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Structural Proteins of *Herpesvirus saimiri*

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Herpesvirus saimiri particles were purified from productively infected owl monkey kidney cell cultures, and the virion polypeptides were analyzed by polyacrylamide gel electrophoresis. A total of 21 predominant proteins were found in lysates of *H. saimiri* 11 particles by Coomassie blue staining or by [^{35}S]methionine labeling and autoradiography; all proteins were between 160,000 and 12,000 daltons in size. They are most probably virion constituents, as most of them were precipitated by immune sera, and no dominant proteins of equivalent sizes were found in mock-infected cultures. Four glycoproteins (gp 155/160, gp 128, gp 84/90, gp 55) and three polypeptides that appeared not to be glycosylated (p71, p35, p28) were assigned to the envelope or matrix of virions, whereas at least four phosphoproteins (pp132, pp118, pp55, pp13) and ten polypeptides without apparent secondary modification (p155/160, p106, p96, p67, p53, p36, p32, p15, p14, p12) were found in the nucleocapsid fraction. Analysis of virion proteins from different *H. saimiri* strains did not reveal appreciable differences in the migration behavior of most polypeptides, including all glycoproteins; however, determination of a strain-specific size pattern was possible for three of four phosphoproteins. The overall similarity in protein architecture of *H. saimiri* strains obviously does not reflect the variability in biology, such as oncogenic properties. In comparison, DNA sequence divergences appear to remain a better taxonomic criterion for strain distinction.

Herpesvirus saimiri is a highly oncogenic agent in numerous species of New World primates and in rabbits (for reviews, see Deinhardt et al. [6] and Fleckenstein [9]). Tamarin marmoset monkeys (*Saguinus* sp.) infected with *H. saimiri* wild-type strains consistently develop rapidly progressing malignant T-cell lymphomas or leukemias, irrespective of the dose of inoculum, the age of the animals, or the virus strains applied. However, in other monkeys, such as common marmosets (*Callithrix jacchus*) and owl monkeys (*Aotus trivirgatus*), oncogenic properties seem to depend on the particular virus strain used for infection (reviewed by Fleckenstein and Desrosiers [11]). *H. saimiri* grows in various monolayer cultures of primate origin in a productive replication cycle, yielding sufficient amounts of virion structural components for biochemical characterizations and strain comparisons.

In contrast to virion proteins, the DNA structure of *H. saimiri* has been described in detail. The genome organization of the virus is very much different from that of all other herpesviruses previously characterized. The predominant type of DNA molecules (M-DNA) consists of a unique segment of L-DNA (112 kilobase pairs [kbp], 36% G+C) which is framed at both ends by various numbers of H-DNA repeat units (1.4 kbp, 70.8% G+C) in tandem orientation (2). Comparative cleavage of DNA from more than 20 independent *H. saimiri* strains with numerous restriction endonucleases yielded distinct but related patterns, indicating that the basic genome structure is the same for all strains (7). Cleavage maps indicated colinear arrangement of DNA sequences within the L-DNA of *H. saimiri* strains, and, except for the leftmost 7 to 10 kbp of L-DNA, very little base pair divergence became evident from determinations of melting temperatures of heteroduplex molecules (16).

When permissive cell cultures are infected with long overlapping restriction fragments of M-DNA from different *H. saimiri* strains by the calcium phosphate technique, a number of recombinant viruses can be isolated (16). This approach allowed the convenient and efficient generation of recombinants between different *H. saimiri* strains, without resorting to using conditionally lethal mutants. Intertypic recombinants of herpes simplex virus have proven to be a useful tool to localize the viral DNA sequences coding for virion polypeptides (19). Aiming at a similar approach to the functional mapping of DNA, we initiated a study of the proteins of *H.*

saimiri, determining number, size, glycosylation, and phosphorylation of the prominent structural polypeptides from three independent virus strains.

MATERIALS AND METHODS

Virus and cell culture. *H. saimiri* prime strain (S295C) originated from a primary squirrel monkey kidney cell culture (18); virus strain 11 was obtained by cocultivation of squirrel monkey lymphocytes with permissive monolayers (8), and strain OMI was isolated from an owl monkey with spontaneous lymphoma (15). The viruses were grown in monolayers of owl monkey kidney (OMK) cells of strains 210 and 637 (5, 23). The cells were checked for mycoplasma at regular intervals by cultivation under anaerobic conditions (12) and found to be free of contamination. To circumvent mycoplasma growth in seed viruses, all stocks were established by transfection with purified virion M-DNA. Virus stocks titrated on OMK cells under 2% (wt/vol) methylcellulose contained ca. 10^6 PFU/ml. Virus particles were purified from cell culture fluids by velocity centrifugation in sucrose gradients as described previously (10).

