Structural Polypeptides of Orthopoxvirus: their Distribution in Various Members and Location Within the Virion

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SUMMARY

The structural polypeptides of accepted species and recently isolated members of the genus Orthopoxvirus have been examined by SDS-polyacrylamide gel electrophoresis. The viruses shared many polypeptides but some differences were found. The viruses could be divided into a vaccinia group (including buffalopox, 'Lenny' and MK-10), an ectromelia group (including elephant virus and Moscow virus), cowpox, camelpox and monkeypox. Minor differences were found in the polypeptides of monkeypox virus strains from human and monkey outbreaks. Controlled degradation of virions showed that the polypeptides which enabled the viruses to be differentiated were located in the surface and sub-surface layers. The cores of the viruses all gave the same complex polypeptide pattern.

INTRODUCTION

Increasing attention is being paid to the structural polypeptides of the variola/vaccinia subgroup (genus Orthopoxvirus) of poxviruses. In particular the method for the controlled degradation of virions devised by Easterbrook (1966) has been used to determine which polypeptides of vaccinia virus were surface, sub-surface or core components (Holowczak & Joklik, 1967; Sarov & Joklik, 1972; Katz & Margalith, 1973).

More recently, comparative studies have been made on the structural polypeptides of smallpox, monkeypox, vaccinia and cowpox viruses. Each virus had a characteristic pattern although the location of the polypeptides within the virion was not determined (Arita & Tagaya, 1977; Esposito et al. 1977).

Although we could not examine smallpox virus we have extended these observations to include all other accepted members of the genus Orthopoxvirus and also some recently-isolated strains of uncertain taxonomic position. In addition we have tried, with some success, to determine the location within the virion of specific polypeptides.

METHODS

Virus strains. (a) Accepted species: vaccinia (Lister, Wyeth, EM63 vaccine strains), cowpox (Brighton, Whipsnade strains), ectromelia (Hampstead), monkeypox (from human infection – Liberian strain, from monkey infection – Copenhagen strain), camelpox (Gorgan), buffalopox (BP4). (b) Unassigned members: (i) Biologically related to vaccinia: ‘Lenny’ virus (Bourke & Dumbell, 1972), MK-10 (Shelukhina et al. 1975). (ii) Biologically

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related to cowpox: ‘elephant’ virus (Baxby & Ghaboosi, 1977), Russian carnivore virus (Marennikova et al. 1978), here designated ‘Moscow’ virus.

**Virus growth and purification.** All viruses were grown on the CAM of 12-day-old chick embryos. Virus, extracted from heavily-infected CAM, was purified using fluorocarbon extraction and density gradient centrifugation, which is known to separate virus from contaminating proteins (Baxby, 1972). Virus was suspended in 0.01 M-phosphate buffer, pH 7.4. Pellets were dispersed by treatment with a Rapidis 150 ultrasonic disintegrator (Ultrasonics).

**Controlled degradation.** A modification of Easterbrook’s method (1966) was used as described by Holowczak & Joklik (1967). Purified virus suspensions (10^{11} virions/ml) in 1 mM-tris-HCl buffer, pH 8.0 were first treated with 0.5% (v/v) NP40 (Shell) for 2 h at 37 °C (Holowczak & Joklik, 1967). The suspension was then centrifuged at 30000 g for 30 min to remove virus. The supernatant, containing the material released by NP40, was dialysed against 0.1% (w/v) SDS in tris buffer and then concentrated tenfold (Minicon B15 Concentrator). This material was then ready for digestion and analysis.

The pellet of NP40-treated virus was resuspended in tris buffer containing 0.5% (v/v) NP40 and 0.12 M-2-mercaptoethanol (2ME) and stirred at room temperature for 1 h. At the end of this period iodoacetamide was added to 0.24 M to stop the reaction and prevent re-aggregation of polypeptides (Holowczak & Joklik, 1967). The material thus released was separated from the virus cores and concentrated as described above. The pellet of cores was washed in tris buffer and deposited by centrifuging at 30000 g for 30 min. Hence for any one virus, four fractions were available: (1) whole virus, (2) material released by NP40, (3) material released by NP40 + 2ME from NP40-treated virus, (4) cores. The fractions were stored at −70 °C until suitable combinations were available for electrophoresis.

**Polyacrylamide gel electrophoresis.** Samples of virus protein (500 µg) were prepared for electrophoresis by boiling for 90 s in 1% 2ME + 2% SDS as described by Pennington (1973). The dissociated proteins were separated by electrophoresis on slabs 82 cm square of 4 to 26% gradients of polyacrylamide (Gradipore Universal). Each sample contained about 12 µg virus protein. This permitted resolution of many minor bands without the major bands being too dense. A continuous buffer system was used of 1% SDS in 0.1 M-phosphate buffer, pH 7.4 (Fairbanks et al. 1971). Electrophoresis was at 80 V for 5 h. After electrophoresis the polypeptide patterns were stained using Coomassie brilliant blue R250 using the method of Fairbanks et al. (1971). Stained gels were photographed through an orange filter to increase contrast.

