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A Phosphorylated Basic Vaccinia Virion Polypeptide of Molecular Weight 11,000 Is Exposed on the Surface of Mature Particles and Interacts with Actin-Containing Cytoskeletal Elements

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A phosphorylated vaccinia virus structural polypeptide of an apparent molecular weight of 11,000 (p11K) was isolated by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis and used for antibody induction. After purification by antigen affinity chromatography, the immunoglobulins detected only one target of a rather basic nature in two-dimensional immune blotting procedures of total virion proteins. By use of a combination of biological, biochemical, and microscopic techniques, p11K could be located on the surface of those vaccinia virus particles, with "classical" morphology and a buoyant density of 1.27 g/cm^3. Upon immunoprecipitation from radioactively labeled infected cells, p11K appeared to be complexed to two additional virus structural proteins, which could be tentatively identified by their molecular weights as precursors for the two major core constituents. When virus assembly was inhibited by rifampin treatment of infected cells, a great part of p11K, either free or in complexed form, was found associated with actin-containing cytoskeletal elements. The ability of p11K to interact with a not-yet-identified, microfilament-associated cellular protein may be related to previous findings showing that assembled vaccinia particles in situ are found in connection with microfilaments. A possible role for the structures precipitated by p11K-specific antibodies in early stages of particle assembly is discussed.

Poxviruses belong to the largest, most complex animal viruses. Thin sections of virus samples obtained from experimentally lysed infected cells and purified by standard procedures reveal three major distinguishable domains within the particles: first, an outer envelope presumably consisting of proteins, glycoproteins, and lipids; second, two lateral bodies with a relatively dense appearance after conventional fixation and staining; and third, a compact nucleoid or core containing the DNA (see, for instance, references 4, 20, and 33). Although many of the major structural proteins can be assigned to one or the other particle substructure (10, 19, 24–26, 29), detailed topographic information is still lacking, and for some proteins even the rough assignment to the different substructures is not clear.

A part of the core components is resistant to dissociation and solubilization by conventional buffer systems even in the presence of nonionic detergents (5, 10, 11, 19, 24, 26). This has hampered purification and characterization of most of the structural proteins, and in consequence an understanding of their arrangement in morphological terms is rather difficult. To circumvent these problems we have isolated some of the major polypeptides of vaccinia virions by preparative sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and have raised antibodies against them. In this report we present results, obtained by a combination of biological, biochemical, and microscopic procedures, which locate a phosphorylatable basic polypeptide of molecular weight 11,000 (p11K) on the surface of virus particles characterized by a buoyant density of 1.27 g/cm^3. This small polypeptide appears to be complexed with two other viral proteins which are tentatively identified as precursors for the two major core constituents. Virus polypeptide p11K, either free or in complexed form, is able to bind to actin-containing cytoskeletal structures in infected cells and may be responsible for the previously detected association of vaccinia particles with microfilament bundles (9).

MATERIALS AND METHODS

Cells and viruses. Preparation and culturing of chicken embryonic fibroblasts, propagation and purification of vaccinia WR virus, and the mode of infection have been described previously (9). To inhibit virus assembly, rifampin (Serva, Heidelberg, Germany) was added to the growth medium (100 μg/ml) from the end of virus adsorption until the time of cell fixation or harvest (6, 16, 17).

Isolation of virion polypeptides and antibody induction. Purified vaccinia virions were dissociated by boiling (4 min) at a final protein concentration of 3 mg/ml in 70 mM Tris-hydrochloride (pH 6.8)–3% SDS–5% 2-mercaptoethanol. Samples corresponding to 3 to 4 mg of total protein were separated on 2-mm-thick gradient SDS-polyacrylamide gels (14 to 20%). After short staining with Coomassie blue, separated polypeptide bands were excised and electrophoretically eluted as described (7).

A 400-μg sample of the protein with an apparent molecular weight of 11K was injected into a rabbit, both subcutaneously and intraperitoneally. This total amount of protein was divided into four injections at 3-week intervals. Appearance and increase of titer were followed by indirect immunofluorescence microscopy, using purified vaccinia virions settled on cover slips (8).

Two-dimensional analysis of virus structural proteins and immune blotting procedures. Virion polypeptides were separated by non-equilibrated pH gradient electrophoresis in the first dimension followed by SDS-polyacrylamide gel (14%) electrophoresis in the second dimension essentially as described by O'Farrell et al. (18). Ampholytes of pH range 3.5 to 10 (Ampholine; LKB) were used. Electrophoresis was for 4.5 h at 400 V. For sensitive protein detection in polyacrylamide gels a silver stain method (34) was employed.

In immune blotting experiments, two different techniques were used which gave qualitatively similar results but differed in the amount of transferred material. Both techniques started with SDS-polyacrylamide gels for protein separation. In the first procedure, excess SDS was removed by intensive washing in Tris-buffered saline (20 mM Tris-hydrochloride, pH 7.2). Then the polyacrylamide gel was brought in direct contact with a 1.5- to 2.0-mm-thick agarose (0.8%) layer containing the antiserum (final dilution 1:80) under examination (see references 3 and 23). The buffer included in the agarose gel was 20 mM Tris-hydrochloride (pH 7.2), 100 mM NaCl, and 0.15% Triton X-100. After incubation for 14 to 20 h at room temperature in a wet chamber, the gel was removed, and the agarose layer was carefully washed with Tris-buffered saline. Specifically bound immunoglobulin G (IgG) species were detected by their affinity for ^{125}I-labeled protein A. In the second procedure, the separated polypeptides were electrophoretically transferred onto a nitrocellulose sheet (28). After saturation of excess protein binding capacities with 5% bovine serum albumin, the nitrocellulose blot was reacted either with crude antiserum to the 11K polypeptide (final dilution, 1:150) or with antigen affinity-purified immunoglobulins (10 μg/ml) stabilized by the addition of 0.5 mg of bovine serum albumin per ml. After intensive washing with several changes of Tris-buffered saline, bound IgGs were again detected by ^{125}I-protein A adherence as described above.

