Dense Particles and Slow Sedimenting Particles Produced by Ultraviolet Irradiation of Poliovirus

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SUMMARY

Low doses of u.v. radiation rapidly inactivate poliovirus, and the virus is progressively converted into dense particles (DPs) of buoyant density 1.44 g/ml in CsCl. The DPs are structurally and antigenically related to standard virus (N-antigen), i.e. they are indistinguishable from virus in their RNA and protein content and in their sedimentation properties. Furthermore, there is no difference in reactivity of the structural proteins of virus and DPs with the monofunctional reagent [3H]N-succinimidyl propionate (3H-NSP). However, DPs differ from virus in that their capsids are permeable to several ions, and they can be degraded by RNase and protease. Increasing the radiation dose causes a successive transformation of DPs into 105S slow-sedimenting particles (SSPs). The SSPs are antigenically related to 76S artificial empty capsids (AECs) or H-antigen, but they differ physically and structurally from them. The SSPs have a higher S value than AECs and contain all the capsid proteins, including VP4, and the RNA, both of these macromolecules being absent from AECs. It is concluded, therefore, that transformation from N- to H-antigenicity by u.v. radiation does not require release of RNA and VP4. Conversion of virus particles to SSPs correlates with altered reactivity of VP2 and to a lesser extent VP1 and VP3, with the monofunctional reagent 3H-NSP.

INTRODUCTION

Ultraviolet (u.v.) radiation inactivates picornaviruses very efficiently. It has been shown for poliovirus that high radiation doses lead to an altered antigenicity (Le Bouvier, 1959; Roizman et al., 1959; Katagiri et al., 1967). Inactivation has mostly been attributed to intramolecular cross-links between nucleotides of the RNA as in the case of mengovirus (Miller & Plagemann, 1974). Recently, photo-induced cross-links between the RNA and coat proteins of poliovirus have been described, and the proteins associated with the RNA were identified (Wetz & Habermehl, 1982). In the course of these investigations it was found that considerable conformational changes, depending on the radiation dose, of the virus capsid occurred.

Structural alterations of picornaviruses, either naturally occurring or artificially produced, have already been described. Formation of dense particles is a common phenomenon of picornaviruses. They naturally occur in harvests of several picornaviruses, such as poliovirus (Yamaguchi-Koll et al., 1975; Wiegert et al., 1977), mengovirus (Perez-Bercoff et al., 1978), swine vesicular disease virus (SVDV) (Rowlands et al., 1975) and eechovirus type 11 (Cova-Baczko & Aymard, 1982). Artificially produced dense particles, caused by β-radiation, were observed in preparations of radioactively labelled poliovirus (K. Wetz, unpublished results). Induction of structural alterations in picornaviruses by different treatments results in several effects. Treatment of poliovirus with heat (Breindl, 1971), urea (Vanden Berghe & Boeyé, 1973) or alkali (Katagiri et al., 1971) produces particles deficient in RNA and VP4 (artificial empty capsids, AECs). Structurally altered particles containing protein and RNA have been described.
for poliovirus after u.v. irradiation (Katagiri et al., 1967) and formaldehyde treatment (Wouters et al., 1973) and for foot-and-mouth disease virus (FMDV) after glutaraldehyde fixation (Sangar et al., 1973).

In this paper we show that, at low doses of u.v. radiation, dense particles (DPs) were produced and with increasing dose a successive modification into slow sedimenting particles (SSPs) of altered antigenicity occurred. We characterized the two kinds of particles and investigated their structural and antigenic relations to untreated poliovirus. We could show that the described alteration from N- to H-antigenicity had occurred at a stage when the particles still contained all the capsid proteins and RNA. We have correlated the gross structural changes to rearrangements of specific polypeptides in the virus capsid.

