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Structural Polypeptides of Rabbit, Bovine, and Human Papillomaviruses

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The number and apparent molecular weight of the structural polypeptides of Shope rabbit papilloma virus (RPV), bovine papilloma virus (BPV), and human papilloma virus (HPV) were estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Up to 10 polypeptides were detected in highly purified BPV and HPV full particles; a close homology was found between the polypeptide composition of both viruses. Purified RPV virions gave a similar polypeptide pattern. The main components of the three papillomaviruses are the major polypeptide (VP1) with a mol wt of approximately 54,000 and the three smaller polypeptides (VP8, 9, 10) with mol wt of about 16,500, 15,500, and 12,500, respectively. VP8, VP9, and VP10 are never detected in empty capsids. When BPV virions were disrupted with alkaline buffer, the six lower-molecular-weight polypeptides (VP5 to 10) remained associated with viral DNA. This suggests that they are internal components of the virions and that the four higher-molecular-weight polypeptides (VP1 to 4) may represent external components. The polypeptide compositions of BPV and polyoma virus, another papovavirus, have been compared. The number of BPV and polyoma virus components (10 and 6, respectively) and the molecular weight of their major polypeptide (54,000 and 44,500, respectively) are different; however, the three main DNA-associated polypeptides of BPV (VP8, 9, 10) and the three histone-like components of polyoma virus (VP4, 5, 6) were shown to have identical apparent molecular weights. The possibility that some of the minor components of papillomaviruses may be proteolytic degradation products or cell protein contaminants is discussed.

Members of the papovavirus group are divided into polyomavirus and papillomavirus subgroups on the basis of the diameter of the capsid and the molecular weight of the viral DNA (4, 5, 32). Numerous studies were reported on the structural polypeptides of some members of the polyomavirus subgroup, i.e., simian virus 40 (SV40), polyoma virus, and human papovaviruses. These viruses contain at least six polypeptides, one major component with a mol wt of 42,000 to 48,000 (VP1) and five minor components with mol wt of 32,000 to 35,000 (VP2), 23,000 to 25,000 (VP3), 14,000 to 19,000 (VP4), 12,500 to 17,000 (VP5), and 10,000 to 15,000 (VP6), respectively (7, 14, 15, 23, 27). In contrast, only a few studies have been reported on the polypeptide composition of papillomaviruses. These viruses induce skin or mucosa benign epithelial tumors under naturally occurring conditions in several animal species, essentially cottontail rabbit, dog, bovine, horse, and man (3, 25, 28). They are usually species specific and do not show a detectable serological cross-reaction (20). They do not replicate in tissue culture, but high amounts of virus may be obtained from papillomas. Previous studies suggested the occurrence of a unique polypeptide subunit in Shope rabbit papilloma virus (17) and of one major component (mol wt, 53,000) with several minor components in human papilloma virus (26).

We report in this paper studies on the structural polypeptides of three papillomaviruses: rabbit Shope papilloma virus (RPV), bovine papilloma virus (BPV), and human papilloma virus (HPV). Ten polypeptides were evidenced by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis in the full particles of these viruses, mainly one major component with an apparent mol wt of 54,000 and three

low-molecular-weight components (VP8, 16,500; VP9, 15,500; VP10, 12,500). Polypeptides VP8, 9, and 10 are not present in empty capsids. They are found associated with the viral DNA after alkaline disruption of BPV and seem to be similar to the three histone-like components (VP4 to 6) evidenced in polyoma virus and SV40 (11, 16, 19, 29).

MATERIALS AND METHODS

Virus purifications. BPV was obtained from bovine cutaneous fibropapillomas collected in six different regions of France and stored at 4 C in a 50% glycerol-0.15 M NaCl solution. Virions and empty capsids were purified according to the procedure detailed previously (8). Briefly, the supernatant of the tumor extract was treated with trypsin and sarkosyl, and then, after high-speed centrifugation, the pellets were incubated with collagenase. After another high-speed sedimentation, full and empty viral particles were separated by equilibrium centrifugation in a CsCl gradient and then further purified by a second equilibrium centrifugation and sedimentation in a sucrose gradient.

HPV was obtained from plantar warts stored at −70 C. Warts were collected and kindly made available to us by P. Agache (Clinique Dermatologique, C.H.U., Besançon, France). Warts (5 g/extraction) were washed in a 0.15 M NaCl solution, minced with scissors, ground with quartz powder in a mortar, and suspended in 50 ml of 1 M NaCl, 0.05 M sodium phosphate buffer, pH 8. The homogenate was centrifuged at 8,000 × *g* for 10 min at 4 C. The pellet was reextracted as before. The two supernatant fluids were pooled and incubated at 37 C for 1 h in the presence of trypsin (1:250, Difco) at a final concentration of 0.01%. After centrifugation at 8,000 × *g* for 10 min, the viral particles were pelleted at 37,500 rpm for 60 min (Spinco 40 rotor) and resuspended in 10 ml of 0.05 M NaCl, 0.01 M EDTA, 0.05 M sodium phosphate buffer, pH 7.4. The viral suspension was centrifuged at 8,000 × *g* for 10 min, and the pellet was homogenized in 2 ml of the same buffer and centrifuged as above. The two supernatant fluids were pooled and solid CsCl was added to a final density of 1.3 g/ml. After centrifugation in a Spinco SW39 rotor at 34,000 rpm for 24 h at 4 C, the two sharply visible bands corresponding to full particles and empty capsids were collected separately by puncturing the bottom of the tube. The homologous bands were pooled and dialyzed against the same buffer. Full and empty particles were further purified by isopycnic equilibrium centrifugation in CsCl, followed by sedimentation in a 21 to 61% sucrose gradient (24) as described for BPV (8).