Labeling of proteins and gel electrophoresis. Virion proteins were labeled with [^{35}S]methionine (10 to 150 μCi/ml, ≥8,000 Ci/mmole; Amersham-Buchler, Braunschweig) by growing the virus in minimum essential medium and 5% fetal calf serum in the absence of methionine or at a reduced methionine concentration (0.05 mM). Phosphoproteins were labeled by the addition of 50 μCi of $^{32}P_i$ per ml (200 mCi/mmole; Amersham-Buchler) in minimal essential medium–5% fetal calf serum.

Before electrophoresis, viral proteins were denatured by boiling them for 5 min in electrophoresis sample buffer (2% [wt/vol]) sodium dodecyl sulfate (SDS)–0.1 M dithiothreitol–10% [vol/vol] glycerol–0.025% bromophenol blue stain–0.08 M Tris-hydrochloride [pH 6.8]). Separation of viral polypeptides was carried out on 7.5 to 20% (wt/vol) gradient gels and on 10 to 15% (wt/vol) linear polyacrylamide gels (1). Fixed gels were processed for staining with Coomassie blue and autoradiography or fluorography with En^{3}Hance (New England Nuclear Corp., Boston, Mass.).

Detection of glycosylated proteins. Glycoproteins were labeled after polyacrylamide gel electrophoresis (PAGE) with ^{125}I bound to concanavalin A (ConA) as described by Burridge (4). A 2-mg amount of ConA was incubated with 5 mCi of Na^{125}I (Amersham-Buchler, Braunschweig) in the presence of 100 μg of chloramine T and 25 mg of D-mannose at room temperature for 30 min. Labeled ConA was purified by chromatography through a Bio-Gel P-10 column in a 50 mM Tris-hydrochloride buffer (pH 7.1) with 250 mM $MnCl_2$, 0.5 mM MgCl, and 0.01% NaN_3. Each slab gel was incubated in 1.5×10^9 cpm ^{125}I-labeled ConA for 16 h, washed extensively, and exposed for autoradiography.

Immunoprecipitation. Precipitations of radioactive viral proteins from purified virions or infected OMK cells with immune sera were essentially done as described by Bodemer et al. (1). Cells were lysed in 50 to 100 μl of RIPA buffer (0.1% SDS–1% sodium desoxycholate–1% Triton X-100–150 mM NaCl–100 μg of phenylmethylsulfonylfluoride per ml–10 mM Tris-hydrochloride [pH 7.2]) (3). The sample cells were sonicated in an ultrasonic waterbath for 1 min and clarified by centrifugation at 10,000 × *g* for 2 min. The extracts were incubated with 1% DL-methionine and with immune serum at a final dilution of 1:10 in a 25-μl reaction volume for 1 h at room temperature. The volume was increased to 100 μl with RIPA buffer before adding 5.0 μl of *Staphylococcus aureus* protein A immunoabsorbent (10% [vol/vol]) (Calbiochem-Behring, Giessen, West Germany). After adsorption for 2 min, bacteria and bound immune complexes were centrifuged at 10,000 × *g* for 2 min. The pellet was washed five times with RIPA buffer and suspended in 20 μl of electrophoresis sample buffer for PAGE. Immune sera and negative control sera from owl monkeys (*Aotus trivirgatus*), cotton top marmosets (*Saguinus oedipus*), squirrel monkeys (*Saimiri sciureus*) and spider monkeys (*Ateles geoffroyi*) were kindly provided by Lawrence A. Falk. The positive monkey sera had antibody titers of 1:128 to 1:512 in indirect immunofluorescence tests with late *H. saimiri* antigens.

Separation of nucleocapsid and envelope proteins. Nucleocapsid and membrane proteins were prepared from purified virus particles by a modification of the method of Sarmiento and Spear (22). ^{35}S-labeled virions (300,000 cpm) were incubated in 50 μl of a solubilization buffer (1% Nonidet P-40–200 mM NaCl–10 mM KCl–5 mM EDTA–50 mM Tris-hydrochloride [pH 7.5]) for 30 min on ice. The nucleocapsids were sedimented by centrifugation in a Beckman Airfuge at 26 lb/in^2 for 20 min, washed twice, and examined by electron microscopy.

