EFFECT OF ETHYLENDIAMINETETRAACETATE ON ESCHERICHIA COLI B RIBOSOMES - BINDING OF PHENYLALANYL sRNA TO 50S PARTICLES CONVERTED FROM 70S RIBOSOMES

Iwao Suzuka

Department of Biophysics
National Institute of Animal Health
Kodaira, Tokyo, Japan

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In preceding communications, studies on the binding of specific sRNA to ribosomal subunits have been described (Suzuka et al., 1965, 1966; Kaji et al., 1966). The 30S subunit itself binds phenylalanyl sRNA and the binding of phenylalanyl sRNA to the 50S subunit is negligible. On the other hand, the addition of 50S subunits to a 30S subunit preparation results in about twofold stimulation of phenylalanyl sRNA binding capacity of the original 30S subunit preparation. The binding of phenylalanyl sRNA is dependent on the presence of poly U (polyuridylic acid), indicating its specific nature.

The conformational changes of 30S and 50S subunits dissociated from E. coli 70S ribosomes were reported by Gavrilova et al., (1965) and by Gesteland (1966). It was therefore of interest to elucidate the relation between structural change of ribosomes and the specific binding capacity for phenylalanyl sRNA of a particle derived from the ribosome. In the present paper I report that the EDTA (ethylenediaminetetraacetate) treatment of ribosomes causes the conversion of 70S ribosomes to a 50S particle in the presence of 0.02 M magnesium acetate and that this 50S particle has the binding activity for phenylalanyl sRNA. Therefore, this 50S particle is different from the normal 50S subunits which is dissociated from 70S ribosomes by reducing the magnesium ion concentration from 0.01 M to 10^{-4} M.

Materials and Methods - Preparation of ribosomes and, 30S and 50S subunits of ribosomes from E. coli B have been described in the preceding communications (Suzuka et al., 1965, 1966; Kaji et al., 1966). E. coli sRNA and C^{14}-phenylalanyl sRNA were prepared according to Kaji et al. (1965). The binding of phenylalanyl sRNA to ribosomes and their subunits or particles was carried out as described by Nirenberg and Leder(1964). Specific radioactivity of C^{14}-phenylalanine was 200 μc/μmole (counting efficiency, 10^6 cpm/μc). Poly U was purchased from the Miles Chemical Laboratory.

Results - Binding of phenylalanyl sRNA to the EDTA treated 70S ribosomes after the sucrose density gradient centrifugation: In the experiment shown in Fig. 1-A, 70S ribosomes were suspended in a buffer containing 0.05 M Tris-HCl (pH 7.1), 0.05 M KCl, 0.006 M β-mercaptoethnol and 0.02 M magnesium acetate, and subjected to the sucrose density gradient centrifugation. The sucrose density gradient contained 0.05 M Tris-HCl (pH 7.1), 0.06 M KCl,0.02 M magnesium acetate and 0.006 M β-mercaptoethnol. The phenylalanyl sRNA binding capacity of each fraction obtained by the centrifugation was tested using C^{14}-phenylalanyl sRNA and poly U. The binding reaction was carried out at a final concentration of 0.04 M magnesium acetate. The absorbance profile at 260 mμ in the centrifuge tube shows two peaks corresponding to 70S ribosomes and 50S subunits. It is clear that the binding capacity for phenylalanyl sRNA resides in the fraction containing 70S ribosomes and not in the fraction containing 50S subunits. As shown in Fig. 1-B, 70S ribosomes were suspended in 0.01 M phosphate buffer (pH 7.0) containing 0.001 M magnesium acetate and subjected to the centrifugation in the presence of 10^{-4} M magnesium acetate instead of 0.02 M magnesium acetate. By this procedure, 70S ribosomes were dissociated into 30S and 50S subunits, and separated from one another (Tissieres et al., 1959). It can be seen that the absorbance profile in the centrifuge tube shows two peaks corresponding to 30S and 50S subunits, and the peak of the binding activity is associated with the position to which 30S subunits sediment, reconfirming the preceding reports (Suzuka et al.,

