Stepwise Degradation of Poliovirus Capsid by Alkaline Treatment

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SUMMARY

The kinetics of the liberation of protein components from purified poliovirus was examined under varying alkaline pH conditions at 40°. The proteins of the liberated components and of the virus capsid were analysed by sucrose gradient centrifugation and polyacrylamide gel electrophoresis.

When the virus particle was treated at pH 10-0, a minor component enriched in the capsid protein VP 4 was liberated from the virus capsid and a remaining capsid structure had the same H antigenicity as intact empty capsids free of virus RNA. A second component consisting mainly of VP 2 was released from the capsid at pH 11-0 and the residual capsid contained VP 1 and VP 3. This altered capsid still possessed H antigenicity and was stable at pH 11-0, but was degraded to smaller components at pH 12-0. This smaller component did not show H antigenicity. The results suggested that the basic matrix of the particle structure is composed of VP 1 and VP 3 and it exhibits H antigenicity.

INTRODUCTION

It has been reported that purified poliovirus with N antigenicity was converted by means of heating (Hinuma et al. 1965; Watanabe et al. 1965) or u.v. irradiation (Katagiri, Hinuma & Ishida, 1967) to a particle with H antigenicity lacking RNA. These treatments led to the production of an empty capsid, but none of the proteins in the virus capsid were liberated from the virus particle. Boeyé & Van Elsen (1967) reported that alkaline disruption of poliovirus yielded RNA-free 78s particles and slowly sedimenting heterogeneous material, and that the 78s particles in turn were slowly degraded to heterogeneous fragments. On the other hand, Maizel (1963) first showed by polyacrylamide gel electrophoresis that the poliovirus particle consisted of four virus capsid proteins (VP 1, VP 2, VP 3 and VP 4). Maizel, Phillips & Summers (1967) demonstrated that when poliovirus was heated at pH 10-5 at 40° for 30 min., two components sedimenting at 73s and about 48, were separated by sucrose gradient sedimentation, and of the four capsid proteins VP 4 was totally absent from the former, while the 4s material from the top of sucrose gradient was greatly enriched with that protein.

In the present communication we describe experiments extending this work by determining the kinetics of alkaline disruption of the virus particle at varying pH and by analysing the protein components associated with or released from the capsid under these conditions. We have also determined the antigenic properties of various intermediate particles during alkaline degradation of the capsid and discuss the structure of the virus capsid.

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METHODS

Virus and cells. The MArlONEY strain of type I poliovirus was used exclusively. Monolayer cultures of HeLa S 3 cells in 500 ml. Roux bottles were used for virus production (Hinuma et al. 1965; Katagiri et al. 1967). For preparation of the radioisotope protein-labelled virus particle, \(^{14}\text{C}\) chlorella protein hydrolysate or \(^{14}\text{H}\) leucine was used. Throughout the studies, highly purified virus particle preparations were used. Procedures for virus production, radioisotope labelling and virus purification have been described previously (Hinuma et al. 1965).

Procedure for alkaline treatment. A modification of the method of Boeyé & Van Elsen (1967) was used. One-tenth volume of Britton-Robinson's buffer (Britton & Robinson, 1931) was added to the virus labelled with \(^{14}\text{C}\) amino acids in 0.01 M-phosphate buffer, pH 7-2. The samples were adjusted with 0.2 N-NaOH to pH 10-0, 11-0 or 12-0 at 20°C, respectively, and incubated at 40°C. These pHs were adjusted every 3 min. during incubation. The samples were quickly transferred to an ice bath and layered on the top of a linear gradient of sucrose (15 to 30 %, w/v) in phosphate buffer and centrifuged in the Hitachi Type 55 P swinging bucket rotor at 13,000 g for 120 min. (4°C). Fractions were collected dropwise from the bottom of the tube.

