JOURNAL OF VIROLOGY, Mar. 1994, p. 1935–1941
0022-538X/94/$04.00+0

Vol. 68, No. 3

Structure of Intracellular Mature Vaccinia Virus Observed by Cryoelectron Microscopy

JACQUES DUBOCHET,[1]* MARC ADRIAN,[1] KARSTEN RICHTER,[1]†
JAVIER GARCES,[2]‡ AND RICCARDO WITTEK[2]

Laboratoire d'Analyse Ultrastructurale,[1] and Institut de Biologie Animale,[2] Bâtiment de Biologie, Université de Lausanne, CH-1015 Lausanne, Switzerland

Received 29 September 1993/Accepted 3 December 1993

Intracellular mature vaccinia virus, also called intracellular naked virus, and its core envelope have been observed in their native, unfixed, unstained, hydrated states by cryoelectron microscopy of vitrified samples. The virion appears as a smooth rounded rectangle of ca. 350 by 270 nm. The core seems homogeneous and is surrounded by a 30-nm-thick surface domain delimited by membranes. We show that surface tubules and most likely also the characteristic dumbbell-shaped core with the lateral bodies which are generally observed in negatively stained or conventionally embedded samples are preparation artifacts.

Vaccinia virus, the prototype orthopoxvirus, is associated with several important discoveries in the history of virology (9). The large size of the virions allowed their visualization with light microscopy by Buist as early as 1886, and these descriptions of small granules founded the concept of the particulate nature of viruses (3a). Vaccinia virus was also the first animal virus to be purified to an extent which allowed accurate chemical analysis. Finally, vaccinia virus also gave its name to vaccination and was closely associated with the development of the science of immunology.

Vaccinia virus was also among the first viruses to be examined by electron microscopy. Our current knowledge of the structure of the virion (Fig. 1 and 2) is based on the work of many scientists and has been reviewed recently (9). Briefly, two forms of virions can be distinguished. Intracellular naked virions (INV), which have recently also been called intracellular mature virions (IMV) (20), remain cell associated and may be recovered after experimental cell lysis. Virions which are naturally released from the cell are surrounded by an envelope derived from an intracellular cisterna and have been designated extracellular enveloped virions (EEV) (18). Both IMV (or INV) and EEV are infectious.

For details of the structure, we refer here to the scheme of Fenner et al. (8), reproduced in Fig. 1, and to typical views shown in Fig. 2. The IMV particles are said to be brick-shaped, measuring ca. 300 by 230 nm. As seen by negative staining (Fig. 2a), the outer membrane of IMV particles consists of randomly arranged tubular elements (surface tubules). The inside of the virion is best visualized in thin sections of infected cells (Fig. 2b and c). They reveal the presence of the viral core which contains the viral genome and which assumes, in the virion, a dumbbell shape owing to the presence of two lateral bodies of unknown function. The core envelope frequently appears associated with a regularly spaced palisade.

Cores released from the virions by treatment of IMV particles with nonionic detergents and reducing agents adopt a more rectangular shape. The isolated core envelope is composed of a smooth membrane, with regularly arranged spikes about 10 nm long and 5 nm in diameter.

Another typical form of the virion is frequently observed in negatively stained preparations. These particles have a smooth outer surface and are delimited by a characteristic 25-nm-thick zone of less-electron-dense material. The core appears homogeneous. This aspect is depicted for example in Fig. 2 and 8 of Müller and Williamson (16). This form of the virion is generally believed to be the consequence of stain penetration and to be less representative of the true structure than the views shown in Fig. 2.

The structure of the virions described above was derived mainly from micrographs obtained by conventional electron microscopy. This procedure involves full dehydration of the material, which can be a source of artifacts (11). Artifacts are particularly severe with membranous structures and enveloped particles whose size and material distribution can be severely affected by the preparation procedure.

Most preparation artifacts are avoided in cryoelectron microscopy of vitrified samples (4). In this technique, the specimens are neither chemically fixed nor strained and they are observed in their fully hydrated environment. The size of virions is precisely conserved, and the structure is well preserved (1, 2, 14, 17). In the present work, we have observed vaccinia virus IMV particles and isolated cores by cryoelectron microscopy of vitrified samples and we have found that the structure of the virion differs markedly from that which is generally accepted to be the true structure.

MATERIALS AND METHODS

Viral strains. The virus strains used were WR, the Western Reserve strain of vaccinia virus that most likely does not express the intact P_{4c} protein (18a), and A392, the Western Reserve strain of vaccinia virus expressing the entire P_{4c} protein (13a).

Virus preparations. IMV were purified by the method of Joklik (10), with minor modifications. After centrifuging the crude virus suspension through 36% sucrose, the virus was resuspended by mild sonication in 10 mM Tris-hydrochloride (pH 9), layered onto a 15 to 40% (wt/vol) sucrose gradient, and centrifuged for 15 min at 13,000 rpm (Kontron, TST 55 rotor). The virus band was extracted, and the virus was collected after

* Corresponding author. Mailing address: Laboratoire d'Analyse Ultrastructurale, Bâtiment de Biologie, Université de Lausanne, CH-1015 Lausanne, Switzerland. Phone: 21 692 2475. Fax: 21 692 2540. Electronic mail address: JDUBOCHE@ULYS.UNIL.CH.

