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Polyamines in Vaccinia Virions and Polypeptides Released from Viral Cores by Acid Extraction

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Vaccinia virions propagated in the presence of [^{3}H]ornithine were found to contain two labeled polyamines, spermine and spermidine. In complete virions the ratio of radioactively labeled spermine to spermidine was about 1:10, whereas in viral cores the ratio was 2:5. This suggests that some spermidine was preferentially lost during the conversion of virions to cores or that spermidine was present in the virions both inside and outside the core structure. Addition of [^{3}H]ornithine to vaccinia virus-infected cells as late as 6 h postinfection demonstrated that, although the conversion of this precursor to polyamines was reduced by 50% or more as compared to mock-infected cells, complete inhibition of polyamine synthesis did not occur. Two percent or less of the total radioactivity associated with virions grown in the presence of [^{3}H]ornithine was found to be acid soluble. Polyacrylamide gel electrophoretic analysis showed that all the structural polypeptides were labeled when virions were propagated in the presence of [^{3}H]ornithine. When cores labeled with a mixture of ^{14}C-labeled amino acids were extracted with 0.25 N H_2SO_4, 12 to 15% of the labeled core polypeptides were released and could be precipitated with acetone. About 40% of [^{3}H]arginine-labeled polypeptides associated with cores were extracted with acid. Four polypeptides or groups of polypeptides were resolved after polyacrylamide gel electrophoresis analysis of the acid-soluble fraction of cores with molecular weights of about 58,000, 34,000, 24,000, and 10,000 to 12,000. About 40% of the [^{3}H]arginine radioactivity extracted from cores coelectrophoresed with the 10,000 to 12,000-molecular weight polypeptide, indicating that this may represent an arginine-rich, histone-like structural polypeptide of the virion.

Polyamines have been demonstrated to interact with acid macromolecules in both eukaryotic and prokaryotic organisms (32). They bind to nucleic acids and phospholipids and may have regulatory as well as structural functions in such cells (9, 26). Polyamines have been reported to occur in bacterial viruses (1, 2), plant viruses (5, 14), and viruses which infect animal hosts (3, 10, 29). Recently, evidence has been presented that the level of ornithine decarboxylase, an enzyme which catalyzes the formation of putrescine, the initial step in polyamine biosynthesis, is increased in cells after infection with vaccinia virus (11). The results suggest that a new enzyme, coded for by the viral genome, may be present in vaccinia virus-infected cells.

We have studied the utilization of [^{3}H]ornithine, a precursor of polyamines (31) and certain amino acids (6, 16), in HeLa cells infected with vaccinia virus. Virions were grown in cells prelabeled with [^{3}H]ornithine and in cells where [^{3}H]ornithine was added only after infection. Evidence will be presented that vaccinia virions propagated in cells prelabeled with [^{3}H]ornithine contain spermidine and spermine in a ratio of about 6:1. When [^{3}H]ornithine was added after infection, the virions were found to contain both polyamines in a ratio of about 10:1. These polyamines could be demonstrated to be associated with viral cores prepared by treatment of virions with Nonidet P-40 and 2-mercaptoethanol.

In addition to polyamines, polypeptides could be released from cores by acid extraction, and these were partially characterized by electrophoresis in polyacrylamide gels. One of the polypeptides resolved by this method could be shown to be an arginine-rich polypeptide with a molecular weight of about 10,000 to 12,000, confirming the observations of Pogo et al. (23). Electron photomicrographs of acid-extracted

cores showed that the principle structural alteration which could be observed after such treatment was the loss of the palisade layer from many but not all the core particles. Finally, the synthesis of polyamines after viral infection was studied and compared to the synthesis of the polyamines in mock-infected control cultures.

MATERIALS AND METHODS

Virus and cells. Vaccinia virus, strain WR, propagated in HeLa cells, was used throughout these studies. A purified virus stock, containing 1.2×10^{11} elementary bodies (EB)/ml and an EB/PFU ratio of 38:1 when titrated on primary chicken embryo fibroblasts, was used in the experiments to be described. Cell growth, purification of virus, and infection of cells were carried out as described previously (4, 20).

Labeling of virions with L-[^{3}H]ornithine. HeLa S3 cells were grown for 18 to 24 h in the presence of L-[^{3}H]ornithine (1 to 5 μCi/ml), collected by centrifugation, washed one time with medium prewarmed to 37 C, and then infected with vaccinia virus at a multiplicity of 1,000 EB/cell and incubated for 24 h at 37 C. Virions purified from such cells were termed prelabeled virions. In other experiments, HeLa cells were infected as described above and 15 min after infection L-[^{3}H]ornithine (1 to 5 μCi/ml) was added, and the cells were collected for purification of virions 24 h later. Virus particles isolated and purified from such cultures were termed postlabeled virions. Virions were purified by banding in 20 to 40% linear sucrose gradients (12) and finally by banding in 20 to 50% preformed tartrate gradients (22). Aliquots of the virus preparations were collected by centrifugation (Sorvall SS34 rotor; 45 min; 15,000 rpm; 4 C), washed once with 10^{-3} M phosphate buffer, pH 7.2, and finally resuspended in 10^{-3} M phosphate buffer, pH 7.2, with the aid of sonic treatment. For determination of total radioactivity associated with the purified virions, 0.1 to 0.2 ml of the resuspended preparations was counted under Aquasol (New England Nuclear Corp.). Estimates of the numbers of elementary bodies, protein, and nucleic acid in virus preparations were made spectrophotometrically (15).