**METHODS**

**Virus and cells.** A large-plaque variant of poliovirus type 1 (Mahoney) was used throughout. HeLa S3 cells were cultivated in suspension using Joklik's minimum essential medium (Gibco) supplemented with 2 g/l bicarbonate, at 56 °C for 30 min; antibiotics added were penicillin G, 100 international units (IU)/ml, streptomycin sulphate, 50 μg/ml, and nystatin, 25 IU/ml.

**Propagation, labelling and purification of virus.** Cells (2 × 10⁶/ml) were infected with virus at a multiplicity of infection (m.o.i.) of 50. To prepare radioactively labelled virus, growth medium lacking amino acids was used. At 2:5 h post-infection, either 3H-labelled amino acids (3-3 μCi/ml) or 14C-labelled amino acids (1-6 μCi/ml) were added (Amersham International). To harvest virus, cells were centrifuged at 60 h post-infection by centrifugation in a Cryofuge 6-6 (Heraeus/Christ, F.R.G.) at 2500 rev/min for 25 min at 4 °C. The cells were resuspended in PBS (0-14 mM-NaCl, 0-49 mM-MgCl₂, 62H₂O, 2.7 mM-KCl, 0-68 mM-CaCl₂, 2H₂O, 6-5 mM-Na₂HPO₄.2H₂O, 0-14 mM-KH₂PO₄, pH 7-2) and lysed by three cycles of freezing and thawing. After the cell debris had been removed by low-speed centrifugation, the virus was precipitated with 10% (w/w) polyethylene glycol (PEG) 6000 (Serva, Heidelberg, F.R.G.), 1% NaCl overnight at 4 °C. Alternatively, the cells were resuspended in PBS containing 0.05% Triton X-100, and after one cycle of freezing and thawing the suspension was clarified in an SW27 rotor (Beckman, Spinco) at 13000 rev/min for 30 min at 4 °C. Thereafter, the virus was sedimented at 27000 rev/min for 150 min at 4 °C. The virus pellet was homogenized in PBS, and CsCl was added to a final density of 1.34 g/ml. After centrifugation (VTi65 rotor, 55000 rev/min, 4 °C, 6 h), the virus band was collected and subjected to a second cycle of isopycnic centrifugation. After dialysis against PBS, the A₂₅₀/A₂₈₀ ratio was usually 1.6.

**Ultraviolet irradiation conditions.** Irradiation was performed at a distance of 10 cm from a mercury germicidal lamp (Osram HNS 10 W, F.R.G.), emitting predominantly at 254 nm with an intensity of 3.2 J/m²/s at the surface of the virus suspension. No correction was made for the scattering of light. Virus was irradiated in ice-cooled plastic containers at a solution depth of 1 mm.

**Trypsin treatment.** Poliovirus-related particles in PBS were reacted with trypsin (Worthington, N.J., U.S.A.) at 1 μg trypsin to 10 μg protein at 20 °C. The reaction was stopped at the time by the addition of a tenfold excess of soybean trypsin inhibitor (Boehringer Mannheim).

**RNase digestion.** Poliovirus-related particles were treated with RNase A (Serva) for 1:5 h at 20 °C (5 μg RNase A to 20 μg RNA). Samples were centrifuged on sucrose gradients (10 to 30% (w/w) for 135 min at 40000 rev/min in an SW40 rotor). All sucrose gradients presented were run under identical conditions.

**SDS-polyacrylamide gel electrophoresis.** For analysis of the proteins by gel electrophoresis (SDS-PAGE), the virus samples were incubated for 1-2 h at 37 °C using a mixture of RNase A and T₁ (Serva) at 5 μg RNase A and 5 μg RNase T₁, to 20 μg RNA, before the proteins were denatured by SDS (2 min, 100 °C). The fluorographic method of Chamberlain (1979) was used to visualize the proteins on slab gels.

**Modification of virus.** Reaction of virus-related particles with the monofunctional reagent [3H]N-succinimidyl propionate (3H-NSP; Amersham International) was performed in 20 mM-triethanolamine–HCl pH 8.2 as described by Wetz & Habermehl (1979). Excess reagent was removed by sucrose gradient centrifugation.