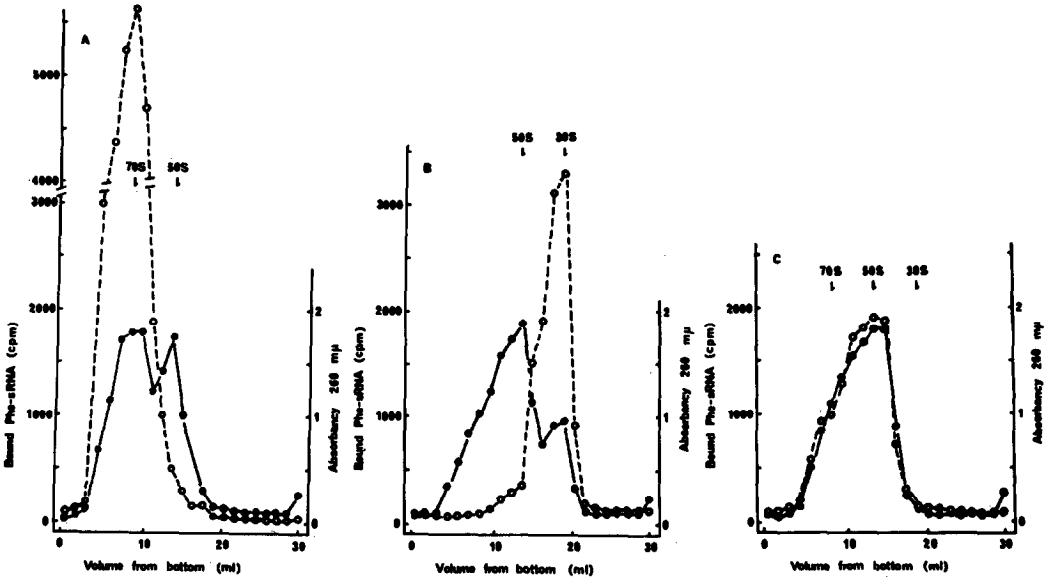

Figure 1. The binding of phenylalanyl sRNA to 70S ribosomes, 30S subunits and EDTA treated 70S ribosomes in the presence of poly U, after the sucrose density gradient centrifugation.

A) Twenty mg of E. coli ribosomes were suspended in 2 ml of solution containing 0.05 M Tris-HCl (pH 7.1), 0.02 M magnesium acetate, 0.05 M KCl and 0.006 M β-mercaptoethanol. The suspension was layered on top of 28 ml of 5-20 % linear sucrose gradient in a buffer containing 0.06 M KCl, 0.02 M magnesium acetate, 0.006 M β-mercaptoethanol and 0.05 M Tris-HCl (pH 7.1) at 0°. The tube was centrifuged in a Spinco SW-25 rotor for 15 hours at a speed of 18,000 rpm at 0°. After the centrifugation, 8 drop fractions were collected from the bottom of the tube. Optical density of each fraction at 260 mμ was measured after 10 fold dilution with water. B) Twenty mg of ribosomes were suspended in 2 ml of solution containing 0.01 M phosphate buffer and 0.001 M magnesium acetate at 0°. This suspension was subjected to the centrifugation as in A) except that the sucrose density gradient contained 10^{-4} M magnesium acetate instead of 0.02 M magnesium acetate. C) Twenty mg of ribosomes were suspended in 2 ml of the same solution as A) except that 0.015 M EDTA was contained, and then subjected to the centrifugation as in A), except that the sucrose density gradient contained 0.015 M EDTA. The binding activity of each fraction for phenylalanyl sRNA was assayed using the reaction mixture containing 20 μg of poly U, 10,000 cpm of C^{14}-phenylalanyl sRNA, 0.1 ml from the fraction, 10 μmoles of Tris-HCl (pH 7.1), 8 μmoles of magnesium acetate and 8 μmoles of KCl in a total volume of 0.2 ml. The binding reaction was carried out for 10 min at 24°. (O---O) bound phenylalanyl sRNA, (●—●) absorbancy at 260 mμ.