Extraction of polyamines. Polyamines were extracted from virions, viral cores, or cell homogenates by rendering such preparations 0.5 N in respect to perchloric acid and incubating for 18 h at 4 C. Acid-insoluble material was separated by centrifugation (Sorvall SS34 rotor; 15,000 rpm; 60 min; 2 C). The supernatants were collected and filtered through membrane filters (type HA; Millipore Corp.). The resulting filtrate was termed the acid-soluble fraction.

Preparation of viral cores. Virions were converted to viral cores by treatment with Nonidet P-40 and 2-mercaptoethanol (8). Cores were further purified by banding in 20 to 60% linear sucrose-D_2O gradients as described previously (25).

Preparation and analysis of dansylated polyamines. The dansyl derivatives of the polyamines present in the acid-soluble fraction were prepared as described previously (3, 7). Briefly, the acid-soluble fraction was adjusted to pH 8.0 with $Na_2CO_3 \cdot 10 H_2O$, 2 volumes of freshly prepared dansyl chloride (30 mg/ml in acetone) were added, and the reaction was allowed to proceed for 16 h at room temperature in the dark. In some experiments spermidine and spermine were added to the acid-soluble fractions before dansylation to provide internal markers. The dansylated polyamines were extracted into benzene, the benzene fraction was dried in vacuo, and the dansyl derivatives were redissolved in a small volume of benzene (1 to 500 μl). Dansylated polyamines were separated by chromatography on Silica Gel G thin-layer chromatography plates (E. Merck, Inc.; Brinkmann Instruments, N.Y.). Plates were developed with ethylacetate:cyclohexane (2:3; vol/vol). The fluorescent dansylated polyamines were located by examination of the plates under a UV light source, marked, scraped into scintillation vials, and counted under Aquasol after storing in the dark for 48 h at 4 C.

Analysis of polypeptides released from viral cores by treatment with acid. Cores were resuspended in water and an equal volume of ice-cold 0.5 N H_2SO_4 was added, and the mixture was incubated at 4 C for 16 h. The acid-insoluble fraction was removed by centrifugation (Sorvall SS34 rotor; 15,000 rpm; 60 min; 2 C). The supernatant was collected and recentrifuged, and the resulting supernatant was again harvested, mixed with 2 volumes of cold acetone, and placed at −4 C for 48 h. Precipitated polypeptides were collected by centrifugation and suspended in a small volume of 0.01 M phosphate buffer, pH 6.8. The precipitated polypeptides were found to be very difficult to solubilize under the standard conditions used to prepare samples for polyacrylamide gel electrophoretic (PAGE) analysis (which will be described below), even with heating. To circumvent this problem, the acid extract was, in some experiments, titrated to neutrality with 1 M Na_2CO_3 and then treated with sodium dodecyl sulfate (SDS) (1%), 2-mercaptoethanol (0.01 M), and urea (1.5 M). Samples were dialyzed against 0.01 M phosphate buffer, pH 7.2, containing 1.0% SDS and 0.01 M 2-mercaptoethanol, and then heated at 80 C for 3 min before PAGE analysis.

Polyamine synthesis in infected and mock-infected cells. To study the synthesis of polyamines in cells infected with vaccinia virus, as compared to mock-infected control cells, the following experiment was carried out. HeLa cells were infected at a multiplicity of 500 EB/cell and, after dilution into complete medium, four replicate cultures containing 10^8 cells were prepared. Mock-infected cultures were similarly established. At 0.15 min, and 2, 4, and 6 h after infection or mock infection, 0.5 μCi of L-[^{3}H]-ornithine was added to the cultures and incubation was continued for 18 h at 37 C. The cells were collected by centrifugation and washed with Earle salt solution, and the cells from each culture were suspended in 10^{-3} M phosphate buffer, pH 7.2. The cell suspension was Dounce homogenized and then sonicated to completely disrupt the cells and their nuclei. Cellular debris was removed by low-speed

centrifugation, and the supernatant was collected, sampled for determination of protein content (18), and then mixed with an equal volume of ice-cold 1 N perchloric acid. After incubating for 16 h at 4 C the acid-soluble fraction from each sample was collected and reacted with dansyl chloride as described above. The dansyl derivatives of the polyamines were extracted into benzene and aliquots were analyzed by thin-layer chromatography.

Electron microscopy techniques. For negative staining, acid-extracted subviral particles were collected by centrifugation, washed, suspended in 10^{-3} M phosphate buffer, pH 7.2, and applied directly to 400-mesh carbon-coated Formvar copper grids. The samples were then stained with 2% potassium phosphotungstate (pH 6.2 to 7.2) and examined in the electron microscope.

SDS-PAGE analysis. Cylindrical, 10% polyacrylamide gels containing 5 M urea, 0.26% *N,N,N'*-methylene-bis-acrylamide, 0.1% SDS, and 0.1 M phosphate buffer, pH 7.2, were employed in the analysis of polypeptides solubilized by treatment of particles or virions with SDS (2.0%), 2-mercaptoethanol (0.2 M), and 1.5 M urea. The conditions for electrophoresis have been described previously (12, 18) and are noted in the appropriate figures. Cylindrical gels were fractionated with the Savant Autogel Divider (Savant Instruments, N.Y.).

