Evidence for Conformational Changes of Poliovirus Precursor Particles during Virus Morphogenesis

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Antisera raised against isolated structural polypeptides VP1, VP2 and VP3 of poliovirus type 1, strain Mahoney, reveal a differential reaction against mature virus and its precursor particles. During virus morphogenesis antigenic sites recognized by VP1 and VP2 antisera are lost stepwise from the surface of precursor particles. These sites are cross-reacting between serotypes and are also lost from precursor particles of type 2 (MEF-1) and type 3 (Saukett). They are absent on the surface of mature virus of all three serotypes. In contrast, the VP3 antiserum recognizes sites expressed maximally on the surface of infectious virus of type 1 (Mahoney). This antiserum did not show significant intertypic cross-reactions with virus particles, empty capsids or 14S particles of poliovirus types 2 and 3. However, it does recognize intertypic cross-reacting sites, like the VP1 and VP2 antisera, on denatured polypeptides and 5S particles of each serotype.

INTRODUCTION

It is generally assumed that poliovirus morphogenesis starts from a protomer sedimenting at 5S containing either uncleaved or cleaved capsid precursor NCVPIa (Rueckert, 1976). The assembly of five protomers leads to pentamers sedimenting at 14S first described by Watanabe et al. (1962). 14S subunits can self-assemble in vitro to form empty capsids sedimenting at 80S (Phillips et al., 1968) or, even more efficiently, can assemble in extracts from infected cells (Putnak & Phillips, 1981). Previously, in pulse-chase experiments a precursor-product relationship between particles sedimenting at 80S and virions was demonstrated (Jacobson & Baltimore, 1968). The particles sedimenting at 80S were therefore called procapsids by these authors. However, recently it was shown that the conditions for the isolation of 80S particles have to be controlled carefully in order to obtain unambiguous results. 80S particles having the properties of procapsids were obtained by a temperature-mediated conversion of unstable 80S particles found in infected cells (Marongiu et al., 1981).

With the aid of monoclonal neutralizing antibodies it was shown that N-antigenic determinants were present not only on virus particles but also on empty capsids and 14S particles (Icenogle et al., 1981; Emini et al., 1982; Rombaut et al., 1983). However, it could be shown that the N-antigenic determinants of empty capsids were destroyed above pH 8.5 (Rombaut et al., 1982).

Apart from these studies, very little is known of how the individual polypeptides are arranged during morphogenesis. We have, therefore, investigated the morphogenetic precursor particles with respect to their antigenic sites recognized by polyclonal monospecific antisera prepared against purified structural polypeptides (Wiegers & Dernick, 1983).

Despite the known type-specificity of the neutralization test, intertypic cross-reactions of heated virus particles were described by Hummeler & Hamparian in 1958. Recently, it was shown that an intertypic cross-reaction was found not only on non-structural but also on structural polypeptides (Romanova et al., 1981). Blondel et al. (1982), who prepared antisera against isolated polypeptides of poliovirus, type 1, strain Mahoney, found an intertypic cross-reaction on completely denatured homologous polypeptides of all three serotypes. They also

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found cross-reacting determinants on heated virus particles, but only their VP1 antiserum reacted with H-particles of each serotype. Using the immunoblot technique, Thorpe et al. (1982) showed that neutralizing antisera raised in rabbits against complete poliovirus, type 3, contained antibodies reacting with VP1 and VP2 of type 3 poliovirus but reacted also with VP1 of type 1 and VP1 of type 2. It was suggested that the intertypic determinants found on denatured polypeptides are masked in the mature virus (Romanova et al., 1981; Blondel et al., 1982). In this paper we will present evidence that during morphogenesis there is a stepwise loss of intertypic determinants indicated by a differential reactivity of the precursor particles with our antiseras raised against individual structural polypeptides.