Radioactive labeling of cells and preparation of extracts for immunoprecipitation experiments. To label preferentially virus-specific proteins, radioactive precursors were included in the culture medium from 5 h postinfection until cell harvest (20 h postinfection). [^{35}S]methionine (specific activity, 136 mCi/mmol) was added at 7 μCi/ml in medium containing one-tenth of the usual unlabeled methionine concentration. $^{32}P_i$ (specific activity, 600 mCi/mmol of phosphorus) was used at 50 μCi/ml in medium with 10% of the usual phosphate content.

For cell harvest, monolayers were briefly washed with phosphate-buffered saline (PBS), scraped off the dish with a rubber policeman, and centrifuged (800 × *g*) at 4°C for 5 min. Cells were lysed (0.2 ml of buffer per dish) in a low-salt solution known to disrupt actin filaments: 10 mM Tris-hydrochloride (pH 7.5), 1% Triton X-100, 0.5% sodium deoxycholate, 0.2 mM $CaCl_2$, 0.5 mM 2-mercaptoethanol, and 1 mM phenylmethylsulfonyl fluoride. The lysate was centrifuged (30,000 × *g*) for 30 min at 4°C. The supernatant obtained was made 80 mM in NaCl, and after addition of affinity-purified p11K-specific IgG (approximately 5 μg/0.2 ml), the mixture was incubated for 1.5 h at room temperature under agitation. Finally, 15 μl of a protein A-Sepharose (Pharmacia, Uppsala, Sweden) slurry was added, and the incubation was continued for another hour. The protein A-Sepharose was harvested by low-speed centrifugation and washed several times with lysis buffer containing 150 mM or 300 mM NaCl. For elution, the protein A-Sepharose was treated with hot SDS buffer (70 mM Tris-hydrochloride, pH 6.8–3% SDS–5% 2-mercaptoethanol). The eluted material was directly analyzed by SDS-polyacrylamide gel electrophoresis, and subsequent fluorography was performed as described by Bonner and Laskey (1).

Indirect immunofluorescence microscopy. The indirect immunofluorescence procedure routinely used has been described in detail for cells grown on glass cover slips (9, 30).

Cytoskeleton preparation and immunoelectron microscopy. Infected cells were briefly washed with warm (37°C) growth medium without serum, prefixed for 5 to 10 s with 0.7% Formalin in PBS, and finally extracted in situ with a nonionic detergent [25 mM sodium phosphate (pH 7.2), 150 mM NaCl, 1 mM $MgCl_2$, 2 mM ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*-tetraacetic acid, 4% polyethylene glycol 6000, 0.5% Triton X-100] for 8 min at room temperature (see also references 2, 9, and 32). After three consecutive washings in buffer without Triton, the resulting cytoskeleton preparations were either scraped off the substratum for further biochemical analysis of their components or fixed with 3.7% Formalin in the same buffer. In the latter case, further processing and immunolabeling with either anti-actin or p11K-specific IgG (60 μg/ml) was done as described previously (9, 32). After embedding and sectioning, the samples were viewed in a Philips 301 electron microscope.

For poxvirus surface labeling experiments, purified native vaccinia WR virions were suspended in PBS by short ultrasonication (approximately 3×10^{10} particles per ml). Samples (20 μl) of particle suspensions were incubated (30 min, 37°C) with either monospecific antiserum to the 11K viral polypeptide or monospecific anti-bovine milk galactosyltransferase (final concentration, 20 μg of IgG per ml) in a total volume of 80 μl.

Viral particles were sedimented (15 min, 20,000 × *g*) and washed three times in 20 mM Tris-hydrochloride (pH 8.8)–80 mM NaCl by cycles of ultrasonic resuspending and pelleting. After final suspension in 80 μl of buffer, ferritin-conjugatd sheep anti-rabbit IgG (50 μg/ml) was added for a further incubation period (45 min, room temperature). Free and particle-bound ferritin conjugates were separated by centrifuging the virions through a 10% sucrose cushion in 20 mM Tris-hydrochloride (pH 8.8)–80 mM NaCl. On the bottom of the centrifuge tubes, Formvar-coated electron microscope grids (100-mesh) had been placed; the virus particles were directly sedimented onto these. Grids were washed three times in PBS, fixed for 5 min with 2.5% glutaraldehyde in PBS, rinsed in water, dried, and, without any further staining, examined in a Philips 301 electron microscope.

Neutralization assay. Samples (0.2 ml) of varying dilutions of either crude antisera or affinity-chromatographed IgG in Hanks salt solution supplemented with 0.5% gelatin were incubated at room temperature for 2 h with 1.3×10^4 elementary bodies of vaccinia WR virus. Unneutralized virus was determined by plaque assay on African green monkey kidney cells (BSC-1) after staining with crystal violet by standard procedures. Each antibody dilution was assayed in triplicate.

