SOME PROPERTIES OF AN ADENINE-RICH POLYNUCLEOTIDE FRAGMENT FROM THE AVIAN REOVIRUS

Fusaoki Koide, Iwao Suzuka, and Kiichi Sekiguchi

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

Received December 11, 1967

The avian reovirus was isolated from chick enteric tract by Kawamura et al.(1965). The viral nucleic acid was found to be double-stranded RNA by physicochemical and biochemical studies(Sekiguchi et al., 1967). Evidence for the heterogeniety of double-stranded RNA of reovirus type 3 has been reported by several workers(Watanabe and Graham, 1967;Shatkin, 1967). Reoviral RNA was separated into three double-stranded portions and a low molecular weight RNA fragment.

The present paper describes some properties of a low molecular weight RNA fragment which was isolated from purified avian reovirus by the sodium dodecyl sulfate(SDS)-phenol method. This low molecular weight RNA fragment contains a large amount of adenylyl residue and has a significant template activity for lysyl sRNA.

Materials and Methods - Avian reovirus was propagated in primary cultures of chicken kidney cell grown in monolayer. The virus was isolated from infected cell suspensions by DEAE cellulose column chromatography and centrifugation at 80,000 x g for 100 min. The pellet was suspended and purified by gel filtration on a Sepharose 2B column(Kawamura et al., 1965; Sekiguchi et al., 1967). Viral RNA fractions were extracted by the SDS-phenol method at room temperature(Homma and Graham, 1963), and sucrose density gradient centrifugation was carried out using a Spinco SW-65 rotor. Preparation of ribosomes from E. coli B, C^{14}-phenylalanyl sRNA and H^3-lysyl sRNA were performed according to Kaji et al. (1965 and 1966). The specific binding of phenylalanyl sRNA and lysyl sRNA to ribosomes was carried out as described by Nirenberg and Leder(1964). Specific activities of radioactive material were as follows: C^{14}-phenylalanine, 200 μc/μmole; H^3-lysine, 167 μc/μmole. Radioactivity was measured using the Packard liquid Scintillation counter. E. coli sRNA was purchased from the General Biochemical Company. Poly A(polyadenylic acid) and poly U (polyuridylic acid) were obtained from the Miles Chemical Laboratory. The determination of base composition of p^{32}- labeled RNA fractions was carried out as described by Tada et al.(1964).

Results and Discussion - P^{32}-labeled viral RNA was separated into two major portions(Peak 1 and Peak 2) by sucrose density gradient centrifugation as shown in Fig. 1-A. The heavier portion(Peak 1) was found to be double stranded RNA by preliminary experiment(Sekiguchi et al., 1967).

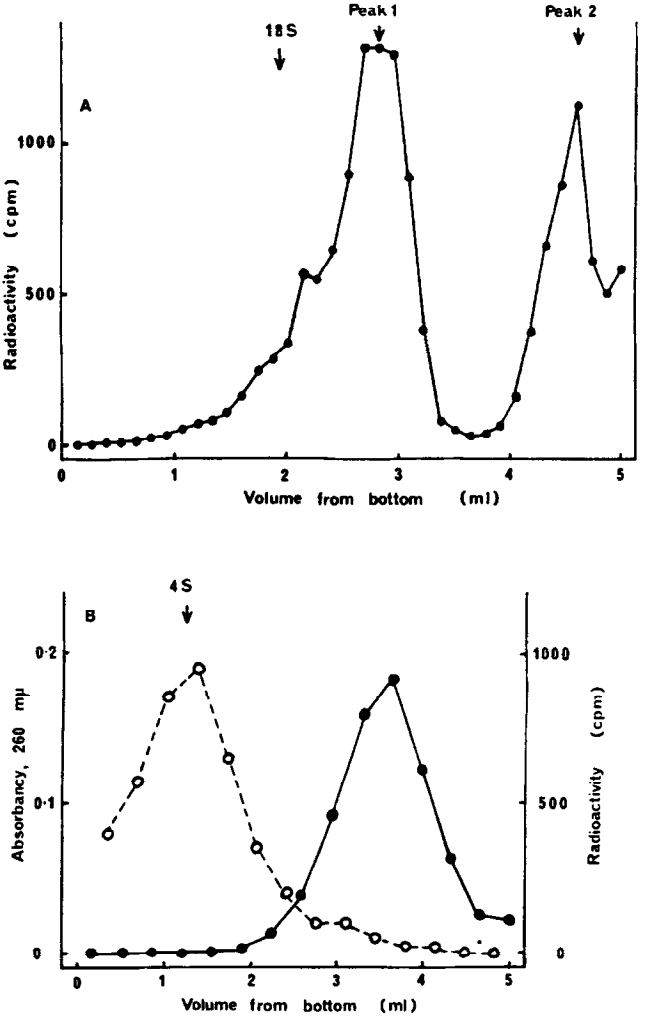

Figure 1-A. Sedimentation behavior of RNA fractions from avian reovirus. P^{32}-labeled purified reovirus was suspended in 0.01 M Tris-HCl(pH 7.1) buffer containing 0.5 mM EDTA and 0.14 M NaCl and extracted by addition of 1/10 volume of 10 % SDS and equal volume of 90 % freshly distilled phenol at room temperature. The RNA fractions were precipitated with alcohol twice and dissolved in 0.01 M Tris-HCl(pH 7.2) buffer containing 0.14 M NaCl and 1 mM $MgCl_2$. P^{32}- RNA (0.3 ml: 2 x 10^7cpm) was layered on top of 4.7 ml of 5-20 % linear sucrose density gradient in a buffer containing 0.01 M Tris-HCl(pH 7.2), 1 mM $MgCl_2$ and 0.14 M NaCl. The tube was centri-