1965; Kaji et al., 1966). The specific activity of 30S subunit fractions was about 30 % of that of the original ribosomes. On the other hand, as shown in Fig. 1-C, 70S ribosomes were treated with the buffer containing 0.015 M EDTA and centrifuged in the presence of 0.015 M EDTA. In this case

the absorbance profile shows only one peak corresponding to 50S position whereas the peaks corresponding to the 70S ribosomes and the 30S subunit could not be detected. It should be pointed out in this figure that the binding activity is associated with the position which 50S particles sediment. Therefore, this particle may become distinguishable from normal 50S subunit which can not bind phenylalanyl sRNA. More than 70 % of the original 70S ribosomes activity is observed in the 50S particles which are transformed from 70S ribosomes by the EDTA treatment. Under the conditions used, there was no appreciable release of soluble materials from the ribosomes.

In a separate experiment, about 85 % of the binding activity of 70S ribosomes was inhibited when 0.015 M EDTA was added to the reaction mixture containing 0.02 M magnesium acetate. This inhibition might be due to the removal of magnesium ion. In the preceding reports (Kaji et al., 1966; Suzuka et al., 1966), the specific binding reaction of sRNA to ribosomes was shown to be dependent on the magnesium ion concentration from 0.01 M to 0.05 M. The binding activity of the EDTA treated ribosomes was completely recovered by the addition of 0.02 M or excessive magnesium acetate after the reaction mixture was treated with 0.015 M EDTA for 20 min at 20° in the presence of 0.02 M magnesium acetate. Thus, this inhibition was reversible. On the other hand, when 0.015 M EDTA was added at 20 min after the onset of the binding reaction of sRNA to ribosomes, only about 15 % of the bound phenylalanyl sRNA was dissociated.

Sedimentation behavior of EDTA treated ribosomes: The results obtained from the sucrose density gradient centrifugation (Fig. 1) were also supported by the sedimentation behavior of the EDTA treated ribosomes in the analytical ultracentrifuge.

As shown in Plate 1-d and f, the peak corresponding to the 70S ribosomes could not be observed, when the ribosomes were treated with 0.015 M and 0.0125 M EDTA in the presence of 0.02 M magnesium acetate. It should be pointed out, however, that while the 70S peak was transformed to a peak

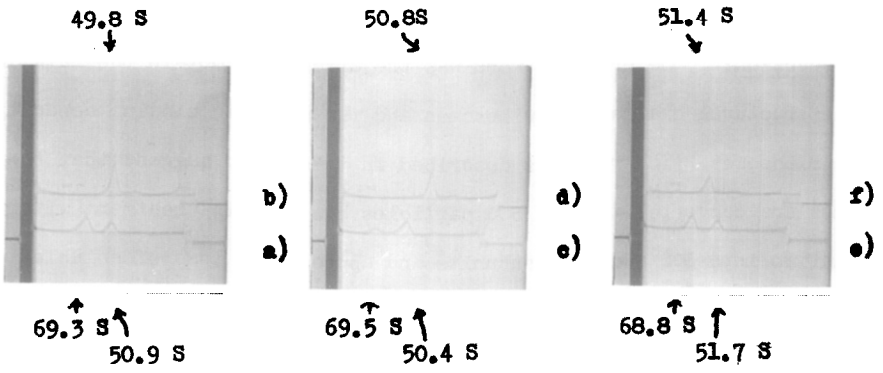

Plate 1. Sedimentation behavior of EDTA treated ribosomes.