RESULTS

Characterization of p11K-specific antibodies. A structural polypeptide of vaccinia particles with an apparent molecular weight of 11K (p11K), isolated by preparative SDS-polyacrylamide gel electrophoresis (see Fig. 1), was used to immunize rabbits. In immune blotting procedures on total virion polypeptides, separated in the presence of SDS according to apparent molecular weights, the different antisera labeled one single band at 11K (Fig. 1, lane c). Since the isolation procedure for the viral polypeptide was solely based on molecular weight, a more accurate further analysis of specificity appeared necessary. For this purpose a combination of nonequilibrated pH gradient electrophoresis (NEPHGE; 18) and SDS-polyacrylamide gel electrophoresis was used to obtain a two-dimensional pattern of virion polypeptides (Fig. 2a). This pattern was electrophoretically transferred to nitrocellulose (28) and reacted with antigen affinity-purified anti-p11K immunoglobulin. Again, only a single polypeptide in a position corresponding to molecular weight 11K was detected. The rather basic nature of the protein was indicated by its position in the two-dimensional system (Fig. 2b). In a similar experiment that used a combination of isoelectric focusing and SDS-polyacrylamide gel electrophoresis for polypeptide separation, the immunoreactive material did not enter the isoelectric focusing gel. This is taken as additional evidence for the basic behavior of the antigen.

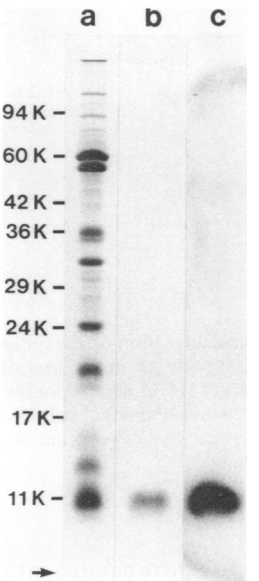

FIG. 1. Analysis of purified 11K virus polypeptide used for antibody induction and characterization of antibodies by an immune blot of separated total virion structural proteins. SDS–14% polyacrylamide gel: (a) Coomassie blue-stained total vaccinia virus (strain WR) polypeptides; (b) purified 11K virus polypeptide (Coomassie blue-stained); (c) decoration of total virion proteins by antiserum to 11K polypeptide. For preparation of the immune blot (c) the same amount of total protein (30 μg) was used as for lane a.

Complexing of p11K to other virus structural proteins. Although we only found one polypeptide species reactive after separation procedures using SDS (see Fig. 1 and 2), immunoprecipitation experiments on extracts of virus-infected cells regularly revealed additional virus-specific proteins (Fig. 3). These results probably indicate an association of the 11K polypeptide with other virion structural proteins of molecular weights 94K and 65K (Fig. 3). Since the cell extracts were prepared in low salt and in the presence of Triton X-100 (1.0%), sodium deoxycholate (0.5%), and 2-mercaptoethanol (1.0 mM), the proposed complex seems insensitive to these conditions.

The antibiotic rifampin is known to prevent poxvirus particle assembly (see, for instance, references 6, 16, 17, and 27) by a mechanism that is still not well understood. It was therefore of interest to examine whether a similar complex with the same viral protein species also existed in rifampin-treated infected cells. Figure 3 (lane d) shows that this is the case. The immunoprecipitation experiments with affinity-purified anti-p11K immunoglobulin also opened the possibility for studying post-translational modification of the 11K virion polypeptide. Figure 3 proves that

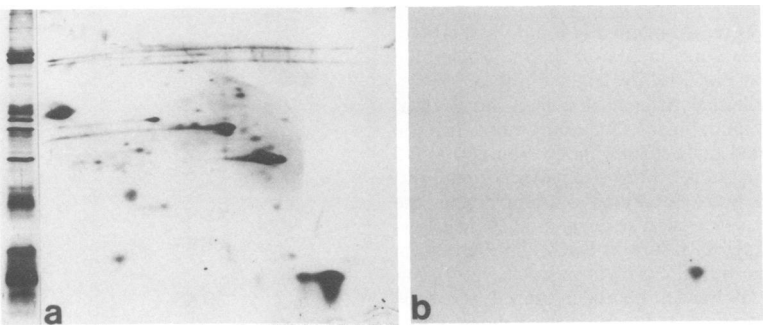

FIG. 2. Characterization of the antigen target by two-dimensional electrophoresis with affinity-purified anti-p11K IgG. For separation in the first dimension, nonequilibrated pH gradient electrophoresis was used. SDS-polyacrylamide gel (14%) electrophoresis was in the second dimension. (a) Gel stained by a silver method; (b) corresponding two-dimensional protein pattern transferred to nitrocellulose and reacted with anti-11K protein antibodies followed by ^{125}I-protein A. One of the most basic polypeptides with molecular weight 11K is specifically labeled.

$^{32}P_i$ is covalently incorporated into p11K, independent of whether particle assembly could have taken place (lane e) or had been inhibited by the presence of rifampin (lane f). Thus incorporation of the 11K polypeptide into virus particles is probably preceded by its phosphorylation.