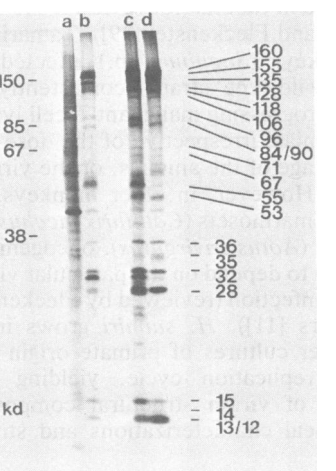

FIG. 1. Separation of [^{35}S]methionine-labeled proteins from cell extracts of *H. saimiri*-infected OMK cells and purified virus particles in a 15% polyacrylamide gel. Lanes: a, Uninfected cells; b and c, infected cell extracts; and d, purified virions. Proteins were labeled with 40 μCi of [^{35}S]methionine per ml in methionine-free medium from 93 to 102 h after infection (a and b) and 90 to 144 h after infection (c and d).

TABLE 1. Sizes, modification, and structural arrangement of virion proteins of three *H. saimiri* strains

	Designation of proteins of strain:			Localization in virion		Precipitation by immune sera
	Prime (S295C)	No. 11	OMI	Envelope	Nucleocapsid	
1	(g)p 160	(g)p 160	(g)p 160			+
2	(g)p 155	(g)p 155	(g)p 155	+	+	+
3	pp 135	pp 135	pp 135		+	+
4	gp 128	gp 128	gp 128	+		+
5	pp 87	pp 118	pp 91		+	+
6	p 106	p 106	p 106		+	+
7	p 96	p 96	p 96		+	+
8	gp 84/90	gp 84/90	gp 84/90	+		+
9	p 71	p 71	p 71	+		+
10	p 67	p 67	p 67		+	+
11	pp 57	pp 55	pp 55		+	+
12	gp 55	gp 55	gp 55	+		+
13	p 53	p 53	p 53		+	
14	p 36	p 36	p 37		+	+
15	p 35	p 35	p 35	+		
16	p 32	p 32	p 32		+	+
17	p 28	p 28	p 28	+		+
18	p 15	p 15	p 15		+	+
19	p 14	p 14	p 14		+	+
20	pp 13/14	pp 13	pp 13		+	+
21	p 12	p 12	p 12		+	+

RESULTS

Number and sizes of virion proteins. Polyacrylamide gel electrophoresis and autoradiography of ^{35}S-labeled denatured proteins from purified *H. saimiri* particles resolved at least 21 clearly discernible polypeptides in the size range between 160 and 12 kilodaltons (K) (Fig. 1d). These polypeptides are listed in Table 1. Each of these polypeptides could also be identified in extracts of virus-infected OMK cells (Fig. 1, b and c), but not in mock-infected cells (Fig. 1a). A number of proteins appeared in the cell extracts in significantly higher proportions than in the corresponding bands from purified particles. Namely, relatively abundant bands of cell extracts were found at the positions of 57, 30, 24, 23, and 18 K. An additional 17-K prominent polypeptide was seen in some, but not all cell extracts. Probably, all of these polypeptides are early viral proteins, precursors of structural proteins, or cellular proteins overproduced in infected cells. Also, all gels of [^{35}S]methionine-labeled proteins revealed a band at the 46-K position which is most predominant in mock-infected cells. Since it corresponded to the known size of cellular actin, this polypeptide was not assumed to be of viral origin.

All except two of the viral proteins listed in Table 1 could be precipitated by immune sera from infected squirrel monkeys (*Saimiri sciureus*), owl monkeys, cotton top marmosets (*Saguinus oedipus*), spider monkeys, or New Zealand white rabbits. Figure 2 gives a survey of the virion proteins precipitated from [^{35}S]methionine-labeled cells with sera from owl and squirrel monkeys and a rabbit. The 53-K protein could not be recognized clearly, probably because of low abundance in the virus or low specific labeling. Also, the 35-K protein was not seen, possibly because of the apparent heterogeneity in migration (Fig. 1d). The 57-K protein was precipitated by the owl monkey serum, however, not by antibodies from an immunized rabbit.