**Antisera.** Antisera against poliovirus (aV) and against 16 min irradiated virus (aSSP) were prepared in rabbits. Five A₂₅₀ units (0-5 ml) of purified virus or SSPs were mixed with 0-5 ml of complete Freund's adjuvant, and the animals were injected subcutaneously. Additional 0-25 ml samples of the respective virus preparations, mixed with 0-25 ml of incomplete Freund's adjuvant, were injected at 4-week intervals. The animals were bled monthly. Antiserum against N-antigen (aN) was prepared by absorption of 200 μl aV with 50 μl AEcs. AEcs were produced by treatment of 50 μl virus (0-5 A₂₅₀ units) for 1 h at 56 °C (Breindl, 1971). Preimmune sera were obtained prior to the first immunization.

**Immunoprecipitation.** The method of Putnak & Phillips (1982) was used with slight modifications. Dilutions of antisera were prepared in NET buffer (0-1 M-NaCl, 1 mM-EDTA, 0-01 M-Tris–HCl pH 7.2) containing 0-2% bovine serum albumin, 0-02% Triton X-100. Five μl of radioactively labelled antigen corresponding to 5000 cts/min were mixed with 15 μl of the serum dilution and incubated for 2 h at 20 °C. To this solution 80 μl of a
Poliovirus-related particles produced by u.v.

20% suspension of Protein A-Sepharose CL-4B (Sigma) in NET was added and incubated for 1-5 h at 20 °C. The suspension was centrifuged and the beads were washed twice with NET. The immunoprecipitate was counted for radioactivity. Preimmune sera were treated in the same way.

Immunodiffusion. Double diffusion (Ouchterlony, 1958) was performed in 1% agarose containing PBS and 0.02% sodium azide. The gel was 2.5 mm thick, and the wells were 3 mm in diameter. Antigens and antisera were diluted in PBS; 10 μl samples were placed into each well, and diffusion was allowed to proceed in a moist chamber for 3 days at 22 °C.

RESULTS

Dense particles (DPs) produced by low u.v. irradiation dose

Irradiation of poliovirus for 15 s caused a loss of infectivity of 5 log_10 units and after 30 s irradiation all the virus was non-infective (Fig. 1). Isopycnic centrifugation in CsCl of virus irradiated for 30 s revealed that about 7% banded as DPs at a density of 1.44 g/ml, whereas the bulk still banded at 1.34 g/ml. Since the latter particles are also non-infective they are designated NIPs. Fig. 2(b) shows that after 1.5 min irradiation, 40% banded as DPs. Thus, it was concluded that, depending on the radiation dose, NIPs were progressively converted into DPs. The kinetics of the formation of DPs as analysed by isopycnic centrifugation in CsCl are shown in Fig. 1.

Comparing the amounts of radioactivity in the RNA and protein moieties of the two bands (Fig. 2b) with those of standard virus (Fig. 2a) it was concluded that DPs and NIPs contained the full complement of RNA and protein. This was confirmed for the structural proteins by SDS-PAGE (Fig. 4c, e, g).

However, centrifugation in CsCl at 25 °C (Fig. 2c), instead of 10 °C (Fig. 2b), yielded more DPs, although the two samples were irradiated with equal doses. This shows an additional temperature dependence of the conversion process.

When virus irradiated for 1.5 min was mixed with standard virus and sedimented through a linear sucrose gradient, all the virus sedimented at 156S. Up to 2 min of irradiation produced no change in sedimentation behaviour (see Fig. 5a and 6a). Isopycnic banding of irradiated virus in CsCl prior to sedimentation in sucrose gradients had no influence on the sedimentation behaviour compared to standard virus (156S). When DPs and NIPs were isolated, dialysed against PBS, and run through sucrose gradients, they sedimented as 156S particles (Fig. 5a to c).

