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Phosphoprotein Component of Vaccinia Virions

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The recent discovery of a protein kinase activity in vaccinia virions led us to search for a viral protein which is phosphorylated in vivo. Vaccinia virus was radioactively labeled by infecting cells in the presence of $^{32}P_i$. A phosphoprotein was isolated from purified delipidated virions by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The phosphoprotein appeared to be a specific viral component induced after infection. More than 60% of the phosphoprotein was associated with viral cores. The electrophoretic mobility of the protein suggested that it has a molecular weight of 11,000 to 12,000. Phosphoserine was liberated by acid hydrolysis and identified by electrophoresis with known standards. Tryptic digests of the purified phosphoprotein were analyzed by two-dimensional electrophoresis and chromatography on thin-layer cellulose plates, and a single major phosphopeptide was resolved. The high selectivity of phosphorylation suggested that the process has a specific function.

Despite the apparently common association of protein kinase activities with enveloped RNA (6, 26) and DNA (5, 18, 19, 21) viruses, nothing is known regarding their biological roles. Paoletti and Moss (18) suggested that the vaccinia viral protein kinase may be responsible for the in vivo phosphorylation of specific virion proteins. To test this possibility it is necessary to (i) grow vaccinia virus in medium containing $^{32}P_i$; (ii) purify the virions; (iii), separate viral proteins from more abundant phosphorous-containing compounds such as nucleic acids, nucleotides, and phospholipids; and (iv) identify the phosphorous derivative. As part of a study on the nature and location of the capsid polypeptides of vaccinia virions, Sarov and Joklik (22) noted that several viral components were labeled with $^{32}P_i$ but did not identify the phosphorous derivatives. Our studies demonstrate that one of the virion polypeptides contains phosphoserine residues. The selectivity of phosphorylation, as determined by peptide mapping of tryptic digests of the virion phosphoprotein, suggests that this process has a specific function.

MATERIALS AND METHODS

Preparation of ^{32}P-labeled vaccinia virions. HeLa S-3 cells were incubated at 37 C in Eagle Spinner medium without phosphate for 1 h. The cells were then collected by centrifugation and infected, at a concentration of 2×10^7 cells/ml, with 3 PFU of vaccinia virus (strain WR) per cell (17). After absorption for 30 min at 37 C, the cells were sedimented and suspended at a concentration of 4×10^5 cells/ml in 250 ml of medium containing 1 to 10 mCi of carrier-free $^{32}P_i$ (New England Nuclear Corp.). At 22 h after infection, the cells were suspended in 0.01 M sodium phosphate, pH 8.2, and disrupted by Dounce homogenization. The nuceli were removed by low-speed centrifugation, and vaccinia virus was purified by sedimentation through a cushion of 36% sucrose and one or two rate zonal sucrose gradient centrifugations essentially as described by Joklik (10). This was followed by equilibrium centrifugation in a 25 to 50% potassium tartrate gradient. The latter was performed at 4 C in an SW50L rotor at $130,000 \times g$ for 60 min. All gradient solutions were made in 0.01 M sodium phosphate, pH 8.2. After each gradient centrifugation, the virus band was diluted threefold in phosphate buffer and sedimented. At all stages in the purification, starting with the cytoplasmic extract, the virus was dispersed with the microtip of a Branson model 140 sonifier. Sonic vibrations were given for five intervals of 15 s while the tubes containing the virus were cooled in an ice bath.

Preparation of viral proteins for electrophoresis. Phospholipids were extracted from the purified virus with 20 vol of chloroform-methanol (1:1) at 4 C, and the insoluble residue was collected by centrifugation. In some experiments the virus was first dissociated with sodium dodecyl sulfate (SDS) and mercaptoethanol and extracted with 3 vol of distilled phenol saturated with 0.25 M Tris-hydrochloride (pH 7.8) to remove DNA. Both layers were re-extracted, and the phenol layers were combined. Two volumes of acetone-0.1 N HCl were added to the phenol layer, and the mixture was stored at −20 C for 2 h. The precipitate was collected by centrifugation and

washed with ethanol-ether (1:1). The dried samples were then dissociated in 0.01 M sodium phosphate, pH 7.2, containing 1 to 2% SDS and 1% mercaptoethanol by heating at 100 C for 1 to 2 min.

Polyacrylamide gel electrophoresis. Polyacrylamide gels (0.6 cm or 0.8 cm by 10 cm) were made of 7.5 or 10% recrystallized acrylamide in 0.1 M sodium phosphate buffer (pH 7.2)-0.1% SDS (14). Electrophoresis was at 3 mA per 0.6-cm diameter gel or 5.5 mA per 0.8-cm diameter gel for 16 h at 18 C. Gels were frozen and sliced into 0.5- or 1-mm sections. Materials were eluted by incubating the gel slices overnight in a solution containing 0.1 M sodium phosphate (pH 7.2)-0.1% SDS-1 mM dithiothreitol. Alternatively, the gels were immersed in cold 25% trichloroacetic acid-0.05 M sodium pyrophosphate, washed with several changes of cold 10% trichloroacetic acid, and transferred to 10% trichloroacetic acid preheated to 90 C. This temperature was maintained for 20 min to hydrolyze nucleic acids, and the tubes were then stored at 4 C for 2 h to allow air bubbles in the gels to redissolve. The gels were then stained with 0.1% Coomassie blue in 25% trichloroacetic acid for 1 h at 37 C (1) and washed overnight in cold 7.5% acetic acid. The gels were either sliced longitudinally, dried, and placed in contact with no-screen X-ray film (Eastman Kodak) to prepare radioautographs (3) or sliced into 1-mm sections, dissolved with hydrogen peroxide, and counted with a Triton-toluene-based scintillation solvent (16). The molecular weights of polypeptides were estimated as previously described (25, 28).