Gel patterns were divided into 12 regions or polypeptide groups, which are designated numerically from 1 to 12 in the appropriate figure. These groups are defined according to their polypeptide content as follows, using the designations of Sarov and Joklik (28): group 1 (VP-1a, b, c, d); group 2 (2a, b, c); group 3 (3a, b, c); group 4 (4a, b, c, d); group 5 (5a, b, c); group 6 (6a, b); group 7 (7a, b); group 8 (single polypeptide); group 9 (9a, b); group 10 (10a, b); group 11 (11a, b); group 12. In general, the polypeptides in groups 1, 2, and 3 can only be observed adequately using radioautographic techniques, or in some cases after staining of gels (28). When gels were fractionated for counting of radioactivity as described above, the component polypeptides within the group often could not be distinguished. Bovine serum albumin (molecular weight 68,000), pancreatic DNase (molecular weight 31,000), and cytochrome *c* (molecular weight 12,000) were employed as molecular weight standards and were detected by staining the gels with Coomassie blue (28).

Reagents and radioisotopes. The L-[G-^{3}H]ornithine (2.54 Ci/mM), ^{14}C-labeled amino acid mixture (0.67 mCi/mg), [^{14}C]thymidine (56.5 mCi/mM), [*methyl*-^{3}H]thymidine (10 Ci/mM), and L-[^{3}H]arginine (570 mCi/mM) were purchased from the New England Nuclear Corp. Polyamines and dansyl chloride were purchased from the Sigma Chemical Co. Nonidet P-40 was a gift of the Shell Oil Co., Ltd., England.

RESULTS

Polyamines in virions and viral cores. Virions prelabeled and postlabeled with [^{3}H]ornithine were analyzed for their polyamine content as described above and summarized in Table 1. Both prelabeled and postlabeled virions could be demonstrated to contain polyamines, which after reacting with dansyl chloride co-chromatographed with dansyl spermine and dansyl spermidine. When virions were propagated in cells prelabeled with [^{3}H]ornithine, the ratio of radioactively labeled spermidine to spermine recovered from purified virions was about 3:1. When [^{3}H]ornithine was added after infection, the ratio of spermidine to spermine was found to be about 10:1; this ratio was not significantly altered when the level of radioactive precursor added to the cells was varied. Part of the radioactivity in the dansylated polyamine preparation analyzed was found to chromatograph with an R_f characteristic of dansylated acetylated polyamines (8). When this fraction was recovered and rechromatographed in the same solvent system, 10 to 30% of the radioactivity remained at the origin, suggesting some cross-contamination of this fraction with materials which normally should remain at the origin. When the zones from thin-layer chromatography plates corresponding to dansyl spermine or dansyl spermidine were eluted with benzene and rechromatographed, no significant loss of the respective dansyl polyamine could be detected (recoveries: 91.7% dansyl spermine; 94.6% dansyl spermidine) and new components were not resolved. We have not attempted to further characterize the fraction which chromatographed with an R_f characteristic of dansylated acetylated polyamines and its significance remains to be determined.

Viral cores prepared as described above were analyzed for their polyamine content, and the results of such an analysis are summarized in Table 2. Two polyamines were detected in cores and their dansyl derivatives co-chromatographed with dansyl spermine and dansyl spermidine, but the ratio of the recovered polyamines was altered as compared to that found when complete virions were analyzed. In cores prepared from virions propagated in cells prelabeled with [^{3}H]ornithine, the ratio of spermine:spermidine was about 5:2, whereas in cores prepared from virions where [^{3}H]ornithine was added after infection the ratio of spermine to spermidine was about 2:5. The data indicated that spermidine had been lost in the conversion of virions to cores. We have considered three interpretations of this result. First, there may be some compartmentalization of the polyamines in the virions, like that reported for herpesvirus (10), with spermidine localized

TABLE 1. *Extraction of polyamines from [^{3}H]ornithine-labeled virions and analysis of dansylated polyamines by thin-layer chromatography (TLC)*

Purified virions labeled with [G-^{3}H]ornithine	Total radioactivity analyzed[a]		% Total counts/min recovered in acid-soluble fraction[b]	% Total acid-soluble counts/min recovered in benzene extract[c]	Distribution of radioactivity in dansylated polyamines analyzed by TLC solvent: ethylacetate-cyclohexane (2:3)[d]		
	Counts/min per ml	Sp act (counts/min per μg of DNA)			Polyamine	R_f	% of total counts/min analyzed
Prelabeled (5 μCi/ml)	34,485	1,379	2.0	73	Acetylated polyamines	0.07	21.3
					Spermine	0.16	12.0
					Spermidine	0.23	41.2
Prelabeled (1 μCi/ml)	4,780	535	2.9	48	Cadaverine	0.42	1.9
					Putrescine	0.40	2.2
Postlabeled (5 μCi/ml)	58,125	2,325	1.8	47	Acetylated polyamines	0.07	17.5
					Spermine	0.16	5.5
					Spermidine	0.23	49.3
Postlabeled (1 μCi/ml)	18,088	962	1.6	39	Cadaverine	0.42	0.2
					Putrescine	0.40	0.6

[a] Virions were propagated in HeLa S3 cells which were prelabeled for 16 h with 5 or 1 μCi of [^{3}H]ornithine per ml, washed with complete medium, and then infected or in HeLa cells where the labeled precursor at the levels indicated was added 15 min postinfection. Infected cells were incubated at 37 C for 24 h and virions were isolated and purified as described.