Two mol. wt. standards (B.D.H.) containing polymers in mol. wt. ranges 14300 to 71500 and 53000 to 265000 were included on each gel. The approximate mol. wt. of the virus polypeptides were determined from the log scale constructed from the migration of these markers.

**RESULTS**

**Polypeptide composition of whole virus**

Each virus gave a complex pattern of at least 25 polypeptides with a range of mol. wt. from 12000 to 130000. Because different electrophoresis methods were used, direct comparison with the results of others was not possible but the patterns were generally similar to those recently reported for some poxviruses (Arita & Tagaya, 1977; Esposito et al. 1977). As might be expected all the virions shared many components. However, careful examination of the gels showed that some groups of viruses were indistinguishable whereas others gave specific patterns. The differences between viruses occurred in three mol. wt. ranges, 20000 to 25000, 30000 to 40000 and 50000 to 60000. The essential results are summarized in
Orthopoxvirus polypeptides

Table 1. Summary of the distribution of those polypeptides which enable Orthopoxviruses to be grouped and characterized

<table>
<thead>
<tr>
<th>Polypeptide†</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>31</th>
<th>34</th>
<th>35</th>
<th>37</th>
<th>53</th>
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<td>Monkeypox H</td>
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<td>Monkeypox M</td>
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<td>Camelpox</td>
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<td>Ectromelia</td>
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<td>Elephant virus</td>
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<td>Moscow virus</td>
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<td>Vaccinia (3)</td>
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<td>Buffalopox</td>
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<tr>
<td>Lenny</td>
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<td>MK-10</td>
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<tr>
<td>Cowpox (2)</td>
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* Number in parentheses indicates number of strains tested.
† Number is approximate mol. wt. of polypeptide x 10^-3.

Table 1. The three strains of vaccinia virus, and buffalopox, Lenny and MK-10, which are closely-related to vaccinia, all produced the same pattern. Ectromelia virus, elephant virus and Moscow virus, which are related biologically, produced the same pattern and could be separated from the vaccinia group by the absence of a polypeptide of mol. wt. 53000 (i.e. p53). Other apparent differences between vaccinia and the ectromelia group at p50 and p70 were not reproducible and will not be considered further. Typical results are shown in Fig. 1 in which, for simplicity, vaccine strain EM63 and elephant virus are used to represent the vaccinia and ectromelia groups respectively. Cowpox virus was separated from the vaccinia group by the absence of p53 and from the ectromelia group, to which it is biologically related, by the presence of p37. Camelpox virus was separated from the vaccinia group by the absence of p53 and from all others by possessing p31 in addition to p32 and p35 instead of p34. Monkeypox virus was characterized by the presence of two polypeptides p22 and p24 instead of the p23 present in all the other viruses.

There were some differences in the proportions of polypeptides produced by viruses in the 13000 to 15000 region. However these were difficult to reproduce consistently and will not be considered further.

Of particular interest was the observation that the two strains of monkeypox gave slightly different patterns. The strain isolated from human monkeypox in Liberia contained p54 whereas the strain isolated from a sick monkey in Copenhagen had p53.

The differences between some viruses were based on small differences in the mol. wt. of certain polypeptides, e.g. p53, p54 and p34, p35. These results were reproducible and that they represent real differences is suggested by the results obtained with partially-degraded virus.

Polypeptides released from whole virus by NP40

NP40, a mild non-ionic detergent releases only very loosely-bound polypeptides from the surface of vaccinia virus (Holowczak & Joklik, 1967; Sarov & Joklik, 1972). Again, the viruses within the vaccinia and ectromelia groups gave similar results and only vaccinia EM63 and elephant virus are included from these groups in Fig. 2 which also includes camelpox, monkeypox and cowpox viruses. Fig. 2 shows that p34, p35 and p37 used to differentiate some of the viruses are loosely-bound surface components. As the majority of polypeptides present in whole virus are not extracted by NP40 this treatment permits some of the differences discussed above and shown in Fig. 1 and Table 1 to be better appreciated.
In particular it is interesting to note that NP40 removed p53 from the monkey monkeypox strain but nothing of similar mol. wt. from the human monkeypox strain. This suggests that the difference between these two strains noted above is probably a real one and shows that p53 in monkey monkeypox is a surface component.

Although as seen above (Fig. 1) both strains of monkeypox contain p32 and p34, NP40 treatment extracted p34 from both strains but p32 from only the Copenhagen strain. This suggests that there are differences in the degree of binding of the same polypeptides in different strains. Similar comments also apply to the poorly-resolved low mol. wt. polypeptides extracted by NP40 only from Copenhagen monkeypox.

