A COMPLEX BETWEEN POLIOVIRUS RNA AND THE STRUCTURAL POLYPEPTIDE VP1

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Received June 28,1976

The isolation of a complex between poliovirus RNA and the structural polypeptide VP1 is reported. This ribonucleo-polypeptide (RNPP) is obtained by dissociation of poliovirus by urea and by subsequent centrifugation in urea containing sucrose gradients in hypotonic phosphate buffered saline. It sediments at about 45S and is sensitive to RNase I.

The specific binding of protein to DNA or RNA is a key for understanding the assembly of nucleic acid-containing particles and cell structures and for elucidating the regulation of a number of biological activities. Poliovirus particles containing 30 % RNA and 70 % protein are an interesting model for the study of virus assembly and of the influence of proteins on infectivity.

We have reconstituted infectious poliovirus particles from a mixture of RNA and protein obtained by dissociation of the virus particle by urea in the presence of mercapto-ethanol or dithiothreitol (1, 2). Since a poliovirus particle contains four different polypeptides, VP1 to VP4 (3), the question arises which of the polypeptides is bound directly to the viral RNA.

MATERIALS AND METHODS

Labeled poliovirus type I, strain Mahoney, and labeled poliovirus RNA were prepared as described (4, 5).

Poliovirus was dissociated by adding 10 to 30 µl of labeled virus in 0.15 M NaCl to 150 µl of 10 M urea in H_2O. In one case ribonuclease at a concentration of 250 µg/ml was present. After incubation for 1 hr at 25° C 150 µl H_2O was added. The sample was layered on top of a sucrose gradient (5 to 20 % sucrose) containing 5 M urea in hypotonic phosphate buffered saline (2 x 10^{-3} M phosphate buffer, pH 7.2 + 1.2 x 10^{-2} M NaCl). Centrifugation was at 257,000xg at 16° C in a Beckman/Spinco SW 60 rotor for 7 hours; 0.2 ml fractions were collected and the radioactivity was determined as described (4). RNA obtained by phenol extraction from poliovirus particles (35S) and ribosomal RNA (28S + 16S) from HeLa cells and bovine serum albumin (4.2S) were used as markers. Their sedimentation coefficients are given in parenthesis.

To the RNPP complex (fraction No. 5 from the sucrose gradient (Fig. 1)) tris-phosphate buffer containing 1 % SDS and 0.1 % 2-mercaptoethanol was added. The sample was heated for 2 min at 100° C and electrophoresed in the polyacrylamide SDS-Disc System (6). Fractionation of gels and measurement of radioactivity have been described (5).

RESULTS AND DISCUSSION

Poliovirus labeled with ^{3}H-uridine in its RNA and with ^{14}C-amino acids (protein hydrolysate) in its protein was dissociated in concentrated urea solution at 25° C for 1 hr (2, 7) and centrifuged in a sucrose gradient containing 5 M urea in hypotonic phosphate buffered saline (see Methods). About one third of the protein label sedimented together with the RNA label (Fig. 1a). Phenol extracted poliovirus RNA (35S) sedimented slightly slower than this peak of RNA and protein. From the position of the markers used in the experiment, the sedimentation coefficient of the RNPP-complex was calculated to be 45S. Two thirds of the protein label were found at the top of the gradient, at a position close to bovine serum albumin (used as 4.2S marker). This slower sedimenting protein peak was free