RPV was obtained from cottontail rabbit cutaneous papillomas provided by Earl Johnson (Rago, Kan.) and stored at 4 C in a 50% glycerol-0.15 M NaCl solution. Papillomas (10 g) were washed, minced, ground, and suspended in 100 ml of 0.15 M NaCl, 0.05 M sodium phosphate buffer, pH 7.2. The homogenate was centrifuged at 8,000 × *g* for 30 min. The viral particles were then pelleted at 37,500 rpm for 60 min and resuspended in 8 ml of 0.05 M NaCl, 0.01 M EDTA, 0.05 M sodium phosphate buffer, pH 7.4. The viral suspension was centrifuged at 8,000 × *g* for 10 min, and the pellet was re-extracted with 2 ml of the preceding buffer. Supernatants were pooled and centrifuged at 37,500 rpm for 60 min, and the pellet was suspended in a small volume of the same buffer; after low-speed centrifugation, viral particles were sedimented in a 21 to 61% sucrose gradient as described for BPV (8). Four visible bands (24) were collected separately. The homologous bands were pooled, dialyzed, and observed under electron microscope. The fraction containing isolated full viral particles was used for electrophoretic analysis.

Purified polyoma virus (large-plaque strain) (6) was a gift of A. Parodi (Département de Biologie Moléculaire, Institut Pasteur, Paris).

All virus preparations were dialyzed against 0.1 M NaCl, 0.001 M EDTA, 0.05 M sodium phosphate buffer, pH 7.3, and stored at −20 C. Protein concentration was determined by the method of Lowry et al. (21) using bovine serum albumin (Pentex, Miles) as a standard. The homogeneity of the preparations was checked by examination of viral particles under a Siemens Elmiskop 101 electron microscope after staining with a 2% sodium phosphotungstate solution, pH 7.2.

Virus dissociation and polyacrylamide gel electrophoresis. Viral preparations (1 to 2 mg/ml) were dissociated by 3-min incubation in boiling water in the presence of 8 M urea, 2% SDS, and 0.2 M 2-mercaptoethanol. In some experiments, the reduced proteins were alkylated with iodoacetamide under the conditions described by Roblin et al. (27). Acrylamide gels (10%) were prepared essentially as described by Maizel (22) and used in an Acrylophor Pleuger apparatus. A mixture of acrylamide and *N*, *N'*-methylenebisacrylamide (ratio, 50:1) was polymerized in the presence of *N,N,N',N'*-tetramethylenediamine (0.08%) and ammonium persulfate (0.08%). Gel and electrode buffer consisted of a 0.1 M sodium phosphate buffer, pH 7.2, containing 0.1% SDS. The gels (0.6 by 7 cm) were prerun for 2 h at 8 mA/gel before the samples were applied. Thirty to 200 μg of viral proteins, in a volume of 100 μl, were applied on each gel in the presence of bromophenol blue (0.0005%) as a tracking dye. After 4 to 5 h of electrophoresis (8 mA/gel), the gels were stained with Coomassie brilliant blue, electrophoretically destained for 3 h, and scanned with a Vernon model TRD 5 densitometer.

Molecular weight determination. SDS-dissociated papillomaviruses (50 μg of protein) were coelectrophoresed with standard proteins (10 μg of each), chymotrypsinogen (mol wt, 25,000), pepsin (mol wt, 36,000), ovalbumin (mol wt, 45,000), and bovine serum albumin (mol wt, 67,000). The molecular weights of the main components VP1, 8, 9, and 10 were determined according to the method of Shapiro et al. (30). One hundred to 200 μg of dissociated viral proteins was simultaneously electrophoresed in another gel, allowing the estimation of the molecular weights of the minor components (VP2 to 7) from the

apparent molecular weights determined in each run for VP1, 8, 9, and 10. The electrophoresis, under identical conditions, of polyoma virus (100 μg), BPV (200 μg), and standard proteins (10 μg of each), cytochrome c (mol wt, 12,400), chymotrypsinogen, pepsin, and ovalbumin allowed the comparison of the apparent molecular weights of the structural polypeptides of both viruses.