Association of p11K structures with cytoskeletal elements. To learn more about the intracellular distribution of p11K, we used affinity-purified IgG in indirect immunofluorescence microscopy of vaccinia virus-infected tissue culture cells. As expected, the antibodies revealed single virions (Fig. 4a) many of which appeared conspicuously close to microfilament bundles easily identified in the corresponding phase-contrast micrograph (Fig. 4b; see also reference 9). In addition to virus particles, microfilament bundles also seemed slightly stained (Fig. 4a; clearer in Fig. 5), and the poxvirus-induced specialized microvilli (Fig. 5, indicated by arrows), which have been characterized previously (8, 9), were clearly seen. The intensity of microfilament labeling by anti-p11K was dramatically increased on cells in which poxvirus assembly was prevented by rifampin (Fig. 6). For instance, the cell in Fig. 6a shows several strongly labeled cytoplasmic "factories" equivalent to rifampin-induced "viroplasm," structures which have been studied in detail at the electron microscopic level (6, 17). Weaker, but clearly specifically labeled filamentous structures are seen within the cytoplasm. Very often these stained filaments run parallel with the longer cell axis, as do microfilament bundles both in normal and infected cells (see part of cell shown in Fig. 6b). Interestingly, the labeling pattern is discontinuous, producing a typical striated appearance along the filament bundles. Similar patterns are known for several actin-associated proteins present in microfilaments of nonmuscle cells (i.e., myosin, tropomyosin, and α-actinin; see references 13, 14, and 31).

In an attempt to identify biochemically the cytoskeletal elements to which the viral 11K protein seems bound, we extracted infected rifampin-treated cells in situ with nonionic detergent-containing buffers (2, 9, 32). Under the conditions employed, approximately 70 to 80% of the total cellular protein was solubilized and removed. Analysis of the remaining structures by SDS-polyacrylamide gel electrophoresis revealed vimentin, actin, and histones as the major protein components of such cytoskeletons. On Coomassie-stained gels no difference between infected and uninfected cytoskeletons could be seen (Fig. 7). When the electrophoretically transferred protein patterns were reacted with antiserum to p11K the presence of cytoskeleton-associated viral polypeptide was indicated for infected but not for uninfected cells.

Similarly prepared cytoskeletons were incubated in situ with p11K-specific antibodies, followed, after adequate washings, by an immune ferritin label. The samples were embedded, sectioned, and examined by electron microscopy. Comparison of Fig. 8a and b shows that the viral 11K protein is bound to actin-containing microfilament bundles, as already suggested by immunofluorescence microscopy on whole cells (see Fig. 4 to 6), and not to vimentin-type intermediate filaments.

p11K localization on assembled virus particles. Having documented an affinity of the viral 11K polypeptide, either free or complexed to some other virus structural proteins, for cytoplasmic microfilamentous structures, we tried to localize the protein in the mature virion. The surface of

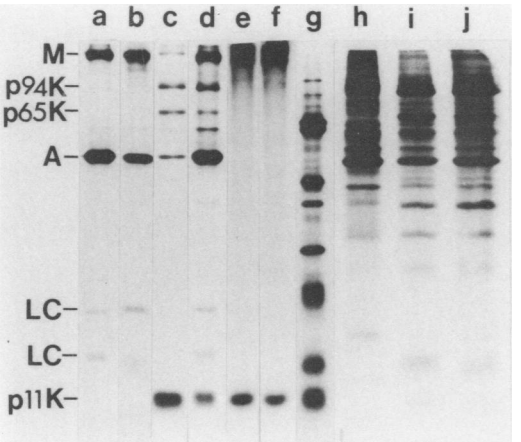

FIG. 3. Post-translational modification of the 11K polypeptide and its association with two other virus structural proteins as revealed by immunoprecipitation experiments. SDS–14% polyacrylamide gel: (a) [^{35}S]methionine-labeled extract from uninfected BSC-1 cells, immunoprecipitated by affinity-purified anti-p11K; (b) extract from vaccinia WR-infected, rifampin-treated cells after immunoprecipitation with preimmune IgG; anti-p11K immunoprecipitates of extracts from infected, untreated (c) or infected, rifampin-treated cells (d). Note that in precipitates a through d actomyosin is found as indicated by the presence of myosin (M), actin (A), and two species of myosin light chains (LC). In addition to the immunspecific target p11K, two further virus proteins (p94K and p65K) are specifically coprecipitated by the affinity-purified IgG to p11K (lanes c and d). Anti-p11K immunoprecipitates of $^{32}P_i$-labeled extracts from infected, untreated (e) or infected, rifampin-treated cells (f). (g) [^{3}H]leucine- labeled purified vaccinia WR virus; (h, i, and j) extracts from [^{35}S]methionine-labeled cells showing the complex polypeptide pattern left behind after immunoprecipitation with the corresponding IgGs: (h) uninfected, anti-p11K; (i) infected, anti-p11K; (j) infected and rifampin-treated, anti-p11K. In every case equivalent parts of total samples were analyzed without consideration of the amount of incorporated radioactivity.

intact vaccinia particles appeared strongly labeled after incubation with immunoaffinity-purified antibodies to the 11K polypeptide followed by a ferritin-labeled second antibody (Fig. 9a). A parallel incubation, using the same procedure but with monospecific anti-galactosyltransferase as the first antibody, did not result in any noticeable surface labeling (Fig. 9b). Additional experiments examining the capacity of anti-p11K to neutralize virus infectivity demonstrated a clear dose-dependent inhibition. The reduction of virus infectivity could be seen in response to either the crude antiserum or the affinity-purifed IgG (Fig. 10). Again, anti-galactosyltransferase, used as crude serum or as the monospecific immunoglobulin, did not give comparable neutralization. Thus a relatively basic, phosphorylatable polypeptide with a molecular weight of 11K is exposed on the surface of that class of infectious vaccinia particles which are characterized by a buoyant density of approximately 1.27 g/cm^3.