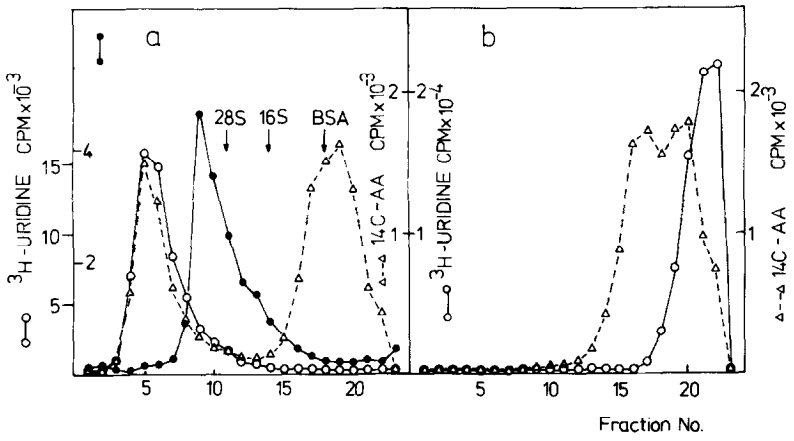

Fig. 1: Centrifugation of urea dissociated poliovirus in a sucrose gradient. ^{3}H-uridine (o ——— o) and ^{14}C-protein-hydrolysate (^{14}C-AA) labeled poliovirus (Δ - - - - Δ) was dissociated and centrifuged in a sucrose gradient containing 5 M urea in hypotonic phosphate buffered saline (see Methods). (a) Dissociation products after incubation without RNase, and (b) in the presence of 250 µg of pancreatic ribonuclease. Separate tubes contained ^{3}H-uridine labeled phenol extracted RNA (● ——— ●) 28S and 16S ribosomal RNA and bovine serum albumin (BSA) as markers.

of any RNA label. It contained the polypeptides VP2 and VP3 (8).

In order to determine whether the RNA in the 45S peak was associated with protein, dissociation of poliovirus by urea was performed in the presence of ribonuclease (RNase I, EC 3.1.4.22 Enzyme Nomenclature 1973). The subsequent centrifugation in an urea containing sucrose gradient revealed the hydrolysis of RNA into small nucleotides found at the meniscus of the gradient (Fig. 1b). The protein label disappeared from fraction 5 and was found in fractions 15 and 16 as a new protein peak. From these experiments it was obvious that the 45S material was sensitive to pancreatic ribonuclease.

It was of interest to see if one of the four viral polypeptides was specifically bound to the RNA or if the RNA-

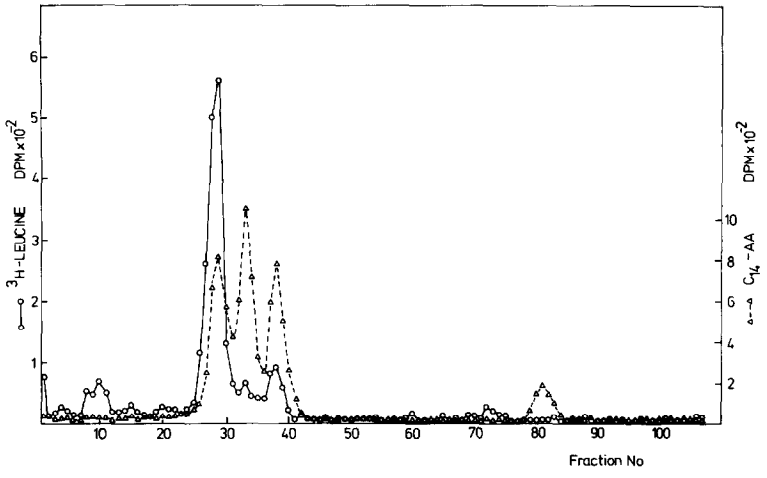

Fig. 2: Polypeptide analysis of the RNPP complex. The ^{3}H-leucine labeled RNPP complex (○——○) was coelectrophoresed with ^{14}C-protein hydrolysate labeled poliovirus (△---△) in polyacrylamide gel (see Methods). Electrophoresis was performed in 13 % polyacrylamide gels at 100 V for 6 hours.

protein complex consisted of all four polypeptides. A fraction of the 45S material obtained after dissociation of ^{3}H-leucine labeled poliovirus from a sucrose gradient was taken and analyzed by polyacrylamide gel SDS-Disc electrophoresis. Fig. 2 demonstrates that the protein found in the RNA-protein complex consisted almost exclusively of VP1. From this data it is concluded that the 45S material is a ribonucleo-polypeptide (RNPP) containing only polypeptide VP1, which with a molecular weight of 35.000 (3) is the largest structural polypeptide of poliovirus.

The dissociation of poliovirus by urea usually results in a separation of RNA and protein (1, 2, 9, 10), unless appropriate precautions are taken. This paper demonstrates that poliovirus polypeptide VP1 is bound to the viral RNA when the dissociation of the virus by urea and the subsequent

centrifugation are performed at low salt concentration. This finding allows a detailed analysis of the interaction between poliovirus RNA and VP1 and of its role in the assembly of poliovirus particles.

Acknowledgement: This work was supported by the Deutsche Forschungsgemeinschaft.

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