Since abundant structural polypeptides 155 to 160 K in size are usually not resolved by gel electrophoresis, some immunoprecipitations were done with reduced amounts of viral proteins. Two proteins (155 K and 160 K) were recognized by sera from a squirrel monkey, a cotton top marmoset, and an owl monkey (Fig. 3). The serum from the spider monkey appeared to precipitate the smaller protein only. Some experiments showed that in contrast to the 160-K polypeptide, the 155-K protein is precipitated by protein A in the absence of serum. Immunoprecipitations with owl monkey and rabbit sera, using gel conditions that allowed separation of small proteins similar in size to cellular histones (12 to 15 K), are shown in Fig. 4. Apparently, at least four polypeptides became discernible upon autoradiography. Virion polypeptides of three different *H. saimiri* strains were analyzed in parallel with regard to their migration behavior in denaturing polyacrylamide gels. The majority

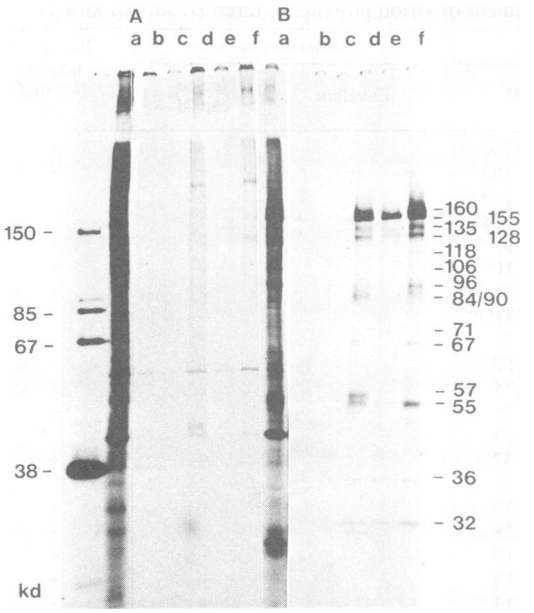

FIG. 2. Precipitation of ^{35}S-labeled *H. saimiri* proteins with immune sera and separation in a 12.5% polyacrylamide gel. Proteins were labeled with 50 μCi of [^{35}S]methionine per ml from 96 to 103 h after infection (40% cytopathic effect) in methionine-free medium. (A) Uninfected cells; (B) infected cells. Lanes: a, Total cellular protein extracts; b, precipitation without IgG; c, precipitation with an anti-*H. saimiri*-negative serum (owl monkey); d, precipitation with an anti-*H. saimiri*-positive serum (owl monkey); e, precipitation with an anti-*H. saimiri*-positive serum (squirrel monkey); and f, precipitation with an anti-*H. saimiri*-positive serum (New Zealand white rabbit). [^{35}S]methionine-labeled phage T7 proteins were used as molecular weight standards.

of virion proteins appear identical (Fig. 5). Only three structural proteins were distinct in size. *H. saimiri* prime strain and strain OMI do not possess a protein equal in size to the 118-K polypeptide of strain 11; however, they have a protein of comparable abundancy at the 87- and 91-K positions, respectively (Fig. 5, Table 1). This suggests that they are equivalent structural elements in the virus particles. Similarly, a limited size variability became apparent with 36- to 37-K proteins and the histone-sized virion proteins (Fig. 5). The strain variability of these small proteins underlines, as does immunoprecipitation, that they are not cellular histones but that they are virus-specific proteins.

Nucleocapsid and envelope proteins. Purified virus particles were partially disintegrated by Nonidet P-40 to separate nucleocapsids from envelope proteins. Six of the structural proteins that were identified previously could be assigned to the membrane protein fraction (128, 84 to 90, 71, 55, 35, and 28 K) (Fig. 6, Table 1). One additional peptide, 155 to 160 K in size, was found in the membrane fraction, and one protein was identified as nucleocapsid polypeptide. Since the gel system did not clearly resolve the size difference, it could not definitely be decided whether the 155-K or the slightly larger protein belongs to the envelope or to the nucleocapsid, respectively. Two comparably faint polypeptides, 111 K and 46 K, became apparent in the fraction of solubilized envelope proteins (Fig. 7). Probably the 111-K protein did not represent a virion component since a dominant protein of this size had been seen in mock-infected cells but not always in purified virions. As outlined above, the 46-K protein is probably cellular

