Dissociation and Reassociation of Poliovirus
II. Protein Components Obtained by Urea Treatment of the Virus Particle

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Dissociation of poliovirus by 9 M urea in 0.015 M NaCl at 25 °C resulted in the liberation of 35S RNA and of polypeptides sedimenting at 2S in sucrose gradients containing 9 M urea. However, a ribonucleopolypeptide (RNPP) complex sedimenting at 45S and oligomers of the viral polypeptides sedimenting at 7—8S were found in addition to the monomers sedimenting at 2S when the urea concentration was lowered to 5 M after the dissociation procedure. Ribonuclease treatment prevents the appearance of the RNPP-complex. The amount of the RNPP-complex decreased, when the dissociation was performed at higher ionic strength. Under these conditions small amounts of empty capsids were detected.

Polyacrylamide gel electrophoresis showed that the RNPP-complex contained the polypeptide VP1. The oligomers (7—8S) contained the polypeptide VP3 and small amounts of VP2. The bulk of VP2 and some VP3 were found in the 2S position together with VP4.

The molecular weight of the dissociation products in urea and phosphate buffer was determined by gel filtration to be about 30,000 for the monomeric polypeptides containing predominantly VP2 and about 70,000 for the oligomeric polypeptides containing predominantly VP3.

Our results demonstrate that the oligomers and the RNPP-complex are not primary products obtained by dissociation of the virus particle by urea but are due to a reassociation of the polypeptides or of VP1 and RNA.

Introduction

Poliovirus morphogenesis is a stepwise and regulated process, during which proteins and RNA are assembled into the very compact and stable poliovirus particle. The morphogenesis in the cell involves cleavage of large precursor proteins into the smaller polypeptides VP1 to VP4 present in the poliovirus particle[1]. Despite the complexity of this process an *in vitro* reconstitution of infectious poliovirus particles was achieved[2, 3]. This reconstitution of infectious particles from a mixture of RNA and protein obtained by dissociation of the virus by urea is at the moment poorly understood. In order to evaluate the mechanism of dissociation we have investigated it in some detail[4]. In this paper we describe small protein components and a complex between poliovirus RNA and the polypeptide VP1, obtained by urea dissociation of poliovirus. A preliminary report on this ribonucleopolypeptide (RNPP) complex was already given[5].

Materials and Methods

[³H]leucine- or [³H]uridine-labeled poliovirus, type I, strain Mahoney, was prepared as described[6, 7].

Dissociation

Poliovirus was dissociated by adding 10 μl of labeled virus in 0.15 M NaCl or in phosphate buffer to 150 μl of 10 M urea in H_2O followed by incubation for 1 h at 25 °C as described[2—4].

Sucrose gradients

After dissociation up to 300 μl samples were layered on top of sucrose gradients containing the indicated amounts of urea and isotonic phosphate buffer (PBS: 0.12 M NaCl, 0.02 M phosphate, pH 7.2) or hypotonic PBS (0.012 M NaCl, 0.002 M phosphate, pH 7.2). Centrifugation was at $257,000 \times g$ at 16 °C in a Beckman Spinco SW 60 rotor for the times indicated in the figures. 0.2 ml fractions were collected and the radioactivity was determined as described[6].

RNA obtained by phenol extraction from poliovirus particles (35S) and bovine serum albumin (BSA: 4.2S) were used as markers.

Gel filtration

For gel filtration a column 88×1 cm of Biogel A5 (Biorad Laboratories, München, Germany) was

used. After dissociation of poliovirus the samples were layered on top of the column and eluted by 8 M urea in isotonic phosphate buffer. 0.57 ml fractions were collected. For gel electrophoresis peak fractions were pooled, 0.2 ml BSA was added and precipitated by 10% trichloroacetic acid (TCA). After washing with TCA and acetone, samples were dissolved in tris-phosphate buffer, pH 6.9, containing 1% SDS and 0.1% mercaptoethanol, and analyzed by gel electrophoresis.

Polyacrylamide gel electrophoresis (PAGE)

The samples were heated for 2 min at 100 °C and electrophoresed in the polyacrylamide SDS Disc-System [8]. Fractionation of gels and measurements of radioactivity have been described [7].

