Iodination of Poliovirus Capsid Proteins

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SUMMARY

Iodination of the surface of poliovirus and its artificial empty capsid demonstrated predominant labelling of polypeptide VP 1 on the intact particle and an increased labelling of polypeptide VP 2 on the artificial empty capsid.

Intact poliovirus particles and the artificial empty capsids (AEC) contain four well defined proteins (Maizel, Phillips & Summers, 1967). Different chemical (McGregor & Mayor, 1968; Martin & Johnston, 1972; Drzeniek, 1975) and physical (Katagiri, Hinuma & Ishida, 1968; Breindl, 1971a) treatments of the infectious particles result in the successive loss of VP 4 and the virus RNA (Katagiri et al. 1968; Breindl, 1971a; Mietens & Koschel, 1971), and a change of their antigenicity. To investigate the localization of the surface-exposed proteins of the virus and its AEC, each was iodinated by the lactoperoxidase reaction (Marchalonis, 1969), a technique which has been applied to several other viruses (Stanley & Haslam, 1971; Katz & Margalith, 1973; Talbot et al. 1973; Rowlands, Sangar & Brown, 1975).

An equal susceptibility of all four virus proteins to iodine is a pre-condition for such an experiment. This was confirmed by disrupting the virus prior to iodination by heating for 5 min at 100 °C in 2 M-urea and 1 % SDS. Subsequent polyacrylamide gel electrophoresis analysis in 15 % acrylamide-SDS-urea (Maizel et al. 1967) showed the typical pattern of the four labelled proteins (Fig. 1a) in comparison with uniformly labelled (with 14C-protein hydrolysate, The Radiochemical Centre, Amersham, England) controls (Fig. 1b). Labelling of the virus with 3H-tyrosine (Radiochemical Centre); Fig. 1c), which is the acceptor of 125I, results in nearly the same pattern as above (see Table I). The self-iodination of lactoperoxidase (Boehringer) itself without addition of other proteins resulted in at least five different labelled proteins (Fig. 1d) which could all be distinguished from the virus proteins by their different mol. wt.

Separation of the iodinated virus samples from lactoperoxidase by ultracentrifugation through sucrose gradients gave results which were similar to those described, but dilution and fragility of the iodinated samples in the gradients made measurement impossible. When intact poliovirus was iodinated (Fig. 1e), 74 % of the virus-protein-associated radioactivity was bound to VP 1 (see Table I); VP 2 and VP 3 showed a very small incorporation of the label. The predominant labelling of VP 1 on infectious picornavirus particles was recently described with the foot-and-mouth disease virus (Talbot et al. 1973; Rowlands et al. 1975) and a bovine enterovirus (Carthew & Martin, 1974), where the authors described a highly accessible VP 2 in the natural empty capsid.

Iodination of the AEC obtained by urea (Fig. 1f; Philipson, Beatrice & Cromwell, 1973; Drzeniek, 1975) or heat treatment (Fig. 1g; Katagiri et al. 1968; Breindl, 1971a), increased the overall iodine incorporation markedly and resulted in a different distribution of 125I-labelling on the three major virus proteins (Table I), suggesting an increased exposure of VP 2 to the lactoperoxidase reaction. This agrees with the results of Lonberg-Holm & Butterworth (1976), but we detected in the AEC a higher rate of iodination of VP 2 and no
Fig. 1. Polyacrylamide gel electrophoresis in a 15% gel. (a) Poliovirus disrupted prior to iodination in 2 M-urea and 1% SDS (5 min, 100°C). (b) Uniformly labelled (¹⁴C-protein hydrolysate) virus capsid proteins. (c) Electrophoresis of ³H-tyrosine-labelled virus. (d) Self-iodinated lactoperoxidase without addition of virus proteins. (e) Intact iodinated poliovirus; the peaks designated by arrows are caused by self-iodinated lactoperoxidase proteins. (f) Iodinated AEC prepared by 2 M-urea (45°C for 1 h) in potassium phosphate buffer, pH 7.4. (g) Iodinated AEC prepared by heating the intact virus (56°C for 1 h) in potassium phosphate buffer, pH 7.4. Poliovirus type I (MAH) was purified in five steps by differential centrifugation and banding on an isopycnic CsCl gradient. For iodination experiments, the purified virus (titre approx. 1 x 10⁴⁰ p.f.u./ml) was pelleted and resuspended to 1/30 volume in 0.05 M-potassium phosphate buffer, pH 7.4.

Table 1. Distribution of virus-protein-bound radioactivity (%)

<table>
<thead>
<tr>
<th>Sample</th>
<th>VP 1</th>
<th>VP 2</th>
<th>VP 3</th>
<th>VP 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uniformly labelled poliovirus (¹⁴C-protein hydrolysate)</td>
<td>24</td>
<td>39</td>
<td>29</td>
<td>8</td>
</tr>
<tr>
<td>Tyrosine-labelled poliovirus (³H-tyrosine)</td>
<td>44</td>
<td>37</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>Poliovirus disrupted prior to iodination</td>
<td>31</td>
<td>35</td>
<td>27</td>
<td>7</td>
</tr>
<tr>
<td>Iodinated intact poliovirus</td>
<td>74</td>
<td>16</td>
<td>10</td>
<td>ND*</td>
</tr>
<tr>
<td>Urea-disrupted iodinated poliovirus</td>
<td>33</td>
<td>57</td>
<td>10</td>
<td>ND</td>
</tr>
<tr>
<td>Heat-disrupted iodinated poliovirus</td>
<td>28</td>
<td>64</td>
<td>8</td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND, Not detected.
additional peak which migrates between VP 2 and VP 3. Both phenomena could be explained by the different techniques in preparing the AEC.

The predominant labelling of VP 2 on the AEC favours the picornavirus structural model of Johnston & Martin (1971) and Philipson et al. (1973), where at each apex of the virus icosahedron a pentamer of VP 4 is surrounded by VP 2 proteins. The liberation of VP 4 from this apical region may expose for iodination further sites of its neighbouring VP 2.

The question of whether VP 4 is an integral part of the spherical array of the capsomers (Maizel et al. 1967; Johnston & Martin, 1971) or carries the antigenic structure (D-antigen) of the intact particle (Breindl, 1971b), i.e. has an external position in the virus capsid, could not be solved by iodination. Although the formation of AEC was demonstrated by polyacrylamide gel electrophoresis and by negative staining in the electron microscope (McGregor & Mayor, 1968, 1971; Martin & Johnston, 1972), the liberated VP 4 could not be iodinated despite the fact that this protein contains tyrosine (Fig. 1c) accessible to labelling after SDS treatment (Fig. 1a). The tyrosine residues of the protein are probably blocked either by aggregation or by its secondary structure, parameters which are certainly influenced by SDS solubilization.

However, our results demonstrate that the antigenic alteration of the mature poliovirus from D- to C-antigenicity is accompanied by a conformational shift in the virus capsid with a significant increased exposure of VP 2 to the outer surface of the AEC. This would suggest that the presence of this protein on the surface of the AEC is at least in part responsible for the new antigenic properties of the particle.

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REFERENCES

Short communications


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