Influence of different ionic and pH environments on structural alterations of poliovirus and their possible relation to virus uncoating

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Poliovirus eclipse products were totally precipitated from infected HeLa cells after different times of infection by using TCA, suggesting that cellular enzymic digestion of parental proteins was not involved in virus uncoating. In an investigation of poliovirus thermal stability in vitro, progressive degradation of native virus into 80S empty capsids occurred upon incubation at 37 °C in a buffer of low ionic strength containing 20 mM-Tris-HCl pH 7.5, whereas in Eagle's medium or in the presence of L cells degradation was very slow. Degradation was faster at alkaline than at acid pH. Furthermore, liberation of the viral RNA was prevented and 135S particles were produced upon treatment of virus at 37 °C in 20-mM-Tris-HCl pH 7.5 containing 2 mM-CaCl2. Although the poliovirus receptor is able to induce conformational alterations of the capsid, low ion concentration could contribute to virus uncoating as well.

Initiation of an infection in susceptible cells by a picornavirus is dependent on the presence of specific receptors on the cell surface to attach the virus, and on a mechanism for virus entry and uncoating. The finding that after adsorption of poliovirus to a cell a portion of virus is eluted in a non-infectious, altered conformational form, while the other portion is internalized, led to the assumption that interactions with cell receptors could initiate uncoating at the cytoplasmic membranes (De Sena & Mandel, 1976; Mandel, 1967), and that the resulting particles could be intermediates of uncoating (De Sena & Mandel, 1977). Moreover, since the conformationally altered particles could also be generated with isolated plasma membranes (Guttman & Baltimore, 1977), or with poliovirus receptors expressed in insect cells (Kaplan et al., 1990), uncoating was thought to occur at the periphery of the plasma membrane (Chan & Black, 1970) or during the following step of virus engulfment (Mandel, 1967). Recently we presented evidence for the kinetics of poliovirus uncoating in HeLa cells (Gromeier & Wetz, 1990). However, these results did not clarify the problem of how the RNA could overcome the membrane barrier towards the cytosol. Although poliovirus receptors are able to produce 135S particles (Kaplan et al., 1990), the influence of buffers of low ionic strength on this mechanism could be of interest, since during virus engulfment by the intact plasma membrane similar low ion concentrations could occur. Low ionic strength has been found to degrade picornavirus capsids (De Sena & Torian, 1980). Analogous to the kinetics of virus uncoating in HeLa cells, we obtained 135S particles in the presence of 2 mM-CaCl2 from which the RNA could easily be released.

From recent work on poliovirus uncoating in HeLa cells it has been suggested that enzymic degradation of the virus shell could be involved (Madshus et al., 1984; Everaert et al., 1989). Therefore, we used precipitation of the parental proteins from infected cell lysates by TCA to test whether they were enzymically digested during uncoating or at later stages of infection (Table 1). The occurrence of only trace amounts of soluble radioactivity in the cellular phase pointed to stable parental proteins during the time course of uncoating. As we have shown recently (Gromeier & Wetz, 1990) uncoating was complete within 2 h of incubation at 37 °C. Up to 4 h post-infection (p.i.) parental proteins remained precipitable, demonstrating that obviously no enzymic degradation had occurred. Furthermore, as shown in Table 1, when virus was adsorbed at 0 °C and the temperature was shifted to 37 °C for different periods, about 50% of the virus associated with cells underwent uncoating, and the rest appeared in the fluid phase, in the form designated 'A' particles (Crowell & Landau, 1983). This experiment also shows that after a 1 h temperature shift no additional virus particles were released into the fluid phase. A similar result was obtained using poliovirus specifically labelled with [3H]myristic acid in VP4.
Table 1. Distribution of parental poliovirus proteins in supernatant and HeLa cells*

<table>
<thead>
<tr>
<th>Time (h) of incubation at 37 °C</th>
<th>Supernatant (c.p.m.)</th>
<th>Recovery in cells (c.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TCA-soluble</td>
<td>TCA-precipitate</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>1</td>
<td>2.7 × 10^3</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>2.5 × 10^3</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>3.2 × 10^3</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>2.5 × 10^3</td>
<td>135</td>
</tr>
</tbody>
</table>

* Cells were incubated with 100 p.f.u. of [3H]leucine-labelled poliovirus (7.5 × 10^4 c.p.m.), corresponding to about 20000 particles per cell, at 0 °C for 1 h and extensively washed. After temperature shift for the times indicated the supernatant medium was counted for radioactivity. The cells were lysed with 0.25% SDS and 0.25% NP40 at room temperature and the radioactivity was precipitated using TCA. Virus preparation was as described (Gromeier & Wetz, 1990).

Table 2. Distribution of VP4 between supernatant and HeLa cells*

<table>
<thead>
<tr>
<th>Time (h) of incubation at 37 °C</th>
<th>Recovery (c.p.m.) in</th>
<th>Percentage in cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supernatant</td>
<td>Cells</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>934</td>
</tr>
<tr>
<td>1</td>
<td>264</td>
<td>974</td>
</tr>
<tr>
<td>2</td>
<td>492</td>
<td>1070</td>
</tr>
<tr>
<td>3</td>
<td>306</td>
<td>848</td>
</tr>
</tbody>
</table>

* Cells were incubated with [3H]myristic acid-labelled poliovirus at 0 °C for 1 h and extensively washed. After temperature shift for the times indicated the supernatant medium and the lysed cells were counted for radioactivity.