Hot trichloroacetic acid precipitation. Carrier serum albumin (250 μg) and 10% trichloroacetic acid were added to each sample. The tubes were incubated in a water bath at 90 C for 20 min and then at 4 C for 15 min with additional trichloroacetic acid and sodium pyrophosphate to final concentrations of 25% and 0.05 M, respectively. The tubes were then centrifuged, and the supernatant fluids were aspirated. The pellets were dissolved in 0.1 ml of 1 N NaOH and reprecipitated with 25% trichloroacetic acid-0.05 M sodium pyrophosphate. The precipitates were collected and washed on membrane filters (type HA, 0.45-μm pore size, Millipore Corp.).

Identification of phosphoserine and phosphothreonine. Proteins labeled with ^{32}P were eluted from polyacrylamide gels, precipitated with cold trichloroacetic acid, washed with ethanol-ether (1:1), and hydrolyzed with or without carrier phosphoserine and phosphothreonine in 2 N HCl for 5 h at 100 C under N_2 in sealed tubes. HCl was removed by lyophilization. Electrophoresis on thin-layer cellulose plates (Eastman Kodak) was performed as previously described (18).

Peptide mapping of tryptic digests. Purified viral proteins were eluted from polyacrylamide gels and precipitated, after addition of 200 μg of carrier rabbit globin, with cold 20% trichloroacetic acid. The protein pellets were washed with alcohol-ether (1:1) and air-dried. Proteolytic digestion was initiated with 10 μg of TPCK trypsin (Worthington Biochemical Corp.) at 37 C in 200 μliters of 0.1 M ammonium bicarbonate containing a small amount of toluene preservative. After 6 h an additional 10 μg of trypsin was added. and at 16 h the sample was centrifuged to remove any insoluble debris. Overall recovery of radioactive material including acid precipitation, washing, and trypsinization steps was greater than 60%. Ammonium bicarbonate was removed by drying under a stream of nitrogen and resuspending the peptides two times in 0.01 N acetic acid. The tryptic peptides were then dissolved in 10 to 15 μliters of pyridine-acetic acid-water (1:10:90) pH 3.5, and spotted near the corner of a 200- by 200- by 0.25-mm cellulose thin-layer sheet (Analtech). Electrophoresis was carried out in the same buffer at 500 V for 195 min on a cold plate apparatus (Savant). After drying at room temperature, the sheet was chromatographed in the second dimension with butanol-pyridine-acetic acid-water (30:20:6:24) for 3 h.

RESULTS

Isolation of ^{32}P-labeled virion components. Vaccinia virus, grown in medium containing $^{32}P_i$ was purified by rate zonal and equilibrium density gradient centrifugations. Essentially all of the radioactive material in the purified preparation cosedimented with the virus band. Phospholipid was removed by chloroform-methanol extraction, and the remaining materials were separated by SDS-polyacrylamide gel electrophoresis. To distinguish between ^{32}P-labeled nucleic acids and ^{32}P-labeled proteins containing phosphoserine or phosphothreonine residues, the eluted materials were counted directly and after hot trichloroacetic acid precipitation. At least three peaks of precipitable material were detected and are indicated by arrows in Fig. 1. The most rapidly migrating component accounted for approximately 4% of the total radioactivity applied to the gel. This viral component will henceforth be referred to as the virion phosphoprotein since it will be shown to contain phosphoserine residues. The more slowly migrating hot trichloroacetic acid-precipitable materials have not been further characterized. The bulk of the radioactively labeled material was DNA. It remained near the origin of the gel and was labile in hot acid. Some additional labile material, just preceding the major phosphoprotein, has not been analyzed but could consist of lower-molecular-weight DNA, RNA, or protein containing phosphohistidine residues (Fig. 1).

It was of interest to compare the virion phosphoprotein with the in vitro reaction product of the vaccinia virion-associated protein kinase. The latter was labeled as previously described (18) by incubating purified vaccinia virus with [γ-^{32}P]ATP, Mg^{2+}, nonionic detergent, and a reducing agent. The in vivo and in vitro ^{32}P-labeled samples were mixed with ^{3}H-