† Present address: Veilchenweg 20, D-68775 Ketch, Germany.

‡ Present address: Laboratorios Clinicos de Puebla, Diaz-Ordaz 808, Puebla, Pue 72530, Mexico.

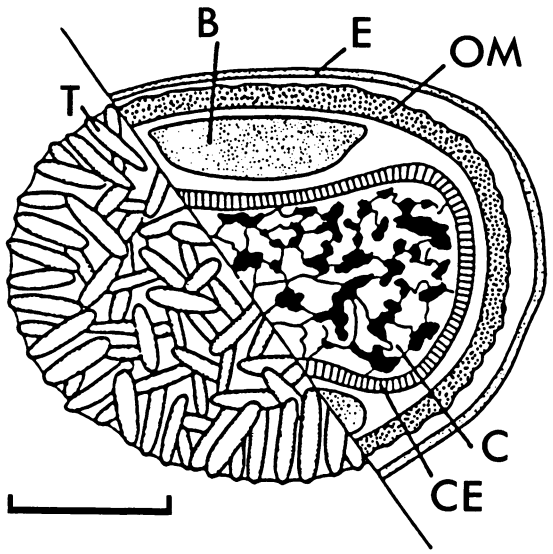

FIG. 1. Schematic representation of the structure of vaccinia virus, derived from conventional electron microscopy. Right-hand side, section of enveloped virion; left-hand side, surface structure of non-enveloped particle revealed by negative staining. T, surface tubules; B, lateral bodies; E, envelope; OM, outer membrane; C, core; CE, core envelope with palisade. Magnification, ×200,000. Bar, 100 nm. Reproduced by permission from Fenner et al. (8; Fig. 2.1, p. 76), with modifications.

a threefold dilution in 10 mM Tris-hydrochloride (pH 9) by centrifugation at 50,000 × *g* for 30 min. After a second round of purification in a sucrose gradient, the virus was finally resuspended in 10 mM Tris-hydrochloride (pH 9) at a concentration of at least 10 mg/ml and stored at 4°C. Before electron microscopic observation, the virus was dispersed by mild sonication.

Isolation of viral cores. Viral cores were prepared as described by Easterbrook (7), with minor modifications. Purified virus were diluted with 50 mM Tris-hydrochloride (pH 7.5) to 2 mg/ml, and 1 volume of 1% Nonidet P-40–2% 2-mercaptoethanol–50 mM Tris-hydrochloride (pH 7.5) was added. After being incubated for 30 min at 37°C, the preparation was centrifuged through 36% sucrose for 30 min at 20,000 × *g* (TST 55 rotor). The cores were gently resuspended in 50 mM Tris-hydrochloride (pH 7.5) and stored at 4°C.

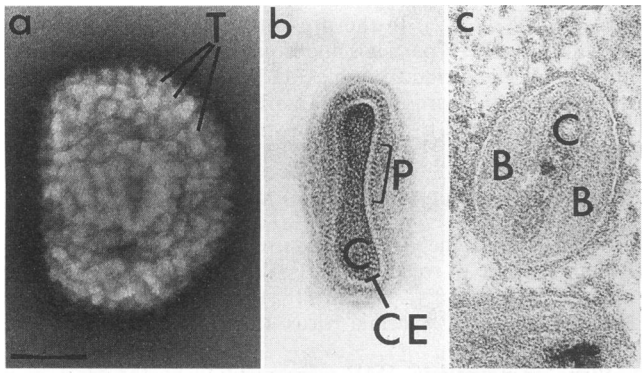

FIG. 2. Electron microscopic view of IMV. (a) Particle negatively stained in 2% uranyl acetate. The general brick shape and the surface tubules (T) are well visible. (b and c) Thin sections of particles embedded in Lowicryl. The core (C), the core envelope (CE), the palisade (P), and the lateral bodies (B) are marked. Magnification, ×100,000. Bar, 100 nm. Panels b and c are used by the courtesy of G. Griffiths.

Preparation of viral core envelopes. $MgCl_2$ was added to the core preparation at a final concentration of 10 mM. After the addition of 10 U of DNase (Promega) per 0.1 mg of cores, the suspension was incubated for 1 h at 37°C. Core envelopes were purified by centrifugation through 36% sucrose at 20,000 rpm (TST 55 rotor) for 1 h. The pellet fraction was gently resuspended in 10 mM Tris-hydrochloride (pH 7.5)–1 mM EDTA and stored at −20°C.

Conventional electron microscopy. Negative staining of specimens and embedding in Epon and in Lowycryl were done by the standard methods (3, 20).

Cryoelectron microscopy. Thin layers of vitrified suspension were prepared by the bare grid method (1, 4). A 3-μl drop of suspension containing ca. 50 mg of particles per ml in 10 mM Tris-hydrochloride (pH 9) was put on a perforated carbon grid mounted on a 200-mesh grid. Most of the drop was removed with blotting paper, and the thin residual film was immediately vitrified by projecting it in liquid ethane cooled in liquid nitrogen. The thin liquid film exists for about 0.1 s before being vitrified in ca. 10^{-4} s.