METHODS

Virus. Poliovirus, type 1 (Mahoney), type 2 (MEF-1) and type 3 (Saukett) were grown in HeLa S3 cells in suspension culture. The labelling of virus-specific proteins in infected HeLa cells has been described (Wiegers & Dernick, 1981). Briefly, suspension cultures of HeLa S3 cells were infected at high multiplicity (100 p.f.u./cell). At 3.5 h after infection the complete medium was replaced by medium lacking amino acids, and 20 to 50 μCi/ml [35S]methionine were added to the culture. Cells were collected 1 h later and resuspended in RSB (0.01 M-NaCl, 0.01 M-Tris-HCl pH 7.4, 1.5 mM-MgCl2) and stored frozen at −20°C.

Preparation of virions and naturally occurring empty capsids (80S) from cytoplasmic extracts. Infected frozen cells were thawed and treated with 0.5% NP40 and 1 mM-dithioerythritol (DTE) for 15 min at room temperature and 45 min on ice. Nuclei were removed by centrifugation for 10 min at 10000 g at 4°C. The supernatant was layered onto a 15 to 30% (w/v) linear sucrose gradient in RSB and centrifuged for 1 h in a Beckman SW60 rotor at 50000 r.p.m. The gradient was fractionated into 0.2 ml portions and assayed for radioactivity. The virion (160S) and empty capsid (80S) peaks were pooled separately. The empty capsids obtained by this procedure had the properties of 80S dissociable particles described by Marongiu et al. (1981) because they were labile at pH 8.5.

Preparation of 14S particles. Cytoplasmic extracts were prepared as above. But the supernatant was layered onto a 5 to 20% (w/v) linear sucrose gradient in RSB and centrifuged for 15 h at 35000 r.p.m. at 4°C in a Beckman SW60 rotor. Fractionation was done as above and the 14S peak was pooled.

Preparation of 5S particles. In contrast to the other types of particles 5S particles were prepared from infected cells which were labelled for only 15 min instead of 1 h with the radioactive amino acid. The shorter labelling time reduced the contamination of 5S particles with non-structural polypeptides especially NCVPX and its precursors.

Preparation of cytoplasmic extracts and centrifugation conditions were identical to those described for the preparation of 14S particles.

Fractions sedimenting at approximately 5S according to a bovine serum albumin marker were analysed by SDS–PAGE. This allowed the determination of the exact position of 5S particles. The fraction containing the highest amount of polypeptides VP0, VP1 and VP3 was used for immunoprecipitation.

Antisera. The viral polypeptides used for immunization were obtained after dissociation of poliovirus, type 1, in 9 M-urea and subsequent isoelectric focusing in 7 M-urea-containing sucrose gradients. Isoelectric focusing and collection of fractions were done as described previously (Wiegers & Drzeniek, 1980).

Fractions containing pure polypeptides were pooled and re-focused in separate columns (Wiegers & Dernick, 1983). About 200 μg of pure polypeptide in 0.5 ml was emulsified with an equal volume of complete Freund’s adjuvant and injected intramuscularly into the hind legs of pathogen-free rabbits. Booster injections containing the same amount of polypeptide in complete Freund’s adjuvant were given after 2 weeks in the same way. The animals received a final boost containing approximately half the amount of polypeptide in complete Freund’s adjuvant after an additional 2 weeks. Fourteen days later the animals were bled and the serum was collected.

Immunoprecipitation of virus-specific particles. Immunoprecipitation of virions and precursor particles was done as described (Wiegers & Dernick, 1983). To 20 μl of pooled fractions of virus or precursor particles, 80 μl RSB containing 0.5% NP40 and 1 mM-DTE was added. After addition of 5 μl antiserum, antibody was always in excess of antigen, the mixture was incubated for 1 h on ice. Then 40 μl of a 10% suspension of inactivated Staphylococcus aureus (Protein A-Bacterial Adsorbent, Miles Laboratories) was added and incubation was continued for 30 min. The adsorbed immune complexes were collected at 10000 g for 3 min and washed with TEN (0.15 M-NaCl, 0.01 M-Tris–HCl pH 7.4, 0.001 M-EDTA, 0.5% NP40) and once with TEN containing high salt (0.6 M-NaCl). The complexes were dissolved by incubation at room temperature for 15 min in 100 μl sample buffer (0.059 M-Tris–HCl pH 6.8, 1% SDS, 1% 2-mercaptoethanol). The bacterial ghosts were removed by centrifugation and the radioactivity in the supernatant was determined.