[b] Virions were collected by centrifugation (Sorvall SS-34 rotor; 15,000 rpm; 40 min; 4 C) and were suspended in water with the aid of sonication. To the suspension an equal volume of cold, 1 N perchloric acid was added, and the mixture was incubated at 4 C for 16 h. The acid-soluble fraction was then prepared as described. Aliquots (1 to 200 μl) were spotted on glass filters, dried, and counted under a toluene-based scintillator.

[c] The acid-soluble fraction prepared from virions was adjusted to pH 8.0 with $Na_2CO_3 \cdot 10H_2O$ and reacted with dansyl chloride for 16 h in the dark at room temperature. The dansylated polyamines were extracted into benzene and aliquots of the benzene fraction were spotted on glass filters and counted in a liquid scintillation spectrometer.

[d] The benzene fraction prepared as described above was spotted onto Silica Gel G thin-layer chromatography plates and the plates were developed in ethylacetate:cyclohexane (2:3; vol/vol) as described by Dion and Herbst (7). Dansylated polyamines were located under a UV light source, scraped into scintillation vials, and counted under Aquasol in a Nuclear-Chicago Isocap/300 counting system with circuitry which automatically detected and rejected samples with high fluorescence. Such samples were incubated in the dark at 4 C until the fluorescence had decayed to acceptable levels.

both outside and inside the core. Secondly, in the conversion of virions to cores, spermidine was selectively eluted from the cores. Finally, dansyl chloride can react with cytesine residues in proteins (13), and the preparation of the dansylated polyamines from core extracts may have been affected by the presence of 2-mercaptoethanol in the reaction mixtures, since the removal of 2-mercaptoethanol even after banding the cores in 20 to 60% sucrose-D_2O gradients may not have been complete. The formation of dansylated polyamines was found to be inhibited in the presence of 2-mercaptoethanol and Nonidet P-40 (>90%). It was not possible, therefore, to determine if spermine was also lost during the preparation of cores. The apparent greater loss of spermidine from cores prepared from prelabeled virions may reflect an altered distribution of polyamines in such virions as compared to virions grown in cells labeled after infection, or a difference in the utilization of [^{3}H]ornithine in the host cells before and after infection.

We can only conclude at this time that both spermine and spermidine could be recovered from viral cores but, as compared to complete virions, the recovery of spermidine relative to spermine was reduced. It should also be noted that the amount of dansylated acetylated polyamines was also decreased in the analyzed core extracts (Table 3).

Nature of the viral polypeptides labeled

TABLE 2. *Extraction of polyamines from [^3H]ornithine-labeled cores and analysis of dansylated polyamines by thin-layer chromatography (TLC)*

Sample[a]	Total counts/min analyzed[b]	% Total counts/min extracted by acid[b]	% Total acid-soluble counts/min recovered in benzene fraction[b]	Distribution of radioactivity in polyamines after TLC analysis[b]		
				Polyamine	R_f	% Total counts/min analyzed
Cores from pre-labeled virions	98,920	4.0	40	Acetylated polyamines	0.07	2
	64,220	3.5	38	Spermine	0.16	52
	53,530	2.8	42	Spermidine	0.23	21
				Cadaverine and putrescine	0.4–0.42	3
Cores from post-labeled virions	69,660	5.0	25	Acetylated polyamines	0.07	9
	50,350	6.3	42	Spermine	0.16	20
	48,950	4.8	37	Spermidine	0.23	46
				Cadaverine and putrescine	0.4–0.42	5

[a] Cores were prepared from virions propagated in HeLa cells prelabeled for 16 h with [^3H]ornithine (5 μCi/ml) or to which [^3H]ornithine (5 μCi/ml) was added 15 min postinfection as described. Results from analysis of three separate preparations are shown.

[b] The acid-soluble fraction of cores was prepared as described in Table 1 for complete virions. The polyamines present in the acid-soluble fraction from cores were reacted with dansyl chloride, and the resulting dansylated polyamines were extracted into benzene and analyzed by TLC as described in Table 1.

TABLE 3. *Extraction of viral cores labeled with [^3H]thymidine, ^{14}C-labeled amino acids, or [^3H]arginine with 0.25 N H_2SO_4*

Labeled virus preparation analyzed[a]	Sp act (counts/min per μg of viral protein)	Radioactivity released from virions by treatment with NP-40 and 2-mercaptoethanol[b]		Total counts/min in cores analyzed[b]	Release of radioactivity from viral cores upon extraction with 0.25 N H_2SO_4, 16 h, 4 C[c]	
		Total counts/min	%		Counts/min released	% Released
^{14}C-labeled amino acids preparation I	1,255.5	333,945	37.8	757,350	86,160	12.1
^{14}C-labeled amino acids preparation II	1,288.8	376,155	34.8	702,765	108,000	15.3
[^3H]arginine	69.7	18,630	43.0	24,690	10,920	44.2
[^3H]thymidine	354.6	1,237.5	0.4	264,322	1,020	0.3

[a] HeLa cells, infected at a multiplicity of 500 EB/cell, were diluted into medium containing 15% of the normal amino acid content. Three cultures were established. [^3H]thymidine (0.5 μCi/ml), ^{14}C-labeled amino acid mixture (0.5 μCi/ml), or [^3H]arginine (0.1 μCi/ml) was added to the appropriate culture 15 min after infection. Infected cells were incubated at 37 C for 24 h and collected by centrifugation, and virions were purified as described.