For thin sections of vitrified material, a low-density pellet of virions in 8% sucrose–7% short DNA fragments was obtained by centrifugation. A small amount of this material (ca. 10 nl) was mounted on a metal pin and immediately projected in liquid ethane. The solution of sucrose-DNA gives an adequate viscosity for manipulation and sufficient cryoprotective effect to allow vitrification of a surface layer of usable thickness. Deeper in the sample, water crystallizes into ice, producing solute precipitation and dehydration of the virion. The frozen sample was cut into thin cryosections in a Reichert FC4E cryochamber of an Ultracut ultramicrotome (Leica, Vienna, Austria), and the sections were flattened on a carbon film on a 200-mesh grid. During the entire procedure, the sample and the sections were kept at or below −160°C.

The cold samples were mounted in a Gatan 626 cryospecimen holder (Gatan, Varrendale, Pa.) and observed at −170°C in a Philips CM 12 cryoelectron microscope equipped with a blade-type anticontaminator. The state of the specimen, whether vitreous or crystalline, was tested by electron diffraction. Observations and image recording were made under low-dose conditions. Kodak SO163 films developed for 12 min in D 19, full-strength (speed, ca. 2 μm^2 per electron × optical density unit) were used for recording micrographs at a typical magnification of ×35,000.

Magnification was calibrated with a cross-grating replica and varied by less than 2% of the nominal value. Size measurements were made directly on the negative or on prints with a ruler or with an optical diffractometer for periodic structures.

RESULTS

General aspects. The general aspect of the freshly prepared IMV particle as observed by cryoelectron microscopy of a vitrified thin layer of suspension is shown in Fig. 3. The virion appears as a smooth, rounded rectangle. One or two adjacent corners are frequently more flattened than the others. At first sight, the virus seems rather homogeneous, with a zone of higher mass in the central region. A 30-nm-thick surface domain (S) is visible in most particles. This domain is delimited by two membranes (Fig. 3, arrows) which, however, are not always visible. On closer examination, the virion has a fine homogeneous grainy texture and its outline is also finely

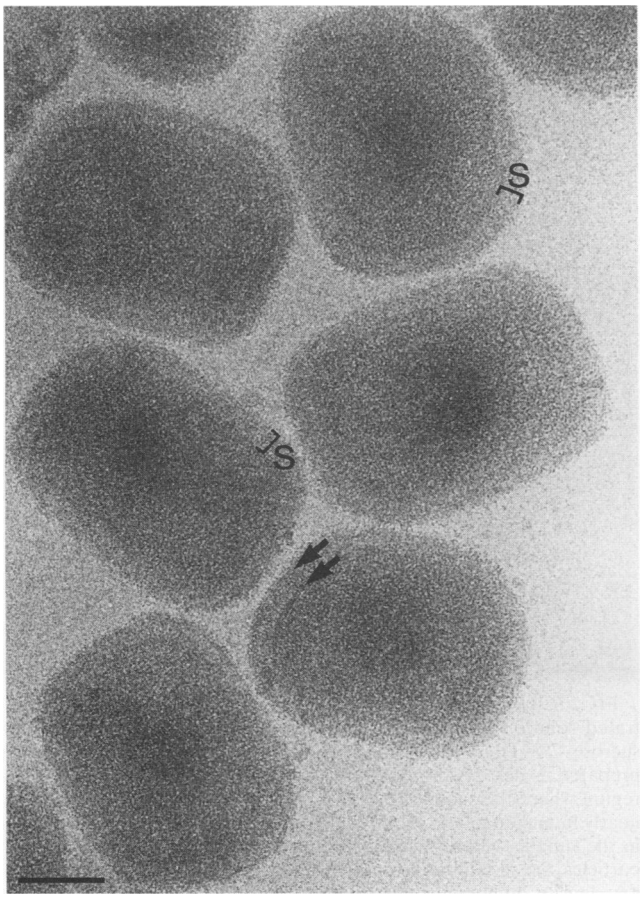

FIG. 3. Vaccinia virus IMV in a thin layer of vitrified suspension, as observed by cryoelectron microscopy. The 30-nm-thick surface domain is marked S. The arrows point toward the two membranes limiting this region. The thickness of the vitrified layer is ca. 300 nm. Magnification, ×112,000. Bar, 100 nm.

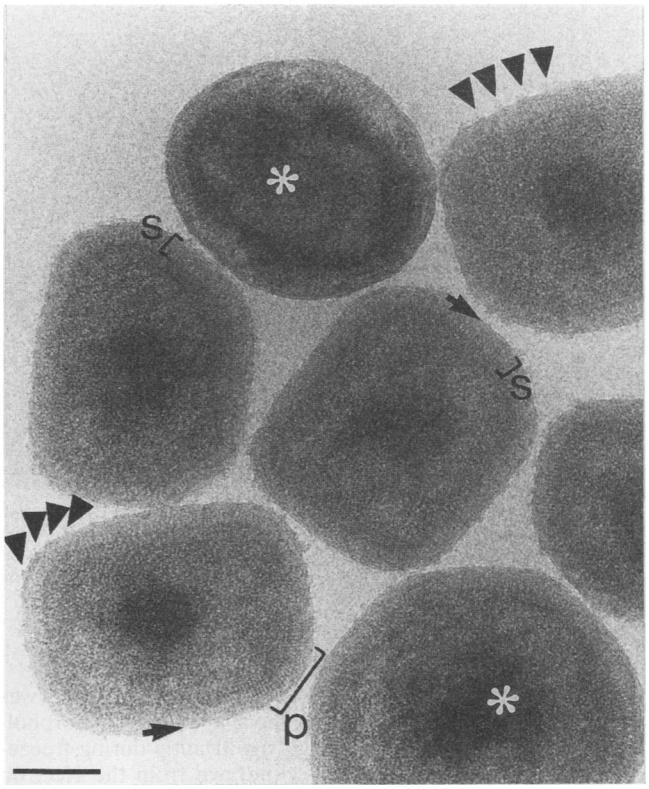

FIG. 4. Aging solution of IMV prepared as described in the legend to Fig. 3. S, surface domain; P, palisade. Arrows point toward a clearly marked unit membrane. Arrowheads indicate discontinuities or ruptures in the outer membrane. Magnification, ×112,000. Bar, 100 nm.