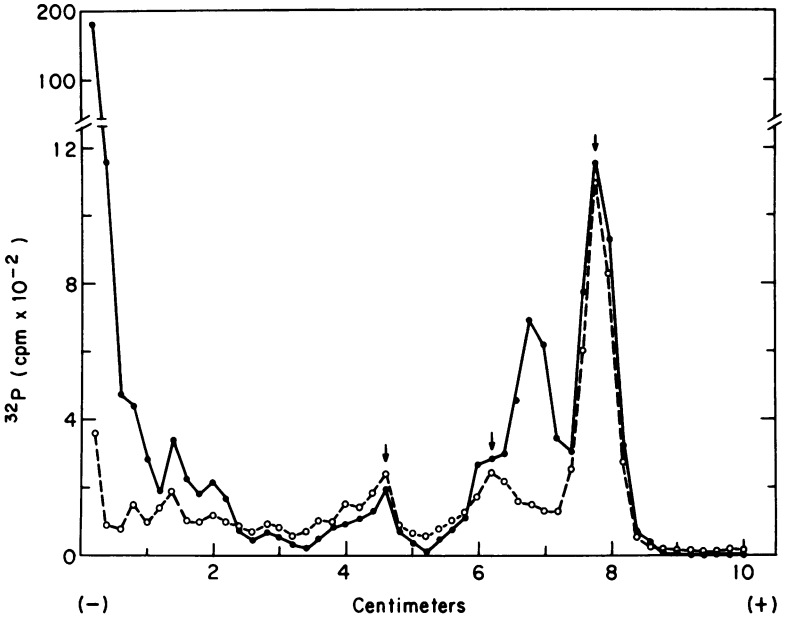

FIG. 1. *Electropherograms of in vivo, ^{32}P-labeled virion components on 7.5% polyacrylamide gels. Vaccinia virus was grown in HeLa cells in medium containing $^{32}P_i$ and purified by rate zonal and equilibrium centrifugations. The virus was extracted with chloroform-methanol and dissociated with SDS. After electrophoresis the gels were sliced, and radioactive materials were eluted in buffer containing SDS. Samples were counted directly (●) and after hot trichloroacetic acid precipitation (○). The positions of hot trichloroacetic acid-precipitable peaks are indicated by arrows. The direction of electrophoresis is toward the anode.*

amino acid-labeled virus to identify the phosphorylated polypeptides. In this experiment proteins were separated from DNA by SDS-phenol extraction prior to gel electrophoresis. After electrophoresis, any remaining nucleic acids were hydrolyzed by heating the gels in trichloroacetic acid at 90 C. The in vitro-labeled proteins appeared to consist of several species with similar electrophoretic mobilities (Fig. 2A). The ^{32}P peak just behind the most rapidly migrating ^{3}H-labeled polypeptide usually was labeled most highly in vitro. The major in vivo-labeled virion phosphoprotein corresponded to the most rapidly migrating viral polypeptide (Fig. 2B). Previous experiments with radioactive amino acid-labeled viral proteins indicated that this peak contains multiple components separable by SDS-hydroxylapatite chromatography (15). The electrophoretic mobility of the in vivo-labeled phosphoprotein was similar to that of cytochrome C which has a molecular weight of 11,700. However, values obtained for SDS-polypeptide complexes of this size may not be reliable (20). Approximately 60% of the in vivo-labeled phosphoprotein was recovered with viral cores (Fig. 3) produced by removal of the envelope with NP-40 detergent and dithiothreitol (2, 8, 13).

Specific association of the phosphoprotein with vaccinia virions. Examination of cytoplasmic extracts by polyacrylamide gel electrophoresis indicated that the virion phosphoprotein was a minor constituent of the total phosphoprotein labeled with $^{32}P_i$ during vaccinia virus infection (Fig. 4). However, this phosphoprotein was specifically co-purified with vaccinia virions (Fig. 4). Reconstitution experiments, performed by adding unlabeled virus to $^{32}P_i$-labeled cytoplasmic extract of uninfected cells, did not suggest significant nonspecific absorption of host phosphoproteins (Fig. 4). In addition, association of phosphoproteins with virus was not observed either when HeLa cells were prelabeled with $^{32}P_i$, washed, and then infected with vaccinia virus in unlabeled medium or when infected cells were labeled with $^{32}P_i$ in the presence of cycloheximide. The presence of the virion phosphoprotein in cytoplasmic extracts of infected cells and its absence from uninfected cells is evident in Fig. 5 and suggests that the phosphoprotein is virus induced.

Identification of phosphoserine residues. Chemical and enzymatic methods used to characterize the purified in vivo-labeled phosphoprotein are summarized in Table 1. Resistance