Results

1. Protein components after urea dissociation

When poliovirus particles are incubated for 60 min at 25 °C with 9 M urea in 0.015 M NaCl and centrifuged in a sucrose gradient (5 to 20%) containing 9 M urea in hypotonic PBS, only one protein peak sedimenting at about 2S is detected. The RNA (uridine-labeled) sediments as one peak at about 35S (Fig. 1).

However, when poliovirus particles are dissociated by 9 M urea in the same manner as described above, then diluted to 5 M urea and centrifuged in sucrose gradients containing 5 M urea in hypotonic PBS, three protein peaks are detected.

The fastest protein peak sediments at about 45S in hypotonic phosphate buffered urea containing sucrose gradients (Fig. 2 a). The slower moving protein peaks sediment at about 7S and 2S.

The 45S peak contains the viral RNA and the polypeptide VP1 as recently reported, and is therefore called the ribonucleopolypeptide (RNPP) complex [5]. Addition of ribonuclease destroys the RNPP complex (Fig. 2 b) by hydrolysis of its RNA. The protein part of the RNPP complex is found under the 7S peak (Fig. 2 b).

The 7S peak consists mainly of the polypeptide VP3 and a small amount of VP2 when the urea dissociation is performed in the absence of ribonuclease (Fig. 3 a). When ribonuclease is present during the dissociation of the virion, this peak contains the polypeptide VP1 in addition to VP3 and low amounts of VP2 (Fig. 3 c).

The 2S peak contains predominantly the polypeptide VP2 and a very small amount of VP3 and VP4 but no VP1, neither in the presence nor absence of RNase (Fig. 3 b).

In order to determine the molecular weight of the protein components obtained by dissociation of poliovirus by urea, the dissociation products were analyzed by gel filtration on a Biogel A5 column (Fig. 4). Protein was found together with viral RNA in the exclusion volume (peak A). The protein disappears from the exclusion volume, when the virus sample is dissociated in the presence of ribonuclease. This result confirms the presence of an RNPP complex in the dissociation products obtained by treatment of poliovirus particles with urea.

In addition to the RNPP complex, three protein peaks (B, C, D) were detected by gel filtration on Biogel A5. From the position of the marker proteins BSA and cytochrome c, the molecular weights were calculated to be roughly 70,000 (peak B), 30,000
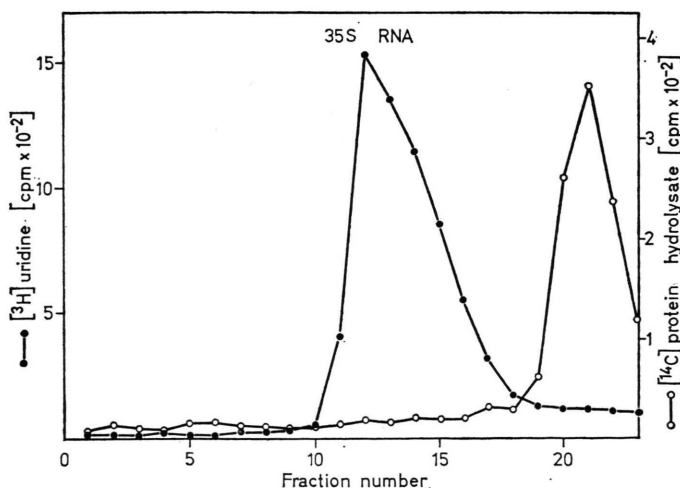