irregular. There is no sign of a structure resembling surface tubules or of a characteristic dumbbell-shaped core or lateral bodies.

Table 1 gives the sizes of isolated IMV particles, as determined in negatively stained and in vitrified preparations. The standard deviations of the measurements, especially for the length, are smaller in vitreous than in negatively stained preparations. No differences could be observed in the size and the aspect of WR and A392 strains.

The smooth surface of the virion shown in Fig. 3 is characteristic of fresh samples prepared without harsh treatment. Samples kept at 4°C for longer periods show a larger variety of structures (degradation). An example is presented in Fig. 4. In this case, the majority of the particles still have the general shape of the intact virion, but the surface domain (S) is more clearly delineated and is frequently marked by a ca. 4.8-nm striation of the palisade (P). The outer membrane is frequently seen as a typical unit membrane with ca. 4 nm of spacing between the leaflets (Fig. 4, arrows). It frequently seems to be broken, creating ridges with a more or less regular spacing of 25 nm (arrowheads). A number of particles appear to be more seriously damaged (Fig. 4, *): they are more irregular in size or more rounded, the outer unit membrane is more visible, and the core frequently seems to separate from the surface domain. In some cases, it resembles the typical dumbbell shape described previously (not shown).

TABLE 1. Sizes of IMV particles and core envelope in negatively stained or vitrified preparations

Virus particle	Length (nm)	Width (nm)
IMV, negative stained	363 ± 51	313 ± 11
IMV, vitreous	349 ± 18	267 ± 9
Core membrane, vitreous	288 ± 20	204 ± 13

Surface tubules. In order to remove any doubts whether the aspect of the virion seen in vitrified preparation (Fig. 3) is more representative of the native state than when the sample is prepared by negative staining (Fig. 2a), we have done the following control experiment. A vitrified sample was prepared similarly to the one shown in Fig. 3 except that a continuous supporting film mounted on a finder grid was used. (A finder grid is such that the observed region can easily be relocated in a subsequent observation). It was verified in the cryoelectron microscope that the virions had the usual aspect, and one region of the grid was irradiated with an electron dose of ca. 3,000 electrons per nm^2. This dose is sufficient to induce considerable chemical transformations, though it is too small to produce bubbling or a significant mass loss and to cause visible structural damage at a dimension larger than about 5 nm. The sample was then slowly allowed to warm up until it reached room temperature after ca. 2 h. Freeze-drying was

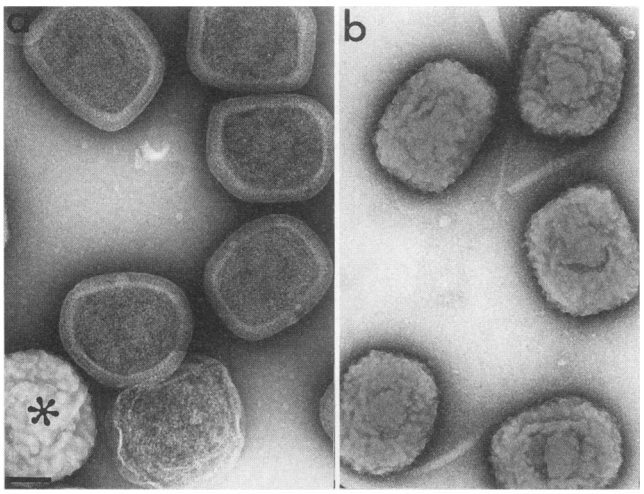

FIG. 5. (a) Negatively stained preparation of IMV freeze-dried in the electron microscope after fixation by electron irradiation (see text for details). (b) Same sample as in panel a but recorded from a region that was not irradiated previously. Magnification, ×60,000. Bar, 100 nm.