Immunoprecipitation of viral polypeptides. Cytoplasmic extracts prepared as described above were dissociated in 1% SDS for 5 min at 100°C. For the immunoprecipitation they were diluted tenfold with cold RSB containing 0.05% NP40 and 1 mM-DTE. Immunoprecipitation was then carried out as described above.

SDS–PAGE. Samples were analysed on 10% or 12.5% polyacrylamide slab gels in SDS using the discontinuous Tris–glycine buffer system described by Laemmli (1970) and Maizel (1971). After electrophoresis the gels were dried and fluorographs were prepared as described by Chamberlain (1979).
RESULTS

We have raised antisera against individual polypeptides of poliovirus type 1 (Mahoney). The structural polypeptides (VP1, VP2, VP3) were obtained by dissociation of poliovirus by 9 M-urea and separation by isoelectric focusing in urea-containing sucrose gradients (Wiegers & Drzeniek, 1980).

These antisera were monospecific because they precipitated only the homologous polypeptide from a mixture of poliovirus polypeptides obtained by SDS dissociation of the virus. Antisera against VP2 also precipitated VP0 from empty capsids. From this set of antisera only the VP3 antiserum precipitated infectious virus. However, empty capsids and heated virus (1 h at 56 °C) were precipitated by VP1, VP2 and VP3 antisera (Wiegers & Dernick, 1983).

When VP1, VP2 and VP3 antisera were used for the immunoprecipitation of radiolabelled infected cell extracts, prepared without the SDS dissociation step at 100 °C, each antiserum precipitated particles comprising VP0, VP1 and VP3, in addition to low amounts of the appropriate precursor molecules. The VP3 antiserum also precipitated virus particles, indicated by the appearance of the VP2 and VP4 bands after SDS-PAGE (Fig. 1). Besides empty capsids, which were precipitated by these antisera, 14S particles and 5S particles are also composed of VP0, VP1 and VP3. In order to answer the question of which particles were precipitated by the monospecific antisera, virus particles, empty capsids, 14S and 5S particles were isolated by sucrose density gradient centrifugation. The homogeneity of 160S virus particles, empty capsids and 14S particles isolated from infected cells by sucrose density gradient centrifugation is shown in Fig. 2(a). The polypeptide content of these particles is shown in Fig. 2(b). As expected, 160S virus particles contained all four structural polypeptides; empty capsids and 14S particles contained VP0, VP1 and VP3. A preparation of 5S particles was also analysed. Though it was prepared after a short pulse, a simple means to reduce the amounts of contaminating non-structural polypeptides, it still contained considerable amounts of NCVP2, NCVPX and NCVP8. Under our experimental conditions, the majority of NCVP1a of 5S particles was already cleaved into VP0, VP1 and VP3.
Fig. 2. Sucrose density gradient centrifugation (a) and polypeptide composition (b) of [35S]methionine-labelled virions (160S), empty capsids (80S) and 14S particles used for immunoprecipitation. (a) Isolated particles from infected cells were analysed in a 15 to 30% (w/v) linear sucrose gradient in RSB for 1 h at 50000 r.p.m, in a Beckman SW60 rotor. Fractions were collected from the bottom of the tube and the radioactivity determined. Left-hand ordinate: O, Virions (160S) and □, 14S particles; right-hand ordinate: △, empty capsids (80S). (b) Polypeptide composition of the particles used for immunoprecipitation. The polypeptides were analysed by SDS-PAGE on a 12.5% polyacrylamide slab gel. 1, Virions (160S); 2, empty capsids (80S); 3, 14S particles; 4, preparation of 5S particles.