[b] Virions were converted to viral cores by treatment with Nonidet P-40 (NP-40) and 2-mercaptoethanol and purified by banding in 20 to 60% sucrose-D_2O gradients as described.

[c] Viral cores were collected by centrifugation (Sorvall SS-34 rotor; 15,000 rpm; 45 min; 4 C) and were suspended in 2 ml of water with the aid of sonication. An equal volume of cold 0.5 N H_2SO_4 was added, and the suspension was mixed and incubated at 4 C for 16 h. The acid-insoluble fraction was removed by two cycles of centrifugation and the clear supernatant was collected; aliquots (100 μl) were applied to glass filters, and the filters were dried and counted by liquid scintillation spectrometry.

with [^3H]ornithine. The precursor relationship of [^3H]ornithine to polyamines and certain amino acids in mammalian cells has been well established (6, 16, 26) so that one might expect that, in addition to the polyamines, viral polypeptides would also be significantly labeled in virions propagated in the presence of the precursor. This was supported by the data pre-

sented in Tables 1 and 2 where only a small percentage of total radioactivity associated with virions or cores was found to be acid soluble. Virions, grown in the presence of [^{3}H]ornithine added after infection, were purified and dissolved in SDS (2.0%), 2-mercaptoethanol (0.2 M), and 1.5 M urea, and the polypeptides were analyzed by PAGE as described in Fig. 1. The results demonstrated that all the major groups of virion polypeptides were labeled, showing that the conversion of [^{3}H]ornithine to amino acids occurred in cells infected with vaccinia virus. The pattern of labeling appeared to be different than that observed when mixtures of ^{14}C-labeled amino acid are used to label the virion polypeptides (28). We are presently carrying out a more careful analysis of the [^{3}H]ornithine-labeled virion polypeptides to determine if in fact a unique pattern of labeling of certain polypeptides can be obtained using labeled ornithine as a precursor.

Release of labeled polypeptides from cores with 0.25 N H_2SO_4. In the course of the experiments described above, it was found that, in addition to polyamines, some polypeptides were released from cores by treatment with acid. In Table 3 are summarized the results of experiments in which virions labeled with [^{3}H]thymidine, ^{14}C-labeled amino acids, or [^{3}H]arginine were sequentially degraded to cores and the cores then were extracted with 0.25 N H_2SO_4 to determine what percentages of the virion nucleic acid and/or polypeptides were released at each step of the procedure. The polypeptides released from the cores by acid were then analyzed by PAGE analysis as described in Fig. 2. As indicated in Table 3, approximately 35 to 40% of the total virion polypeptides (in terms

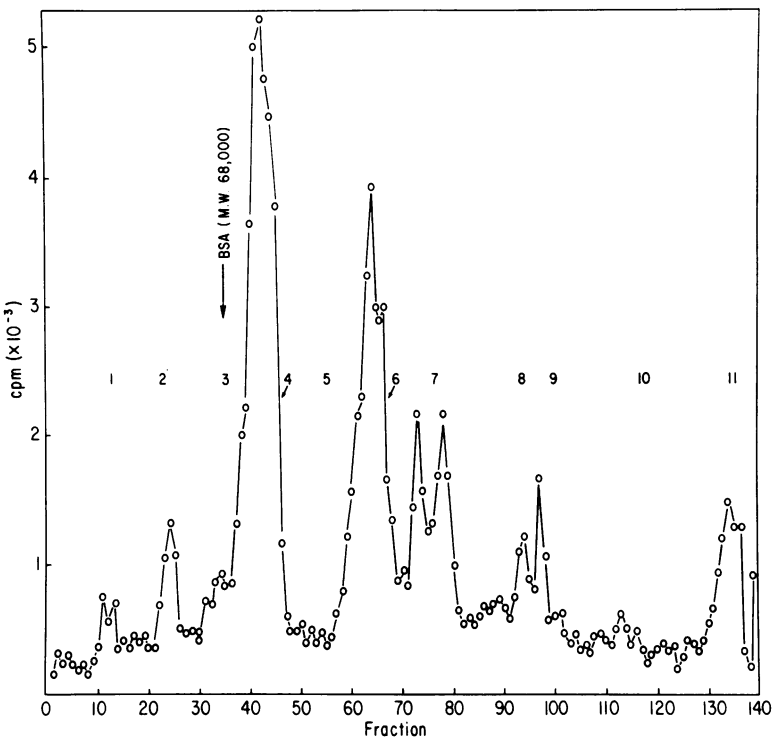

FIG. 1. *PAGE analysis of polypeptides labeled with [^{3}H]ornithine in purified vaccinia virions. Vaccinia virions were isolated and purified from HeLa cells to which [^{3}H]ornithine (5 μCi/ml) had been added 15 min after infection. Aliquots of the purified virions were collected by centrifugation and resuspended in 0.01 M phosphate buffer, pH 6.8, with the aid of sonication. The virions were dissolved in SDS (2.0%), 2-mercaptoethanol (0.2 M), and urea (1.5 M), and the sample was incubated at 37 C for 16 h and 80 C for 3 min. An aliquot (250 μl) of the preparation was applied to a polyacrylamide gel, 25 cm in length. The electrophoresis buffer was 0.1 M phosphate buffer, pH 7.2, containing 1% SDS. Electrophoresis was for 25 h at room temperature, 4.5 mA per gel. Gels were fractionated into about 150 samples and the samples were frozen, thawed, and counted under Aquasol by liquid scintillation spectrometry. The position of bovine serum albumin run in parallel in a separate gel and detected by staining with Coomassie blue is indicated. The groups of structural polypeptides are numbered from 1 to 12 and the polypeptides present in these groups have been defined. M. W., Molecular weight.*