Alkaline disruption of BPV. Preparations of full BPV particles (1 to 1.5 mg of viral proteins, corresponding to about 10^8 hemagglutinating units [8]) were sedimented at 37,000 rpm for 1 h (Spinco 40 rotor). The pellet was suspended in 1 ml of 0.2 M sodium carbonate buffer, pH 10.6, containing 0.01 M dithiothreitol (13) and then incubated for 6 h at 0 C with frequent shakings. The degradation products were applied on an agarose (A 1.5 m, Bio-Rad) column (1 by 45 cm), equilibrated at 4 C with 0.2 M sodium carbonate buffer, pH 9.0, and then eluted with the same buffer at a flow rate of 10 ml/h. The column had been previously calibrated with standard proteins: catalase (mol wt, 250,000), bovine serum albumin, and cytochrome c. The void volume was determined using PM_2 phage DNA (mol wt, 6×10^6) and T_5 phage. Fractions (0.5 ml) were collected and assayed for hemagglutinating activity (8). Their absorbance at 260 and 280 nm was checked on a PMQII Zeiss spectrophotometer. The fractions corresponding to each peak were pooled and dialyzed against 0.01 M NaCl, 0.005 M sodium phosphate buffer, pH 7.3. Pools were concentrated by evaporation and then analyzed by polyacrylamide gel electrophoresis in the presence of SDS as described above.

RESULTS

Virus purifications. Full and empty particles of BPV and HPV, purified according to the procedures described above, including two equilibrium centrifugations in CsCl and one sedimentation in a sucrose gradient, are homogeneous and constituted of isolated particles, as illustrated for HPV in Fig. 1. The recovery of full and empty BPV particles ranged, respectively, from 0.2 to 200 μg and from 0.1 to 30 μg of viral proteins per g of tumor tissue; for full and empty HPV particles, it ranged, respectively, from 200 to 400 μg and from 75 to 100 μg of viral proteins per g of plantar warts. Since only small amounts of cottontail rabbit papillomas were available, RPV was only purified by two cycles of differential centrifugations and one sedimentation in a sucrose gradient. The viral preparation obtained consisted mainly of isolated full particles but contained a small percentage of isolated empty capsids and small aggregates of empty and full particles.

Polypeptide composition of full BPV, HPV, and RPV particles. The results obtained after SDS-polyacrylamide gel electrophoresis of SDS-dissociated BPV and HPV virions are shown in Fig. 2a and c. Both viruses show very close polypeptide compositions (Fig. 3). Up to 10 polypeptides are detected when 100 to 200 μg

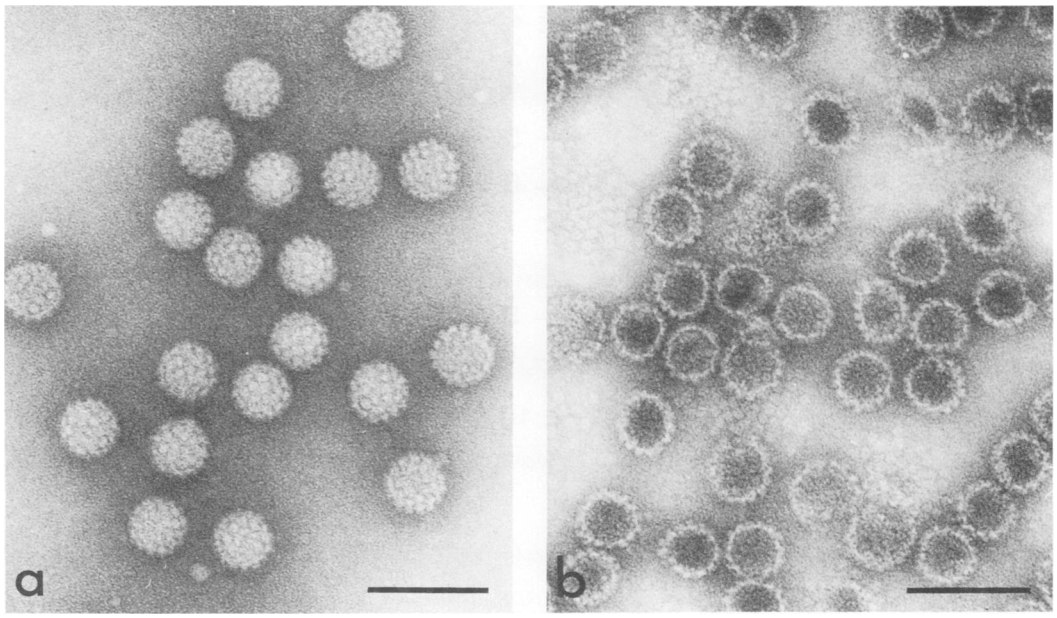

FIG. 1. *Electron micrographs of full and empty HPV particles. Full (a) and empty (b) HPV particles were purified as described in Materials and Methods and observed after staining with a 2% sodium phosphotungstate solution. Bar, 0.1 μm.*