Polyacrylamide gel electrophoresis. After alkaline treatment \(^{14}\text{C}\) amino acid labelled virus was mixed with virus labelled with \(^{3}\text{H}\) leucine, and each mixture was acidified with 0.1 vol. each of acetic acid and 10 × reticulocyte standard buffer (0.1 M-NaCl, 0.15 M-MgCl₂, 0.1 M-tris, pH 7.4: 10 × RSB), and then made 0.5 M in urea and 1 % in sodium dodecyl sulphate. After 1 hr at 37°C the samples were dialyzed for 15 to 20 hr at 25°C against 1000 to 2000 vol. of 0.01 M phosphate buffer containing 0.5 M-urea, 0.1 % sodium dodecyl sulphate and 0.1 % 2-mercaptoethanol. The method of polyacrylamide gel electrophoresis was as described by Summers, Maizel & Darnell (1965) except that the final concentration of ammonium persulphate was 0.05 % and the gel was in a 4 × 150 mm. column. After electrophoresis, gel slices were collected into scintillation vials containing 0.5 ml. of water. Each vial was allowed to stand at room temperature overnight, and \(^{3}\text{H}\) and \(^{14}\text{C}\) radioactivity was determined in a Packard liquid scintillation counter after the addition of 10 ml. of Bray's solution (Bray, 1966).

Titration of N and H antigen. N and H antigens were assayed by the complement fixation test using monoreactive N and H antisera (Katagiri et al. 1967).

Source of materials. \(^{14}\text{C}\) chlorella protein hydrolysate (80 mc./m-mole) was kindly supplied from the Institute for Applied Microbiology, University of Tokyo. \(^{3}\text{H}\) leucine (15 c./m-mole) was purchased from the Daiichi Pure Chemicals Co Ltd (Tokyo).

RESULTS

Kinetics of the release of proteins from virion on alkaline treatment

The \(^{14}\text{C}\) protein-labelled virus preparations were treated at pH 10-0, 11-0 or 12-0 at 40°C and samples were withdrawn at intervals, and immediately 1-0 ml. of each sample was layered on the top of a linear gradient of sucrose and centrifuged. Usually 18 fractions were obtained by puncture of the bottom of the tube.

Distributions of \(^{14}\text{C}\) radioactivity and H antigenicity after 15 min. and 60 min. treatment followed by fractionation are shown in Fig. 1. No N antigenicity was detected in all samples. The preparation of virus treated at pH 10-0 showed two peaks of \(^{14}\text{C}\) radioactivity; a peak of H antigenicity (H peak) in fraction 10 (about 80%) and a slower sedi-
menting peak without H antigenicity in fractions 15 to 17 (S peak), as shown in Fig. 1a and 1d. The H peak showed some heterogeneity by 60 min. treatment at this pH.

When the virus preparation was treated at pH 11.0, H peak was in fraction 11, as shown in Fig. 1b and 1e. H antigenicity was not detected in the S peak (Fig. 1b, 1e). As shown in Fig. 2, the H to S change of virus reached a plateau within 10 min. at both pH 10.0 and

Fig. 1. Sucrose gradient (15 to 30% w/w) centrifugation of poliovirus labelled with [14C] amino acid mixture after the alkaline treatment at varying pH values at 40°: (a) 15 min. at pH 10.0; (b) 15 min. at pH 11.0; (c) 15 min. at pH 12.0; (d) 60 min. at pH 10.0; (e) 60 min. at pH 11.0; (f) 60 min. at pH 12.0; •—•—•—•—•—• 14C radioactivity (counts/min.); △—△ H-complement fixing antigenicity (CF units/ml.).
I1.0, giving the conversion values of about 10 and 40%, respectively, throughout the incubation period up to 60 min. In another experiment (not shown) this was so up to 120 min. When the virus preparation was treated at pH 12.0, the peak of 14C radioactivity in fraction 10 and 11 was very small or completely absent within 10 min. and most or all of the 14C radioactivity was recovered in fraction 15, 16 and 17 (Fig. 1c, 1f, 2). H antigenicity was not found in any fractions after treatment at pH 12.0.

Thus, stepwise degradation of capsid suggested that some protein components were released from the virus particles with a certain regularity at pH 10.0 and 11.0.