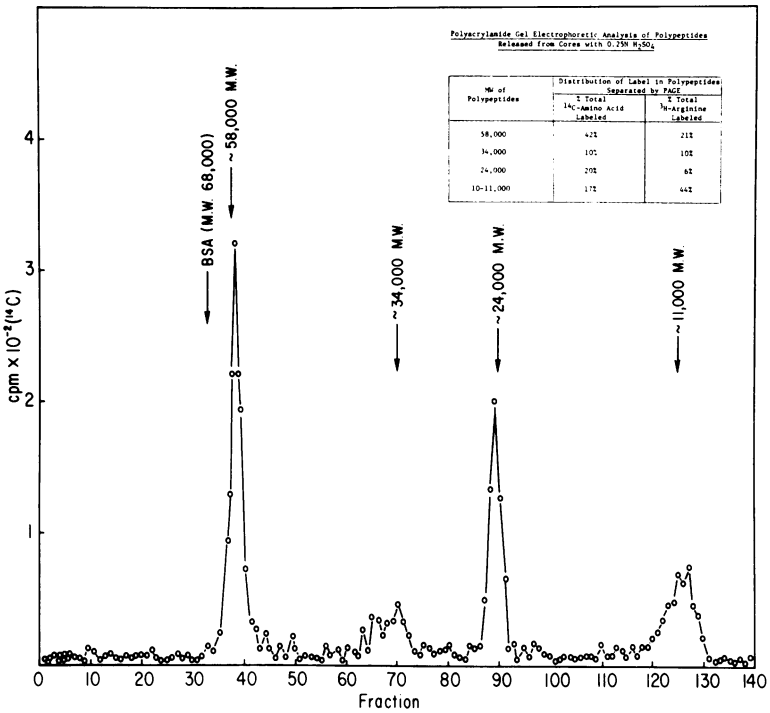

FIG. 2. *PAGE analysis of polypeptides extracted from viral cores with 0.25 N H_2SO_4. Viral cores, labeled with ^{14}C-labeled amino acids or [^{3}H]arginine, were prepared and purified as described and extracted with 0.25 N H_2SO_4 as described in Table 3. The acid-soluble fraction was neutralized with Na_2CO_3, treated with SDS (2%), 2-mercaptoethanol (0.2 M), and urea (1.5 M), and dialyzed against 0.01 M phosphate buffer, pH 6.8, containing 1% SDS and 0.01 M 2-mercaptoethanol for 24 h at 37 C. Aliquots were removed, heated for 3 min at 80 C, and analyzed by electrophoresis in 10% acrylamide gels as described in Fig. 1. The molecular weight (M. W.) of the polypeptides was estimated by comparison with the protein standards analyzed in a parallel gel. In the insert, the distribution of radioactivity in the four major polypeptides or groups of polypeptides resolved by PAGE analysis of the acid-soluble fraction from cores labeled with ^{14}C-labeled amino acids or [^{3}H]arginine (gel pattern not shown) is indicated.*

of radioactivity) were released from virions in their conversion to cores. This was true of virions labeled with a mixture of ^{14}C-labeled amino acids or with [^{3}H]arginine. When cores labeled with ^{14}C-labeled amino acids were extracted with acid, 12 to 15% of the core-associated radioactivity was released. Acid treatment of cores labeled with [^{3}H]arginine resulted in release of 38 to 45% of the total core-associated radioactivity, demonstrating that polypeptides enriched with arginine could be extracted from viral cores with acid. Analysis of the polypeptides in the acid-soluble fraction by PAGE produced the result shown in Fig. 2. Four polypeptides or groups of polypeptides were resolved. The major species had molecular weights of 58,000 and 24,000, and the minor species had molecular weights of 34,000 and 10,000 to 12,000. The relative distribution of radioactivity in these polypeptides is summarized in the insert of Fig. 2. Of interest was the fact that, when the [^{3}H]arginine-labeled acid-soluble fraction from cores was analyzed, 37 to 48% of the total radioactivity analyzed by PAGE migrated with the 10,000- to 12,000-molecular-weight polypeptides. Both the migration of these species in the SDS buffer system (23) and their high content of arginine suggests that this fraction represents one or more histone-like polypeptides, 10,000 to 12,000 in molecular weight. Electron photomicrographs of cores before and after acid extraction are shown in Fig. 3. Approximately 55 to 60% of the particles recovered after acid extraction were lacking the outer components or palisade layer normally found on cores prepared by treatment of virions with Nonidet P-40 and 2-mercaptoethanol (8; Fig. 3B). The remaining particles examined retained all or part of this outer layer. The results showed that some of the polypeptides released by acid treatment of cores may be structural components of the palisade layer.

