Structural Subunits of Poliovirus Particles by Electron Microscopy

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(Accepted 2 May 1977)

SUMMARY

Electron microscopy of poliovirus particles and empty capsids under various conditions of specimen deposition and staining visualizes the dissociation products of these particles. The dissociation proceeds in steps; it begins with the expansion of particles and leads to the final product of the dissociation—a cluster of several sub-particles of equal size (approx. 100 Å in diam.). A scheme of the dissociation is proposed on the basis of the observed intermediates.

INTRODUCTION

Poliovirus particles prepared for electron microscopic study by conventional procedures, using phosphotungstic acid (PTA) negative staining, appear as small compact spheres with a diam. of approx. 280 Å, only rarely showing a fine structure (Schaffer & Schwerdt, 1959; Rueckert, 1971; Brown & Hull, 1973). Differences in the number of capsomers assigned to the poliovirus particle, ranging from 32 (Mayor, 1964) or 42 (Mayor, 1964; Agrawal, 1966) to 60 (Horne & Nagington, 1959), reflect the difficulty in determining the fine structure of poliovirus particles by electron microscopy. From X-ray studies, Finch & Klug (1959) suggested that the poliovirus particle contains 60 identical subunits.

Further insight into the structure of picornaviruses was obtained from biochemical studies by gradual dissociation of virus particles (Katagiri, Aikawa & Hinuma, 1971; Rueckert, 1971; Talbot & Brown, 1972; Philipson, Beatrice & Crowell, 1973; Vanden Berghe & Boeyé, 1973; Drzeniek, 1975) which led to models for the virus particle (Rueckert, 1971; Talbot & Brown, 1972; Philipson et al. 1973).

We have attempted to visualize the fine structure of the poliovirus particle by a number of electron microscopic techniques. The use of Cs⁺ ions as a contrasting stain revealed RNA-containing cores with a diam. of 180 Å in poliovirus particles (Boublik & Drzeniek, 1976). In the electron microscopy study presented here we have obtained direct evidence for substructures of the poliovirus particle measuring about 100 Å in diam.

METHODS

Standard poliovirus particles (density = 1.34 g/ml) type I, strain Mahoney (Wiegers, Yamaguchi-Koll & Drzeniek, 1977) and naturally occurring empty capsids (top component, density = 1.29 g/ml) were obtained and characterized as described previously (Drzeniek & Bilello, 1974; Yamaguchi-Koll, Wiegers & Drzeniek, 1975; Boublik & Drzeniek, 1976). They were stored at 4 °C in 3 M-CsCl (at −18 °C for longer periods), and diluted for the electron microscopy in 0.05 M-tris-HCl buffer, pH 7.4, containing 0.1 M-NaCl and 3 M-CsCl.
Electron microscopy. Poliovirus particles were studied in combinations of two modes of deposition on the carbon support film and two types of staining: 1 % aqueous solution of PTA (pH adjusted to 7·0) and 1 to 2 % aqueous solution of uranyl acetate (pH 4·0). Deposition of the specimen on the single carbon film is referred to as 'adsorption', whereas deposition between two carbon films is called 'sandwich'. Both techniques have been described elsewhere (Boublik, Hellmann & Kleinschmidt, 1977). Specimens were observed in a JEM-100B electron microscope, operated at 80 kV and using a direct magnification of \( \times 70000 \).

RESULTS AND DISCUSSION

Poliovirus particles prepared for electron microscopy by the classic adsorption technique – deposited on a carbon film support and negatively stained with 1 % PTA (Fig. 1) – show the usual features of the virion: sphere-like particles of uniform size (approx. 280 Å in diam.), compact and without any indication of a fine structure. The number of empty capsids (e) is less than 1 %. The electron microscopic image of particles from the same preparation, also stained with 1 % PTA but deposited on the support by another procedure, looks quite different (Fig. 2). The specimen in this experiment was supposed to be deposited between two thin carbon films; however, because of the partial break of the additional carbon film, poliovirus particles were deposited in two different modes on the same grid. In the darker
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Fig. 2. Poliovirus particles, stained with 1 % PTA, in (a) sandwich, (b) non-sandwich, (c) heavily stained non-sandwich area.

area (a), the virions were 'embedded' in the carbon sandwich; in the lighter area, in the non-sandwiched (b) region, the virus particle deposition corresponded to adsorption to a single carbon film. Conformation of virions in the sandwich area (a) was completely changed and the originally compact sphere-like particle dissociated into several sub-particles. Particles in the non-sandwiched area (b) with the light deposit of staining solution, showed disintegration similar to that of particles in the sandwich. Particles which retained a structure typical of poliovirus (Fig. 1) were observed only in the heavily stained area (Fig. 2, c) in the non-sandwich region. This proves that dissociation of virions is not caused by the additional carbon film, due, for example, to the cohesive forces in the sandwich. It is the distribution of stain which seems to be the important factor for the preservation of the virion structure. The overall view of a sandwiched area (Fig. 3) supports this interpretation. Particles in the areas with heavier staining (H) appear intact while the structure of those in the lightly stained areas (L) is greatly affected. Careful evaluation of these areas shows that the number of different shapes (a to g) of the dissociation products is restricted and indicates that the dissociation of virion proceeds in steps. The suggested scheme of the dissociation is shown in Fig. 4. After the initial swelling by about 15 to 20 % (b) a notch appears, first on
Fig. 3. Poliovirus particles, stained with 1% PTA, sandwich technique in a heavy (H) and light (L) stain deposit. (a) Control poliovirus particle, (b) swollen particle, (c and d) swollen particles with notches, (e and f) intermediate forms of dissociated particles, and (g) sub-particles.
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Fig. 4. Scheme of the dissociation of poliovirus particles; symbols a to g correspond to that of Fig. 3.

