Location of Vaccinia Virus Structural Polypeptides on the Surface of the Virus Particle

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Vaccinia virus has a complex structure. Thin sections of virus particles show three structural components: the outer envelope, the lateral bodies and a central structure, known as the 'core', which contains the virus DNA (Dales, 1963). Easterbrook (1966) showed that treatment with the nonionic detergent NP40, in the presence of 2-mercaptoethanol (2 ME) leads to the dissociation of the outer membrane and release of the core.

By polyacrylamide gel electrophoresis, Holowczak & Joklik (1967) identified at least 17 structural polypeptides, three of which were associated with the core. Katz & Moss (1970) showed that some of these polypeptide peaks could be further separated into two to three polypeptides, thus increasing the total number of resolved structural polypeptides to 22.

Our knowledge concerning the arrangement of vaccinia virus polypeptides on the particle surface is very limited. The present paper attempts to determine which of the virus polypeptides is situated on the surface. We have used iodination of vaccinia virus polypeptides

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Fig. 1. Polyacrylamide gel electrophoresis of vaccinia virus structural polypeptides. [3H]-leucine-labelled virus was disrupted and the polypeptides were separated by electrophoresis on 7.5% polyacrylamide gel at 3 mA for 19 h. A diagram of the Coomassie-brilliant-blue-stained gel (up) and the [3H]-leucine-labelled polypeptides of the same gel (down) are shown. Marker proteins: bovine serum albumin and cytochrome C were applied to a separate gel. Their positions, indicated by arrows were determined by staining with Coomassie blue.
Fig. 2. Polyacrylamide gel electrophoresis of virus particle and envelope (released) polypeptides. Following electrophoresis, the gels were immersed in TCA (20%), stained with Coomassie blue (0.1% in 10% TCA), washed in acetic acid (7.5%), sliced longitudinally, dried and exposed to X-ray film (Fairbanks, Levinthal & Reeder, 1965). Tracings of the developed X-ray films were made with a Joyce-Loebl microdensitometer.

Vaccinia virus (strain wr) was grown on HeLa cell monolayers in Eagle’s media supplemented with 5% calf serum. Two days after infection the cells were homogenized and virus particles were purified as described by Joklik (1962). Labelled virus was prepared by the addition of 10 μCi/ml of [3H]-leucine (52 Ci/m-mol, Amersham, England) to infected cells in Eagle’s media containing 1/50 the regular concentration of leucine and 2% dialysed calf serum. The structural polypeptides of vaccinia virus were analysed by electrophoresis on polyacrylamide gels after disruption of the virus with 2% SDS and 1% 2-ME, for 1 min at 100 °C (Katz & Moss, 1970).

A pattern of the structural polypeptides of vaccinia virus is shown in Fig. 1. The upper part of the Fig. indicates the position of the Coomassie-blue-stained polypeptides and the lower part the [3H]-leucine labelled polypeptides.

A modification of the method of Easterbrook (1966) was used for dissociating virus particles into envelope and core components. This procedure was also used by Holowczak &
Fig. 3. Lactoperoxidase labelling of purified vaccinia virus. (A) Vaccinia virus was disrupted with SDS (2% final concentration) for 30 min at 37 °C, dialysed overnight against 0.15 M-NaCl, buffered at pH 7.3 with 0.2-tris (to remove most of the detergent) and iodinated using lactoperoxidase. The iodinated polypeptides were treated with SDS and 2-ME and analysed by electrophoresis on 10% polyacrylamide gel at 4 mA for 17 h. (B) Pure vaccinia virus was iodinated using lactoperoxidase, then treated with SDS and 2-ME and analysed by electrophoresis as in (A). The gels were stained with Coomassie-brilliant blue, in order to determine the position of the main virus polypeptides and then sliced into 1 mm fractions which were counted in a Packard Tri-Carb Auto-Gamma spectrometer.

Joklik (1967) to localize core and membrane proteins. Purified virus was incubated for 30 min at 37 °C in a solution containing 50 mM-tris buffer (pH 8.6), 0.5% NP-40 (Shell, London) and 50 mM-dithiothreitol (dTT). The mixture was then treated with ultrasonic vibrations and layered on to 36% (w/v) sucrose in 10 mM-tris buffer (pH 8.6) containing 2 mM-dTT. After centrifuging in an SW 50L rotor at 25,000g for 80 min, the top fraction (released envelope) and the pellet (cores) were collected as previously described (Katz & Moss, 1970). It can be seen (Fig. 2) that at least three polypeptides: 4c, 6a and 6b are released from the virus by NP-40 and dTT. Similar results were previously observed by Katz & Moss (1970) and by Garon & Moss (1971).

Purified vaccinia virus was iodinated using lactoperoxidase, which was prepared by
U. Olshevsky from our laboratory, following the method of Morrison, Hamilton & Stotz (1957). To 2 ml of purified virus (75 µg protein) in 0.15 M-NaCl, buffered at pH 7.3 with 0.2 M-tris, 4 µl of lactoperoxidase ($E_{280} = 0.5; E_{412} = 0.3$), 100 µl of $[^{125}I]$ ($= 400$ µCi, free from reducing agents, Amersham, England), 20/µl of 0.1 mm-KI and 20 µl of 1 mm-H$_2$O$_2$ (Merck, Germany) were added. The reagents were vigorously mixed and allowed to react for 10 min at room temperature. In a control reaction mixture, from which lactoperoxidase was omitted, protein iodination could not be detected. When intact virus was iodinated, the reaction was stopped by diluting the mixture in 10 mm-tris, pH 9, and sedimenting the virus in 50 L rotor at 13500 rev/min for 1 h.

The purified vaccinia virus iodinated using lactoperoxidase, was dissociated with SDS and 2-ME and analysed by gel electrophoresis. As a control, the virus was disrupted with SDS before iodination. The two gel patterns thus obtained are shown in Fig. 3. Virus iodinated after disruption (Fig. 3A) gave a similar polypeptide pattern shown in Fig. 1. Clearly, all the main virus polypeptides contain tyrosyl and/or histidyl residues susceptible to iodination. Iodination of intact virus gave a markedly different result (Fig. 3B). Peaks 4c and 6b were the major electrophoretic components to be labelled. Two additional polypeptides of lower mol. wt. than 6b were also iodinated to great extent. This suggests that these four polypeptides have an external location in the virus particle structure. The finding that both 6a and 6b are polypeptides of the envelope (Fig. 2) but only 6b is iodinated (Fig. 3B) suggests that 6b has an external position as compared to 6a in the envelope. The latter is a major glycosylated polypeptide of the virus (Holowczak, 1970; Garon & Moss, 1971).

To Dr Dov Karpas – in memoriam.

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