The morphological integrity of DPs was demonstrated by electron microscopy of negatively stained samples (Fig. 3b). DPs were similar in size to virus (Fig. 3a) but they showed a ring inside the particles. Their outer diameter was estimated to be 28 nm, which is in reasonable agreement with the published diameter of 27 nm for virus (Schaffer & Schwerdt, 1959). The inner ring of 17 nm diameter was attributed to the RNA core, which agrees reasonably with the published data of 18 nm for poliovirus (Boublik & Drzeniek, 1976) and 19 nm for small spherical RNA viruses (Jacrot et al., 1977). Therefore, we calculated 5.5 nm for the thickness of the protein shell.

Structural changes of the capsid could be established by the sensitivity of DPs to protease. In contrast to standard virus, DPs were sensitive to trypsin while NIPs, although irradiated with the same dose, were not. Fig. 4(a to g) shows that the capsid proteins of DPs were degraded to smaller, faster migrating polypeptides, while the polypeptides of NIPs migrated similarly to those of virus.

It had previously been shown that u.v. irradiation renders poliovirus sensitive to RNase (Katagiri et al., 1967). Therefore, we tested the sensitivity of DPs and NIPs to RNase A. Subsequent sucrose gradient centrifugation revealed that only DPs (Fig. 5c) were degraded to 76S AECs, lacking RNA and VP4 (Fig. 4k), whereas NIPs and standard virus were resistant (Fig. 5a, b).

Slow sedimenting particles (SSPs) produced by high u.v. irradiation dose

Irradiation times up to 2 min did not influence the sedimentation coefficient of the virus in sucrose gradients (Fig. 6a). However, increasing irradiation times resulted in a severe conformational change of the virus, which caused a reduction in its S value and a progressive
Fig. 1. Kinetics of poliovirus inactivation and conversion of particles with time of u.v. irradiation. ■, Infectivity; ○, conversion into DPs; ●, conversion into SSPs. Infectivity was estimated by plaque titration. The percentage of DPs was estimated from isopycnic centrifugation profiles such as that in Fig. 2(b), and the percentage of SSPs from velocity sedimentation profiles such as that in Fig. 6.

conversion into SSPs. After 8 min irradiation 50% of the virus sedimented as SSPs, while the other half sedimented at 156S (Fig. 6b). After 16 min irradiation all the virus sedimented as SSPs (Fig. 5d). The dose dependence of the formation of SSPs, as analysed by sucrose gradient centrifugation, is shown in Fig. 1. Using the relation that in a sucrose gradient particles migrate approximately in proportion to their S values (Martin & Ames, 1961), we calculated a value of 10^4S for SSPs. The same result was obtained when irradiated virus was centrifuged in CsCl (where all the virus banded as DPs), prior to sedimentation on a sucrose gradient.
The SSPs had the same ratio of RNA to protein as untreated virus as analysed on sucrose gradients (Fig. 5a, d). SDS–PAGE confirmed that the SSPs contained the four structural proteins (Fig. 4i). It was therefore concluded that the SSPs had the same mass as untreated virus.

In sedimentation velocity experiments in the analytical ultracentrifuge SSPs sedimented monodispersely with constant velocity in the gravitational field (Fig. 7) (see Svedberg & Pedersen, 1940). It was possible, therefore, to estimate their sedimentation coefficient. Referring to standard conditions we obtained the value $s_{20,w} = 105.2$.