DISCUSSION

The very complex pattern of poxvirus structural proteins and the no less complicated particle morphology of the virus have hampered studies concerned with topography and virion morphopoiesis. Given this situation, well-characterized antisera to single defined polypeptide components should prove helpful. Such an antiserum, directed against a basic and phosphorylatable virion polypeptide of molecular weight 11K, was used in this study to locate the target antigen both in infected cells and in assembled particles.

Previous studies using differential extraction of virions under a variety of conditions have given ambiguous results for 11K polypeptide localization within the virus particles. In their pioneering work, Sarov and Joklik (24) concluded that p11b (corresponding to the examined 11K phosphopolypeptide) is neither a core component nor a reactive virion surface protein. A careful recent study by Oie and Ichihashi (19) showed that approximately 50% of methionine-labeled material with the apparent molecular weight of 11,000 can be extracted either by 0.5% Nonidet P-40 alone or by a combination of 0.5% Nonidet P-40 and 80 mM 2-mercapoethanol, indicating an association with the outer envelope. About the same amount of 11K methionine label, however, remained core associated. A very similar situation was found by Sagot and Beaud (22) when they assayed vaccinia virions for phosphorylating activity at pH 10. Under these extreme pH conditions few proteins dissociated from the particles and appeared in the supernatant. Among these proteins was an 11K polypeptide whose removal, again, was incomplete. All other removed major proteins could be characterized as envelope components.

The immunoferritin labeling experiments (Fig. 9) prove that a basic phosphorylatable polypeptide of apparent molecular weight 11K is exposed on the surface of intact vaccinia particles. In addition, we have shown that binding of specific antibodies to this target antigen neutralizes virus infectivity (Fig. 10). The basic nature of the polypeptide may be important for its interaction with acidic phospholipids within the virus envelope.

A polypeptide with almost identical properties has been described as a constitutive component

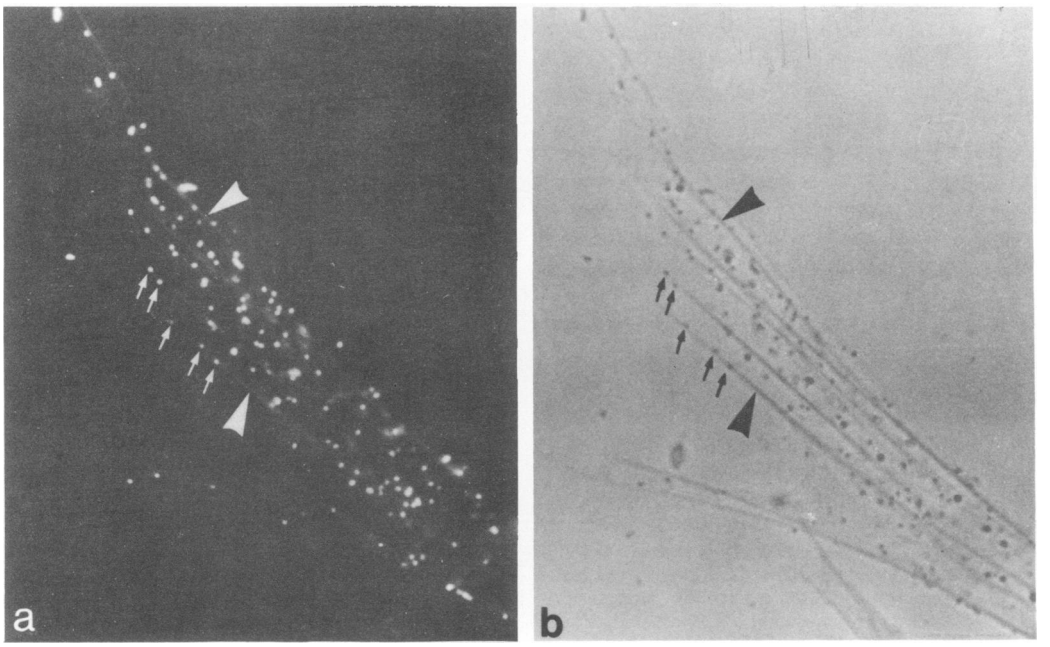

FIG. 4. Immunofluorescence microscopic detection of 11K polypeptide-carrying structures within infected chicken embryonic fibroblasts. (a) Labeling with antibodies to the 11K viral protein; (b) phase-contrast micrograph of the same cell. Some apparently microfilament-associated vaccinia particles are indicated by small arrows. Large arrowheads point to faintly stained stress fibers in (a). ×1,300.

of both factories and virions. Mainly because of its basic character and its post-translational phosphorylation, a role in viral DNA binding or packaging within assembling particles, or both, has been assigned to this polypeptide in analogy to histone H1 function in eucaryotic chromosomes (12, 21). Since factories with a homogeneous appearance in electron microscopy but lacking any unit-size particulate structures are not labeled by our antiserum (data not shown), a direct relationship to the latter reports is currently not possible. However, on the grounds of the basic nature of p11K a certain affinity for polyanionic structures like nucleic acids can be assumed. Thus the tentative localization of a very similar virus-specific polypeptide within factories (21) could be explained by a redistribution upon biochemical homogenization of infected cells. That p11K indeed can bind to DNA, especially to superhelical structures, has been shown by Kao et al. (12). In contrast to histone H1, however, which reveals maximal DNA binding at 0.1 M salt, the optimal salt concentration in the case of p11K is 0.03 M, and binding is virtually abolished at 0.1 M (12). Thus the biological importance of this DNA binding remains unsettled. Furthermore, although it seems unlikely, we cannot completely exclude the possibility that two different basic virion polypeptide species of apparent molecular weight 11K exist, one associated with virus cores and the other with the outer envelope (compare the differential extractability discussed above). Upon analysis of total virion proteins on heavily loaded two-dimensional polyacrylamide gels, using