![Fig. 2. The kinetics of the liberation of protein components from the [14C] protein-labelled poliovirus at varying alkaline pH at 4o°. • •, pH 12.0; • •, pH 11.0; •, pH 10.0.](image)

Gel electropherograms of the proteins from H peaks and S peaks

Polyacrylamide gel electrophoresis was used to study the polypeptide composition of the released components and the residual capsids obtained after treatment at various pH. Procedures for the samples are shown in Table 1. First, the 14C protein-labelled virus particle was treated at pH 10.0 at 4 o° for 10 min. and chilled rapidly. The treated virus was divided into two parts. One part was fractionated by sucrose gradient centrifugation, the other was adjusted to pH 11.0 and incubated at 4 o° for 10 min. and then fractionated by sucrose gradient centrifugation. 14C radioactivity of each sample showed the same pattern as shown in Fig. 1a and 1b. The H or S peak in virus treated at pH 10.0 or 11.0 was designated 10 H, 10 S, 11 H or 11 S, respectively. A portion of the 11 H was treated at pH 12.0 at 4 o° for 10 min. and fractionated by sucrose gradient centrifugation. The distribution of 14C radioactivity consisted with that shown in Fig. 1c. The S peak in the sample treated at pH 12.0 was designated 12 S. After the addition of the virus particle labelled with [3H]leucine as a marker for electrophoretic analysis, each sample (10 H, 10 S, 11 H, 11 S or 12 S) was solubilized by the treatment described in Methods, and then analysed by polyacrylamide gel electrophoresis.

The results are summarized in Fig. 3. The 10 H was found to lack VP 4 (Fig. 3a), whereas the 10 S was enriched in VP 4 (Fig. 3b), though the latter consistently contained a small amount of other peptides. The 11 H contained VP 1 and VP 3 but no VP 4 or VP 2 (Fig. 3c), whereas the 11 S contained predominantly VP 2 as well as VP 4 (Fig. 3d). Amounts
Table 1. Procedures of protein analysis of H or S peaks in alkaline-treated poliovirus samples

\[ \text{Sucrose gradient centrifugation} \]

\(11\text{H} \) Polyacrylamide gel electrophoresis (Fig. 3c)

\(11\text{S} \) Polyacrylamide gel electrophoresis (Fig. 3d)

\(10\text{H} \) Polyacrylamide gel electrophoresis (Fig. 3a)

\(10\text{S} \) Polyacrylamide gel electrophoresis (Fig. 3b)

\(12\text{S} \) Polycrylamide gel electrophoresis (Fig. 3e)
of both VP 1 and VP 3 in the 11 S were also not negligible but were consistently very small. In the 12 S, the polypeptide pattern was indistinguishable from that of the 11 H as shown in Fig. 3e.

Fig. 3. Polypeptide analysis by polyacrylamide gel electrophoresis of H or S peaks derived from 14C labelled virus capsid. (a) 10 H; (b) 10 S; (c) 11 H; (d) 11 S; (e) 12 S (see Table 1).

Purified virus labelled with [3H] leucine was added to each sample, and each mixture was solubilized as described in Methods and analysed in 10% acrylamide gel. The electrophoresis was for 24 hr at 5 mA/column. The anode is to the right in these figures. O-----O 3H radioactivity; •----------• 14C radioactivity.

