Isoelectric Points of Polypeptides of Standard Poliovirus Particles of Different Serological Types and of Empty Capsids and Dense Particles of Poliovirus Type 1

(Accepted 6 October 1977)

SUMMARY

The isoelectric points of polypeptides of standard and dense poliovirus particles and of empty capsids have been determined by isoelectric focusing in urea and by two-dimensional analysis. Comparing virus strains belonging to the three serological types of poliovirus, differences in the pI of some, but not all of the structural polypeptides are found. The pI of polypeptides of dense particles and of empty capsids are identical with those of standard particles. Polypeptide VP0 present in empty capsids has a pI between those of VP4 and VP2.

Poliovirus has been recognized by gel electrophoresis in the presence of SDS to consist of four polypeptides, differing in mol. wt. (Maizel, 1963). By this technique, small size differences can be seen between virus strains belonging to different serological types of poliovirus (Holland & Kiehn, 1968; Cooper, Summers & Maizel, 1970; Fennel & Phillips, 1974; Beckman, Caliguiri & Lilly, 1976).

In a preceding communication, we have reported that the structural polypeptides of poliovirus type I differ markedly in their isoelectric point when examined by isoelectric focusing (IF) in urea and by two-dimensional separation (Hamann, Wiegars & Drzeniek, 1977). It was of interest to determine whether differences exist in the isoelectric points of polypeptides of other virus strains as well as of naturally occurring empty capsids (Maizel, Phillips & Summers, 1967) and of dense poliovirus particles (Rowlands et al. 1975; Yamaguchi-Koll, Wiegars & Drzeniek, 1975; Wiegars, Yamaguchi-Koll & Drzeniek, 1977).

The results of isoelectric focusing and two-dimensional analysis of standard poliovirus particles differing in serological type, as well as of empty capsids and of dense poliovirus particles, are documented in Fig. 1. The isoelectric points (pI) of the main bands detected by isoelectric focusing are tabulated in Table 1. The correlation of the bands found by isoelectric focusing (Fig. 1a) with the virus polypeptides VP1 to VP4 was derived from SDS-polyacrylamide gel electrophoresis in the second dimension (Fig. 1b to e). The preparation of standard virus, dense poliovirus particles and empty capsids was previously described (Drzeniek & Bilello, 1974; Yamaguchi-Koll et al. 1975). The following poliovirus strains were examined: poliovirus type I, strain Mahoney; poliovirus type II, strain Lansing; and poliovirus type III, strain Saukett.

The isoelectric points of the structural polypeptides of poliovirus are given in Table 1: (1) the order of the isoelectric points is the same for all three strains examined, VP1 is always the most basic and VP3 the most acid polypeptide, VP4 is near neutrality and VP2 is slightly less acidic than VP3. (2) Differences exist in the isoelectric points of some of the corresponding polypeptides of the three virus strains. (3) No differences exist in the isoelectric points of the corresponding polypeptides of standard and dense poliovirus particles and of empty capsids.
Fig. 1. Isoelectric focusing and 2D-analysis of poliovirus. (a) Isoelectric focusing of poliovirus in urea (slab gel): (1) standard particles, type III; (2) standard particles, type II; (3) standard particles, type I; (4) dense particles, type I; (5) empty capsids, type I. (b to e) Two-dimensional analysis of poliovirus. First dimension (horizontal): isoelectric focusing in gel rods. Top: reference gel. Second dimension (vertical): SDS polyacrylamide gel electrophoresis. A reference sample of SDS-dissociated poliovirus is shown on the left. (b) Poliovirus type III; (c) type II; (d) type I (X: contaminant, not labelled in radioactively labelled virus preparations, Y: unknown component, 35S-methionine labelled); (e) empty capsids, type I. Virus was dissociated in 9 M-urea + 0.1 mg/ml RNase I for 1 h at 25 °C (type I and II) or 37 °C (type III) (Drzeniek, 1975). Isoelectric focusing (first dimension) was done in 5% polyacrylamide gel rods, 2.5 mm in diam., containing 9 M-urea and 1.5% ampholyte, pH 5 to 9, and 0.5%, pH 2 to 11. Samples were layered on top of the gel, which was the anode for (b), (c), (d), and the cathode for (e). SDS disc electrophoresis (Maizel, 1971) for the second dimension was carried out in 13% polyacrylamide gels. Gels were stained with Coomassie blue. All materials and techniques were described in detail by Hamann & Drzeniek (1977).
Table I. Isoelectric points of poliovirus polypeptides in 9 M-urea