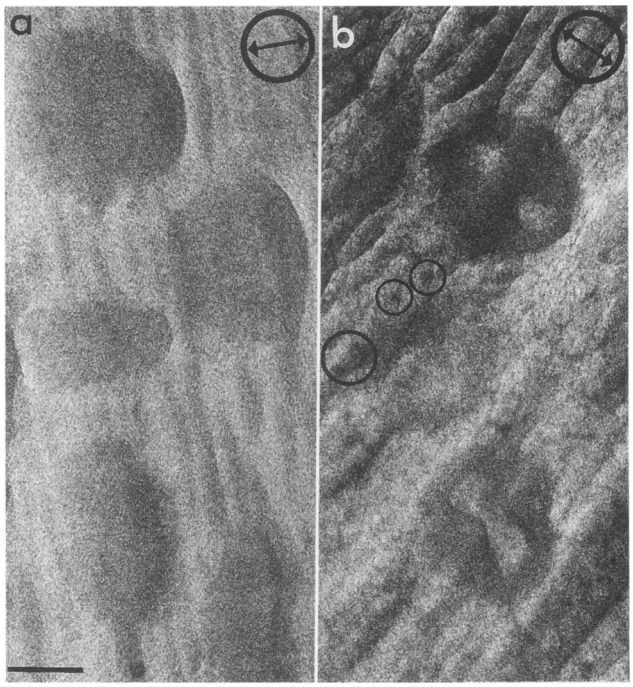

FIG. 6. Unstained and chemically unfixed cryosection of a concentrated solution of IMV. In order to make vitrification possible, 8% sucrose–7% DNA was added to the solution. The specimen was prepared as described in Materials and Methods. (a) Micrograph of a region where the sample is vitrified. The virion appears with a dense, nearly homogeneous body. (b) Micrograph of the same section deeper in the sample, where the water is crystallized in hexagonal ice. Most particles are nonhomogeneous, and many of them have a central low-density dumbbell-shaped region. The quality of the specimen is degraded by cutting-induced compression along the cutting direction (marked by a circled double arrow). Crevices and deformation lines are other visible cutting artifacts. The segregation pattern formed by material excluded from the growing ice crystals and the Bragg reflections (circled) indicate that water is crystallized in this region. The section thickness is ca. 100 nm. Magnification, ×100,000. Bar, 100 nm.

essentially finished before the temperature of −100°C was reached, and it was observed that the shape and the morphology of the particle did not change significantly during freeze-drying. The specimen was then withdrawn from the electron microscope, immediately stained negatively with 2% uranyl acetate, and observed again under normal room temperature conditions. An obvious difference was noticeable between the region previously irradiated at low temperature and that which was not. In preirradiated regions, illustrated in Fig. 5a, most of the virus particles resemble those observed in the vitreous state though the surface domain is more visible. Only a small minority of the particles have the usual aspect of negatively stained virions with surface tubules (*). In nonpreirradiated regions, nearly all the particles have the usual aspect, with surface tubules of negatively stained particles (Fig. 5b).

This experiment allows us to monitor the transformation of the virion during the different steps of the preparation procedure. It demonstrates that the structure shown in Fig. 5a resembles that observed in previous preparation steps and is therefore likely to be a faithful representation of the native virion. On the contrary, the characteristic structure with surface tubules seen in Fig. 5b appears only after staining. The difference between panels a and b must therefore be attributed to a staining artifact in panel b.

This experiment demonstrates further that electron irradiation can prevent the artifactual formation of surface tubules upon negative staining. This can be the consequence of a porosity induced by electron beam damage in the surface domain, making it insensitive to osmotic effect. It also suggests that the surface domain is normally sensitive to osmotic stress, either because it is delimited by two nonpermeable membranes or because it has the structure of an osmotically active gel.

Dumbbell-shaped core. As for the dumbbell shape of the core presented in Fig. 2b and c, some of the above observations suggest that it is also a preparation artifact. Supporting this view is the fact that dumbbell-shaped cores are rarely observed in vitrified samples of freshly prepared virions, whereas they are more numerous in older preparations or after chemical fixation.

However, the possibility remains that in the thin vitrified layer, the particles are all oriented in such a way that the dumbbell-shaped core could be visualized only in extreme lateral views which are not feasible in the electron microscope. In order to exclude this possibility, we have observed thin cryosections of a vitified concentrated solution of isolated virions. In such specimens, the orientation of the particles is random and all projections are equally probable. The result, presented in Fig. 6, shows, first of all, that vaccinia virus particles are not well suited for cryosections and do not compare favorably to other samples such as liver tissue (12), muscle (13), DNA crystals (19), or apple leaves (15) prepared by the same method. In particular, they suffer from severe cutting damage due to compression along the cutting direction (double arrows) and most of the fine details of virus structure are lost. As for the aspect of the virus, two cases can be distinguished. When the section is truly vitrified, as is the case in Fig. 6a, most of the virus particles have a homogeneous mass distribution without recognizable internal structure. In contrast, the characteristic dumbbell-shaped core is visible in many particles in regions where ice is crystallized. This is the case for the region shown in Fig. 6b, in which the crystalline state of the ice is apparent from the segregation pattern of the solute visible in the background and from the dark spots (circled) due to Bragg reflections in the hexagonal ice crystals. In addition,