From these results, it is most probable that a substructure composed of VP 4 is readily released from the virus capsid at pH 10·0, and when virus particles lacking VP 4 are treated at pH 11·0, a substructure containing VP 2 is also liberated from the virus capsid. On
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When poliovirus preparations were analysed by sucrose gradient centrifugation after heating at 50° (Hinuma et al. 1965) or u.v. irradiation at pH 7.2 (Katagiri et al. 1967; S. Aikawa, S. Katagiri & Y. Hinuma, unpublished results), the 14C protein-labelled virus particle with N antigenicity, 160 s, was converted to an empty particle with H antigenicity, 80 s, and concomitantly virus RNA was released from the capsid, while none of 14C protein was detected in slower sedimenting fractions. However, on heating at 40° at pH 10.0, virus RNA was released from the capsid (Van Elsen & Boeyé, 1966) and a minor component enriched in VP 4 was simultaneously liberated from the capsid. In this connection, the present report suggests that VP 4 is readily removed from the capsid without a measurable change in the sedimentation coefficient of the capsid (Fig. 1a, 1d; Fig. 3a, 3b). Furthermore, it was evident that virus samples after treatment at pH 10.0, which contained capsids lacking RNA and VP 4, were incubated at pH 11.0, the second component consisting mainly of VP 2 was released from the altered capsid. The remaining capsids which contained VP 1 and VP 3 (Fig. 3c) had a slightly smaller sedimentation coefficient than the VP 4-deficient particle (Fig. 1b, 1e) in all of 8 repeated experiments. Thus the VP 1 and VP 3 are bound to each other in a way that is stable at pH 11.0. This particle deficient in VP 2 and VP 4 was however unstable at pH 12.0 and was converted to smaller components (Fig. 1c, 1f). However, at least the peptide-bonds in the 12 S were never broken by the treatment at pH 12.0 because the electrophoretic pattern of the 12 S was the same as that of the 11 H (Fig. 3c, 3e). Although a small amount of VP 1 and VP 3 in the S peak liberated at pH 10.0 and 11.0 could be accounted for by complete degradation of a small part of virus particles at these pH values (Maizel et al. 1967), an association of these proteins as necessary components of the liberated substructure cannot be ruled out.

These results show that it is probable that the poliovirus capsid consists of the three kinds of substructural protein components, i.e. a minor substructure composed of VP 4, the second substructure composed of VP 2 and the third substructure composed of VP 1 and VP 3.

It was of particular interest that the H antigenicity of the virus capsid was shown not only in the RNA-deficient particles produced by heating or u.v. irradiation at neutral pH, but also in the particles lacking in both RNA and VP 4 after treatment at pH 10.0 and in the 60 s particles lacking in RNA, VP 4 and VP 2 after treatment at pH 11.0. Clearly, the H antigenicity on the surface of the virus capsid (Katagiri, Hinuma & Ishida, 1968; Hinuma, Katagiri & Aikawa, 1970) is determined by a structure composed of two virus proteins, VP 1 and VP 3, and apparently VP 2 and VP 4 are not involved. That H antigenicity is determined by a certain conformation brought about by combination of VP 1 and VP 3 is supported by the fact that the S peak produced at treatment at pH 12.0 did not react with anti-H serum.

Combining the previous and present data, the properties of various forms of virus particle produced by various treatments are summarized in Table 2. This may lead to a conclusion that the basic matrix of the virus capsid is constructed only by VP 1 and VP 3. The arrangement of these proteins in the architecture of the particle may be one of most important keys to the determination of the fine structure of poliovirus. Studies to this end are now in progress in our laboratory.
Table 2. Properties of various forms of poliovirus particles produced by physicochemical treatments

<table>
<thead>
<tr>
<th>Particle</th>
<th>Approximate sedimentation coefficient</th>
<th>Antigenicity</th>
<th>Peptide composition</th>
<th>Produced by treatment with</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus particle</td>
<td>160 s</td>
<td>N</td>
<td>VP 1, 2, 3, 4</td>
<td>pH 7.2, 50°* or u.v.†</td>
</tr>
<tr>
<td>RNA deficient particles</td>
<td>80 s</td>
<td>H</td>
<td>VP 1, 2, 3, 4</td>
<td>pH 10.0, 40°§</td>
</tr>
<tr>
<td>RNA . VP4 deficient particles</td>
<td>80 s</td>
<td>H</td>
<td>VP 1, 2, 3</td>
<td>pH 11.0, 40°§</td>
</tr>
<tr>
<td>RNA . VP4 . VP2 deficient particles</td>
<td>60 s</td>
<td>H</td>
<td>VP 1, 3</td>
<td></td>
</tr>
</tbody>
</table>

* Hinuma et al. 1965.
† Katagiri et al. 1967; Aikawa, Katagiri & Hinuma, unpublished results.
‡ Maizel et al. 1967.
§ The present report.
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REFERENCES


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