**Polypeptides released from NP40 treated virus by NP40+2ME**

Simultaneous treatment with NP40+2ME will release those polypeptides not released by NP40 alone and which are not present in the core. Some of the polypeptides will be superficial, even surface components; others will be located further inside the virion.
18 polypeptides were released by NP40+2ME treatment and representative results are shown in Fig. 3. Polypeptides p23, p24 and p53 which differentiated some viruses (Table 1) were removed by this treatment. In addition p32 which had been removed from Copenhagen monkeypox by NP40 alone, was extracted from the other viruses by NP40+2ME. Similarly p53 removed from Copenhagen monkeypox by NP40 was extracted from members of the vaccinia group by the slightly more drastic treatment with NP40+2ME. P34 and p37 had evidently been incompletely extracted by NP40 for these polypeptides were also extracted by NP40+2ME.

Apparent differences, both qualitative and quantitative, were found with p76. However this polypeptide was not present in whole virions (Fig. 1), which suggests that it may be an artefact perhaps produced by degradation of high mol. wt. polypeptides by the extraction process.
Polypeptides released from cores

The cores left behind after NP40 + 2ME treatment contained about 25 polypeptides. They contained two major polypeptides p27 and p56; the others were present in much smaller amounts (Fig. 4). There are apparent differences between the core polypeptides of camelpox and the other viruses. However p23, absent from camelpox in Fig. 4 was present in camelpox in other experiments and p68 and p72 were not seen in whole virus and like p76, as discussed above, may be artefacts.

The cores apparently contained most of the polypeptides found in whole virus. However cores are easily lysed by vigorous washing and in avoiding this it is possible that they were contaminated by other structural polypeptides. In any event, it is clear that the differences detected between the viruses were not reflected in the polypeptide composition of the cores.
DISCUSSION

The results presented here confirm and extend the work of others which indicate that Orthopoxvirus members may be characterized by their polypeptide patterns. Previous workers who examined smallpox, monkeypox, cowpox and vaccinia viruses noted the importance of the 30000 to 40000 mol. wt. region (Arita & Tagaya, 1977; Esposito et al. 1977). We found this region important but also found differences in the 20000 to 25000 and 50000 to 60000 regions. Some viruses which are known to be biologically distinct, for instance buffalopox and different vaccinia strains had indistinguishable polypeptide patterns. However polypeptides of identical mol. wt. are not necessarily identical and different native proteins may be dissociated into polypeptides of identical mol. wt. Also our technique detected slightly fewer polypeptides than other workers and it is possible that different techniques and the increased sensitivity of radiolabelling would separate these viruses. It was interesting to find that cowpox was different from both elephant and Moscow virus, to
both of which it is biologically very closely related (Baxby & Ghaboosi, 1977; Marennikova et al. 1978).

By controlled degradation of the viruses we have been able to show that most of the polypeptides which separated the viruses were located in the surface layers of the viruses. This is of interest in view of increasing evidence that there are minor immunological differences among poxviruses. Unfortunately we were unable to determine the location of p54 and p22 found in monkeypox.

Indistinguishable polypeptides in closely-related viruses differed in the ease with which they could be extracted by NP40. Thus NP40 extracted p32 and p53 from Copenhagen monkeypox but not from other viruses which contained them. This indicates that these polypeptides may have slightly different locations or be rather less firmly bound in this virus. The ease with which polypeptides are released will be affected by their bonding to other polypeptides.

Although monkeypox virus strains from different sources have been shown to be biologically homogeneous (Rondle & Sayeed, 1972), we found minor differences in the structural polypeptides of strains from human and simian outbreaks. We believe these differences to be real because differences have also been found between both the DNA endonuclease fragments (Esposito et al. 1978) and the polypeptides from infected cells (L. Harper, personal communication) of human and monkey isolates of monkeypox virus.

Little information is available about the function of polypeptides in different poxviruses. It has been suggested that a polypeptide designated 4c directs the incorporation of virions into A-type inclusions (Shida et al. 1977). Estimates of the mol. wt. of 4c vary from 55000 (Sarov & Joklik, 1972) to 58000 (Stern & Dales, 1976). However, we were unable to detect differences in this mol. wt. range between viruses with empty (i.e. V-) A-type inclusions, e.g. Moscow virus, and those (i.e. V+) which incorporate virions, e.g. elephant virus. Also V+ strains of cowpox (Whipsnade) gave the same pattern as V- strains (Brighton).

Increasing interest is being shown in the minor antigenic differences among Orthopoxvirus species and the polypeptides in immunoprecipitates have been examined by electrophoresis (Esposito et al. 1977). One problem is that of retaining antigenic specificity, although Stern & Dales (1976) have recently isolated an antigenic tubule protein from vaccinia virus by treatment with NP40+2ME. It is possible that the mild NP40 extraction employed here may be useful, particularly as it releases a small number of polypeptides which aid differentiation of the viruses.

REFERENCES


Orthopoxvirus polypeptides


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