The results of the immunoprecipitation of 160S virus particles, empty capsids and 14S particles is shown in Table 1. Empty capsids and 14S particles were precipitated by all three antisera. Antisera against VP1 and VP2 precipitated about 60% of 14S particles and 30 to 40% of empty capsids, but only small amounts (7 to 15%) of virus particles. However, 80% of virions, 70% of empty capsids and 60% of 14S particles were precipitated by the VP3 antiserum. We have also immunoprecipitated the preparation of 5S particles (Table 2). In this case VP1 and VP2 antisera precipitated about 50%, the VP3 antiserum about 40% of the radioactivity of the polypeptides NCVP1a, NCVP3a, VP0, VP1 and VP3 present in the preparation of 5S particles. These values had to be calculated differently, because the 5S fraction was heavily contaminated by non-structural polypeptides. The amount of non-structural polypeptides exceeded 60 to 70% in different 5S preparations. Therefore the percentage of radioactivity precipitated can only be roughly compared with the percentage of radioactivity directly determined of almost pure 14S, 80S and 160S particles.

Our results obtained with monospecific antisera demonstrate that during morphogenesis antigenic sites recognized by VP1 and VP2 antisera gradually disappear from the surface of the precursor particles examined and they are absent on 160S virus particles. On the other hand antigenic sites recognized by the VP3 antiserum are expressed maximally on the surface of 160S virus particles, but are also present on precursor particles although to a lesser extent.

Recently several investigators have shown that poliovirus structural polypeptides share common intertypic determinants (see Introduction). Our results for immunoprecipitation of viral polypeptides of type 2 (MEF-1) and type 3 (Saukett) virus-infected cells are shown in Fig. 3 and confirm these observations. Our three monospecific antisera recognized intertypic determinants on SDS-denatured polypeptides. Not further differentiated, type 2 and type 3 particles consisting of VP0, VP1 and VP3, which were obtained from infected cells without
Table 1. Immunoprecipitation of poliovirus, type 1 (Mahoney), type 2 (MEF-1) and type 3 (Saukett) virions (160S), empty capsids (80S) and 14S particles with monospecific antisera raised against type 1 polypeptides

<table>
<thead>
<tr>
<th>Component</th>
<th>Virions (160S)</th>
<th>Empty capsids (80S)</th>
<th>14S particles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Preimmune serum</td>
<td>1.1* (0.6)†</td>
<td>0.7 (0.4)</td>
<td>0.7 (0.2)</td>
</tr>
<tr>
<td>VP1 antiserum</td>
<td>7.2 (0.9)</td>
<td>2.4 (1.1)</td>
<td>3.5 (1.8)</td>
</tr>
<tr>
<td>VP2 antiserum</td>
<td>14.4 (3.5)</td>
<td>2.6 (1.1)</td>
<td>3.9 (1.1)</td>
</tr>
<tr>
<td>VP3 antiserum</td>
<td>80.6 (6.8)</td>
<td>1.5 (0.5)</td>
<td>2.1 (1.3)</td>
</tr>
</tbody>
</table>

* The data represent the percentage of radioactivity which was precipitated. The values given are averages from at least three experiments.
† Standard deviation in parenthesis.

Table 2. Immunoprecipitation of 5S particles of poliovirus, type 1 (Mahoney), type 2 (MEF-1) and type 3 (Saukett) with monospecific antisera raised against type 1 polypeptides

<table>
<thead>
<tr>
<th>Virus type</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preimmune serum</td>
<td>2.6* (2.1)†</td>
<td>1.0 (0.1)</td>
<td>1.0 (0.1)</td>
</tr>
<tr>
<td>VP1 antiserum</td>
<td>48.5 (0.1)</td>
<td>47.3 (1.1)</td>
<td>55.0 (5.0)</td>
</tr>
<tr>
<td>VP2 antiserum</td>
<td>48.7 (3.7)</td>
<td>50.6 (2.5)</td>
<td>57.8 (4.7)</td>
</tr>
<tr>
<td>VP3 antiserum</td>
<td>38.2 (2.1)</td>
<td>24.5 (6.6)</td>
<td>32.6 (6.2)</td>
</tr>
</tbody>
</table>