of viral proteins are electrophoresed. The major polypeptide VP1 and the lower-molecular-weight components VP8, 9, and 10 are regularly detected in the 19 preparations of BPV and five preparations of HPV tested. The other components are not always evidenced (Table 1). Moreover, the relative proportions of the minor components may differ in various preparations of the same virus and, as illustrated for BPV and HPV in Fig. 3, from one virus to the other. Scans of the stained gels may be used to quantitate approximately the amount of each polypeptide, providing that the protein content of each band does not exceed 20 μg, to follow the Beer-Lambert law after staining with Coomassie brilliant blue (7, 9). When 30 to 50 μg of viral proteins are electrophoresed, only the major polypeptide VP1 and the lower-molecular-weight components VP8, 9, and 10 are evidenced. Since VP8, 9, and 10 are not well separated, only the weight ratio of VP8, 9, and 10 to VP1 can be estimated. It was found to be 0.27 ± 0.06 and 0.28 ± 0.05 for BPV and HPV, respectively, as evaluated from the scans of the electrophoregrams obtained with six different BPV preparations and five different HPV preparations. A similar polypeptide pattern was observed for RPV when compared to BPV (Fig. 4), except for the absence of VP2 and the presence of two additional polypeptides (VP4′ and 7′). After electrophoresis of SDS-dissociated polyoma virus, only the six structural polypeptides already described (15, 27, 29) are evidenced (Fig. 2e).

The occurrence of additional peaks between the top of the gel and the major component VP1 was shown to depend on dissociation conditions. A relatively important amount of these proteins, as compared to that of VP1, remained after a 90-min incubation at 60 C in the pres-

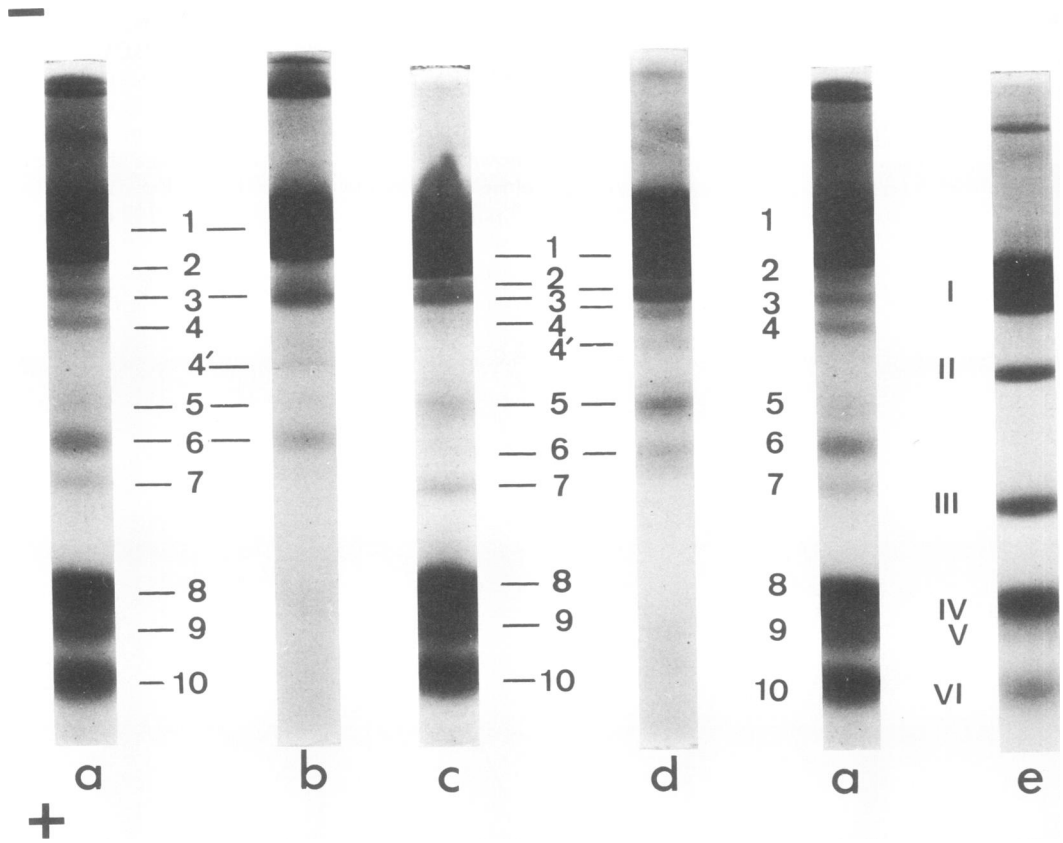

FIG. 2. *SDS-polyacrylamide gel electrophoresis of SDS-dissociated papillomaviruses. Viral particles were dissociated in the presence of urea, 2-mercaptoethanol, and SDS, electrophoresed, and stained as described in Materials and Methods. Full (a) and empty (b) BPV particles (200 μg of proteins) and full polyoma virus (e) particles (100 μg of proteins) were electrophoresed in the same run for 5 h. Full (c) and empty (d) HPV particles (200 μg of proteins) were electrophoresed in another run for 5 h.*

ence of 8 M urea, 2% SDS, and 0.2 M 2-mercaptoethanol. This amount was minimized when dissociation was performed by a 3-min incubation in a boiling water bath, but was not further eliminated by alkylation of the reduced proteins. These additional peaks could correspond to aggregates of polypeptides, as reported for SV40 (7) and polyoma virus (27).