Fig. 1. Centrifugation of dissociated poliovirus in a sucrose gradient containing 9 M urea in hypotonic PBS ($\mu = 0.015$). [³H]uridine (●—●) and [¹⁴C]protein hydrolysate (○—○) labeled poliovirus was mixed, dissociated in 9 M urea and centrifuged in the same tube. Centrifugation time was for 10 hours at 18 °C.
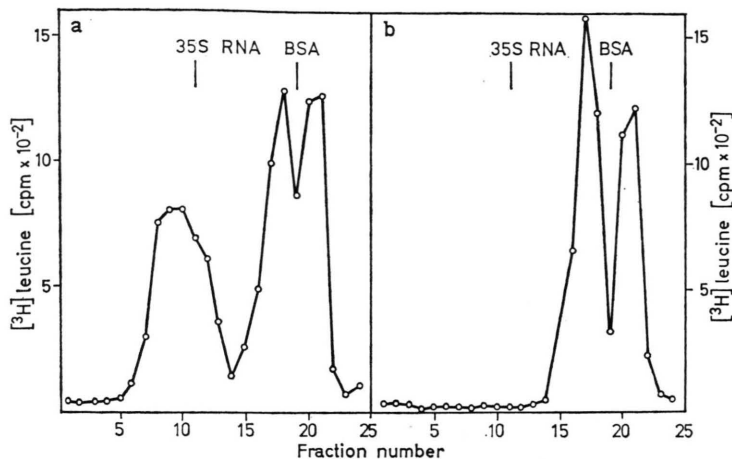
Fig. 2. Centrifugation of dissociated polio-virus in an urea-sucrose gradient containing hypotonic phosphate buffer. Labeled polio-virus was dissociated and centrifuged in a sucrose gradient containing 5 M urea in hypotonic phosphate buffer for 6 hours at 50,000 rpm. (a) Dissociation products after incubation without RNase and (b) in the presence of 250 μg of pancreatic ribo-nuclease.
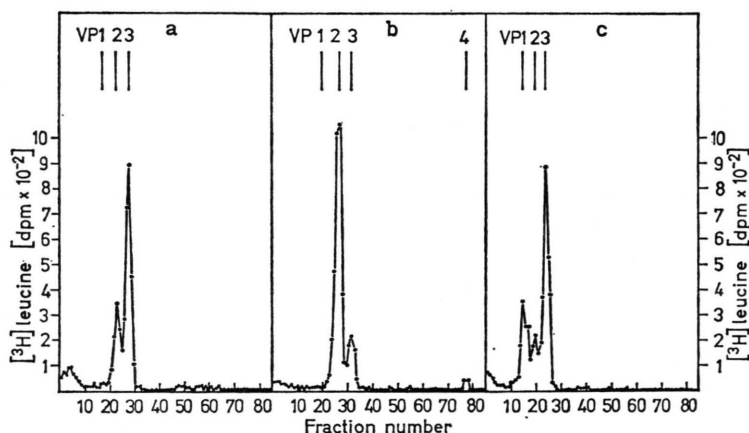

Fig. 3. Polypeptide composition of the urea dissociation products isolated from a su-crose gradient. Dissociation products were taken from a sucrose gradient as described in Fig. 2 and electrophoresed on poly-acrylamide gels (see Methods). Figure 3 a and b show the polypeptide compositions of the fraction 18 (a) and 20 (b) from the sucrose gradient described in Fig. 2 a. Fig. 3 c shows the polypeptide analysis of fraction 16 of the gradient from Fig. 2 b, when pancreatic ribonuclease had been present during dissociation.
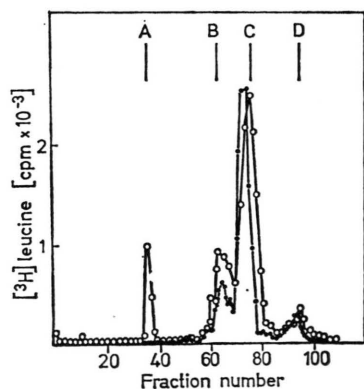

Fig. 4. Gel filtration of dissociated poliovirus on Biogel A5. Labeled poliovirus was dissociated and analyzed on a Bio-gel A5 column (see Methods). Dissociation was in absence (○—○) and in presence of 250 μg pancreatic ribo-nuclease (●—●).

(peak C) and 7000 (peak D). Peak B consists pre-dominantly of the polypeptide VP3, small amounts of VP2 and traces of VP4 (Fig. 5 a). Peak C con-tains predominantly VP2 and a certain amount of VP3 and VP4 (Fig. 5 b). Peak D contains the polypeptide VP4 as judged from its molecular weight of 7000.