* The values represent the percentage of radioactivity precipitated of NCVP1a, NCVP3a, VP0, VP1 and VP3. The data are mean values from two experiments. The ratio of structural to non-structural polypeptides in our 5S preparation was determined by densitometry of an X-ray film after SDS-PAGE of the preparation.
† ± Standard deviation in parenthesis.
dissociation by SDS at 100°C, were precipitated by VP1 and VP2 antisera to the same extent as particles obtained from type 1-infected cells, but to a lesser extent by VP3 antiserum. A comparison of intertypic antigenic determinants of precursor particles of poliovirus type 1, type 2 and type 3, is given in Tables 1 and 2. The results obtained demonstrate that intertypic antigenic determinants detected by VP1 and VP2 antisera, which are found on denatured polypeptides, are present on all precursor particles, but they gradually disappear and are finally absent on 160S poliovirus particles. Intertypic antigenic determinants detected by the VP3 antiserum on denatured polypeptide VP3 and on 5S particles are greatly reduced on 14S particles and are practically absent on 160S virus particles, because of the evident lack of reaction of type 2 and type 3 virions. The strong precipitation of 160S particles of type 1 is due to the presence of homotypic antibodies directed against type 1 in the VP3 antiserum.

**DISCUSSION**

Despite the fact that morphogenetic precursors of picornaviruses and their polypeptide compositions have been known for some time (Rueckert, 1976), little information about their antigenic relationship has been obtained.

Antibodies against degraded virions (S-antigen) reacted with nascent viral polypeptide chains associated with polyribosomes and also with an antigen of infected cells, sedimenting at 5S (Scharff & Levintow, 1963; Scharff et al., 1964). After immunization with 14S particles from infected cells, antisera were obtained which reacted neither with empty capsids nor virions. Because the antigen which was recognized was different from the S-antigen described above, it was designated 'S'-antigen (Rueckert, 1976).

Our results demonstrate that antisera prepared against isolated purified structural polypeptides can be used for the investigation of the antigenic relationship between precursor particles found in infected cells. For the set of antisera described here it is shown that all three monospecific anti-polypeptide sera recognize antigenic sites on denatured polypeptides. These sites are also recognized on the structural precursor polypeptide NCVP1a (Wiegers & Dernick, 1983).
It is evident that these sites recognized on denatured polypeptides are conserved between all three serotypes. Furthermore, they seem to be strong immunogens because they are also found with other antisera raised against individual polypeptides isolated and purified by different methods (Romanova et al., 1981; Blondel et al., 1982).

It is clear that antigenic sites recognized by our monospecific antisera disappear from precursor particles during the morphogenesis of infectious poliovirus particles. The most dramatic change in the antigenicity is seen between empty capsids and the mature, infectious 160S virus particles. It has already been proposed that encapsidation of the viral RNA is accompanied by a gross conformational change of the protein shell (Carthew & Martin, 1974; Rueckert, 1976). Our investigation confirms this general phenomenon of a conformational change. But it demonstrates such changes much more precisely and shows differences in antigenicity which occur during morphogenesis at different levels of poliovirus precursor particles.

Our monospecific antisera also demonstrate different type-specific and intertypic antigenic determinants of virions and their precursors. The VP1 and VP2 antisera used in our investigation recognized intertypic antigenic determinants by being directed against highly conserved regions of the structural polypeptides. In the case of the VP3 antiserum, we found an increasing expression of type-specific determinants reacting with this antiserum during virus maturation. These determinants are present on the virus surface but not on 80S or 14S particles. In addition, VP3 antiserum contains antibodies directed against sites found on the denatured polypeptide VP3 and on 5S particles. These sites, however, are intertypic antigenic determinants because of their demonstrated cross-reaction between serotypes. VP3 antiserum therefore exerts two specificities: one type-specific reaction directed against antigenic determinants present only on 160S virus particles, and the other an intertypic reaction directed against sites present on the very early precursors, i.e. 5S particles and on denatured VP3.

The use of polyclonal monospecific antisera directed against isolated polypeptides therefore revealed type-specific and intertypic antigenic reactions and relationships between mature poliovirus particles and their morphogenetic precursors not seen so far with polyclonal antiserum directed against intact virus particles. It further allowed the correlation of these antigenic sites to a defined structural polypeptide of this virus.

REFERENCES


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