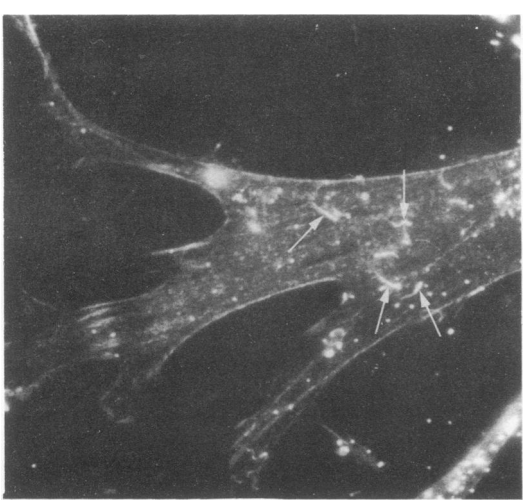

FIG. 5. Detection of poxvirus-induced microvilli by 11K protein-specific antibodies. Some of these specialized structures known to be present on the cell surface are marked by arrows. ×1,100.

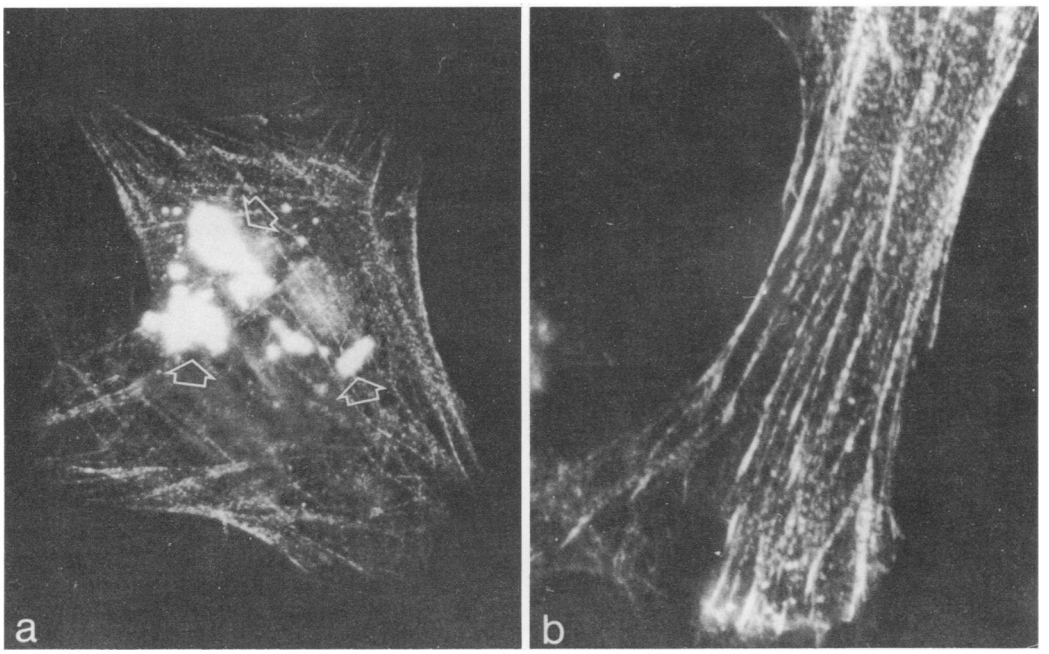

FIG. 6. Appearance of microfilament labeling by anti-p11K serum in rifampin-treated, vaccinia virus-infected chick cells. A whole cell is shown in (a). Drug-induced cytoplasmic "viral domains" are strongly labeled (open arrows). In addition, cellular filaments are also specifically revealed by the antiserum. Part of another infected cell is shown in (b). Note the discontinuous labeling of filament bundles, giving rise to a striated staining pattern. Due to rifampin treatment, assembled virus particles are virtually absent. (a) ×1,000; (b) ×1,500.

non-equilibrated pH gradient electrophoresis (NEPHGE) in the first dimension for separation of basic polypeptides, one or two additional minor spots as well as one more prominent spot directly adjacent to the antibody-detected antigen were revealed (see Fig. 2). At the moment it is unknown whether these two spots represent the same polypeptide species, differing only in net charge (phosphorylation), or whether they are unrelated molecular species.

Although the anti-p11K immunoglobulin detected only one target on immune blots after protein separation by SDS-polyacrylamide gel electrophoresis (see Fig. 1 and 2), in immunoprecipitation experiments at least two additional virus structural polypeptides coprecipitated specifically in extracts from infected cells (Fig. 3). By their molecular weights (94K and 65K) they could tentatively be identified as precursors for the two major core components (see, for instance, reference 16). The cleaved products (bands 4a and b according to Sarov and Joklik [24]) were, however, not precipitated. A complex consisting of these three virus proteins, revealed by the use of monospecific antibodies to p11K, might play a role in early stages of assembly which are not influenced by rifampin.