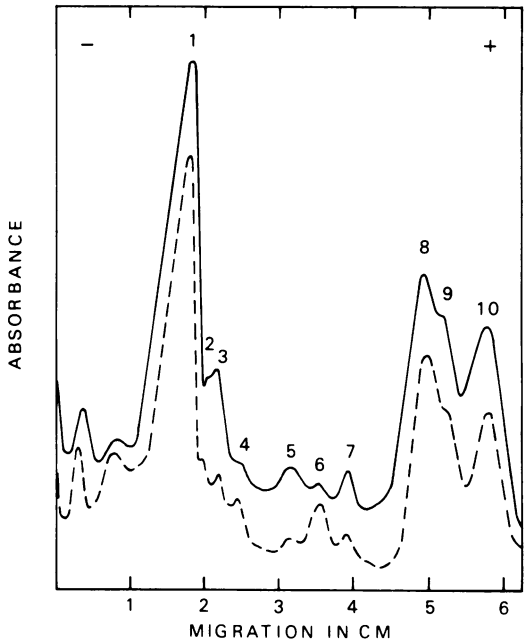

FIG. 3. *Densitometric recordings of stained electropherograms of SDS-dissociated full BPV and HPV particles. Full BPV (- - -) and HPV (——) particles (200 μg of proteins) were dissociated, electrophoresed in the same run for 5 h, and stained as described in Materials and Methods. Gels were scanned on a Vernon densitometer.*

Polypeptide composition of empty BPV and HPV particles. Up to seven polypeptides were detected in empty BPV capsids (Fig. 2b). When compared to full BPV particles (Fig. 5), the major and five minor components of empty capsids show the same electrophoretic mobilities as VP1, 2, 3, 5, 6, and 7, VP2, 5, and 7 being irregularly detected (Table 1). The minor component (VP4'), which migrates between VP4 and VP5, is always evidenced. Six polypeptides were detected in empty HPV particles (Fig. 2d). The major and four minor components have the same electrophoretic mobilities as VP1, 2, 3, 5, and 6 of full HPV particles; one minor component migrates like the VP4' component of empty BPV particles. The low-molecular-weight components VP8, 9, and 10 were never detected in 13 different preparations of empty BPV capsids and five preparations of empty HPV capsids.

Molecular weight determination of the polypeptides of RPV, BPV, and HPV. The molecular weights of the structural polypeptides of RPV, BPV, and HPV were estimated by electrophoresis on SDS-polyacrylamide gels in the presence of standard proteins (30) and compared to those of the polypeptides of polyoma virus. The apparent molecular weights of the polypeptides of the three

TABLE 1. *Molecular weights of structural polypeptides of papillomaviruses*

Viral polypeptides	BPV				HPV				RPV	
	Full particles		Empty particles		Full particles		Empty particles		Full particles	
	Mol wt[a]	N[b]	Mol wt	N	Mol wt	N	Mol wt	N	Mol wt	N
VP1	54,200 ± 1,500	19	54,800 ± 1,500	13	53,500 ± 600	5	53,600 ± 550	4	53,700 ± 600	3
VP2	49,200 ± 1,700	14	49,100 ± 1,850	7	47,900 ± 250	4	47,700 ± 600	3		
VP3	44,700 ± 1,700	11	44,100 ± 1,000	13	44,000 ± 900	5	44,700 ± 1,150	4	42,700 ± 1,500	3
VP4	40,600 ± 1,950	17			40,600 ± 950	4			39,000 ± 1,400	3
VP4'			36,200 ± 1,500	13			35,700 ± 750	4	35,000 ± 1,700	3
VP5	33,700 ± 1,500	9	32,500 ± 1,250	2	32,750 ± 1,700	5	30,700 ± 600	3	31,000 ± 1,000	3
VP6	29,350 ± 750	13	30,100 ± 1,500	10	29,600 ± 1,700	3	28,500 ± 500	4	28,200 ± 1,050	3
VP7	24,700 ± 550	14	25,000 ± 1,300	2	25,000 ± 1,500	5			23,700 ± 1,150	3
VP8	16,100 ± 700	19			16,600 ± 250	5			17,700 ± 1,500	3
VP9	15,500 ± 850	19			15,300 ± 250	5			16,200 ± 1,050	3
VP10	12,300 ± 850	19			12,300 ± 350	5			13,700 ± 1,000	3

[a] Molecular weights were determined by SDS-polyacrylamide gel electrophoresis as described by Shapiro et al. (30). Mean values ± standard deviations were determined from the scans of stained electrophoregrams of 19 and 13 preparations of full and empty BPV particles, of five and four preparations of full and empty HPV particles, and from three different electrophoregrams of the same RPV preparation.

[b] *N*, Number of electrophoregrams where each polypeptide was detectable; the total number of electrophoregrams is given in footnote *a*.