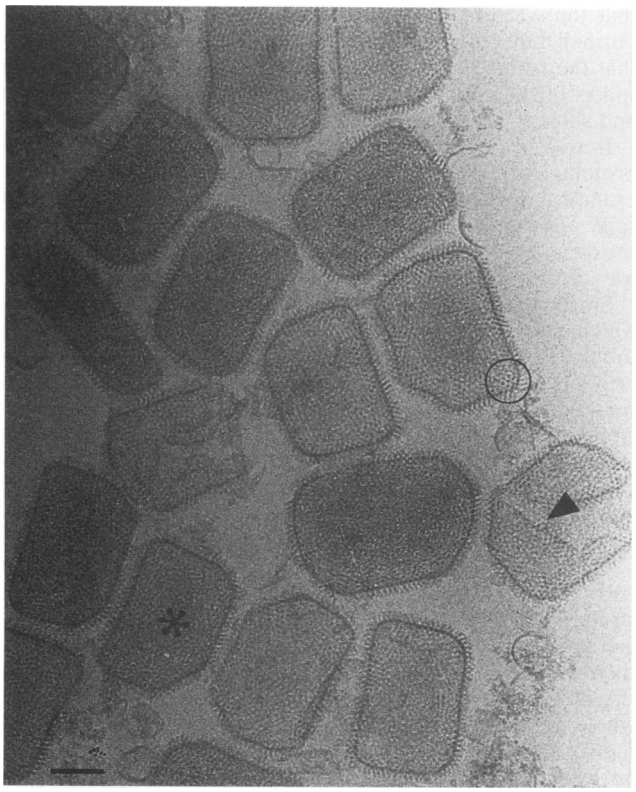

FIG. 7. Thin layer of vitrified isolated core envelopes. The membrane marked with an asterisk has a characteristic shape, whereas the one marked by an arrowhead is torn off. Ordered spikes are visible around the particle or, in some places, at the surface (circled area). The thickness of the film is ca. 200 nm. Magnification, ×70,000. Bar, 100 nm.

the crystalline versus vitreous state of the water was tested directly by electron diffraction. Since the two views presented in Fig. 6 are from the same section, the difference must be attributed to freezing damage, probably induced by a dehydration effect.

Core envelope. Isolated cores are rarely intact. They lose variable amounts of their content and are heterogeneous in shape and dimension. Removal of the residual core material by digestion with DNase results in apparently empty core envelopes which can be precisely characterized. Such a preparation is shown in Fig. 7. The envelope seems to be formed by a membrane with more or less regularly arranged spikes pointing outward. The envelopes have roughly the same general shape as IMV particles, but they are less rounded and they are smaller. The length and the width of the envelope, measured at the level of the membrane, are both ca. 60 nm smaller than the corresponding dimensions in the IMV particle (Table 1). It is a frequent occurrence that one or two adjacent corners of the envelope are flattened, giving the very characteristic shape of the particle marked by an asterisk. Some of the envelopes are not fully intact but the general shape is preserved (arrowhead).

The spikes seem to be packed closely on the envelope in a disordered or an ordered way. When they are ordered, they form a hexagonal lattice of 9.7-nm spacing. One example in which a hexameric arrangement is clearly visible just at a corner of the particle is circled. The order seems to improve in specimens kept at 4°C for longer periods. The spikes protrude from the membrane by ca. 20 nm. Seen from the side at high resolution, the spikes seem to have the shape of a hollow tube, resulting, when many spikes are tightly aligned, in a periodic striation of half dimension (ca. 4.8 nm). The spikes are easily lost from the core envelope. In many preparations, large regions of the envelopes are devoid of spikes or small clusters of few spikes are scattered over the envelope. In some cases, the spikes are lost during preparation of the thin vitrified layer and are oriented by the thin film surface. Such a case is illustrated in Fig. 8. This orientation effect, which has been observed frequently with other viruses (5, 6), is due to the fact that spike proteins tend to adsorb to the surface of the thin liquid film in which the particles are imprisoned for a fraction of a second before vitrification. They remain in the vicinity of the particle from which they originate, because they have not had time to diffuse far away. The spikes are probably attached to the liquid surface through their hydrophobic tails and therefore all have the same orientation. In this projection, the spike proteins of the core envelope appear as hollow cylinders with an outer diameter of ca. 10 nm.

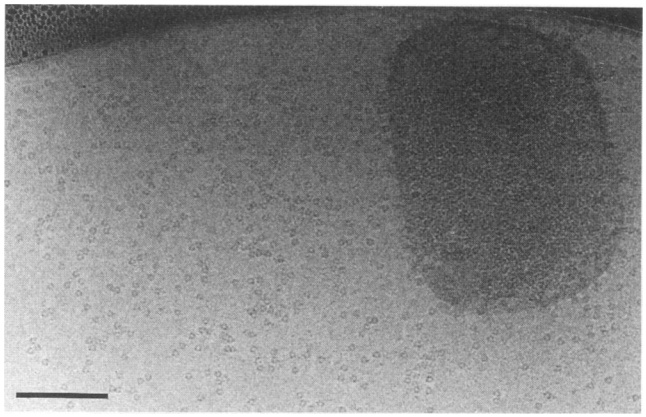

FIG. 8. Vitrified thin layer of isolated core envelopes. The particle shown has lost many of its spikes during preparation. They are still in the vicinity of the particle but are oriented in respect to the thin film surface. Magnification, ×112,000. Bar, 100 nm.

DISCUSSION

Shape and structure of the virion. The results presented above demonstrate that the native freshly prepared IMV particle is more faithfully represented in Fig. 3 than in Fig. 1 or 2. The difference between these two representations is mainly due to preparation artifacts. The major features deduced from the present work are schematized in Fig. 9. The 30-nm-thick surface domain is osmotically sensitive and is delimited by two membranes which can be recognized as thin zones of high density. In aging preparations, the material seems to be more segregated in this compartment, and the membranes frequently appear with the typical double leaflet aspect of a unit membrane. The palisade forming a regular, ca. 5-nm striation is attached outward from the core envelope. The core appears dense and homogeneous.