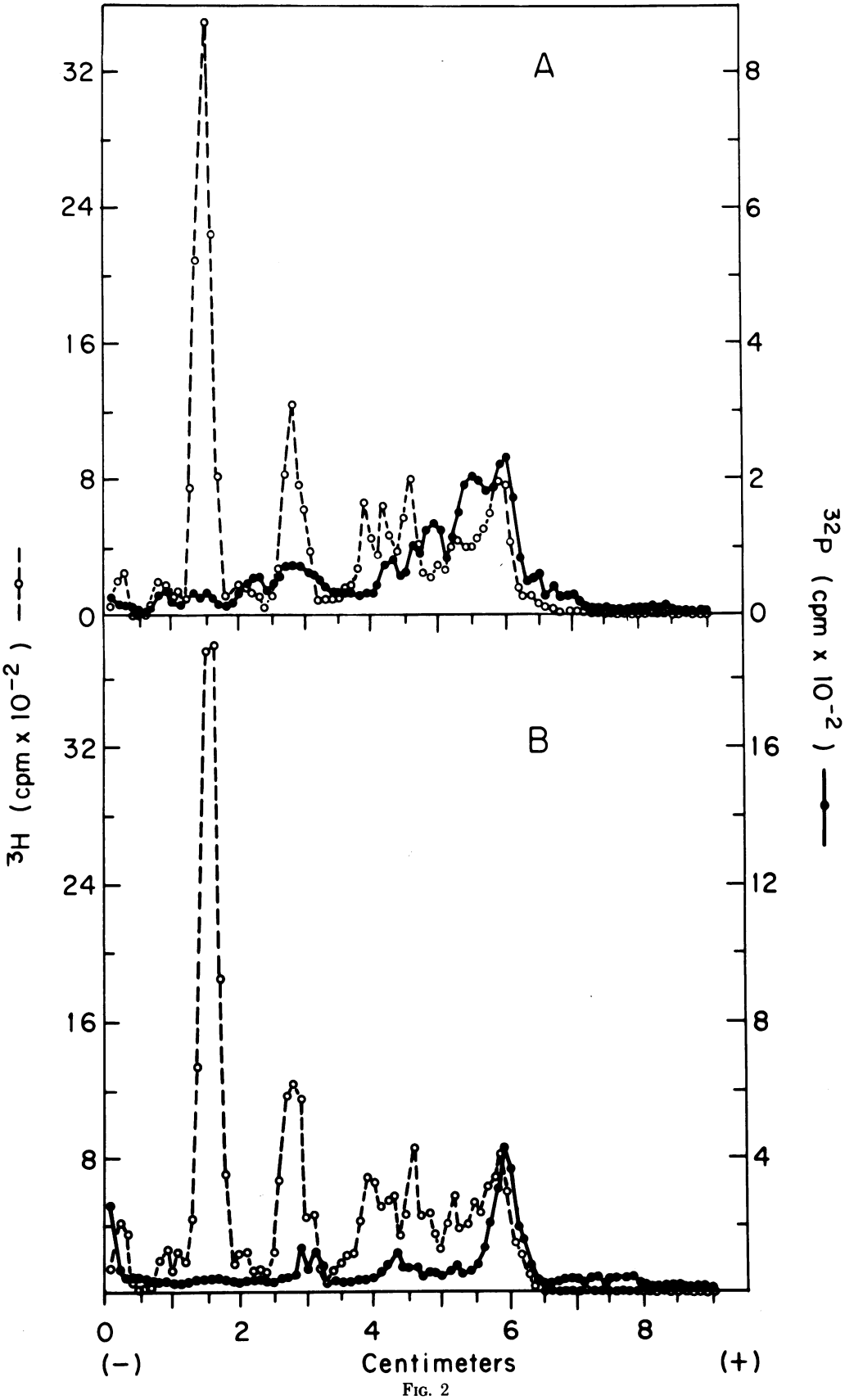
A
B
32
24
16
8
0
8
6
4
2
0
16
12
8
4
0
2
4
6
8
(−)
Centimeters
(+)
^{3}H (cpm x 10^{-2})
^{32}P (cpm x 10^{-2})

FIG. 2

to hot trichloroacetic acid, deoxyribonuclease, and ribonuclease all indicated the absence of phosphodiester bonds; resistance to extraction by chloroform-methanol suggested that the label was not associated with phospholipid; resistance to hydroxylamine indicated that acylphosphate bonds were not present. In contrast, the sensitivity to alkali and Pronase were consistent with ^{32}P-labeled phosphoserine or phosphothreonine residues in peptide linkage.

