB) all the 100S dimers were converted to the 70S form (Fig. 1a). This conversion could be reversed by the addition of β-mercaptoethanol (Fig. 1b lower), but not by 2×10^{-2} M Mg^{+2} (Fig. 1b upper) or 5×10^{-4} M spermine.

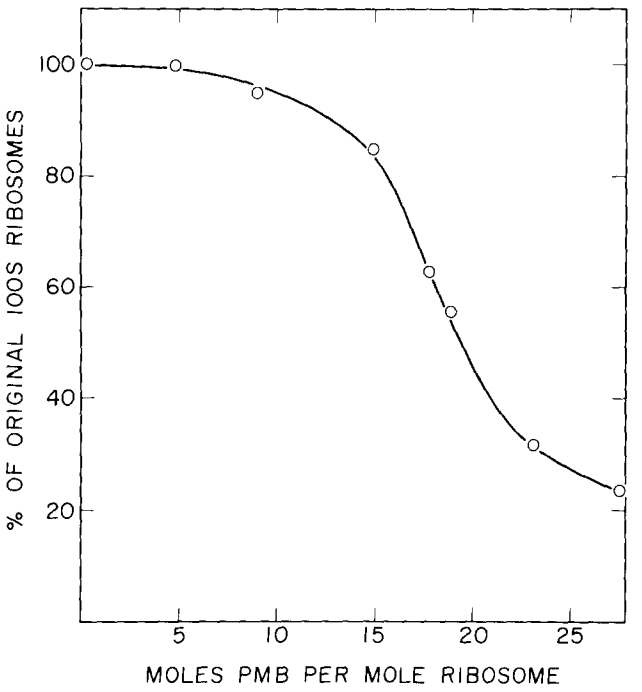

Fig. 2 Effect of PMB on the dissociation of 100S ribosomes. The ribosome samples were incubated for 90 min. with various concentrations of PMB prior to ultracentrifugal analysis. The amount of 100S ribosomes in the samples was determined by measuring the area under each peak. The molar concentration of the ribosomes was calculated on a 70S basis assuming a molecular weight of 2.7×10^6 (Tissieres et al, 1959).

Figure 2 shows the disappearance of 100S ribosomes plotted against the number of PMB molecules per ribosome. Approximately 5-6 moles of PMB is bound per mole of ribosome before dissociation of 100S particles begins. As further PMB is added the 100S particle begins to dissociate into the 70S form.

Other sulfhydryl reagents tested were I_2, iodoacetate, N-ethylmaleimide and 5,5'dithiobis (2-nitrobenzoic acid) (DTNB); of these only treatment with I_2 (2×10^{-4} M at pH 7.5 at 0°C) converted the 100S ribosomes into the 70S form. Since PMB at low concentrations (Boyer, 1959) and I_2 treatment under the conditions used (Cunningham and Nuenke, 1959) are believed to be specific for sulfhydryl groups, the data suggests that sulfhydryl groups may be important in the maintenance of the dimeric form of E. coli ribosomes.

The nature of the ribosomal component containing the SH groups involved in this reaction is unknown although our initial data strongly suggests the SH groups are on the ribosomal proteins. If Mg^{+2} is responsible in the binding of 70S ribosomes to form dimers (Tissieres et al, 1959) it is unlikely that the ribosomal proteins are directly involved since Goldberg (1966) has shown that all the Mg^{+2} in ribosomes is associated with the r-RNA. Furthermore, Marcot-Queiroz and Monier (1965) have reported the dimerization of r-RNA at high Mg^{+2} concentrations which they believe may be equivalent to the 100S ribosome. It is possible, however, that the ribosomal proteins can regulate the dimer formation through their interactions with the RNA moiety. Addition of PMB (presumably onto the SH groups of the ribosomal protein) leads to conformational changes in the protein and prevents the formation of the 100S dimer.

A more detailed account of these and related experiments will be published shortly.

References

Boyer, P.D. in "The Enzymes" (Boyer, P.D., Lardy, H., and Myrback, K., Editors), Vol. I, p. 539, Academic Press, New York (1959).
Capecchi, M., as reported by Watson, J.D., Bull. Soc. Chim. Biol., 46, 1399 (1964).

Cohen, S.S., and Lichtenstein, J., J. Biol. Chem., 235, 776 (1960).
Cunningham, L.W., and Nuenke, B.J., J. Biol. Chem., 234, 1447 (1959).
Feiss, M., and DeMoss, R.D., J. Mol. Biol., 14, 283 (1965).
Goldberg, A., J. Mol. Biol., 15, 663 (1966).
Marcot-Queiroz, J., and Monier, R., J. Mol. Biol., 14, 490 (1965).
Matheson, A.T., and Tsai, C.S., Can. J. Biochem., 43, 323 (1965).
McCarthy, B.J., Biochim. Biophys. Acta, 39, 563 (1960).
Roberts, R.B., Abelson, P.H., Cowie, D.B., Bolton, E.T., and Britten, R.J., Carnegie Inst. of Wash. Public. No. 607 (1955)
Tissieres, A., Watson, J.D., Schlessinger, D., and Hollingworth, R.B., J. Mol. Biol., 1, 221 (1959).