Synthesis of polyamines after infection of

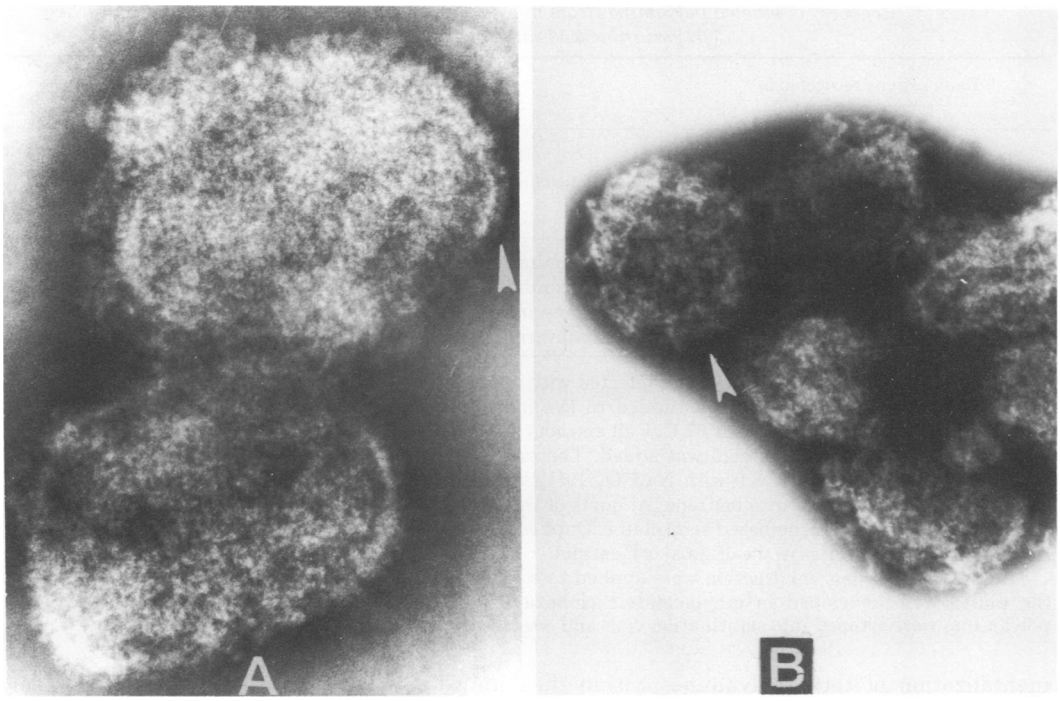

FIG. 3. *Electron microscopy of negatively stained cores before and after extraction with 0.25 N H_2SO_4. Viral cores were isolated, purified, and extracted with acid as described (Table 3). Cores or acid-extracted cores were collected by centrifugation and suspended in 10^{-3} M phosphate buffer, pH 6.8, and aliquots were removed and examined in the electron microscope after negative staining as described. (A) Cores before acid extraction. Arrow indicates the regular array of cylindrical subunits on the surface of the particles called the palisade layer. Magnification ×180,000. (B) Cores after acid extraction. Arrow indicates irregular surface and lack of palisade layer. About 50 to 60% of the particles examined had this morphology. The remaining particles retained all or part of the palisade layer. Magnification ×113,000.*

cells with vaccinia virus. To measure the effect of viral infection on the synthesis of polyamines the experiment described above was carried out. The rationale for the experiment was based on two factors: (i) vaccinia virus infection of cells at high multiplicities results in a rapid inhibition of host protein synthesis (19); and (ii) studies in a variety of animal cell systems have demonstrated that ornithine decarboxylase has a relatively short half-life (27, 30). Thus, unless this enzymatic activity was uniquely spared in the face of a general inhibition of host protein synthesis, by 4 to 6 h postinfection, enzymatic activity and therefore the conversion of [^{3}H]ornithine to polyamines should have been inhibited. Part of the results of such an experiment are summarized in Table 4. The total radioactivity recovered from infected cells in the form of polyamines, as compared to control, mock-infected cells, was reduced by 50% or more when the radioactive precursor was added at 6 h postinfection. The distribution of label in the polyamines which were labeled in infected cells was similar to that in control cells and, in particular, 35 to 40% of the label could be recovered as spermidine. These results show that, although the conversion of [^{3}H]ornithine to polyamines was reduced in infected cells, it was not completely inhibited, and the synthesis of those polyamines which were found in virions continued after infection.

DISCUSSION

The naturally occurring polyamines, spermine, spermidine, and putrescine, are found in many plants, bacteria, and animal tissues. Polyamines have been isolated from herpes simplex virus (10), human type 5 adenovirus (29), and myxoviruses (3). In the case of the herpes simplex virus, exogenous ornithine could only function as a precursor to host and viral polyamines when added before infection. Although complete herpes virions contained both spermidine and spermine in a molar ratio of 1.6 ± 0.2, disruption of the viral envelope resulted in a loss of spermidine, suggesting a compart-

TABLE 4. *Recovery of labeled polyamines from mock-infected and vaccinia virus-infected cells when [^3H]ornithine was added 6 h after infection*

Radioactivity recovered in benzene fraction[a]			Radioactivity recovered in dansyl polyamines separated by TLC[b]				
Mock-infected control	Infected	Counts/min infected per counts/min control (%)	Polyamine	R_f	Infected total counts/min	Control total counts/min	Counts/min infected per counts/min control (%)
7,038	3,823	54.3	Spermine	0.16	737	1,408	62
8,934	3,652	41.0	Spermidine	0.23	3,339	4,072	82
			Putrescine	0.40	754	2,137	36
			Cadaverine	0.42	1,080	2,892	41