(Table 2). Since VP4 is suggested to have a specific function upon virus uncoating by mediating particle binding to hydrophobic cell membranes (Kräusslich et al., 1990; Marc et al., 1990), the release into the fluid phase was investigated after a temperature shift. As shown in Table 2, about 75% of VP4 was associated with the cellular phase compared to about 50% of the total virus protein (Table 1), and even after 3 h p.i. the polypeptide was bound to the cells. However, its function in mediating membrane binding remained unclear, particularly as the resulting 135S particles are hydrophobic and bind to liposomes (Frick & Hogle, 1990).

Recently it has been shown that the receptor is able to induce 135S particles at physiological temperature (Kaplan et al., 1990). However, thermal stability of the virus capsid under the influence of reduced ion concentration and pH environments could also influence virus uncoating. This seemed a reasonable phenomenon for investigation. Incubation of radioactively labelled virus in 20 mM-Tris–HCl pH 7.5 for 1 h at 37 °C resulted in a decrease of infectivity. Sucrose gradient centrifugation of the mixture revealed 80% conversion into 80S empty capsids and about 20% remaining native virus (Fig. 1c). Also, degradation occurred in hypotonic phosphate buffer at 37 °C (Fig. 2), whereas the virus was completely stable in these buffers at room temperature. In contrast, treatment of the virus at 37 °C in an isotonic buffer, e.g. Eagle’s medium, resulted in high stability (Fig. 1b). Likewise the virus was stabilized to a level as high as 96% in Eagle’s medium in the presence of non-permissive L cells (Fig. 1a), and a similar result was obtained with HeLa cells (not shown). Although poliovirus penetrated HeLa cells, the amount of 135S particles in the supernatant was not significantly greater than that obtained with L cells. Surprisingly, virus treatment in Eagle’s medium resulted in a greater yield of 135S particles than with the two cell lines, suggesting the cell
membranes had a stabilizing effect (Fig. 1). With Eagle's medium and with both cell lines only low amounts of 80S particles were detected. These experiments in hypotonic buffers show that low ionic concentrations together with elevated temperature could be responsible for irreversible alterations of the capsid, leading to the liberation of VP4 and the RNA. The fact that there was no conversion at room temperature suggests a temperature dependence, like that of poliovirus uncoating in HeLa cells (Mandel, 1967; Rombaut et al., 1990). Similar results after treatment in hypotonic buffers have been obtained with rhinovirus and poliovirus (Lonberg-Holm et al., 1976) and with coxsackievirus (Cords et al., 1975).

Since divalent cations have been suggested to be important for virus adsorption to cell membranes (Crowell & Landau, 1983), we investigated the effect of calcium on in vitro uncoating. Treatment of virus for 1 h at 37 °C in hypotonic buffer containing 2 mM-CaCl2 and subsequent centrifugation in sucrose gradients also containing 2 mM-CaCl2 resulted mainly in 135S particles (Fig. 1d). In general, transformation of virus into 135S particles was reproducible under these conditions. However, in some cases few 135S particles were obtained and the virus was further degraded to 80S. The reason for this remained unknown. After calcium was replaced with magnesium, the resulting particles were not unimolecular and sedimented as a broad band with peak fractions between 120S and 110S (not shown). The 135S particles are thought to represent an expanded form of poliovirus (Gromeier & Wetz, 1990; Wetz et al., 1983), which is essential and capable of releasing the RNA, whereby the generation of the relatively compact and stable empty capsids may be the driving factor. Reversible swelling of many icosahedral plant viruses under the influence of divalent cations seems to be a general phenomenon and it has been shown for southern bean mosaic virus that a swollen virus particle is RNase-sensitive, whereas the compact form is not (Krüse et al., 1982).

It has been suggested that low pH is a prerequisite for poliovirus uncoating (Madschus et al., 1984). Therefore we investigated the structural alterations of poliovirus at 37 °C in buffer of low ionic strength at different physiological pH values. However, as demonstrated in Fig. 2, the virus showed a greater stability at acid than at alkaline pH values which is in agreement with results regarding the stability of poliovirus capsid at similar pH values (Rombaut et al., 1982). The rate of virus conversion into empty shells was somewhat slower than that of virus uncoating in HeLa cells, which seemed almost complete after 2 h (Gromeier & Wetz, 1990).

Although there is no evidence for similar low ion concentration at the cell surface at present, it is conceivable that after binding of the virus to its specific receptor, the cytoplasmic membrane engulfs the virion at physiological temperature (Crowell & Landau, 1983). This trapping could be accompanied by a strong reduction of the ion concentration during formation of a vesicle, which in turn could induce conformational alteration of the capsid. This hypothesis is supported by the observation that antibody-neutralized virus is structurally stable in similar chemical and temperature conditions, which could be important for the elucidation of the mechanism of antibody-mediated virus neutralization (K. Wetz & T. Kucinski, unpublished results).

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References


Short communication


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