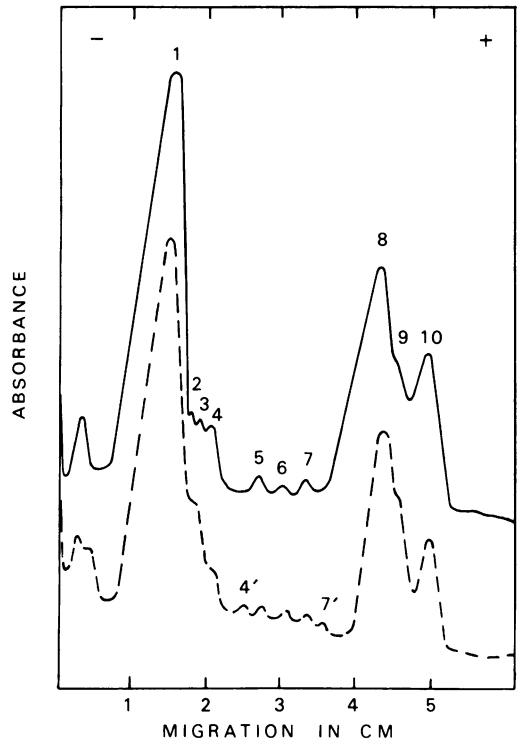

FIG. 4. *Densitometric recordings of stained electropherograms of SDS-dissociated full BPV and RPV particles. Full BPV (——) and RPV (- - -) particles (200 μg of proteins) were dissociated, electrophoresed in the same run for 4 h, and stained as described in Materials and Methods. Gels were scanned on a Vernon densitometer.*

papillomaviruses are reported in Table 1; those of the polyoma virus components obtained under the same conditions (Fig. 6) (VP1, 44,500; VP2, 35,000; VP3, 23,000; VP4, 16,700; VP5, 15,000; VP6, 12,500) are in close agreement with the values already reported (15, 27, 29). The results summarized in Table 1 illustrate the similitude between the polypeptides of BPV and HPV particles. They further show that RPV, BPV, and HPV share in common (i) a major component which has a higher apparent molecular weight (53,000 to 54,000) than that of the major polyoma virus polypeptide; (ii) three smaller components (VP8, 9, and 10), not detected in empty capsids, which have similar molecular weights (16,000 to 17,000; 15,000 to 16,000; 12,000 to 13,000, respectively) to those of the histone-like components of polyoma virus (VP4, 5, and 6) (Fig. 2a and e; Fig. 6) when BPV and polyoma virus are electrophoresed under identical conditions.

Evidence for the association of some of the polypeptides with viral DNA in BPV particles. Exposure to alkaline pH of either SV40 (1, 7) or polyoma virus (13) particles leads to the disruption of virions and allows the separation of external proteins from the low-molecular-weight proteins associated with viral DNA. When BPV preparations were incubated at pH 10.6 in the presence of dithiothreitol, their hemagglutinating activity was lost almost instantaneously. After 6 h of incubation, neither viral particles nor capsomeres could be detected under the electron microscope. Filtration of the disruption products on an agarose column, previously calibrated with standard proteins, separated the material into two peaks (Fig. 7). The first peak, eluting in the void volume of the column, had a 260- to 280-nm absorbance ratio of 1.72, suggesting the presence of a DNA-protein complex. The second broad peak showed a 260- to 280-nm absorbance ratio of 0.9, characteristic of proteins. The elution volume of this peak, which is slightly higher than that of bovine serum albumin, suggests that it contains mainly the major polypeptide VP1.

The polypeptides contained in each of these

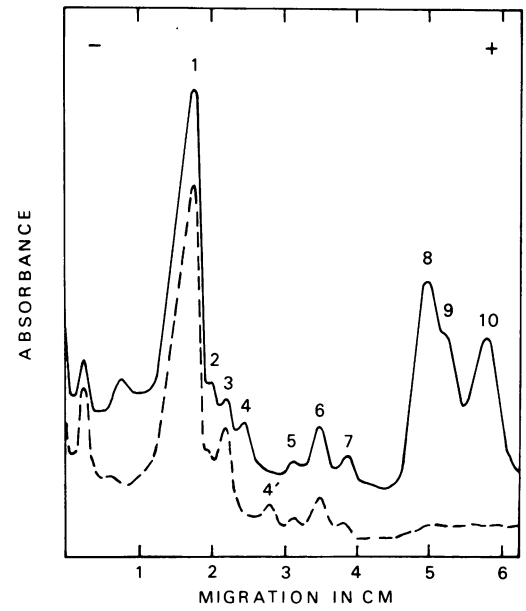

FIG. 5. *Densitometric recordings of stained electropherograms of SDS-dissociated full and empty BPV particles. Full (——) and empty (- - -) BPV particles (200 μg of proteins) were dissociated, electrophoresed in the same run for 5 h, and stained as described in Materials and Methods. Gels were scanned on a Vernon densitometer.*