In contrast to DPs and NIPs, the SSPs were rather unstable. Recycling on a sucrose gradient caused partial loss of VP4 and RNA (not shown). Treatment with RNase A produced 76S AECs.
Fig. 4. Effect of trypsin on poliovirus-related particles. As shown in Fig. 2(b), NIPs, fraction 20, and DPs, fraction 6, were obtained from a CsCl gradient of virus irradiated for 1-5 min and dialysed against PBS. SSPs were obtained by irradiation of virus for 16 rain. AECs were prepared as described in Methods and subsequently purified on sucrose gradients as in Fig. 5(d). Digestion conditions are described in Methods. Electrophoresis was performed on 15% SDS-polyacrylamide gels. The radioactively labelled proteins were visualized by fluorography. (a) DPs +, trypsin-treated for 60 rain; (b) DPs +, trypsin-treated for 30 min; (c) DPs -, untreated; (d) NIPs +, trypsin-treated for 60 min; (e) NIPs -, untreated; (f) V +, standard virus trypsin-treated for 60 min; (g) V -, standard virus untreated; (h) SSPs +, trypsin-treated for 30 min; (i) SSPs -, untreated; (j) AECs +, trypsin-treated for 30 min; (k) AECs -, untreated.

(Fig. 5 d). Digestion with trypsin caused rapid degradation of the structural proteins of SSPs and AECs. The decrease in VP1 to VP3 of DPs, SSPs and AECs after trypsinization and the concomitant increase in degradation products showed that SSPs and AECs were more sensitive than DPs (Fig. 4a, b, h, j). The even faster degradation of AECs compared to SSPs might be due to protection by the RNA in the latter.

Electron microscopical examination of negatively stained SSPs from the peak fraction of a sucrose gradient revealed that the SSPs had a larger diameter and a more diffuse shape than untreated virus (Fig. 3c).

Immunological properties of DPs and SSPs

The antigenic properties of the different virus particles were examined by radial immunodiffusion against antiviral serum (αV), anti-N serum (αN) and anti-SSP serum (αSSP). DPs had the same antigenicity (N) as standard virus, whereas SSPs were H-antigenic, as were AECs.

Completely fusing precipitin bands of standard virus and virus irradiated for 2 min (containing mostly DPs plus a few NIPs) showed antigenic identity when tested against αV and αN (Fig. 8a, b). Crossing over between the bands of virus and SSPs and of virus and AECs respectively revealed non-identity when diffusing against αV (Fig. 8a), whereas identity was deduced from fusing bands between AECs and SSPs against αV or αSSP (Fig. 8a, c). As, firstly, SSPs had the same antigenicity as AECs and, secondly, AECs were defined as having H-antigens (Breindl, 1971), it was concluded that SSPs were also H-antigenic.

Intermediate times of irradiation led to progressive conversion of the virus from N- to H-antigenicity. Virus irradiated for 6 min possessed both N- and H-antigenicity, while 10 min...
Poliovirus-related particles produced by u.v.

Fig. 5. Sucrose gradient centrifugation of RNase A-treated virus-related particles in comparison to untreated particles. RNase A treatment of double-labelled particles (3H-labelled RNA and 14C-labelled protein) and centrifugation were as described in Methods. Sedimentation is from right to left. Fractions were collected and counted for radioactivity. (a) Standard virus; (b) NIPs; (c) DPs; (d) SSPs. Preparation of NIPs, DPs and SSPs is described in Fig. 4. □, 3H-labelled RNA of RNase A-treated particles; ■, 14C-labelled protein of RNase A-treated particles; O, 3H-labelled RNA of untreated particles; ●, 14C-labelled protein of untreated particles.

Fig. 6. Sedimentation velocity analysis of poliovirus-related particles on sucrose gradients. (a) Virus irradiated for 2 min; (b) virus irradiated for 8 min. O, [3H]Uridine-labelled RNA; ●, 14C-labelled protein.

Irradiation produced mostly H-antigenicity. Fusing precipitin bands between SSPs and 10 min irradiated virus and between 10 min and 6 min irradiated virus showed antigenic identity when tested against αSSP (Fig. 8c). The partial N-antigenicity of virus irradiated for 6 min was revealed by the incomplete fusion and spur formation of the bands of standard virus and virus irradiated for 2 min over the band of virus irradiated for 6 min, when tested against αN (Fig. 8b).
Fig. 7. Sedimentation velocity patterns of SSPs in the analytical ultracentrifuge (Beckman model E) equipped with a photoelectric scanner. The traces were monitored, at 264 nm 10 min after reaching a maximum speed of 28000 rev/min, at intervals of 4 min. Sedimentation is to the right. Sedimentation was performed in PBS at a rotor temperature of 7.2 °C using a charcoal-filled Epon double-sector centre piece. The S_{apparent} value was corrected for viscosity and density of the solution. The partial specific volume of the solute was assumed to be 0.687 ml/g (Rueckert, 1976).

