INTERACTION OF RIBOSOMES AND POLYDEOXYRIBONUCLEOTIDES

Mituru Takanami* and Toshio Okamoto
Research Institute for Nuclear Medicine and Biology
Hiroshima University, Hiroshima, Japan

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In the course of study to analyze conditions permitting the interaction between messenger RNA and ribosomes, we reached the conclusion that ribosomes could interact with polyribonucleotide chains in a "freely rotating" configuration, resulting in the formation of ribosome aggregates(Okamoto & Takanami,1963, Takanami & Okamoto,1963). Similar observation was made in several laboratories(Barondes & Nirenberg,1962, Spyrides & Lipmann,1962, Warner et al.,1963, Gierer,1963, Staehelin et al.,1963, Gilbert, 1963), and the name "polysome" was proposed for such aggregates (Warner et al.,1963). To elucidate the mechanism of this interaction further, it was of great interest to know whether the polymer of deoxyribonucleotides shows similar affinity toward ribosomes or not. We thus examined the affinity of two kinds of phage DNA, those of ϕX174 and T2, under experimental conditions similar to the ones used in a previous study(Takanami & Okamoto, 1963).

Results of the experiment clearly demonstrated that, like polyribonucleotides, single strand DNA alone could be associated with ribosomes, forming a heavy complex.

A solution of (^{32}P)-ϕX174 phage was kindly provided by Dr. K.Matsubara, University of Kanazawa, Japan. (^{32}P)-T2 phage was

*Present address: Brookhaven National Laboratory, Upton, L.I. New York.

prepared from T2 phage infected E.coli B(H), which had been exposed to a medium(Tris-glucose) containing (^{32}P)-phosphate for about one generation time after infection. DNA was extracted from the respective phages by the phenol method. Each DNA solution was then treated by ultrasonic oscilator for 5 min. to reduce its viscosity. Since more than 90% of the radioactivity of these preparations became acid-soluble by DNase treatment, it was confirmed that very little labeled RNA was present as a contaminant in these preparations.

The procedures for preparing ribosomes from E.coli B(H), and the technique used for sedimentation analysis in sucrose density gradient were the same as those described in a previous paper (Takanami & Okamoto,1963). About 2 mg of ribosomes, in 5 mM Mg^{2+}-0.01M Tris, pH 7.6, and 2 to 10 µg of DNA, in 0.05M NaCl-0.01M Tris, pH 7.6, was mixed at a room temperature(in the vicinity of 15°C), and Mg^{2+}concentration of the mixture was adjusted to 5 mM. Then, the mixture was layered on top of a linear sucrose density gradient(5-20%), and sedimentation analysis was carried out. Approximate sedimentation coefficient of unknown peaks was estimated assuming that the ratio of the distance traveled from the meniscus by any two components is nearly constant (Martin & Ames, 1961).

First, the affinity of ϕX174 DNA toward ribosomes was tested. The sedimentation profile of the ϕX174 DNA-ribosome mixture is given in Fig.1 (a). As can be seen from the figure, almost all the radioactivity was found in a heavy region, the sedimentation coefficient of the main peak being around 110s. ϕX174 DNA alone distributed in a region lighter than 70s ribosomes under the same conditions of centrifugation. It was thus strongly suggested that the shift of the radioactivity was due to the complex formation with ribosomes. It was noted, however, that, unlike the mixture

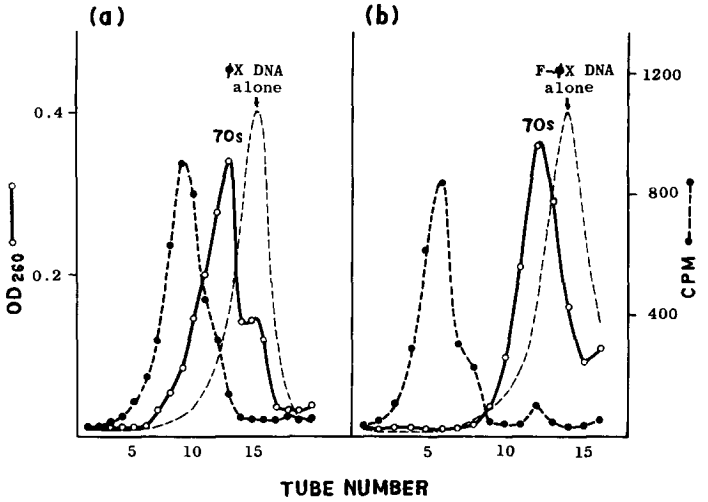

Fig.1 Sedimentation analysis of ribosome-φX174 DNA mixtures. (a) Untreated φX174 DNA. Centrifuged at 25,000rpm for 3 h. (b) Formaldehyde-treated φX174 DNA (F-φX DNA). Centrifuged at 25,000rpm for 1 h.

of poly-U and ribosomes, the single strand DNA failed to form much more rapidly sedimenting complexes, whereas its molecular weight was assumed to be much larger than the poly-U molecule employed. For this reason, we assumed that, like natural RNA, φX174 DNA in solution might contain some secondary structure stabilized by hydrogen bonds within its own strand, and that such a secondary configuration prevented the formation of the polysome structure. Accordingly, φX174 DNA was treated with 2% formaldehyde for 20 h at 37°C, as described previously(Takanami & Okamoto,1963). When the affinity of the modified DNA was tested under similar conditions, it was found that the modified DNA could form much larger aggregates, approximate sedimentation coefficient of the main peak being around 210s(Fig.1(b)). It was confirmed that the formaldehyde-treated DNA alone gave a sedimentation profile similar to that of original φX174 DNA. Assuming that the aggregate is a linear chain of spherical 70s ribosomes, which is approximated by an equivalent elipsoid(Gierer,1963), it was estimated that the

complex consisted of 9 to 10 ribosome particles. On the contrary, original untreated ϕX174 DNA might be combined with only two or three 70s ribosomes. It has been assumed that reaction of formaldehyde with amino groups of bases, involved in hydrogen bondings, brings about a dissociation of secondary structure(Doty et al., 1959). Accordingly, above results would provide good evidence for the validity of our speculation.