fuged in a Spinco SW-65 rotor for 3 hours at a speed of 55,000 rpm at 15°. After the centrifugation, 5 drop fractions were collected from the bottom of the tube. The radioactivity of 2 µl aliquots was counted. (●——●), distribution of P^{32}-labeled RNA fractions.

Figure 1-B. Sedimentation behavior of Peak 2 RNA compared with E. coli B sRNA.

Peak 2 RNA(11,800 cpm) from Fig. 1-A was mixed in 0.3 ml of solution containing 100 µg of E. coli sRNA, 0.01 M Tris-HCl(pH 7.5), 0.14 M NaCl and 1 mM $MgCl_2$. The mixture was layered on top of 4.7 ml of 5-20 % linear sucrose density gradient in a buffer containing 0.01 M Tris-HCl(pH 7.2), 0.14 M NaCl and 1 mM $MgCl_2$. The tube was centrifuged in a Spinco SW-65 rotor for 16.5 hours at a speed of 60,000 rpm at 8°. After the centrifugation, 2 drop fractions were collected from the bottom of the tube. Optical density of even-numbered fractions at 260 mµ was measured after 5-fold dilution with water. The radioactivity of 0.1 ml aliquots from odd-numbered fractions was counted. (O----O), absorbancy at 260 mµ: (●——●), distribution of P^{32}-labeled Peak 2 RNA.

On the other hand, about 30 % of radioactivity of total RNA was detected in the lighter fraction (Peak 2). It was possible that the Peak 2 could be due to contamination with sRNA from the cultured cells. To elminate this possibility, Peak 2 was subjected to a second sucrose density gradient centrifugation with E. coli sRNA as a marker. As shown in Fig. 1-B, it is clear that the radioactivity of Peak 2 was found near the 2S position which was distinctly separated from the E. coli sRNA(4S). In a separate experiment, amino acid acceptor capacity of the Peak 2 RNA fraction could not be observed. Therefore, these observations indicate that Peak 2 RNA is distinguishable from the usual control sRNA.

The base composition of Peak 2 RNA was determined by the radioactivity of the separated mononucleotide on a Dowex - 1 column chromatography as shown in Table 1. More than 80 % of radioactivity was found in AMP and about 10 % for UMP, 5 % for GMP and 2 % for CMP respectively. However, the radioactivity of CMP is around of counting error, that is, probably no C contains in Peak 2 RNA molecule.

Table 1. Base composition of Peak 1 and Peak 2 RNA isolated from avian reovirus

	Base composition in per cent			
	C	A	U	G
Peak 1 RNA (double stranded)	22.0	27.4	29.1	21.5
Peak 2 RNA	1.8	82.8	10.6	4.8

After centrifugation of P^{32}-labeled viral RNA(Fig. 1-A: 124,000 cpm), the fractions were pooled into two portions(Peak 1 and Peak 2). Peak 2 RNA was

purified by gel filtration on a Sephadex G-25 column. RNA was precipitated with two volumes of cold ethanol after addition of 1 mg of yeast RNA as a carrier, was hydrolyzed in 0.3 N KOH for 18 hours at 37° and neutralized with perchloric acid. The hydrolysate of RNA was fractionated on a Dowex 1 column (Tada et al., 1964) and each fraction of nucleoside monophosphate was confirmed spectrophotometrically. The base composition was calculated from the radioactivity of each collected nucleotide.

It should be pointed out that Peak 2 RNA consists of a large amount of adenine (A) in its nucleotide chain, suggesting the presence of an A region.

If this RNA contains an A region, lysyl sRNA will bind to ribosomes in the presence of Peak 2 RNA as a template, since AAA is the codon for lysyl sRNA. The template activity of Peak 2 RNA was tested by the E. coli ribosome-sRNA binding system as described by Nirenberg and Leder(1964). As shown in Fig. 2-A, the binding of lysyl sRNA to ribosomes was increased effectively during the incubation period in the presence of Peak 2 RNA. The amount of the bound lysyl sRNA was approximately twice as much as that of lysyl sRNA directed by poly A. No appreciable binding took place in the absence of poly A and Peak 2 RNA.