One difficulty in deducing the three-dimensional shape of the virion comes from the fact that the sample is not homogeneous. It probably contains particles at various stages of intracellular maturation. Differences between particles observed on the micrographs also result from the fact that different projections are observed. It appears however that the virion is not randomly oriented in the thin vitrified layer. Most particles have one long and one short dimension with a ratio of

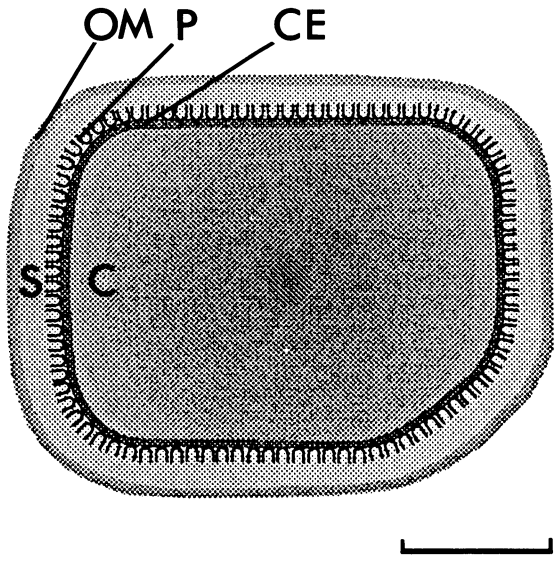

FIG. 9. Schematic view of an IMV particle as it is deduced from the present work. S, surface domain; OM, outer membrane; P, palisade; CE, core envelope; C, core. Magnification, ×200,000. Bar, 100 nm.

ca. 1.3. Intact particles with two equally long or equally short axes are practically nonexistent. From this fact, it can be concluded that the long axis of the virion is always approximately parallel to the plane of the thin vitreous film. This fact is not surprising since the dimension of the virus is large and with the kind of electron microscope used in this study, it is not possible to observe vitrified films thick enough for free orientation of the virion. As a consequence, a whole set of projections are never observed and important information concerning the third dimension is missing. We have tried to compensate for this lack of data by recording projections from strongly tilted grids and large-angle stereographic views. The results have been disappointing because of the lack of easily observable structural features on the surface of the virion and because its roundish shape does not favor three-dimensional reconstruction (a sphere looks the same in all directions).

We are therefore left with the question of the third dimension of the virion. Has the virion the general shape of a rounded barrel or that of a rounded brick? In the latter possibility, what is the thickness of the brick? These questions could probably be solved by observing thicker films of suspensions which would require that the observations are made in a high-voltage electron microscope.

A hint is provided by observation of the core envelope (Fig. 7). Here, whether the envelope is brick- or barrel-shaped depends on whether the top and bottom sides of the observed particles are parts of a cylinder seen perpendicularly to its axis or if they are two parallel flat surface elements. The second possibility seems to be correct, since undistorted symmetrical hexameric arrangement of the spike proteins can be seen even at the edge of the core envelope (circled region in Fig. 7). Direct observation of stereographic pairs of tilted images also gives the impression that the core membrane is made of flat surface elements (not shown).

The core envelope with its more or less regularly arranged spikes observed in purified form in the present work (Fig. 7) certainly corresponds to the internal membrane with the palisade that forms the limit between the surface domain and the core of the intact virion (Fig. 3). This is proved by the fact that the size of the core of the intact virion and that of the purified core envelope are very close (Table 1, keeping in mind that the surface domain is 30 nm thick) and also because the spikes in the core envelope are in the same relative position and show the same periodicity as the palisade.

It will be interesting to identify by immunolabelling the proteins forming the various structures of the virion. For example, we need to know what is the protein forming the spikes of the core envelope? Such data, together with that of the structure will help in elucidating the morphogenetic pathway of the virus in the cell.

Surface tubules and dumbbell-shaped core—two artifacts which need to be understood. We have shown that surface tubules and probably also the dumbbell-shaped core are artifacts due to dehydration of the virion. They are however so remarkable and reproducible that they must also be understood in terms of the underlying structure. It is amazing to note that the more real structure revealed by cryoelectron microscopy has also been observed in previous studies made by conventional methods, but it has not been considered to be significant. (For example, see Fig. 2 and 8 of reference 16.)

Our failure to detect surface tubules by cryoelectron microscopy is not a consequence of the absence of P_{4c} in our WR strain, because these structures were also absent in the A392 isolate expressing intact P_{4c}. The surface tubules are probably the result of dehydration of the surface domain. The extent of the phenomenon shows that the collapse takes place in the whole volume of the surface domain, suggesting that in the native state, this region has the consistency of a gel. A hint at an explanation of the reproducible aspect and the frequent regularity of the surface tubules is offered by the image of the aging preparations of virions showing apparent breaking points, sometimes regularly spaced, in the outer membrane of the virus. The nature of these breaking points is not known, but one can imagine that they are related to some kind of scaffold, involved in the development of the virion.

The dumbbell shape of the core and the formation of the lateral bodies probably result from a nonisotropic drying collapse. We suggest that the gel-like core material formed by a more or less homogeneous mixture of internal proteins and DNA collapses under the effects of dehydration. The DNA could precipitate together with part of the proteins. The characteristic dumbbell-shaped core would derive from the previous arrangement of the DNA. Part of the original core material, expelled during the collapse, would form the lateral bodies.