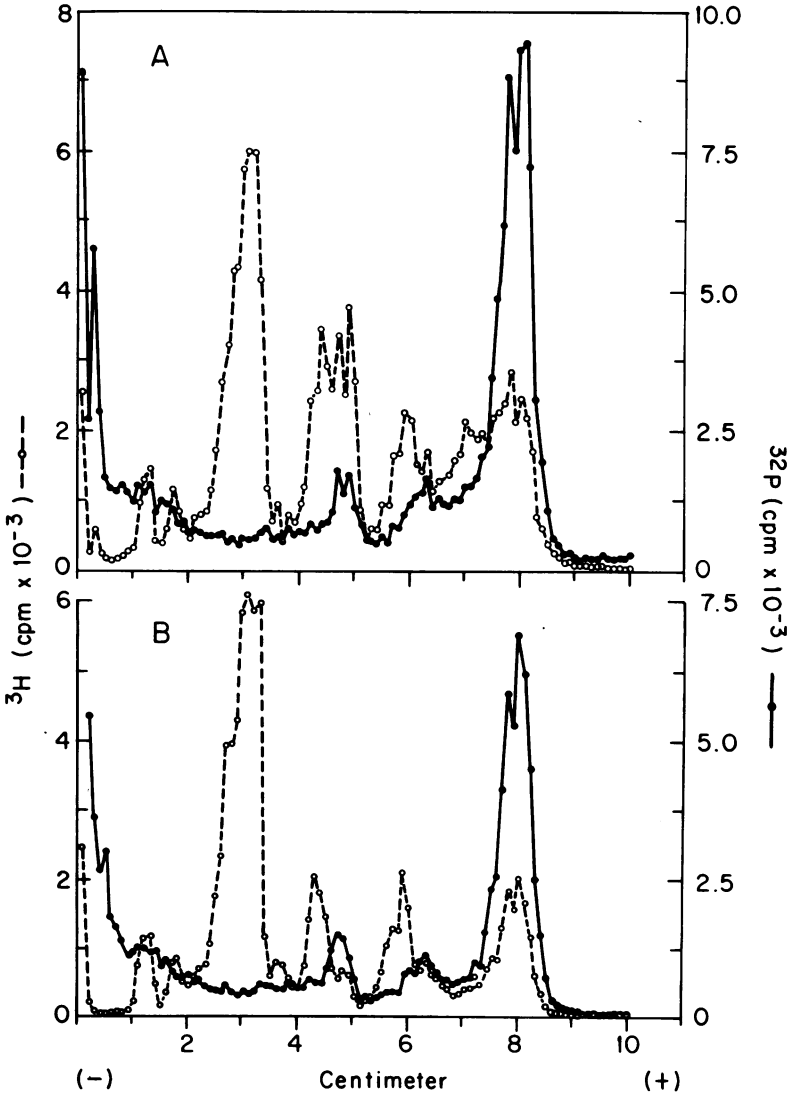

FIG. 3. *Association of phosphoprotein with viral cores. Vaccinia virions, labeled in vivo with $^{32}P_i$, were mixed with ^3H-amino acid-labeled virions and incubated at 37 C with NP-40 detergent and dithiothreitol. The cores were recovered by centrifugation through a sucrose cushion. Electropherograms of unfractionated virus (A) and cores (B) were prepared as in Fig. 2.*

FIG. 2. *Electropherograms of in vitro and in vivo, ^{32}P-labeled viral proteins. The in vitro reaction product of the viral protein kinase was obtained by incubating purified vaccinia virions with [γ-^{32}P]ATP as previously described (18). In vivo ^{32}P-labeled virions were obtained by growing vaccinia virus in medium containing $^{32}P_i$. Both ^{32}P-labeled samples were mixed with ^3H-amino acid-labeled vaccinia virions and then dissociated with SDS and mercaptoethanol and extracted with phenol as described in Materials and Methods. The proteins were washed with ethanol-ether and analyzed by SDS-polyacrylamide gel electrophoresis. The gels were incubated in hot trichloroacetic acid to hydrolyze nucleic acids, stained with Coomassie blue, and sliced into 1-mm sections for scintillation counting. A, In vitro protein kinase reaction product; B, in vivo-labeled virion proteins.*

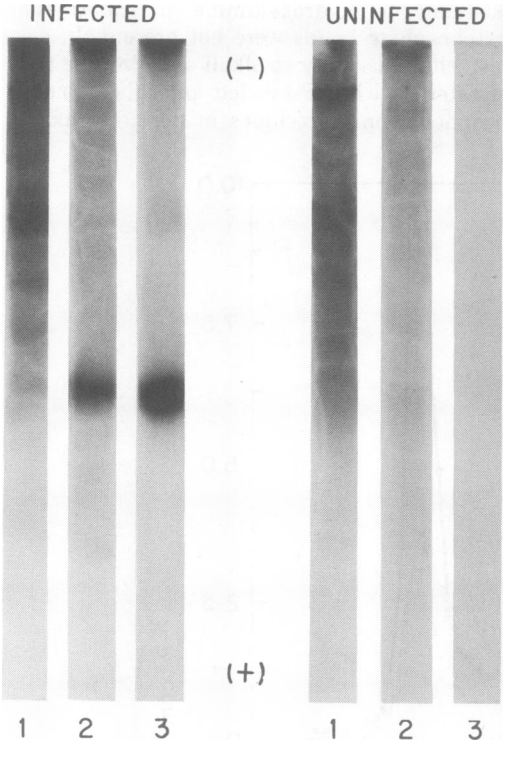

FIG. 4. *Polyacrylamide gel radioautographs of ^{32}P-labeled proteins at different stages of virus purification. Vaccinia virus-infected and uninfected cells were labeled with 4 mCi of $^{32}P_i$. After 22 h both cultures were harvested and mixed with unlabeled carrier virus. Vaccinia virus was purified from the cytoplasmic extracts and samples were saved at the following steps: (1) cytoplasmic extract; (2) pellet from 36% sucrose cushion; (3) virus band from equilibrium density potassium tartrate gradient. The samples were extracted with chloroform-methanol and dissociated with SDS and mercaptoethanol. The amount of ^{32}P-labeled protein was determined by hot trichloroacetic acid precipitation of a small amount of each sample. Similar amounts of ^{32}P-labeled protein from each of the fractions derived from virus-infected cells were then applied to one set of gels. Corresponding volumes of material from uninfected cells were applied to a second set of gels. After electrophoresis, the gels were incubated in hot trichloroacetic acid to hydrolyze nucleic acids and then processed as described in Materials and Methods.*

Similar results were previously obtained during characterization of the in vitro reaction product of the vaccinia virion-associated protein kinase (18). Controls for the various procedures were carried out with solubilized virus in which the radioisotope was primarily in DNA. As expected, the labeled DNA was resistant to alkali, Pronase, and ribonuclease and sensitive to hot trichloroacetic acid and deoxyribonuclease (Table 1).