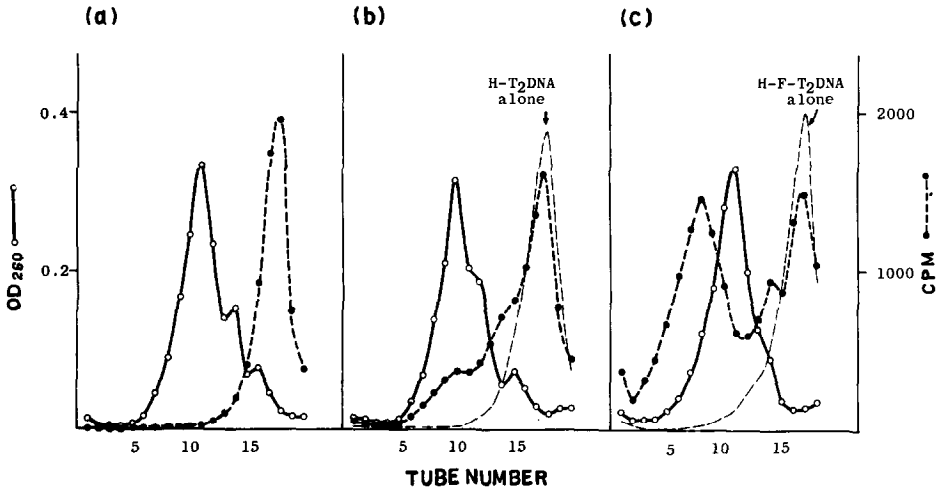

Fig.2 Sedimentation analysis of ribosome-T2 DNA mixtures. (a) Untreated T2 DNA. (b) Heated T2 DNA (H-T2 DNA). (c) Heat- and formaldehyde-treated T2 DNA (H-F-T2 DNA). Centrifuged at 25,000rpm for 3 h.

Similar type of experiments was carried out using T2 DNA, in place of ϕX174 DNA. In contrast to ϕX174 DNA, however, the double strand DNA showed no significant affinity to ribosomes(Fig.2 (a)). Then, a solution of T2 DNA in 0.05M NaCl was heated for 5 min. at 100°C and cooled rapidly, in order to abolish the double-stranded configuration. As can be seen in Fig.2 (b), such a denatured DNA gave an indication of significant interaction with ribosomes. When the heated DNA was treated further with 2% formaldehyde for 20 h at 37°C, the quantity involved in the interaction became much

larger, and more than 60% of the radioactivity was transferred to the heavy complex region(Fig.2 (c)).

In a previous paper, we reported that 30s subunit alone could form a complex with polyribonucleotides, and suggested that the polyribonucleotide, as messenger RNA, would be attached to ribosomes on their 30s subunit(Okamoto & Takanami,1963). In order to test the affinity of DNA toward each ribosome subunit, then, two kinds of subunits were prepared and sedimentation analysis was performed on the mixtures of the heat- and formaldehyde-treated T2 DNA and individual subunits. As the results, it was demonstrated that the denatured DNA showed interaction with 30s subunit alone(Fig.3).

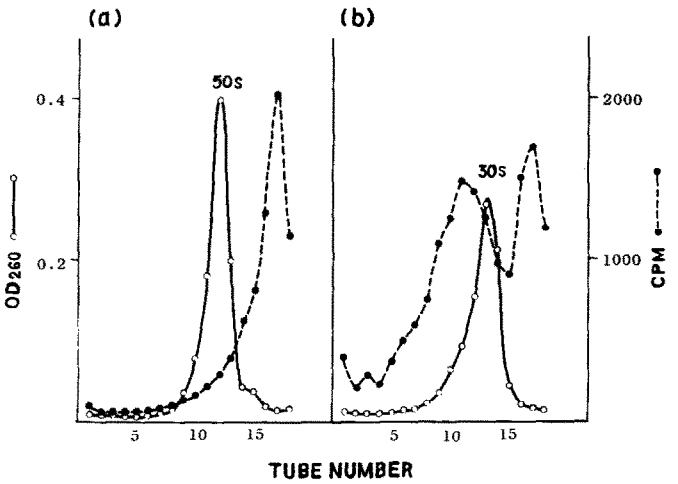

Fig.3 Sedimentation analysis of mixtures of heat- and formaldehyde-treated T2 DNA and individual ribosome subunits. (a) 50s subunit. (b) 30s subunit. Centrifuged at 25,000 rpm for 3h.

It seems possible to conclude, therefore, that the polymer of deoxyribonucleotides, like that of ribonucleotides, possesses an ability to associate with ribosomes, if the chain exists in a single-stranded and freely rotating form. In other words, affinity of a polynucleotide chain toward ribosomes does not depend upon the nature of its sugar component.

Still, we can say little about the mechanism of this interaction. The fact that the double strand DNA had no affinity with ribosomes appears to suggest that the base side of the nucleotide chain has an important role in this interaction. However, we can not rule out the possibility that the chain is associated to ribosomes by its phosphate groups, since the double-stranded configuration is not so flexible as a single-stranded one and will not easily accomodate itself upon the surface of the ribosomes.

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