In conclusion, we note that artifacts similar to those observed in the present study are likely to take place whenever large enveloped viruses are observed by conventional preparation methods of electron microscopy. The results of such observations should be considered with caution unless they are confirmed by observations in the vitrified state.

ACKNOWLEDGMENTS

We thank G. Griffiths for useful discussions and for Fig. 2b and c, D. Pickup for the virus isolate A392 and for information on P_{4c}, and F. Fenner for permission to reproduce Fig. 1.

This work was supported by the Swiss National Fonds for Scientific Research (grant 31-26562.89 to R.W.) and by the Etat de Vaud.

REFERENCES

1. **Adrian, M., J. Dubochet, J. Lepault, and A. W. McDowall.** 1984. Cryo-electron microscopy of viruses. Nature (London) **308**:32–36.
2. **Booy, F. P., and A. G. Fowler.** 1985. Cryo-electron microscopy reveals macromolecular organization within biological liquid crys-

tals seen in the polarizing microscope. J. Biol. Macromol. **7:**327–335.

3. **Bremer, A., C. Henn, A. Engel, W. Baumeister, and U. Aebi.** 1992. Has negative staining still a place in biomacromolecular electron microscopy? Ultramicroscopy **46:**85–111.

3a. **Buist, J. B.** 1886. The life-history of the micro-organisms associated with variola and vaccinia. Proc. R. Soc. Edinburgh **13:**603–615.

4. **Dubochet, J., M. Adrian, J.-J. Chang, J.-C. Homo, J. Lepault, A. W. McDowall, and P. Schultz.** 1988. Cryo-electron microscopy of vitrified specimens. Quart. Rev. Biophys. **21:**129–228.

5. **Dubochet, J., M. Adrian, J. J. Chang, J. Lepault, and A. W. McDowall.** 1987. Cryo-electron microscopy of vitrified specimens, p. 114–131. *In* R. A. Steinbrecht and K. Zierold (ed.), Cryotechniques in biological electron microscopy. Springer-Verlag, Heidelberg, Germany.

6. **Dubochet, J., M. Adrian, J. Lepault, and A. W. McDowall.** 1985. Cryo-electron microscopy of vitrified biological specimens. Trends Biochem. Sci. **10:**143–146.

7. **Easterbrook, K. B.** 1966. Controlled degradation of vaccinia virions in vitro: an electron microscopic study. J. Ultrastruct. Res. **14:**484–496.

8. **Fenner, F., D. A. Henderson, I. Arita, A. Jezek, and I. D. Ladnyi.** 1988. Smallpox and its eradication. World Health Organization, Geneva.

9. **Fenner, F., R. Wittek, and K. Dumbell.** 1989. The orthopoxviruses. Academic Press, Inc., New York.

10. **Joklik, W. K.** 1962. The purification of four strains of poxvirus. Virology **18:**9–18.

11. **Kellenberger, E., and J. Kistler.** 1979. The physics of specimen preparation, p. 1–14. *In* W. Hoppe and E. Mason (ed.), Structure research VII. Friedr. Vieweg and Son, Wiesbaden, Germany.

12. **McDowall, A. W., J.-J. Chang, R. Freeman, J. Lepault, C. A. Walter, and J. Dubochet.** 1983. Electron microscopy of frozen hydrated sections of vitreous ice and vitrified biological samples. J. Microsc. **131:**1–9.

13. **McDowall, A. W., W. Hofmann, L. Lepault, M. Adrian, and J. Dubochet.** 1984. Cryo-electron microscopy of vitrified insect flight muscle. J. Mol. Biol. **178:**105–111.

13a. **McKelvey, T., and D. Pickup.** Personal communication.

14. **Metcalf, P., M. Cyrklaff, and M. Adrian.** 1991. The three-dimensional structure of reovirus obtained by cryo-electronmicroscopy. EMBO J. **10:**3129–3136.

15. **Michel, M., T. Hillmann, and M. Müller.** 1991. Cryosectioning of plant material frozen at high pressure. J. Microsc. **163:**3–18.

16. **Müller, G., and J. D. Williamson.** 1987. Poxviridae 3, p. 421–433. *In* M. V. Nermut and A. C. Steven (ed.), Animal virus structure. Elsevier, Amsterdam.

17. **Olson, N. H., and T. S. Baker.** 1989. Magnification calibration and the determination of spherical virus diameters using cryo-microscopy. Ultramicroscopy **30:**281–298.

18. **Payne, L. G., and E. Norrby.** 1976. Presence of haemagglutinin in the envelope of extracellular vaccinia virus particles. J. Gen. Virol. **32:**63–72.

18a. **Pickup, D.** Personal communication.

19. **Richter, K., and J. Dubochet.** 1990. High resolution study of DNA in vitrified sections, p. 488–489. *In* L. D. Peachey and D. B. Williams (ed.), Proceedings of the XIIth International Congress on Electron Microscopy. San Francisco Press, Inc., San Francisco.

20. **Sodeik, B., R. W. Doms, M. Ericsson, G. Hiller, C. E. Machamer, W. van't Hof, G. van Meer, B. Moss, and G. Griffiths.** 1993. Assembly of vaccinia virus: role of the intermediate compartment between the endoplasmic reticulum and the Golgi stacks. J. Cell Biol. **121:**521–541.