ASSOCIATION OF SUBUNITS IN PURIFIED PREPARATIONS OF *ESCHERICHIA COLI* RIBOSOMES

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1. Introduction

Previous experiments by Miskin et al. [1] and by Zamir et al. [2] have demonstrated that *Escherichia coli* 30 S and 50 S ribosomal subunits, isolated according to conventional techniques, show very low activities with respect to functions related to protein synthesis. These activity losses could be ascribed to exposure to low concentrations of monovalent (NH_4^+ or K^+) or divalent (Mg^{2+}) cations used at some steps during ribosome purification and fractionation. Isolated subunits could be restored to higher levels of activity by a short incubation at elevated temperatures in a proper ionic environment.

Two particular functions were tested by Miskin et al. [1] and Zamir et al. [2]: the non-enzymatic binding of aminoacyl-tRNA to the 30 S subunit and the peptidyl-transferase activity of the 50 S subunit as measured by the "fragment reaction" [3]. We wish to report here our own observations on another function involving both subunits, namely their ability to associate together in the presence of 10 mM Mg^{2+} ions at 0°.

2. Experimental

Cells of the strain *E. coli* RNase I_{-0}, originally isolated by Gesteland [4], were grown in a fermentor in large batches, harvested in early log phase and kept as a frozen paste at −20° until used. Ribosomes were purified according to the procedure of Kurland [5] with the modifications introduced by Likover and Kurland [6]. In further manipulations, all buffers contained 10 mM tris-HCl, pH 7.8, and 6 mM β-mercaptoethanol. The final high-speed sedimentation was done after over-night dialysis against a buffer containing 30 mM NH_4Cl and 10 mM magnesium acetate (standard buffer); the pellets were resuspended into the same buffer and the ribosome suspension was kept in small batches in a liquid nitrogen conservator. Isolation of 30 S and 50 S ribosomal subunits was performed by zonal sedimentation on 5%–20% sucrose gradients prepared in a buffer containing 30 mM NH_4Cl and 0.3 mM magnesium acetate (dissociation buffer). After fractionation, the Mg^{2+} concentration was increased to 10 mM and the subunits concentrated either by high-speed centrifugation or by precipitation with 0.7 volume of 95% ethanol at 0° [7]. The pellets were resuspended in standard buffer and kept in liquid nitrogen.

The distribution of subunits and 70 S ribosomes was determined by sedimentation on 5%–20% linear sucrose gradients prepared in standard buffer at 1° in a Spinco SW 27 or SW 25-3 rotor at 20,000 rpm for 15 hr. The gradients were analysed with an ISCO model D fractionator coupled with an ISCO 610 recorder and the percentage of 70 S was calculated after measurement of peak areas with a planimeter.

3. Results

Sucrose gradient analysis of various preparations of purified ribosomes always showed the simultaneous presence of free subunits and 70 S ribosomes (fig. 1, A) although the percentage of 70 S ribosomes varied between 40 and 65% from preparation to preparation,

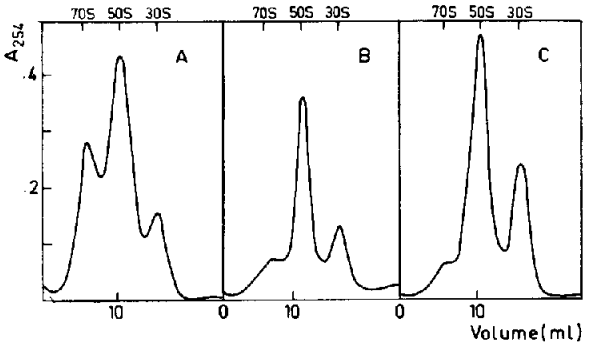

Fig. 1. Distribution of subunits and 70 S particles in various preparations.
A) 10 A_{260} units of purified ribosomes
B) 2.4 A_{260} units of 30 S subunits and 5.0 A_{260} units of 50 S subunits mixed and kept at 0° for 60 min before analysis.
C) 10 A_{260} units of purified ribosomes were dialyzed for 20 hr at 4° against dissociation buffer. After raising the Mg^{2+} concentration to 10 mM, they were kept at 0° for 60 min before analysis.

even when they were prepared from the same batch of cells.