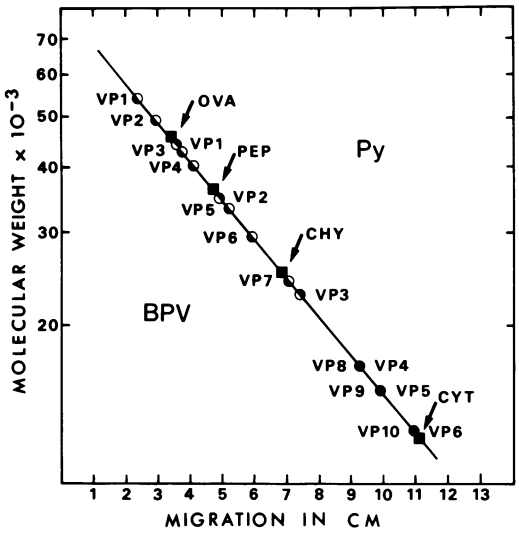

FIG. 6. *Migration of SDS-dissociated BPV, polyoma virus, and standard proteins in SDS-polyacrylamide gels as a logarithmic function of molecular weights. BPV polypeptides (◔, 200 μg), polyoma virus (Py) polypeptides (◔, 100 μg), and standard proteins (■, 10 μg of each), ovalbumin (OVA), pepsin (PEP), chymotrypsinogen (CHY), and cytochrome c (CYT), were electrophoresed in the same run under the conditions described in Materials and Methods. The migration of the polypeptides was measured on the scans of the stained gels.*

peaks were further characterized by polyacrylamide gel electrophoresis in the presence of SDS. The low-molecular-weight peak contained the major component VP1, the minor components VP2, 3, and 4, and traces of VP5, 6, and 7 (Fig. 8). The DNA-containing peak contained most of VP5, 6, and 7, the lower-molecular-weight polypeptides VP8, 9, and 10, and a small amount of VP1. Identical results were obtained with two viral preparations. This suggests that the higher-molecular-weight polypeptides (VP1 to 4) are capsid components and that the lower-molecular-weight polypeptides, especially VP8, 9, and 10, are internal components of the viral particle.

DISCUSSION

Papovaviruses are divided into polyomavirus and papillomavirus subgroups on the basis of the size of their capsid and of the molecular weight of their genome (4, 5, 32).

Polyomaviruses have a capsid with a diameter of about 45 nm and a DNA with a mol wt of 3.5×10^6, giving a coding potential for about seven medium-sized polypeptides with a total mol wt which does not exceed 175,000. Six polypeptides, at least, are evidenced in the virions (7, 14, 15, 23, 27). The higher-molecular-weight components VP1, 2, and 3 are presumably external components and may constitute the capsomeres (7, 13). They are detected in SV40 lytically infected cells only after the onset of viral DNA replication and are probably virus coded (2, 29, 31). The minor components of low-molecular-weight VP4, 5, and 6 were reported to be associated with viral DNA (7, 13, 16, 19) and may be host cell histones (11, 19, 29), the role of which in the regulation of the expression of the viral genome (16) or in the packaging of viral DNA (13) has been considered.

Papillomaviruses have a capsid of about 55 nm in diameter and a DNA with a mol wt of 5×10^6, giving a coding potential for about 11 medium-sized polypeptides with a total mol wt of about 250,000. The number and molecular weights of the structural polypeptides of RPV, BPV, and HPV reported in this paper may contribute to further distinguish between papil-

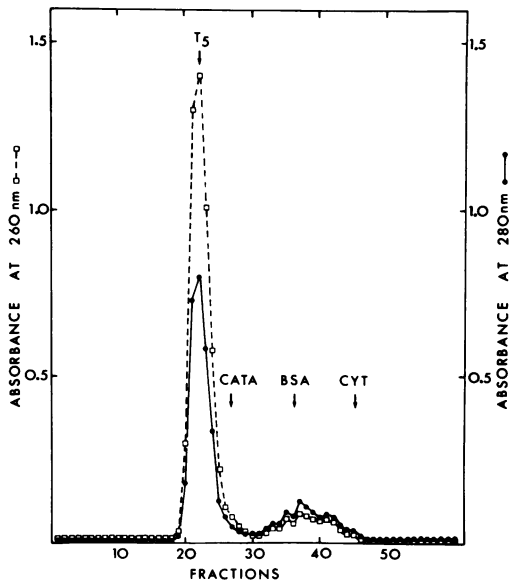

FIG. 7. *Agarose gel filtration of alkali-disrupted full BPV particles. Full BPV particles (1.5 mg) were alkali disrupted, and the disruption products were filtered on an agarose column as described in Materials and Methods. The column had been previously calibrated with T_5 phage (T_5), catalase (CATA), bovine serum albumin (BSA), and cytochrome c (CYT). The fractions (0.5 ml) were collected, and the absorbance at 260 nm (□) and 280 nm (●) was determined.*