[a] HeLa S3 cells were mock infected or infected with vaccinia virus at a multiplicity of 500 EB/cell. Six hours later [^3H]ornithine (0.5 μCi/ml) was added to the infected or mock-infected cultures and incubation was continued for an additional 12 h at 37 C. Cell extracts were prepared as described and to the extracts an equal volume of cold 1 N perchloric acid was added. The mixture was incubated at 4 C for 16 h. The acid-soluble fraction was adjusted to pH 8.0 with $Na_2CO_3 \cdot 10H_2O$ and reacted with dansyl chloride, and the dansylated polyamines were extracted into benzene. Aliquots of the benzene fraction were spotted onto glass filters, dried, and counted under a toluene-based scintillator. Duplicate samples were analyzed and the results were corrected for total protein recoveries in the original cell extract.

[b] Aliquots of the benzene fraction were applied to Silica Gel G thin-layer chromatography (TLC) plates and the plates were developed in ethylacetate:cyclohexane (2:3; vol/vol). Zones corresponding to the various polyamines were scraped into scintillation vials and counted under Aquasol.

mentalization of these polyamines within the virion (10). Our results demonstrate that both spermidine and spermine are present in vaccinia virions and that [^3H]ornithine can serve as precursor to these polyamines when added before or after infection of the cells with virus. The recent report that a unique ornithine decarboxylase activity can be measured in the cytoplasm of vaccinia-infected HeLa cells (11) may explain why this precursor continues to be incorporated into polyamines and amino acids, whereas host protein synthesis in general is being rapidly shut-off (19). Our results showed that polyamine synthesis was, in fact, reduced but not completely inhibited in infected cells. Moreover, all the viral structural polypeptides were labeled when [^3H]ornithine was provided as a precursor after infection, suggesting that its conversion to amino acids and subsequently the utilization of these amino acids for polypeptide synthesis readily occurred in infected cells. It remains to be determined whether there is a greater utilization of [^3H]ornithine for amino acid biosynthesis as compared to polyamine biosynthesis in cells infected with vaccinia virus.

When virions were converted to cores by treatment with Nonidet P-40 and 2-mercaptoethanol, an alteration in the ratio of spermine to spermidine could be measured which suggested that, during the preparation of cores, there was some preferential loss of spermidine. As in the case of the herpes simplex virus (10), this may indicate a compartmentalization of the polyamines within the vaccinia virion. However, as discussed above, these experiments remain open to a number of different interpretations.

The polyamine content of type 5 adenovirus, reported by Shortridge and Stevens (29) to contain both spermine and spermidine, has recently been investigated by Pett and Ginsberg (21). The data of Pett and Ginsberg (21) indicates that the polyamines reported to be found in type 5 adenovirions may actually be due to the nonspecific binding of host polyamines to virions. They further suggest that the neutralization of DNA phosphate in adenovirus virions may be accomplished by the arginine-rich internal polypeptide associated with the virus DNA (17, 24) rather than by polyamines.

The problem of nonspecific binding or trapping of polyamines within vaccinia virions was a problem we were cognizant of throughout this work. Virions propagated in cells where [^3H]ornithine was provided as the precursor were carefully purified to a constant specific activity (counts per minute per micrograms of viral protein) and we could find no evidence for release of polyamines by repeated washing of virion preparations with 10^{-3} M phosphate buffer, pH 6.8. The fact that only spermine and spermidine were found in a constant ratio within virions suggests that the incorporation of these polyamines in the structure of the virus may be controlled and not accidental. We have not yet critically determined the content of

polyamines within virions to determine what percentage of the DNA phosphate in the virus particles could be neutralized by the polyamines which are present. On the basis of the radioactivity recovered in the two polyamines from virions where [^{3}H]ornithine was added after infection, we have estimated the ratio of polyamine nitrogen to DNA phosphate to be approximately 0.64, sufficient to neutralize about 60 to 70% of the DNA phosphate in the virion (using the constants described previously [10] and assuming a molecular weight of 150×10^6 for the vaccinia genome). This figure, however, is only an estimate and disregards the possible compartmentalization of the polyamines and the contribution of preformed polyamines existing in the cell at the time of infection which could be utilized by the virus. If only spermine is present in association with the DNA, about 20% or less of the DNA phosphate would be neutralized on the basis of these calculations.

Recently, Pogo et al. (23) have demonstrated that an arginine-rich basic polypeptide of molecular weight 11,000 to 12,000 occurs both in virus cores and cytoplasmic factories from vaccinia-infected cells. The results of the experiments described here are in agreement with the findings of these investigators in regard to the arginine-rich polypeptides found in virions. Our results also suggest that, in addition to basic polypeptides, polyamines may play a role in neutralizing the charges of DNA and could function in the folding and packaging of vaccinia DNA within the core. Pogo et al. (23) found that only one polypeptide or group of polypeptides was released in significant quantities from cores labeled with [^{3}H]arginine. It may be that some of the polypeptides we have found in acid extracts of the ^{14}C-labeled amino acid-labeled cores in addition to the 10,000 to 12,000-molecular-weight, arginine-rich polypeptides are not acid soluble but are released or dissociated from the core under acidic conditions.

Studies are now underway to determine which of the released polypeptides are associated with the surface layers of the core and more particular the palisade layer, which appeared to be removed (Fig. 3) when cores were extracted with acid.

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