one side (c) and then on the opposite side (d). Products of further dissociation are intermediates (e, f), which are not as well defined as the previous forms (b to d). However, the last step of the dissociation (g) is characterized quite well. The final product is a typical cluster of sub-particles, which all seem to be about 100 Å in diam.

Results comparable to the PTA staining (Fig. 1 to 3) were obtained with poliovirus particles contrasted with uranyl acetate and deposited on the grid by either the adsorption or sandwich technique. Poliovirus particles are quite well preserved in the areas with heavier staining; expanded and dissociated forms are mostly observed in the lightly stained regions (Fig. 5a). The structural arrangement of the sub-particles, which are the final product of the dissociation, can be better seen on the gallery of selected particles (Fig. 5b). The highest number of sub-particles which we were able to resolve clearly was eight. This observation does not exclude the possibility that the actual number of sub-particles is higher because some of them might be hidden. Electron micrograph 10 in Fig. 5(b) most probably shows extrusion of RNA from the virion particle, which explains the presence of the empty capsids (e) occasionally seen in the preparation of poliovirus particles (Fig. 1).

Similar results were obtained with empty capsids (Fig. 6) with both PTA and uranyl acetate staining. The stability of empty capsids is also affected by the stain distribution: in the heavily stained region empty capsids retain their characteristic shape (Fig. 6a) whereas, in the lightly-stained areas, they dissociate into sub-particles (Fig. 6b). The sub-particles are less well defined, but still comparable to those derived from poliovirus particles. The electron micrograph in Fig. 6(c) with unevenly stained areas shows the effect of the stain distribution on the preservation of empty capsids similar to Fig. 3 for poliovirus particles.

In summary, dissociation of poliovirus particles and of empty capsids proceeds in steps, starting with expansion of the virus particles (about 15 to 20% increase in diameter), followed by notches in the protein coat, and finally, after passing several intermediates, by dissociation of the virus particle (empty capsid) into a cluster of several (8 or more) equal sub-particles approx. 100 Å in diam. The mechanism of virion dissociation is not understood but this process can be affected by the amount and the distribution of the staining medium. Both uranyl acetate and PTA have comparable stabilizing effects on virus particles and empty capsids.

The mol. wt. of the poliovirus capsid is 4 to 6 × 10^6 (Rueckert, 1971). Assuming the lowest mol. wt. and the lowest number of sub-particles visible, i.e. 8, a mol. wt. of about 5 × 10^5 for each of the 100 Å sub-particles can be calculated. However, the uncertainty of
Fig. 5. (a) Poliovirus particles, stained with 1% uranyl acetate, sandwich technique. (b) Selected sub-particles after dissociation of poliovirus (1 to 9). Poliovirus particles with RNA leaving the capsid (10).
Fig. 6. (a) Empty capsids of poliovirus, stained heavily with 1% PTA, sandwich technique. (b) Empty capsids of poliovirus, stained lightly with 1% PTA, sandwich technique. (c) Empty capsids of poliovirus, stained unevenly with 1% PTA, sandwich technique.
the mol. wt. of the poliovirus and possible superposition of dissociation products imply that the number of sub-particles may be 12. It is therefore suggested that the 100 Å sub-particles are analogous to the 13 to 15S particles described by Rueckert (1971).

An expanded form of poliovirus was postulated as the first step in the dissociation of poliovirus by urea (Drzeniek, 1975). Treatment of foot-and-mouth-disease virus with 4% glutaraldehyde increased its diam. by 25% (Sangar et al. 1973). A stepwise mechanism of picornavirus dissociation based on biochemical data was suggested by a number of investigators (Breindl, 1971; Katagiri et al. 1971; Rueckert, 1971; Philipson et al. 1973; Vanden Berghe & Boeyé, 1973; Drzeniek, 1975).

The data presented in this paper visualize the dissociation of a virus into its sub-particles enabling a better characterization of small and compact RNA viruses by electron microscopic techniques.

The excellent technical assistance of Miss Marianne Hilbrig and Miss Wilhelmine Hellmann is gratefully acknowledged. One of us (R.D.) was supported by the Deutsche Forschungsgemeinschaft.

REFERENCES


(Received 11 March 1977)