# BINDING OF SPECIFIC sRNA TO 30S SUBUNITS - COMPARISON WITH THE BINDING TO 70S RIBOSOMES*

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During the course of studies on the role of ribosomal protein in the binding of specific sRNA to ribosomes (Kaji and Kaji, 1965a), it was found that the 30S subunit by itself binds specific sRNA in the presence of synthetic polynucleotides (Kaji et al., 1965a). The binding was specific in that only the aminoacyl sRNA coded for in the messenger RNA was induced to bind to 30S subunits. This binding of specific sRNA to the 30S subunits is rather surprising in view of the current concept that the 50S subunit has the binding site for sRNA (Cannon et al., 1963). In this communication we compared some of the characteristics of binding of specific sRNA to 30S subunits with characteristics of the binding to 70S ribosomes.

Materials and Methods - E. coli ribosomes free of endogenous messenger RNA were prepared as described previously (Kaji and Kaji, 1964a). The 70S ribosomes were dissociated into 30S and 50S subunits by the addition of phosphate (Tissieres et al., 1959) and separated by sucrose density

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gradient centrifugation (Cox et al., 1964). The binding of aminoacyl sRNA to ribosomes and subunits were carried out as described by Nirenberg and Leder (1964). $C^{14}$-phenylalanyl sRNA or $H^3$-phenylalanyl sRNA were prepared as described (Kaji et al., 1965b). E. coli sRNA was prepared according to Ofengand et al. (1961). $C^{14}$-ribosomes were prepared as follows: E. coli cells were grown in a minimal salt media which was modified by omission of $(NH_4)_2SO_4$ and by the addition of 0.104 g of casein hydrolysate and 0.17 ml of $C^{14}$-protein hydrolysate (0.1 mc/0.072 mg, 100 μc in 0.1 ml of 0.1 N HCl) in 1 l. The radioactive cells were harvested and mixed with carrier non-labeled E. coli cells. The radioactive ribosomes (5,400 cpm/mg) were prepared from the $C^{14}$-labeled E. coli as described above. Specific activities of radioactive material were as follows: $C^{14}$-phenylalanine, 200 μc/μmole (counting efficiency, $10^6$ cpm/μc); $H^3$-phenylalanine, 1,000 μc/μmole (counting efficiency, 3 x $10^5$ cpm/μc).

Results - Binding of phenylalanyl sRNA to 30S subunits: In the experiment shown in Fig. 1, $C^{14}$-labeled ribosomes were suspended in a phosphate buffer and subjected to sucrose density gradient centrifugation. The $H^3$-phenylalanyl sRNA binding capacity of each fraction obtained by the centrifugation was tested using $H^3$-phenylalanyl sRNA and poly U (polyuridylic acid). The distribution of $C^{14}$-radioactivity in the centrifuge tube shows two peaks corresponding to 50S and 30S positions. It is clear that the binding capacity for $H^3$-phenylalanyl sRNA in the presence of poly U resides in the fraction containing 30S subunits and not in the fraction containing 50S subunits. Additional evidences for binding of sRNA to 30S subunits are presented in a separate publication (Kaji et al., 1965a). After these observations were made, our attention was recently brought to a paper by Matthaei et al. (1964) which described their independent finding that the 30S subunit by itself binds sRNA.

Using the radioactive 30S subunits obtained in this experiment, the absorption of 30S subunits to the Millipore filter (HA 0.45 μ) was tested.

This was necessary because the Millipore filter method is based on the absorption of 70S ribosomes to the Millipore filter (Nirenberg and Leder, 1964). It was found that 30S subunits, 50S subunits, and 70S ribosomes are similarly absorbed by the filter paper under the present experimental conditions.

