Internal Components Released from Rhinovirus and Poliovirus by Heat

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Picornaviruses in general are quite heat-labile in the absence of cations (Wallis & Melnick, 1962). McGregor & Mayor (1968) showed that heating purified preparations of poliovirus and rhinovirus at 50° for 2 min. led to the extrusion of ribonucleoprotein strands. These results suggested that gentle heating of the virus particles at slightly lower temperatures might offer a more easily controllable method of degradation.

Suspensions of purified rhino- and poliovirus (0.1 ml.) were heated at 45° or 50° for various periods. After heating, the preparations were cooled immediately in an ice bath and specimens were prepared for electron microscopy. Poliovirus (LSC) when heated at 45° for 30 to 60 sec. appeared to lose portions of its capsid structure leaving ‘holes’ in the particle (Fig. 1). A diffuse amorphous core resembling a ‘puffball’ was then released from within the capsid leaving an empty shell (Fig. 2). This component was not demonstrated previously by McGregor & Mayor (1968). Upon further heating the core became progressively extended, and after 10 min. at 50° only long strands and empty capsids were observed.

In contrast, rhinovirus preparations were much more stable morphologically at 45° than poliovirus preparations. When heated for 1 min. at 45° the majority of rhinovirus particles appeared to be still intact. Even when heating at 45° was continued up to 10 min., only about half of the particles had released their RNA. Many of these empty particles were degraded into smaller subunits and individual capsomeres (Fig. 3). At 50° the degradation of the rhinovirus particles progressed rapidly. After 1 min. few intact particles were found and many of the empty particles were degraded. As heating was continued up to 10 min., strands similar to those found with the poliovirus became progressively extended. The empty particles formed after the release of the virus RNA seemed to be very unstable and were generally degraded into smaller subunits. In an effort to slow down this process the volume of the virus suspension was increased to 0.5 ml. This material was then incubated at 50° for 1 min. followed by rapid cooling. Under these conditions results were obtained similar to those observed with poliovirus. Amorphous cores appeared to be released from within the capsids, leaving empty capsids which appeared, in addition, to have portions of their structure missing (Fig. 4). The material released from within the capsids was associated with partially extended strands.

Samples at various stages of degradation were treated with specific enzymes in order to determine the chemical composition of the components (Table 1). When the cores were treated with RNase before extension occurred they seemed to be completely digested. Once extension occurred, however, the strands were completely resistant to digestion with RNase unless they were first treated with pepsin. These observations suggested that the virus RNA was first released from within the particle. This was followed by its unfolding and association with a protein which appeared to protect it from the action of RNase (Fig. 2).

Van Elsen, Boeyé & Teuchy (1968) described the release of a fibrillar structure from poliovirus when it was treated at pH 10 or heated at 56° or 50°. This structure resembled the RNP strands described here and in our previous study (McGregor & Mayor, 1968), but did not appear to be sensitive to ribonuclease. This finding is still unexplained but could
Fig. 1. Poliovirus (LsC) heated at 45° for 30 to 60 sec. Some capsids appear to have 'holes' (arrows).

Fig. 2. Poliovirus (LsC) heated at 45° for 30 to 60 sec. Arrows show internal material ('puffballs' or cores) released from the capsid and also extension to form the RNP strand.
represent a particularly strong protection afforded by the protein under the experimental conditions used. However, we are currently planning to resolve this point by studying the fate of radioactively labelled uridine in heat-degraded preparations of purified poliovirus.

Addition of 1 M-MgCl₂ to enteroviruses stabilizes their infectivity at 50°C (Wallis & Melnick, 1962). The Mg²⁺ ions presumably preserve the protein structure of the virus particle. Preliminary morphological experiments with Mg²⁺ ions suggest the stabilization of the poliovirus particle at two structural locations. The capsid remained intact when it was heated for 10 min. at 50°C in the presence of 1 M-MgCl₂. In addition, Mg²⁺ ions stabilized the core component released after heating the virus at 45°C for 30 sec. and extension into RNP strands did not occur with continued heating. Neither the complete particle nor the core component of rhinovirus 14 appeared to be stabilized to heating by the addition of 1 M-MgCl₂. These results are in agreement with the variable response of rhinoviruses to stabilization by Mg²⁺ ions (Plummer, 1965). This suggests that heat degradation of rhinovirus and poliovirus in the presence of Mg²⁺ ions might be useful in revealing the location of structural differences in the particles.

Jacobson, Asso & Baltimore (1970) showed that when poliovirus-specific polypeptides are synthesized in the presence of amino acid analogues, a very large polypeptide of mol. wt

Fig. 3. Rhinovirus 14 heated at 45°C for 1 min. Numerous empty and degraded capsids are present (arrows).
greater than 200,000 is produced. Their results suggest that the poliovirus genome is translated into this single polypeptide, which is then divided to yield specific virus structural proteins, including those of the procapsid. One of these proteins may stabilize the poliovirus core component.

Fig. 4. Rhinovirus 14 preparation heated at 50° for 1 min. The internal core component has been extruded from most particles and is also found free from the capsid (arrows).

Table 1. Enzymic digestion of internal virus components in heat-degraded preparations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Poliovirus internal component</th>
<th>Rhinovirus internal component</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>Strand</td>
</tr>
<tr>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>RNase</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Pepsin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pepsin, RNase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pepsin, PVS*, RNase</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pepsin, DNase</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ : Morphological structure still present after treatment; - : structure no longer detectable after treatment.
* PVS (polyvinyl sulphate) is a known inhibitor of RNase activity.

We have shown that when 1 M-MgCl₂ was added at the time when the poliovirus RNP was released from the particle, the core was stabilized and remained as an intact structure during continued heating. Stabilization of these virus cores with 1 M-Mg²⁺ ions may allow the core components to be separated and purified, so that they may be studied further.
Short communications

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REFERENCES


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