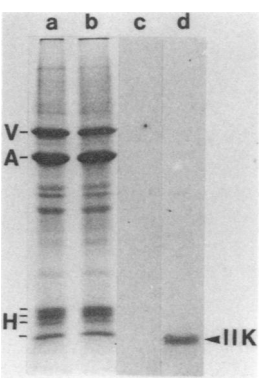

FIG. 7. Association of the 11K viral protein with cytoskeletal elements after in situ extraction of whole infected cells. Lanes a and b reveal the protein composition of cytoskeletons obtained by in situ extraction of rifampin-treated uninfected (a) or infected (b) chick cells. In lanes c and d the immune blots of corresponding samples after reaction with anti-p11K serum, followed by ^{125}I-protein A treatment, are shown. In virus-infected cells the 11K polypeptide appears cytoskeleton associated (d), whereas cytoskeletons from uninfected cells do not contain p11K (c). V, Vimentin; A, actin; H, histones.

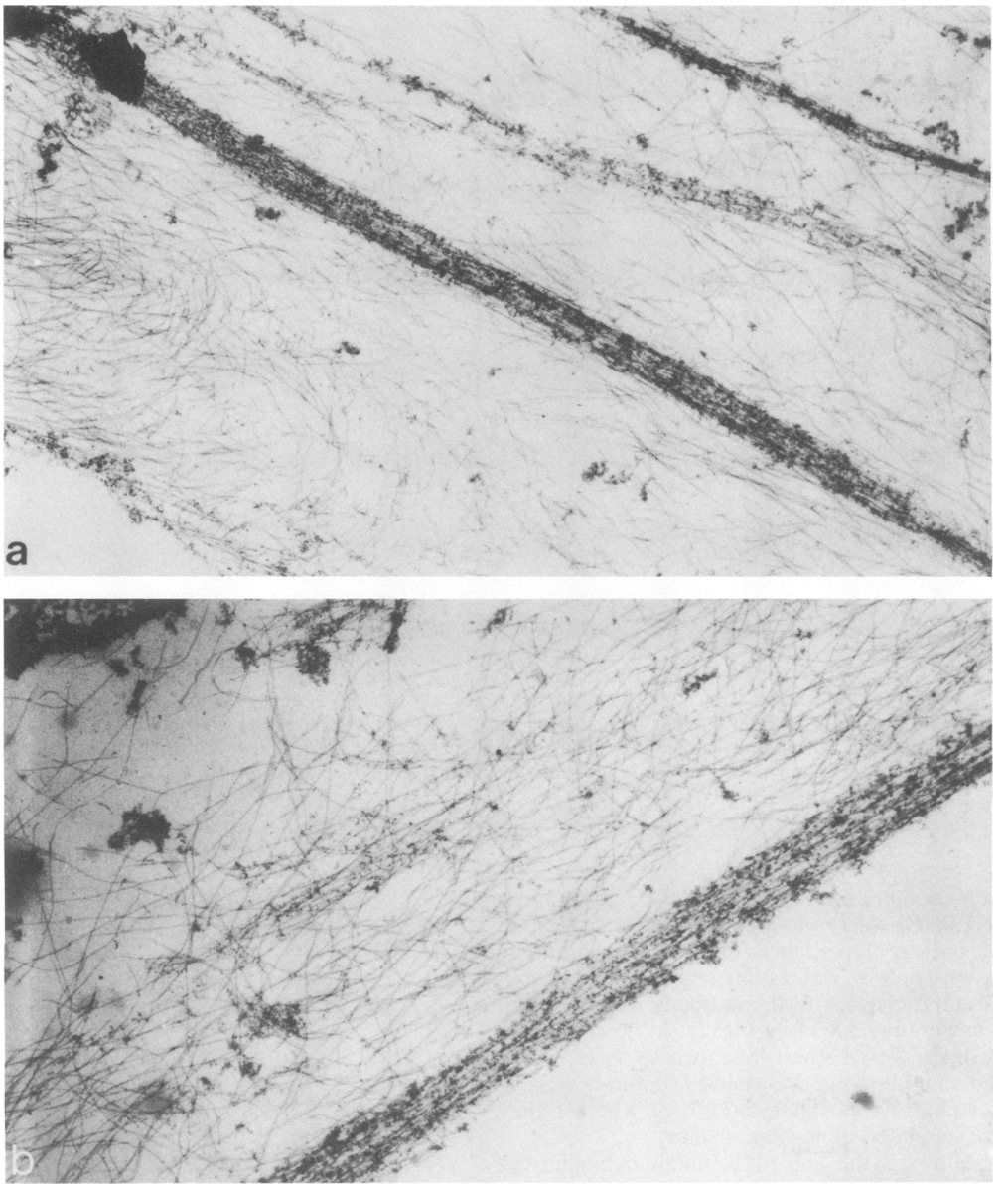

FIG. 8. Ultrastructural identification of actin-containing microfilament bundles as the cytoskeletal elements to which the 11K viral protein seems bound in rifampin-treated infected cells. (a) Cytoskeleton marked with rabbit p11K-specific immunoglobulins; (b) cytoskeleton labeled with affinity-purified rabbit anti-actin antibodies. Note that in both cases similar structures are stained by the indirect technique employed, which uses ferritin-conjugated IgG for final antigen localization. ×26,000.

The ability of precursors p94K and p65K to interact with surface component p11K seemed to be lost upon proteolytic processing.

When further particle assembly was blocked by the presence of rifampin, a pronounced association of p11K with cytoskeletal elements of the infected cell was indicated (see Fig. 6, 7, and 8). Only the actin-containing microfilament part of the cytoskeleton is involved in p11K binding, as shown by light and electron microscopy (see Fig. 6 and 8). p11K does not seem to interact with the two other virtually independent filamentous structures of the cytoskeleton, microtubules and intermediate-sized filaments.