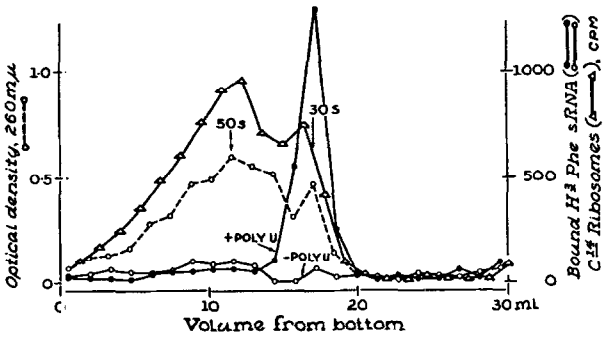


Figure 1. Binding of $H^3$-phenylalanyl sRNA to 30S subunits.

$C^{14}$-labeled ribosomes (16.8 mg) were suspended in 1 ml of solution containing 0.01 M phosphate buffer (pH 7.0) and 0.001 M magnesium acetate at 0°. This procedure dissociates 70S ribosomes into 30S and 50S subunits (Tissieres et al., 1959). Using this ribosome suspension and 1 ml of 5% sucrose solution in the same buffer, inverse sucrose gradient (5-0%) was prepared and layered on top of 28 ml of a 5-20% linear sucrose gradient in a buffer which contained 0.05 M KCl, $10^{-4}$ M magnesium acetate, and 0.1 M Tris-HCl (pH 7.8). The tube was centrifuged in a Spinco SW-25 rotor for 15 hours at a speed of 19,500 rpm at 6°. After the centrifugation, 7-drop fractions were collected from the bottom of the tube. Optical density of odd numbered fractions at 260 mμ was measured after 33-fold dilution with water. The binding capacity of odd numbered fractions for $H^3$-phenylalanyl sRNA was assayed. The reaction mixture for measuring the binding activity of each fraction contained the following in μmoles per 0.1 ml: 6.25 Tris (pH 7.1), 2.5 KCl, 2 magnesium acetate. In addition, it contained 20 μg of poly U, 9,800 cpm of $H^3$-phenylalanyl sRNA, and 0.03 ml of the fractions obtained by the sucrose density gradient centrifugation. The binding reaction was carried out for 15 min at 23°. The radioactivity of the $C^{14}$-ribosomes and subunits were measured with 0.2 ml aliquots from even numbered fractions. ●——●, bound $H^3$-phenylalanyl sRNA; O——O, bound $H^3$-phenylalanyl sRNA in the absence of poly U; Δ——Δ, distribution of $C^{14}$-ribosomes; O- - - O, optical density of each fraction at 260 mμ.

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Comparative studies on the effect of LiCl: In a preceding communication, we reported that LiCl inhibits the binding of specific sRNA to ribosomes without appreciably inhibiting the binding of poly U to 30S subunits (Kaji and Kaji, 1964b). It is of interest therefore to compare the effect of LiCl on binding of specific sRNA to 70S ribosomes with the

effect on the binding of specific sRNA to 30S subunits. As shown in Fig. 2, binding of sRNA to 30S subunits is much more sensitive to LiCl action. The binding activity of 30S subunits in 0.2 M LiCl is only 14.6% of the binding activity of 30S subunits in the absence of LiCl, while more than 66% of the original 70S ribosome activity is present at this concentration of LiCl. In a control experiment, it was found that absorption of subunits and 70S ribosomes to the Millipore Filter was not influenced by the presence of LiCl.

It has been found that relatively high concentration of LiCl dissociates 70S ribosomes into 30S and 50S subunits (Kaji and Kaji, 1964b). The dissociation of 70S ribosomes by LiCl is reversible and antagonized by the presence of $K^+$ or $NH_4^+$ ions (unpublished observation). The sedimentation analysis using Spinco Model E ultracentrifuge revealed that about 40% of the 70S ribosome in the original ribosome suspension was dissociated into 30S and 50S subunits in this reaction mixture containing 0.2 M LiCl. Therefore, the activity observed with the 70S ribosome preparation in 0.2 M LiCl is the sum of the activities of residual 70S ribosomes and the 30S subunits formed by the dissociation of 70S ribosomes. However, since the activity of 30S subunits is very low at 0.2 M LiCl, the contribution of the 30S subunits to the binding activity of the 70S ribosome preparation is rather small. It should be pointed out in this figure that no appreciable binding activity was observed with 50S subunits. In the absence of poly U, there was no binding of phenylalanyl sRNA either to 30S subunits or 70S ribosomes indicating the specific nature of this binding.