The polyacrylamide gel electrophoresis of the protein components separated by centrifugation or by gel filtration and their relative positions in the gradient or on the column indicate that the faster sedimenting protein peak (7S) obtained by centri-fugation corresponds to peak B (molecular weight 70,000) of the gel filtration and the 2S peak cor-responds to peak C (molecular weight 30,000). The RNPP complex (45S) corresponds to peak A, the molecular weight of which was too large to be determined by the use of Biogel A5. From the com-
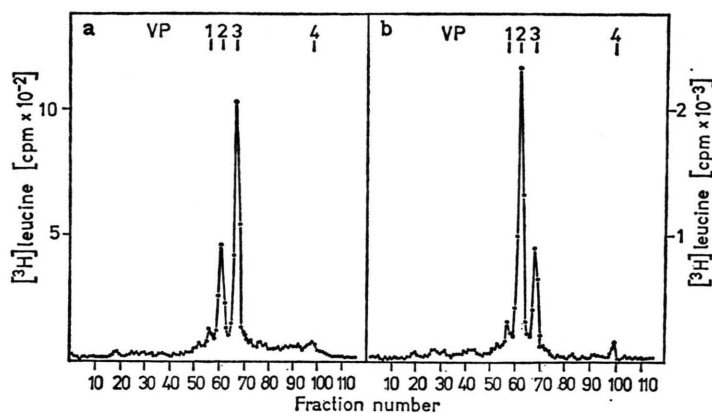
Fig. 5. Polypeptide composition of the urea dissociation products isolated from the Biogel A5 column. Fractions as indicated by B and C in Fig. 4 were pooled and analyzed by polyacrylamide gel electrophoresis (see Methods). (5a) shows the polypeptide pattern of peak B and (5b) that of peak C.

position of the RNPP complex its molecular weight can be calculated to be about 4×10^6 (RNA + 60 VP1 molecules).

2. The RNPP complex and empty capsids

For the understanding of the reconstitution process of poliovirus[3, 4] it is important to know the conditions for the existence of the RNPP complex and empty capsids after the dissociation of poliovirus particles by urea. From the experiments described above (Fig. 1) it is obvious that neither empty capsids (sedimenting at 80S, containing the viral polypeptides VP1, 2, 3, see ref. 7), nor the RNPP complex are found when the procedure is performed at hypotonic salt concentrations and the urea concentration is maintained at 9 M during dissociation and centrifugation in the sucrose gradient. The RNPP complex is detected when the urea concentration is lowered to 5 M after the dissociation. The influence of the salt concentration on the appearance of the RNPP complex and of empty capsids is illustrated in Fig. 6. The amount of the RNPP complex is dependent on the salt concentration present in the gradient (Fig. 6 a, b), and

during the dissociation procedure preceding the centrifugation (Fig. 6 c). High salt concentrations decreased the amount of the RNPP-complex but increased the amount of empty capsids. Thus, the amount of empty capsids was increased from less than 0.3% at low salt concentrations (0.030 M) to 3% if the dissociation of poliovirus was performed in 0.34 M NaCl. No RNPP complex was found at this high ionic strength (Fig. 6 c). Another effect of the salt concentration was its influence on the sedimentation velocity of the RNPP complex in urea containing sucrose gradients. The samples in Fig. 6 were centrifuged at the same conditions. The RNPP complex sediments slower at low ($\mu = 0.015$) than at high ($\mu = 0.15$) ionic strength. A parallel effect of the ionic strength was exerted on the sedimentation velocity of poliovirus RNA but not on proteins. The difference in the sedimentation velocity of the RNPP complex therefore reflects the effect of salts on its RNA. The above given sedimentation coefficient for the RNPP complex was determined to be 45S at an ionic strength of 0.015. Poliovirus 35S RNA and ribosomal 28S and 16S RNA were used as markers.
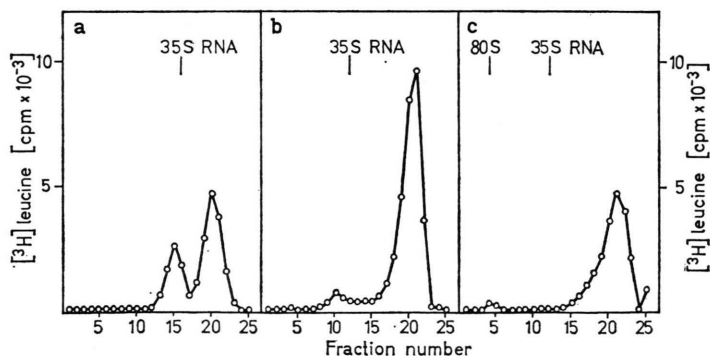