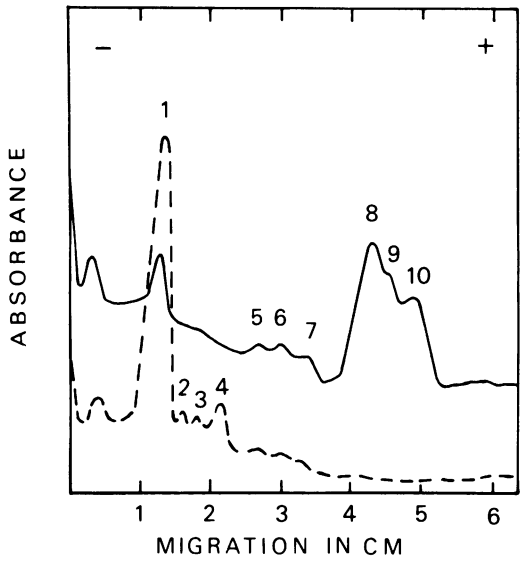

FIG. 8. *Densitometric recordings of stained electropherograms of the viral components obtained after agarose filtration of the alkali-disrupted BPV virions. The fractions corresponding to the excluded peak (——) and the included peak (- - -) were pooled, dialyzed, and concentrated. Samples of each pool (75 μg of proteins) were dissociated, electrophoresed for 4 h, and stained as described in Materials and Methods. Gels were scanned on a Vernon densitometer.*

lomaviruses and polyomaviruses. Whereas six structural polypeptides are detected in the full particles of polyomaviruses, 10 components were evidenced in BPV and HPV virions. Whereas the major component of polyomaviruses has a mol wt of 42,000 to 48,000 (7, 14, 15, 23, 27), the major polypeptide component of papillomaviruses, VP1, has an estimated mol wt of 53,000 to 54,000, in agreement with the previously reported data for HPV (26). The number of VP1 molecules per virion may be evaluated to 370, taking into account that VP1 constitutes 80% of the main viral polypeptides, as estimated from the scans, and that the total mol wt of structural polypeptides is about 25×10^6, as calculated from the mol wt of the DNA (5×10^6) (4) and the percentage of DNA per virion (17.5%) (17). This value is consistent with the number of subunits required (420) to constitute the icosahedral capsid of the papovaviruses made of 72 capsomeres (10, 18, 32). The higher molecular weight of the subunit accounts for the larger size of the capsid of papillomaviruses as compared to that of polyomaviruses. However, like polyomaviruses, papillomaviruses contain three polypeptides with apparent mol wt between 12,000 and 17,000 (VP8, 9, and 10) which are not detected in empty capsids and are presumably associated with the viral DNA. Taking into account that they constitute approximately 20% of the main viral polypeptides and affording a mean value of 15,000 for their mol wt, the weight ratio of VP8, 9, and 10 to the viral DNA can be estimated to 1.4. This is higher than the value (about 1) calculated from the data reported for radiolabeled SV40 (7) and polyoma virus (13, 27). This difference may be explained by the overestimation of the amount of VP8, 9, and 10, due both to the conditions of their evaluation by optical scanning of the stained gels, as discussed for SV40 (7), and to the inadequacy of the technique to quantitate the amount of the minor components (VP2 to 7).

The total molecular weight of the structural polypeptides of BPV and HPV (310,000) exceeds the total coding capacity of the viral genome. This suggests that some of the polypeptides are either cleavage products of viral proteins or cell-coded proteins as discussed previously for SV40 and polyoma virus (2, 7, 11, 12, 19, 27, 29, 31). For instance, some of the minor polypeptides of papillomaviruses, which occur in small and variable amounts, may result from in vivo degradation of the major polypeptide VP1 by the proteolytic enzymes involved in the disappearance of the nucleus and cytoplasmic organelles occurring in the last step of the keratinization process in the epidermal host cell. Proteolytic degradation may also occur in vitro during the grinding of the tumors or at the stage of the tryptic treatment of the tumor extracts. This treatment, which was shown to favor the obtention of two well-defined bands of full and empty papillomavirus particles after equilibrium centrifugation in a CsCl gradient (5), was omitted in the purification procedure of RPV. Under these conditions, all the polypeptides found in BPV and HPV particles are evidenced in RPV, except one corresponding to VP2. Moreover, some polypeptides may be the result of a natural cleavage of a single viral gene product, as recently reported for three polyoma virus components (VP1, 2, 3) (12). Finally, some of the polypeptides of papillomaviruses could be host cell proteins either contaminating the viral preparations or being a structural component of the virions. The first hypothesis cannot be excluded for some of the minor components, although highly purified BPV and HPV preparations were used in this study. Indeed, additional minor polypeptides, believed to be cellular contaminants, are detected in highly purified radiolabeled preparations of SV40 (31) and polyoma virus (13). The second hypothesis is

most likely for polypeptides VP8, 9, and 10, which share common properties with the three histone-like components (VP4, 5, 6) of SV40 and polyoma virus. Three other components of papillomaviruses (VP5, 6, and 7), which were shown to be associated with viral DNA in the case of BPV, may have a similar origin. The regular occurrence of some of these components in empty capsids (VP6 for BPV and VP5 and 6 for HPV) suggests that they might be involved in DNA-protein, as well as protein-protein, interactions and might play a role in the assembly of viral components as suggested for VP3 of SV40 and polyoma virus (7, 13).

Experiments on the in vitro reassembly of viral particles after alkaline disruption of the virions, as reported for polyoma virus (13), may contribute to ascertaining the structural role of the polypeptides evidenced in papillomaviruses.

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