The very faint precipitation obtained with SSPs and AECs against αN (Fig. 8b) could be due to some degradation of the particles during the diffusion time and reaction of cryptotopic antigenic determinants with corresponding antibodies in the antiserum (Stöfler et al., 1973). The diffuse precipitation of standard virus and 2 min irradiated virus against αSSP could be explained by H-antigenic particles constantly present in virus preparations (Fig. 8c).

Differentiation between the antigenicities of virus-related particles could also be established by immunoprecipitation using αN and protein A. When virus irradiated for 2 min was separated into DPs and NIPs, the two kinds of particles were immunoprecipitated up to high serum dilutions, while SSPs and AECs were scarcely precipitated (Fig. 9). The 156S particles of virus irradiated for 6 min, isolated from a sucrose gradient, were precipitated to a higher extent than a normal mixture of 156S particles and SSPs from virus irradiated for 8 min, showing that as long as the particles are 156S, they are antigenically more related to virus than to SSPs. The two different tests showed that AECs and SSPs on the one hand and native virus and DPs on the other hand were immunologically related particles.

Chemical modification of virus-related particles

Since we suggested that conformational changes of the capsid were responsible for the generation of DPs and SSPs and for their different immunological and physical properties, we attempted to relate these changes to rearrangements of the structural proteins. We could show, by chemical modification of lysine groups of the capsid proteins with the monofunctional reagent 3H-NSP, that surface alterations of VP2 were mainly responsible for the N to H transformation: SSPs were reacted with 3H-NSP and excess reagent was separated by sucrose gradient centrifugation. As shown in Fig. 10(a) the sedimentation coefficient of the modified SSPs was unchanged but the band was slightly broadened, when compared to unmodified SSPs (Fig. 5d). The extent of modification of the capsid proteins was examined by SDS–PAGE. VP1 to VP3 were modified, while VP4 was absent from the electrophoresis pattern (Fig. 10b). Since untreated SSPs contained VP4 (Fig. 4i), loss of this protein must be caused by the chemical treatment, either by modification or solvent conditions, followed by sucrose gradient centrifugation and subsequent SDS–PAGE of double-labelled SSPs (14C-protein and 3H-RNA). Treatment with the solvent (triethanolamine) alone, followed by sucrose gradient centrifugation and subsequent SDS–PAGE of double-labelled SSPs (14C-protein and 3H-RNA) revealed that the particles became deficient in VP4, but still contained VP1 to VP3 and the RNA in normal amounts (not shown). Therefore, we suggest that absence of VP4 in the 3H-NSP-modified SSPs was due to triethanolamine treatment.
Fig. 8. Immunological relationship of virus-related particles by radial immunodiffusion. Each of the radial wells of (a), (b), and (c) contained 9 x 10^10 particles as antigen. V: standard virus; 2': virus irradiated for 2 min; 6': virus irradiated for 6 min; 10': virus irradiated for 10 min. SSPs and AECs. The centre wells contained as antisera: (a) aV, anti-virus serum (diluted 1:3); (b) aN, anti-N serum; (c) aSSP, anti-SSP serum.
Fig. 9. Reactivities of poliovirus-related particles to anti-N serum (αN) using Protein A. ●, Virus; ▽, NIPs; ○, DPs. NIPs and DPs were prepared as described in Fig. 4. ▲, 156S peak of virus irradiated for 6 min, isolated from a sucrose gradient such as that in Fig. 6(b), fraction 14, and dialysed against PBS; △, virus irradiated for 8 min, unfraccionated; ■, AECs; □, SSPs. The percentage of immunoprecipitation (% ip) was calculated using the following formula: % ip = (ct/min ip – ct/min pip)/(ct/min v – ct/min pip) × 100, where ct/min ip = amount of radioactivity immunoprecipitated using αN, ct/min pip = amount of radioactivity immunoprecipitated using preimmune serum, and ct/min v = amount of input radioactivity of the virus.