Comparative studies on the effect of incubation: In preceding communications from our laboratory, we reported that the binding of sRNA, as well as charged sRNA (aminoacyl sRNA) takes place even at 0°. With crude extract, the binding of sRNA to ribosomes could not be observed upon incubation of the reaction mixture at 37° for 10 minutes. This was interpreted to be due to the breakdown of polysomes in a crude extract. As shown in Fig. 3, the specific binding of phenylalanyl sRNA to poly U - 70S ribosome complex

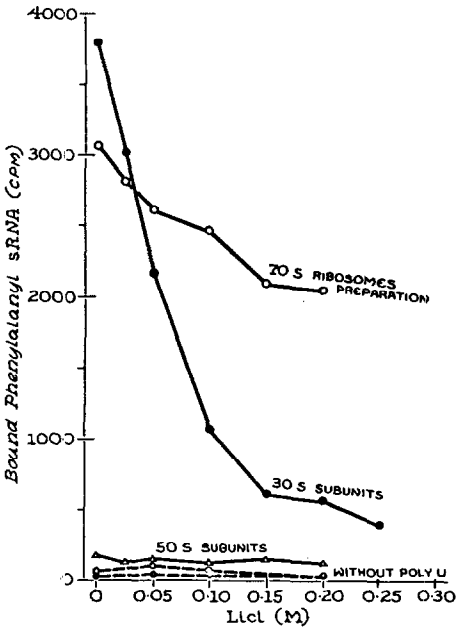


Figure 2. Comparative studies of LiCl effect on the binding of phenylalanyl sRNA.

Twenty mg of E. coli ribosomes were suspended in 1.0 ml of solution containing 0.01 M phosphate buffer (pH 7.0) and 0.001 M magnesium acetate at 0°. To obtain subunits, this suspension was subjected to the sucrose density gradient centrifugation as in Fig. 1 (except that centrifugation was carried out at a speed of 23,000 rpm for 10 hrs, and the density gradient contained 0.006 M β-mercaptoethanol and 0.01 M Tris instead of 0.1 M Tris). The complete reaction mixture for binding of phenylalanyl sRNA contained the following in μmoles/0.2 ml: 18 Tris (pH 7.1), 4 magnesium acetate, 3 KCl, 0.125 puromycin, 0.2 phosphoenolpyruvate (Na salt). In addition it contained 40 μg of poly U, 60 μg of 70S ribosomes or 53.5 μg of 50S subunits or 48 μg of 30S subunits, 5,900 cpm of $C^{14}$-phenylalanyl sRNA, and LiCl as indicated in the figure. The binding reaction was carried out for 15 min at 24°. O——O, 70S ribosomes, poly U was added; O- - -O, 70S ribosomes, poly U was omitted; ●——●, 30S subunits, poly U was added; ●- - -●, 30S subunits, poly U was omitted; △——△, 50S subunits, poly U was added.

takes place at 0° in confirmation of preceding reports (Kaji and Kaji, 1963 and 1964a; Nirenberg and Leder, 1964). On the other hand, the binding of specific sRNA to 30S subunits is strictly dependent on incubation at higher temperatures. No binding of aminoacyl sRNA took place to 30S subunits at 0°, whereas at 35° there was no difference in the binding capacity of both of these 70S ribosomes and 30S subunits preparations. It should be pointed out, however, that the specific activity of 70S ribosomes (bound sRNA/unit weight of ribosomes) is higher than that of 30S subunits.