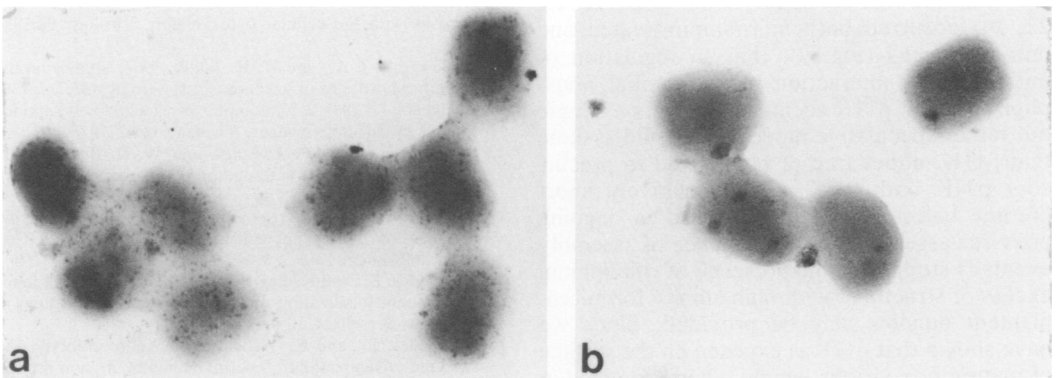

FIG. 9. Monospecific antibodies to the 11K polypeptide decorate the surface of purified native vaccinia virions. As described for Fig. 8, indirect immunoferritin labeling was used for specific antigen localization. (a) Surface localization of p11K is indicated by particle-bound ferritin conjugates; (b) virus particles incubated with the same concentration of monospecific antibodies to bovine milk galactosyltransferase appear unlabeled. ×60,000.

Whether p11K binding to microfilaments is direct or indirect via complexing with precursors p94K and p65K could not be established at this time.

To identify the cellular component of microfilament bundles to which viral p11K is bound also needs further experiments. Due to the discontinuous distribution of p11 K along the bundles one might speculate that not filamentous actin itself, which is labeled in a continuous way by corresponding actin antibodies (see, for example, reference 15), but one of the actin-associated proteins like α-actinin, tropomyosin, or myosin is responsible for p11K binding. All three proteins have been shown, by indirect immunofluorescence microscopy with corresponding affinity-purified antibodies, to distribute discontinuously along microfilament bundles (13, 14, 31). Alternatively, we cannot overlook the possibility that p11K because of its basic amino acid sequence may bind to polyanions as discussed above for DNA and therefore recognizes the relatively acidic F-actin filament directly.

There are several reports pointing to a microfilament-virion interaction in the infected cell. Of particular interest are the virus-induced microvilli in which a virion supported by a filament bundle is found underneath the tip of the structure (see reference 9 for further references). Unfortunately it is not known whether this cytoskeletal interaction reflects a true functional importance rather than being purely fortuitous. Our finding that a virus-coded 11K protein is found in situ to be bound either directly or indirectly to microfilaments may provide a novel approach. Unfortunately we currently do not know why this association seems only weak under normal infection conditions allowing particle assembly and maturation, but is strongly enhanced in the absence of poxvirus assembly when cells are treated with rifampin (compare Fig. 4 and 5 with Fig. 6). The well-documented

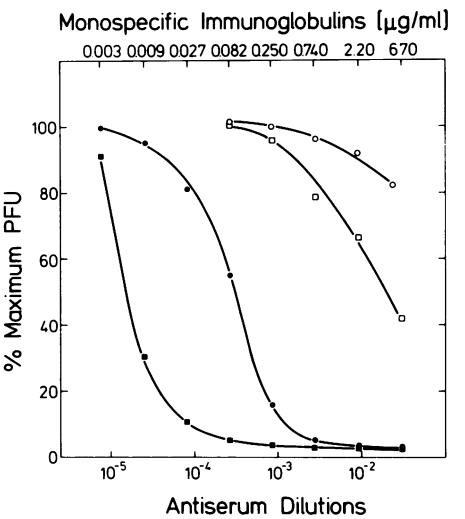

FIG. 10. Neutralization of virus infectivity by antiserum to the 11K polypeptide. Varying dilutions of either crude antisera (■, anti-p11K; □, anti-bovine milk galactosyltransferase) or affinity-purified IgG (●, anti-p11K; ○, anti-galactosyltransferase) were incubated with a fixed amount (1.3×10^4 particles) of vaccinia WR virus at room temperature for 2 h. Residual infectivity was determined by plaque assay on monkey BSC-1 cells. Maximum PFU correspond to 225 counted plaques per dish. Neutralizing activity is associated with anti-p11K IgG but not with anti-galactosyltransferase IgG.

post-translational phosphorylation of p11K (21, 22, 24) occurred both in rifampin-treated and untreated cells (Fig. 3); thus, a regulation of microfilament interaction by differential phosphorylation of p11K seems unlikely. In a simple but rather speculative model, one could assume that p11K, either free or complexed to precursors p94K and p65K, has a relatively short lifetime before being quickly used in ongoing poxvirus assembly. If the sequence of assembly events is stopped in the presence of rifampin an excess of structures with high affinity for microfilament bundles may be provided. Since we have shown that p11K is exposed on the surface of mature pox virions which, when examined in situ in the infected cell, are preferentially found associated with microfilament bundles (see Fig. 4 and reference 9), p11K is probably also a candidate for the interaction of assembled particles with actin-containing filamentous structures. This is only valid for virions with "classical" appearance in thin sections, lacking additional membraneous structures (7), and sedimenting at a buoyant density of 1.27 g/cm^3 when purified.

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