Fig. 10. Analysis of SSPs after modification with 3H-NSP. (a) Sucrose gradient centrifugation; (b) SDS-PAGE of SSPs isolated from the peak fractions of the sucrose gradient. The gel was cut into 1 mm slices; b, bromophenol blue. ○, 14C-labelled capsid protein; ●, 3H-NSP bound to the proteins.

Likewise, chemical modification of standard virus, NIPs and DPs led to modification of VP1 to VP3, but in no case was VP4 modified, although present on the electrophoresis pattern (not shown). Similarly VP1 to VP3 of AECs were modified.

From the specific radioactivity of the reagent (3H) and from the radioactive label of the virus capsid (14C), it is possible to calculate the number of modified lysine residues in each capsid protein (Table 1), assuming that one A260 unit corresponds to 9.4 × 1012 virions (Rueckert, 1976). Virus, NIPs and DPs contained equal numbers of modified lysines in their polypeptides.
Poliovirus-related particles produced by u.v.

Table 1. Modified lysine residues per capsid protein in poliovirus-related particles using $^3$H-NSP*

<table>
<thead>
<tr>
<th>Capsid protein</th>
<th>VP1</th>
<th>VP2</th>
<th>VP3</th>
<th>VP4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus</td>
<td>3.4</td>
<td>0.6</td>
<td>2.0</td>
<td>0.03</td>
</tr>
<tr>
<td>NIPs</td>
<td>3.4</td>
<td>0.6</td>
<td>2.0</td>
<td>0.02</td>
</tr>
<tr>
<td>DPs</td>
<td>3.4</td>
<td>0.6</td>
<td>2.0</td>
<td>0.01</td>
</tr>
<tr>
<td>SSPs</td>
<td>4.3</td>
<td>2.3</td>
<td>2.6</td>
<td>- $^\dagger$</td>
</tr>
<tr>
<td>AECs</td>
<td>5.1</td>
<td>3.3</td>
<td>3.5</td>
<td>- $^\ddagger$</td>
</tr>
</tbody>
</table>

* The number of modified lysine residues was calculated as follows. One $A_{260}$ unit of virus corresponds to $9.4 \times 10^{12}$ virions (Rueckert, 1976) and was determined to be equivalent to $3.05 \times 10^{6}$ d/min of $^1$C-amin-o acid-labelled virus. After modification of the particles with $^3$H-NSP (sp. act. 66 Ci/mmol) the amount of modification of each capsid protein was determined from a graph like that in Fig. 10(b). Taking into account that a virion contains 60 copies of each polypeptide, the number of modified lysine residues per polypeptide in one particle could be calculated. The total number of lysine residues in each polypeptide is: VP1, 14; VP2, 4; VP3, 10; VP4, 4 (Kitamura et al., 1981).

$^\dagger$ Detached from SSPs due to solvent conditions during modification.

$^\ddagger$ Not present in AECs (see Fig. 4k).

However, the extent of modification of SSP polypeptides was approximately fourfold higher in VP2 and 1.3-fold higher in VP1 and VP3 than in the corresponding proteins of standard virus particles. The higher modification rate in the AEC proteins is probably due to additional accessible lysines in the interior of the shell.