All sedimentation was from right to left and was performed at 20° in a Beckman model E ultracentrifuge. The approximate sedimentation value of each peak is shown in the plate. a) ribosomes (1.88 mg) were suspended in 0.4 ml of solution containing 0.05 M Tris-HCl (pH 7.1), 0.02 M magnesium acetate, 0.006 M β-mercaptoethanol and 0.05 M KCl. The picture was taken 18 min after the rotor reached the running speed (35,600 rpm). b) The same as (a) except that the buffer contained 0.02 M EDTA. c) As in (a) except that the buffer contained 0.01 M EDTA. d) As in (c) except that the buffer contained 0.015 M EDTA. e) The ribosomes were suspended in the buffer as in (c) for 20 min at 20°, and then magnesium acetate was added to this mixture to give a final concentration of 0.04 M. f) As in (a) except the buffer contained 0.0125 M EDTA.

corresponding to the 50S subunits, no appreciable amount of 30S subunits was detected. On the other hand, in the control ribosomes, the major portions of particles were 70S ribosomes and 50S subunits (Plate 1-a). Thus, the 70S ribosomes were converted to 50S particles by EDTA treatment without dissociation into 30S and 50S subunits, and the EDTA action on the ribosomes might not be due to a simple removal of magnesium ion. When the concentration of EDTA was lowered to 0.01 M, an appreciable amount of 70S was present (Plate 1-c), indicating that the critical concentration of EDTA which is necessary for transformation of 70S ribosomes to the 50S particle lies between 0.0125 M and 0.015 M. At a higher concentration (0.02 M), some dissociation occurred as shown in Plate 1-b. When the 70S ribosomes were treated first with 0.015 M EDTA, followed by the addition of 0.02 M magnesium acetate after 20 min at 20°,

it was noted that the converted particles could be restored to the 70S ribosomes (plate 1-e).

Discussion: The present work was the attempt to obtain the relation between structural changes of ribosomes and the specific binding capacity of sRNA to ribosomes. The results described in this paper suggest that the 70S ribosomes are converted to the 50S particles by the EDTA treatment without dissociation into 50S and 30S subunits and appreciable loss of soluble materials from ribosomes. An important point is that the specific binding for phenylalanyl sRNA of the converted 50S particles is not lost significantly compared with the original 70S ribosomes. One can therefore conclude that these 50S particles are different from normal 50S subunits which are dissociated from 70S ribosomes by simple reducing of magnesium ion. Thus, they may be characterized as an unfolded state derived from a compact structure of 70S ribosomes. It has been shown by Kaji and Kaji (1965) that the pretreatment of ribosomes with pancreatic RNase abolished binding of sRNA completely. Cammack and Wade (1965) observed that, at magnesium concentrations lower than 1 mM in 0.15 M NaCl, autodegradation by RNase of E. coli B (RNase+) ribosomes occurred. The possibility that the RNase autodegradation occurs under conditions where the EDTA treatment are carried out in a buffer containing 0.02 M magnesium acetate, 0.05 M KCl, 0.05 M Tris-HCl (pH 7.1) and 0.006 M β-mercaptoethanol was made unlikely on the basis of the following observations. a) As shown in Fig. 1-C, the converted particles had the binding activity. b) The 70S → 50S conversion was reversible by the addition of excessive magnesium acetate as shown in Plate 1-c. c) In a separate experiment, the converted particles were shown to contain still ribosomal RNA in nature by the Spinco E analytical centrifuge. Gesteland (1966) found that 21S particles via a 36S intermediate are derived from 50S subunits and 16S particles via a 26S intermediate from 30S subunits of RNase 1 free ribosomes by the dialysis against 0.01 M Tris (pH 7.4) and 0.001 M EDTA, but a direct comparison with his findings is difficult because of the widely different

conditions on EDTA concentration, on salt concentration and on the treatment. Additional measurements are necessary to characterize the unfolded state of ribosomes by electron microscope and by optical rotatory dispersion. Further experiments are in progress along this line.

Summary - E. coli 70S ribosomes were converted to 50S particles by the treatment with 0.015 M EDTA solution containing 0.02 M magnesium acetate, 0.05 M KCl, 0.006 M β-mercaptoethanol and 0.05 M Tris-HCl (pH 7.1). Under this condition, no appreciable dissociation of ribosomes into 30S and 50S subunits could be observed. The specific binding capacity for phenylalanyl sRNA of the 50S particles converted from 70S ribosomes had more than 70 % of untreated 70S ribosomes. Therefore, this 60S particle is different from the normal 50S subunit which is dissociated from 70S ribosomes by the absence of magnesium ion.

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