Discussion - It has been found that the response of the binding activity of 30S subunits to the change of magnesium ion concentration was quite different from that of the 70S ribosomes (Kaji et al., 1965a). This finding,

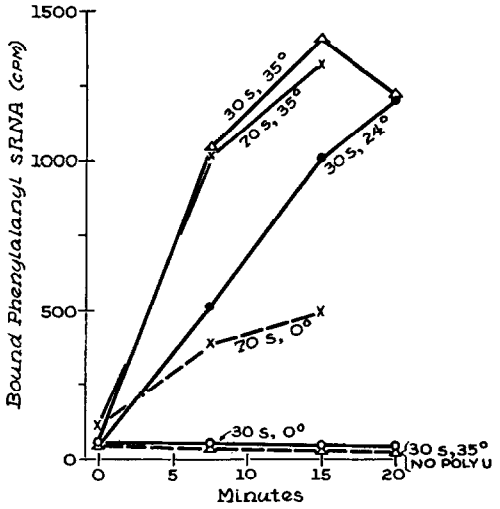


Figure 3. Effect of incubation on the binding of phenylalanyl sRNA to 30S subunits and to 70S ribosomes.

The reaction mixture for binding of phenylalanyl sRNA contained the following in μmoles/0.46 ml: 40 Tris-HCl (pH 7.1), 10 magnesium acetate, 0.25 puromycin, 30 KCl, 0.42 phosphoenolpyruvate (sodium salt), 1.8 β-mercaptoethanol. In addition, it contained 120 μg poly U, 10.6 μg of pyruvate kinase, 120 μg of 70S ribosomes or 160 μg of 30S subunits, and 23,600 cpm of $C^{14}$-phenylalanyl sRNA. The mixture was incubated at various temperatures and 0.1 ml aliquots were taken at the time intervals and the bound phenylalanyl sRNA was measured. The reaction mixtures were incubated as indicated below: O——O, 30S subunits at 0°; ●——●, 30S subunits at 24°; Δ——Δ, 30S subunits at 35°; Δ- - -Δ, 30S subunits at 35°, but without poly U; X——X, 70S ribosomes at 35°; X- - -X, 70S ribosomes at 0°. The radioactivity of the bound phenylalanyl sRNA in 0.1 ml of the reaction mixture is plotted against the time of incubation.

together with the observations reported in this communication suggest that the binding of aminoacyl sRNA to 30S subunits is qualitatively different from the binding of specific aminoacyl sRNA to 70S ribosomes. On the other hand, specificity of the binding, and inhibitory effects of streptomycin were observed with the sRNA binding to 30S subunits in a similar fashion to those with 70S ribosomes (Kaji et al., 1965a, Kaji and Kaji, 1965b). In these aspects, the binding of specific aminoacyl sRNA to 30S subunits is similar to the binding to 70S ribosomes.

The observation that 30S subunits can by itself bind specific aminoacyl sRNA raises many questions about the role of 50S subunits in the binding of specific sRNA. Our preliminary results suggest that 50S subunits act as a stabilizer of the bound sRNA. Therefore, for the maximum binding of sRNA, 50S subunits are necessary. Detailed studies on the role of 50S subunits will be described in a subsequent publication.

The finding that specific sRNA binds to the 30S subunits makes it clear that it is qualitatively different from the non-specific binding of sRNA described originally by Hoagland and Comly (1961) and, later extended by Cannon et al. (1963). Our recent finding that non-specific binding of sRNA to 50S subunits is less sensitive to LiCl than that of specific binding to the ribosomes strengthens this notion (unpublished observation). Further experiments are in progress to elucidate the relationship between specific binding of sRNA and non-specific binding of sRNA.

Summary - Binding of specific sRNA to 30S subunits alone is more sensitive to LiCl than the binding to 70S ribosomes. At 0°, binding of specific sRNA to 70S ribosomes takes place while the binding to 30S subunits does not.

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