**DISCUSSION**

We have shown in this paper that a low u.v. radiation dose leads to the formation of DPs, which are antigenically related to native virus. Therefore, the differences in capsid structure between them should be minimal. These differences were not detectable by chemical modification using the monofunctional reagent $^3$H-NSP. Similar results have been published upon iodination of SVDV dense particles (Rowlands et al., 1975). However, there are at least four ways in which poliovirus DPs can be distinguished structurally from native virus: (i) penetration of caesium ions into the particles, which has been shown by Mapoles et al. (1978) to be a prerequisite for their higher density; (ii) penetration of small amounts of uranyl acetate as analysed by negative staining; (iii) sensitivity of DPs to RNase; (iv) sensitivity to protease.

From the first three features of DPs, it is reasonable to conclude that small holes are generated in the capsid, which allow the passage of ions. These holes may also be the reason why the RNA becomes accessible to RNase. However, it is difficult to imagine that an RNase molecule can pass through a 5-5 nm-long hole, which is the thickness of the protein shell. If we assume that the RNA, at certain repeated positions, is embedded into the shell then relatively minor conformational changes of the capsid may expose the RNA. A similar structural model for the RNA core has been proposed by Jacrot et al. (1977) for cucumber mosaic virus (CMV), a small RNA-containing isometric plant virus, which is also sensitive to RNases. These authors showed that the RNA of CMV penetrates, by about 2 nm, into the protein shell which has a total thickness of 6 nm, and that the protein packaging of the capsid leaves room for holes. The fourth criterion suggests that the shell of the DPs differs structurally from that of native virus and from NIPs in that it is sensitive to trypsin. This requires that at least lysine- or arginine-containing regions of the polypeptides become more exposed in the DPs. However, the exposure of lysine may be excluded, because DPs, NIPs and virus are similarly modified by the lysine-specific reagent.

Higher radiation doses caused a reduction of the S value of the particles, leading to the transformation of the virus (N-antigen) to SSPs (H-antigen). This process implies severe conformational changes of the virus capsid. The capsid of SSPs should be structurally related to AECs, since both are H-antigens. However, unlike AECs, SSPs contain VP4 and RNA.
In contrast to virus, SSPs have more than four times as many surface-directed lysine groups in VP2 and about 1.3 times as many in VP1 and VP3 respectively, as judged by reaction with $^3\text{H}$-NSP. It is therefore reasonable to assume that the transformation of poliovirus from N- to H-antigenicity is mainly accompanied by conformational alterations of VP2 and to a lesser extent VP1 and VP3. A contribution of VP4 to this conformational change could not be deduced from the modification studies, since modified SSPs became deficient in VP4 due to solvent conditions.

The greater extent of chemical modification of AECs compared to SSPs may be due to reactive lysines in the interior of the shell, which are covered in the SSPs by the RNA. Similar results were obtained by modification of poliovirus AECs, using an enzymic iodination reaction (Beneke et al., 1977), and by using acetic anhydride as a labelling reagent (Lonberg-Holm & Butterworth, 1976). The latter authors obtained similar results with rhinovirus (HRV-2) both in the transformation from virus to AECs and the predominant labelling of VP2.

The reduction of the S value of SSPs to 105S as compared with that of virus (156S) can be attributed to a swelling of the particles. The sedimentation velocity of a particle depends on both mass and shape. Since SSPs have the same mass as virus, the slower sedimentation of SSPs may be due to their larger diameter. This assumption is supported by the larger diameter of SSPs in electron micrographs. The degree of swelling has yet to be verified, especially since some specimens of SSPs also showed empty shells of the same size as virus or broken shells in the electron micrographs. One may speculate that SSPs represent a transition state, which facilitates the extrusion of the RNA, resulting in formation of AECs of diameter similar to virus. Poliovirus particles expanded like SSPs were obtained under special specimen deposition conditions for electron microscopical visualization (Boublik & Drzeniek, 1977). Likewise, treatment of FMDV with glutaraldehyde resulted in a swelling of the particles and a reduction of their S value (Sangar et al., 1973). We assume that swelling of picornaviruses after several treatments is a general phenomenon. Our data indicate that swelling is accompanied by marked structural changes including the change from N- to H-antigenicity. Hence, release of RNA and VP4 is not an absolute prerequisite.

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