To identify ^{32}P-labeled phosphoserine and phosphothreonine residues, the purified virion phosphoprotein was hydrolyzed with HCl and subjected to electrophoresis with authentic standards on thin-layer plates. Labeled materials corresponding to phosphoserine and P_i were detected by radioautography (Fig. 6). Inorganic phosphate is formed by partial hydrolysis of phosphoserine under these conditions. The radioactive materials migrating more slowly than phosphoserine and phosphothreonine standards are presumably phosphopeptides produced by incomplete hydrolysis (24). Phosphoserine, eluted from the thin-layer plates, accounted for 24% of the applied radioactivity. This recovery is similar to that obtained by other workers for a variety of phosphoproteins (23). The absence of phosphothreonine was of interest since the in vitro protein kinase reaction product contained small amounts of the latter amino acid (Fig. 6, reference 18).

Analysis of tryptic phosphopeptides. We considered it important to determine whether the virion protein was phosphorylated at a specific site. Accordingly, the purified in vivo, ^{32}P-labeled protein was digested with trypsin, and the phosphopeptides were analyzed by thin-layer techniques. Only one major and a few minor phosphopeptides were resolved by electrophoresis at pH 3.5 (Fig. 7), two-dimensional electrophoresis, and chromatography (Fig. 8), and two-dimensional electrophoresis at pH 3.5 and 6.4 (data not shown). The tryptic phosphopeptides were soluble in trichloroacetic acid, and the major phosphopeptide accounted for more than 60% of the radioactive material. The in vitro reaction product of the virion-associated protein kinase was isolated by polyacrylamide gel electrophoresis and was analyzed in a similar manner. The major peak containing predominantly material migrating just behind the virion phosphoprotein was pooled and digested with trypsin. More than 15 phosphopeptides were resolved by two-dimensional techniques; however, the peptide indicated by an arrow (Fig. 8) corresponded to the major phosphopeptide derived from the virion phosphoprotein. These two phosphopeptides also had similar electrophoretic mobilities at pH 6.4.

DISCUSSION

We have demonstrated that one of the proteins of purified vaccinia virions contains phosphoserine residues. Analysis of ^{32}P-labeled cytoplasmic extracts and fractions from the various stages of virus purification indicated that the phosphoprotein is virus induced and is specifi-

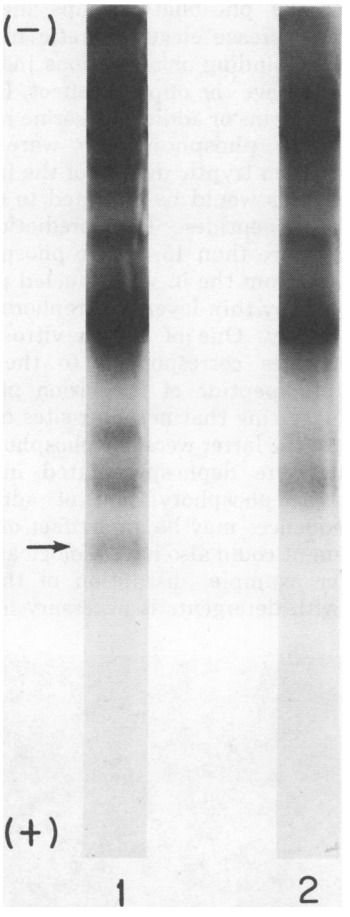

FIG. 5. *Polyacrylamide gel radioautographs of ^{32}P-labeled cytoplasmic extracts from infected and uninfected cells. Cytoplasmic extracts from infected and uninfected cells were prepared as described in Fig. 4. Equal amounts of material from infected and uninfected cell fractions were analyzed on 7.5% gels. The gels were washed with hot trichloroacetic acid, and radioautographs were prepared. The arrow indicates the position of a band corresponding to the phosphoprotein from purified virus analyzed on a separate gel. 1, Cytoplasmic extract from infected cells; 2, cytoplasmic extract from uninfected cells.*

callv associated with virions. The phosphoprotein has a molecular weight of 11,000 to 12,000 and corresponds to the lowest-molecular-weight vaccinia viral core polypeptide. The nature of several other minor ^{32}P-labeled components has not yet been determined.

Peptide mapping of tryptic digests of the virion phosphoprotein revealed only one major phosphopeptide. Assuming that the protein has multiple serine or threonine residues, this result implies that phosphorylation is highly

TABLE 1. *Properties of vaccinia virion phosphoprotein*

Treatment	% Acid insoluble[a]	
	Phosphoprotein[b]	Virus[c]
Treatment A[d]		
Untreated	100	100
10% trichloroacetic acid, 90 C	85.7	5.5
1 N NaOH, 100 C	2.9	83.9
Chloroform: methanol (1:1)	91.9	74.2
Succinic acid	81.3	103.2
Succinic acid-hydroxylamine	81.9	105.6
Treatment B[e]		
Untreated	100	100
Deoxyribonuclease	81.8	36.0
Ribonuclease	85.2	91.6
Pronase	22.0	96.9

[a] Carrier serum albumin (250 μg) was added to each sample which was then precipitated with trichloroacetic acid and collected on membrane filters (Millipore Corp.).

[b] The ^{32}P-labeled virion phosphoprotein was purified as described in Fig. 1.

[c] SDS-dissociated, ^{32}P-labeled vaccinia virus.

[d] Treatment A: Duplicate portions of each sample were treated with 10% trichloroacetic acid for 20 min; 1 N NaOH at 100 C for 15 min; 1 M succinic acid (pH 5.5) at 37 C for 1 h; 1 M succinic acid-1 M hydroxylamine at 37 C for 1 h.