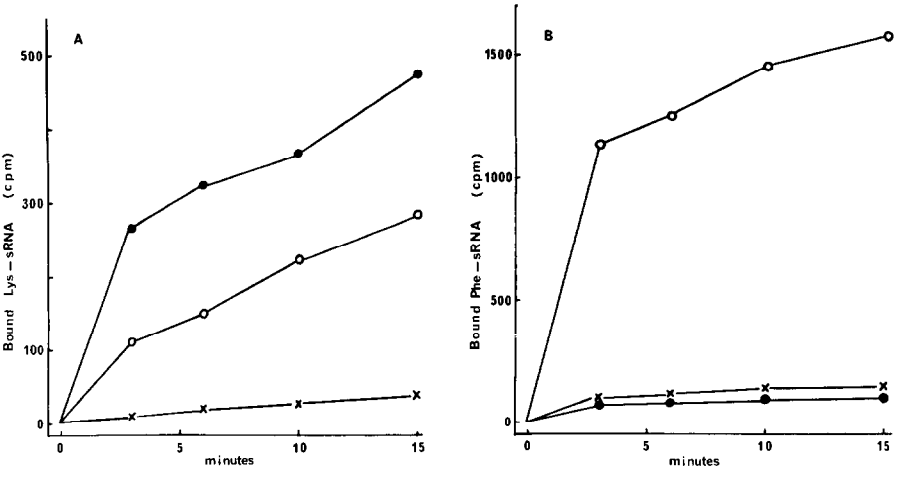

Figure 2. Time course of specific binding of lysyl sRNA to E. coli B ribosomes in the presence of Peak 2 RNA

Peak 2 RNA was prepared as described in Fig. 1-A, except that 0.01 M sodium phosphate was added to the culture medium instead of P^{32}-phosphate, and purified by gel filtration on a Sephadex G-25 column(1 x 22 cm). A) The binding activity for lysyl sRNA was assayed using the reaction mixture containing 40 µg of poly A or Peak 2 RNA, 7850 cpm of H^3-lysyl sRNA, 200 µg of E. coli B ribosomes, 20 µmoles of Tris-HCl(pH 7.1), 16 µm of KCl and magnesium acetate in a total volume of 0.86 ml. The binding reaction was carried out at 24°. At various time intervals, 0.2 ml aliquots were taken, passed through a cellulose nitrate millipore filter and the radioactivity of the bound lysyl sRNA was counted. B) The binding reaction for phenylalanyl sRNA was carried out in the same reaction mixture as A), except that 20 µg of poly U and 10,000 cpm of C^{14}-phenylalanyl sRNA were added instead of poly A and H^3-lysyl sRNA. (O——O), Poly A(Fig. 2-A) or poly U(Fig. 2-B) was added; (●——●), Peak 2 RNA was added: (x——x), Template was omitted from the mixture.

On the other hand, as shown in Fig. 2-B, phenylalanyl sRNA could not be bound to the Peak 2 RNA-ribosome complex. In the control experiment, phenylalanyl sRNA was bound to the poly U-ribosome complex. These results indicate that Peak 2 RNA has the code for lysyl sRNA. Thus, Peak 2 RNA may be concerned exclusively with the A region in its nucleotide chain.

In a separate experiment, Peak 2 RNA was fractionated to a size corresponding to a molecular weight of approximately 20,000 by gel filtration on a Sephadex G-50 column. It is not clear whether Peak 2 RNA holds functional activity as a template in viral specific protein synthesis or lysyl sRNA can be simply bound to Peak 2 RNA-ribosome complex without peptide formation under our experimental conditions. Furthermore, an important point is to elucidate a mutual relationship between the double stranded RNA(Peak 1) and the A-rich RNA fragment(Peak 2) in the structure of reoviral RNA. Watanabe and Graham(1967) reported that messenger RNA of reovirus infected cells has base complementation to the double stranded RNA. The present observations, however, would suggest that the binding of lysyl sRNA to the Peak 2 RNA-ribosome complex has some significant function in reovirus formation. Full details concerning properties of the A-rich RNA fragment are currently under investigation and will be reported elsewhere.

Summary - Avian reoviral RNA was fractionated into two portions which were found to be a double stranded RNA and an A-rich RNA fragment. The A-rich RNA fragment is different from the usual sRNA and contains more than 80 % of AMP and 10 % of UMP as nucleotide. This A-rich RNA fragment was found to bind lysyl sRNA to E. coli ribosomes.

REFERENCES

Homma, M. and A. F. Graham, J.Cellular Comp. Physiol., 62, 179(1963)
Kaji, A., H. Kaji and G. D. Novelli, J. Biol. Chem., 240, 1185(1965)
Kaji, H., I. Suzuka and A. Kaji, J. Biol. Chem., 241, 1251(1966)
Kawamura, H., F. Shimizu, M. Maeda and H. Tsubahara, Nat. Inst. Hlth. Quart., (Japan) 5, 115(1965)
Nirenberg, M. W. and P. Leder, Science 145, 1399(1964)
Sekiguchi, K., F. Koide and H. Kawamura, (Manuscripts in preparation)
Shatkin, A. G., (Personal communication)
Tada, M.,M. Tada and K. Yagi, J. Biochemistry(Tokyo) 55, 136(1964)
Watanabe, Y. and A. F. Graham, J. Viology 1, 665(1967)