Fig. 6. Dissociation products obtained in the presence of different salt concentrations. a. Labeled poliovirus was dissociated as usual in 9 M urea and 0.015 M NaCl and centrifuged 150 min at 50,000 rpm in a sucrose gradient containing 5 M urea in hypotonic phosphate buffered saline. b. After dissociation the sample was centrifuged in a sucrose gradient containing 5 M urea in isotonic phosphate buffered saline. Centrifugation time was as in a. c. Before dissociation NaCl at a final concentration of 0.34 M was added to the sample. The dissociated virus was centrifuged under isotonic conditions as in b.

Discussion

The destruction of the infectivity of poliovirus particles by urea results in the dissociation of the virion into its components, namely RNA and protein [2, 3]. The mechanism of this dissociation as well as the influence of urea concentration, pH, temperature and time were investigated in the foregoing work [4]. The experiments reported in this paper characterize the protein components obtained by treatment of poliovirus particles with urea.

In agreement with previous findings [3] a complete absence of poliovirus particles and of empty capsids is demonstrated when the dissociation is performed with 9 M urea in 0.015 M NaCl solution and the products are centrifuged in a sucrose gradient containing 9 M urea in hypotonic PBS. Under these conditions only one protein peak is demonstrated sedimenting at about 2S, although its asymmetry indicates some material sedimenting faster than 2S (Fig. 1). The 2S polypeptide peak contains mainly VP2 and small amounts of VP3 and VP4. It has the size of single polypeptide molecules of a molecular weight of about 30,000 as determined by gel filtration. These values are in agreement with published results: thus poliovirus polypeptide-SDS-complexes or polypeptides oxidized with performic acid sedimented at 2S and had a molecular weight of $27,000 \pm 4,000$ [9, 10].

Dilution of the dissociated sample to 5 M urea results in the appearance of two additional protein components. The protein peak sedimenting at about 7S consists predominantly of the polypeptide VP3. This and the molecular weight of 70,000 determined by gel filtration suggest the occurrence of different oligomers (dimers to pentamers) in the 7S peak.

A 45S peak, designated as ribonucleopolypeptide (RNPP) complex contains all of the VP1 polypeptide bound to viral RNA as demonstrated recently [5]. The RNPP complex is sensitive to pancreatic RNase (Fig. 2) and high salt concentrations (Fig. 6). Small amounts of empty capsids are also detected under these conditions but virus particles are completely absent.

In previous studies the degradation of poliovirus either at alkaline pH at 40 °C [11] or at 37 °C in the presence of 7.2 M urea [12] led to a release of VP4 and VP2 leaving a complex between VP1 and VP3. A degradation by urea was also described for Coxsackie B3 virus and poliovirus. Treatment of the virions for 5 min at 37 °C at pH 9 yielded empty capsids and components sedimenting at 20S and 5S [13]. However, these authors could not exclude irreversible denaturation and the possibility of reaggregation of the viral polypeptide. The Mouse Elberfeld virus, another member of the picornavirus group, was dissociated into 14S subunits at slightly acidic pH. The 14S subunits could be further dissociated into 5S components by incubation in 2 M urea [14]. Identical results were obtained with mengovirus [15].

Although these data were interpreted in favour of distinct substructures of the picornavirus particle, our results demonstrate that larger components result from a reassociation of primarily obtained single molecules (polypeptide and RNA).

Further characterization of poliovirus components are underway, which will give some insight into the interactions of the polypeptides and RNA in the virus particle and determine the reactions of viral polypeptides and RNA after the dissociation of the virion.

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