[e] Treatment B: Duplicate portions of each sample were incubated in a final volume of 1 ml with: 50 μg of deoxyribonuclease I (ribonuclease free) in 0.2 M Tris-hydrochloride (pH 7)-1.5 mM $MgCl_2$ at 37 C for 30 min; 50 μg of pancreatic ribonuclease in 0.2 M Tris-hydrochloride (pH 7.7) at 37 C for 30 min; 50 μg of Pronase (nuclease free) in 0.2 M Tris-hydrochloride (pH 7.8)-1.5 mM $CaCl_2$ at 37 C for 30 min. Pronase had been preincubated for 15 min at 37 C.

selective. Presumably, the previously described viral protein kinase (18) is responsible for phosphorylation. The association of the enzyme with the virion suggests that phosphorylation occurs during viral maturation. Possibly, the protein kinase as well as other maturation enzymes remain associated with enveloped vaccinia virions after completion of their primary functions.

It was important to compare the viral proteins phosphorylated by the protein kinase in vitro with the virion phosphoprotein. The in vitro reaction was carried out by incubating purified virions with [γ-^{32}P]ATP in the presence of a nonionic detergent, reducing agent, and Mg^{2+}

Analysis by polyacrylamide gel electrophoresis indicated that the in vitro-labeled components had similar and slightly lower electrophoretic mobilities than the virion phosphoprotein. This suggests that additional polypeptides are phosphorylated in vitro. A possible alternative is that additional phosphates added to the virion phosphoprotein alters its electrophoretic mobility. Although the additional negative

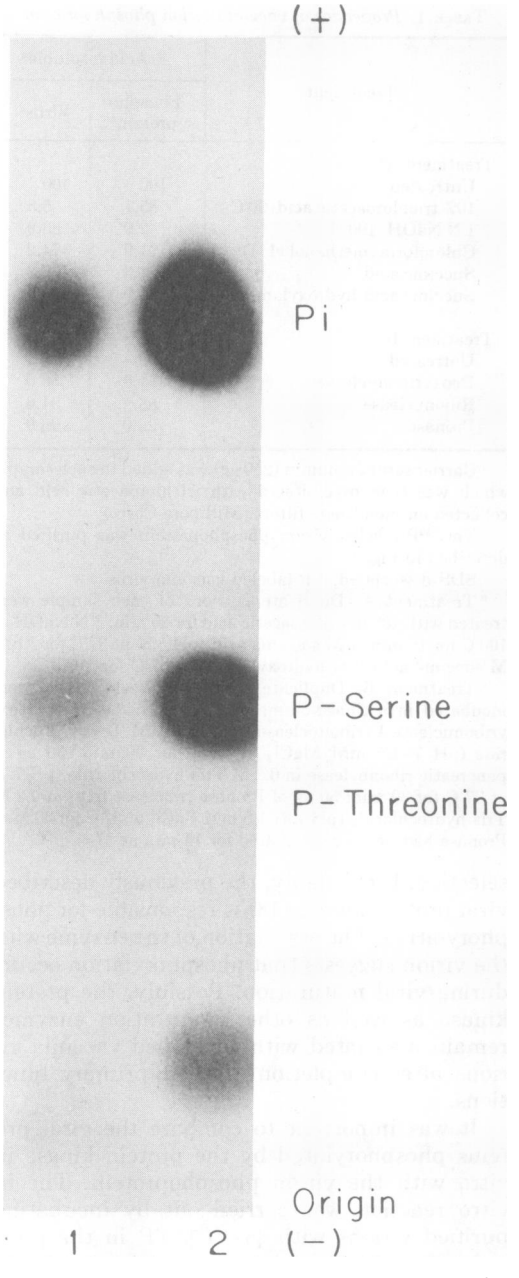

FIG. 6. *Identification of ^{32}P-labeled phosphoserine by thin-layer electrophoresis. The in vivo-labeled virion phosphoprotein and the in vitro reaction product of the virion protein kinase were purified by polyacrylamide gel electrophoresis. Samples were hydrolyzed with HCl and analyzed by electrophoresis as described. The positions of phosphoserine and phosphothreonine standards were determined by staining with ninhydrin; $^{32}P_i$ was localized by radioautography. 1, In vitro protein kinase reaction product; 2, in vivo-labeled virion phosphoprotein.*

changes of the phosphate groups might be expected to increase electrophoretic mobility, reduced SDS binding or alterations in conformation could have the opposite effect. If either additional proteins or additional serine residues on the virion phosphoprotein were phosphorylated, then tryptic digests of the in vitro-labeled proteins would be expected to contain many phosphopeptides. This prediction was confirmed. More than 15 tryptic phosphopeptides derived from the in vitro-labeled product were resolved by thin-layer electrophoresis and chromatography. One of the in vitro-labeled phosphopeptides corresponded to the single major tryptic peptide of the virion phosphoprotein, suggesting that acceptor sites on some molecules of the latter were not phosphorylated in vivo or were dephosphorylated in vitro. Although the phosphorylation of additional peptide sequences may be an artifact of the in vitro system, it could also have biological significance. For example, disruption of the viral envelope with detergents is necessary for opti-