<table>
<thead>
<tr>
<th>Polypeptide (main component)</th>
<th>Standard poliovirus particles</th>
<th>Poliovirus, Type I, Mahoney</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Type I, Mahoney</td>
<td>Type II, Lansing</td>
</tr>
<tr>
<td></td>
<td>Dense particles</td>
<td>Empty capsids</td>
</tr>
<tr>
<td>VP0</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>VP1</td>
<td>8-1</td>
<td>8-6-8.8</td>
</tr>
<tr>
<td>VP2</td>
<td>6-4</td>
<td>6-25</td>
</tr>
<tr>
<td>VP3</td>
<td>6-o</td>
<td>6-o</td>
</tr>
<tr>
<td>VP4</td>
<td>7-3</td>
<td>7-2-7.5</td>
</tr>
</tbody>
</table>

* Not detected. † Present in trace amounts.

The differences observed in the relative strength of some bands and the failure to detect VP4 in one-dimensional gels (Fig. 1 a) are due to losses in the isoelectric focusing and staining procedure as discussed previously (Hamann & Drzeniek, 1977). In the two-dimensional analysis of poliovirus particles, secondary spots having the same mol. wt. are found for VP1, VP2 and VP3 (Fig. 1 b to c). In a detailed analysis of the occurrence of multiple bands of VP2, evidence was presented that charge modifications during preparation and storage of the virus are responsible for the apparent heterogeneity of this polypeptide (Hamann & Drzeniek, 1977). An additional spot (Y) was detected in some preparations of poliovirus type I (Fig. 1 d), which cannot be assigned to any of the polypeptides. Because it is labelled with 35S-methionine to approximately the same extent as the other polypeptides, it is not clear up to now whether it represents a new component of the virus particle or is a contaminant of the preparation.

Recently, Chlumecka, D'Obrenan & Colter (1977) analysed the polypeptides of Mengo virus. Their data also show small differences in the pI of the respective polypeptides of the three virus variants tested. Interestingly, in Mengo virus the isoelectric points of the four polypeptides lie in the same range, no neutral or basic proteins being found. Yet, the authors isolated the polypeptides before focusing using SDS, and it cannot be completely excluded that this treatment alters their apparent isoelectric point.

It can be seen from Fig. 1 and Table I that polypeptides present in standard virus particles and in dense particles of poliovirus type I have the same isoelectric points. Analysis of dense particles by SDS-electrophoresis revealed no differences in the polypeptide pattern compared to standard poliovirus particles (Yamaguchi-Koll et al. 1975). From the analysis of dissociation products of dense particles, it was suggested that structural changes are responsible for the altered physical properties and infectivity (Wiegers et al. 1977). The data presented here confirm this hypothesis on the basis of the additional parameter of the charge of the polypeptides.

In empty capsids only traces of VP2 are detected, as is to be expected. VP1 and VP3 show the same isoelectric point as in the virion. VP0 has a pI between VP2 and VP4 and shows, like VP3, a limited heterogeneity. It could be argued that it is susceptible to the same modification process as that found for VP2 (Hamann & Drzeniek, 1977).

The techniques of isoelectric focusing and 2D-analysis presented in this and the previous paper give new information on poliovirus polypeptides. They point to a sensitive method which can detect small differences in the proteins of related virus strains and mutants. The reported results are a first step towards a more detailed description of charge differences of poliovirus polypeptides necessary for a better understanding of the structure of the virion and its morphogenesis.
We thank Miss M. Hilbrig for the preparation of poliovirus. This work was supported by the Bundesministerium für Forschung und Technologie (BCT 90) and the Deutsche Forschungsgemeinschaft (Dr 57/6).

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REFERENCES


(Received 5 August 1977)