On the contrary when similar analyses were performed on mixtures of isolated subunits kept in standard buffer at 0°, the percentage of 70 S ribosomes was always reduced to about 10%, irrespective of the percentage of 70 S ribosomes present in the preparation from which they were derived (fig. 1,B).

Sucrose gradient sedimentation was not necessary for the occurrence of this loss of afinity between the two subunits, since a sample of unfractionated ribosomes simply dialyzed for 20 hr against the dissociation buffer and returned to the standard buffer at 0° also showed the same reduced percentage of 70 S ribosomes (fig. 1,C). Comparison of the effects of various buffers established that, as was the case with the loss of the aminoacyl-tRNA binding activity of the 30 S subunits [2], the important factor in our case also is the concentration of Mg^{2+} ions. The loss of affinity between the two subunits was regularly observed upon prolonged dialysis against buffers containing either 100 mM or 30 mM NH_4^+ and concentrations of Mg^{2+} equal to or lower than 1 mM.

On the other hand restoration of the affinity could be obtained by incubation at elevated temperatures in standard buffer (table 1). Similar results were also

Table 1
Effect of temperature on the reassociation of 30 S and 50 S subunits.

Temperature °C	percent 70 S
0	13
30	24
40	51
50	63
55	72
60	61

A purified sample of ribosomes was dialyzed at 4° for 20 hr against dissociation buffer. After raising the Mg^{2+} concentration to 10 mM, aliquots were incubated for 10 min at the indicated temperatures, cooled and kept at 0° for 60 min before sucrose gradient analysis.

Table 2
Effect of NH_4Cl concentration on the reassociation of 30 S and 50 S subunits.

NH_4Cl concentration (M)	percent 70 S
0	13
0.1	15
0.2	12
0.5	10
1.0	46
1.5	73

Aliquots of a sample of purified ribosomes dialyzed for 20 hr against the dissociation buffer were dialyzed for 4.5 hr against buffers containing 10 mM Mg^{2+} and the indicated concentrations of NH_4Cl. They were finally dialyzed for 3 hr against standard buffer before sucrose gradient analysis.

obtained upon exposure at 4° to buffers containing 10 mM Mg^{2+} and various concentrations of NH_4Cl (table 2). The percentage of reassociation was sharply dependent upon temperature for ribosomes kept in standard buffer, and upon NH_4^+ concentration for ribosomes kept at low temperature in the presence of 10 mM Mg^{2+}. It should also be pointed out that, upon heating or NH_4Cl treatment, the percentage of 70 S particles always reached a maximum of 70–75% for all our ribosome preparations. This observation taken together with the variation in 70 S percentage from preparation to preparation indicates that a variable loss of affinity between the two subunits occurs during purification of ribosomes in our conditions.

The same maximum level of 70 S ribosomes was obtained with mixtures of isolated subunits af-

ter incubation for 60 min at 40° in standard buffer. This last observation indicates that if any RNA or protein factors are eventually lost during sucrose sedimentation, they are not required for the reassociation.

Experiments in which isolated subunits were heated separately demonstrated that heated 30 S subunits readily reassociate with unheated 50 S subunits, while the reverse is not true. Therefore the loss of affinity between the two subunits, which is produced by prolonged exposure to low Mg^{2+} concentrations, mainly if not exclusively, results from a modification of the 30 S subunit.

4. Discussion

It can be concluded from our own observations, as well as from previous observations by others [1, 2] that conventional conditions used for the separation of ribosomal subunits can lead to modifications of their functional properties as compared with those of the original unfractionated ribosomes. In our case, these modifications manifested themselves by a loss of the ability of the two subunits to associate together into a 70 S particle. In view of the conditions which lead to the loss of affinity between the two subunits and of those which permit the restoration of the original level of 70 S particles, we propose that these modifications are essentially conformational in character.

Although it is not completely clear whether the 70 S ribosomes found in purified preparations made according to Kurland [5,6] are equivalent to run-off empty ribosomes released from polysomes after peptide chain termination [8], or whether they still contain some component of the translation machinery responsible for their stability [9]; it is clear, however, that the interpretation of experiments on the state of association of ribosomal subunits should be made with proper consideration of the exact conditions under which the subunits have been prepared and handled.

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