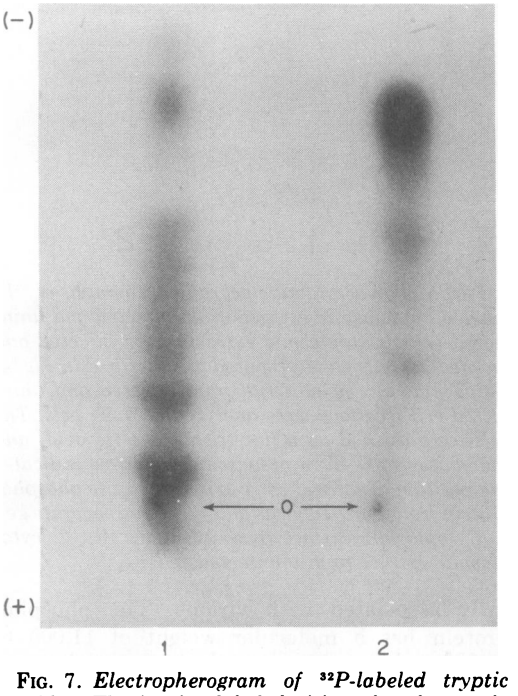

FIG. 7. *Electropherogram of ^{32}P-labeled tryptic peptides. The in vivo labeled virion phosphoprotein and the in vitro reaction product of the virion protein kinase were purified by polyacrylamide gel electrophoresis and digested with trypsin. Electrophoresis of the tryptic phosphopeptides was carried out at pH 3.5, and radioautographs were prepared. The origins are indicated by arrows. 1, In vitro reaction product; 2, in vivo-labeled virion phosphoprotein.*

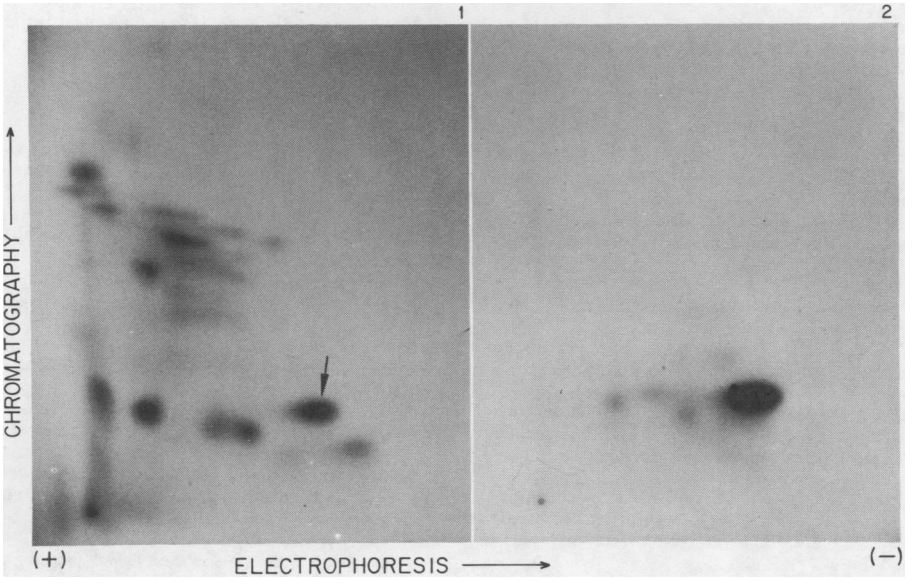

FIG. 8. *Two dimensional analysis of ^{32}P-labeled tryptic phosphopeptides. Tryptic peptides derived from the purified in vivo-labeled virion phosphoprotein and the purified in vitro reaction product of the viral protein kinase were analyzed on thin-layer cellulose plates by electrophoresis at pH 3.5 in the first dimension and by chromatography in the second dimension. Radioautographs are shown. The origins can be seen as small spots in the lower left corners of the plates. 1, In vitro protein kinase reaction product, the arrow indicates the phosphopeptide which corresponds to the major one derived from the in vivo-labeled virion phosphoprotein. 2, In vivo-labeled virion phosphoprotein.*

mal in vitro protein kinase and RNA polymerase activities and may be analogous to events occurring during the uncoating stage of infection. It remains to be determined whether the inoculum virus undergoes additional phosphorylation in vivo after it is uncoated.

Our finding that a specific polypeptide of vaccinia virions is phosphorylated in vivo is quite different from a recent report with SV40 virus (27). When SV40 was grown in medium containing $^{32}P_i$, all of the structural proteins were labeled. SV40 does not contain detectable protein kinase activity, and the significance of this relatively nonspecific phosphorylation is unknown. The major capsid protein of rabies virus can be labeled in vivo with ^{32}P, suggesting that it also may be a phosphoprotein (F. Sokol and H. F. Clark, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 225, 1972).

Phosphorylation is the third modification of vaccinia virion polypeptides that has been recognized. Some virion polypeptides are formed by cleavage of higher-molecular-weight precursors (12, 13), and at least one is a glycoprotein (4, 7, 16). These modifications can significantly alter the biological properties of proteins. Phosphorylation, in particular, is an important process which regulates the activities of a variety of enzymes (9). The high selectivity of in vivo phosphorylation of vaccinia viral polypeptides suggests that the modification may have a specific role. Further studies of the enzymatic and structural components of vaccinia virions may help define this viral function.

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