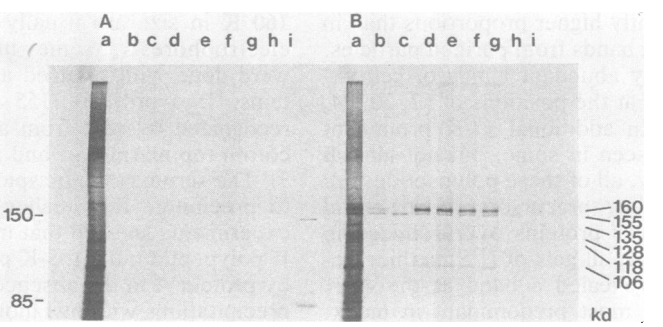

FIG. 3. Precipitation of ^{35}S-labeled *H. saimiri* proteins with immune sera and separation in a 10% polyacrylamide gel. Proteins were labeled with 50 μCi of [^{35}S]methionine per ml from 96 to 113 h after infection (50 to 70% cytopathic effect). (A) Uninfected cells; (B) infected cells. Lanes: a, Total cellular protein extracts; b, precipitation with an anti-*H. saimiri*-positive serum (squirrel monkey); c, precipitation with an anti-*H. ateles*-positive serum (spider monkey); d and e, precipitation with two anti-*H. saimiri*-positive sera (owl monkey); f and g, precipitation with two anti-*H. saimiri*-positive sera (marmoset); and h and i, precipitation with two anti-*H. saimiri*-negative sera (owl monkey). [^{35}S]methionine-labeled phage T7 proteins were used as molecular weight standards.

actin. All other proteins appeared to be constituents of nucleocapsids. The proteins of strain-specific size variability were found in the group of nucleocapsid proteins; this seems to be remarkable, since surface polypeptides of viruses are usually expected to be subject to a faster evolutionary divergence than internal capsid proteins or DNA-associated polypeptides.

Glycosylation. Glycosylated viral proteins are usually detected by adding labeled glucosamine or mannose to the cell cultures as precursors of the carbohydrate moieties. However, there remains always the intrinsic problem that the compounds are metabolized and radioactive label is incorporated, to a certain extent, into the amino acids of nonglycosylated proteins. When *H. saimiri* was grown for 3 to 5 days in the presence of D-[1-^{14}C]glucosamine or D-[1-^{3}H]mannose, we found most polypeptides to be radioactively labeled. Shorter labeling periods, however, did not result in sufficient incorporation. Thus, we preferred a different approach for identifying the glycoproteins. Unlabeled virion polypeptides were separated by PAGE, and thereafter, the glycoproteins in the gels were marked with ^{125}I-labeled ConA. Four bands were identified at the positions where [^{35}S]methionine-labeled polypeptides had been seen before (155 to 160, 128, 84 to 90, and 55 K) (Fig. 7). Each of these polypeptides had been assigned to the envelope of *H. saimiri*; however, based on ConA labeling, not all membrane proteins appeared to be glycosylated.

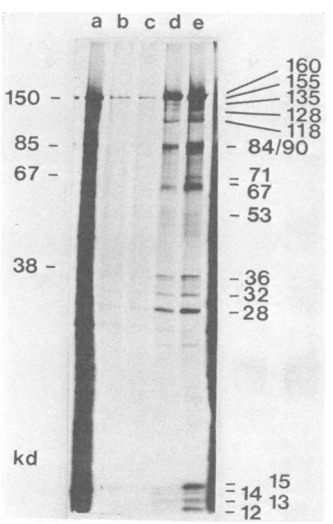

FIG. 4. Precipitation of ^{35}S-labeled *H. saimiri* proteins with immune sera and separation in a 15% polyacrylamide gel. Proteins were labeled with 55 μCi of [^{35}S]methionine per ml from 72 to 76 h after infection (60% cytopathic effect) in methionine-free medium. Lanes: a, Total cellular protein extract from infected cells; b, precipitation without immunoglobulin G; c, precipitation with an anti-*H. saimiri*-negative serum (owl monkey); d, precipitation with an anti-*H. saimiri*-positive serum (owl monkey); and e, precipitation with an anti-*H. saimiri*-positive serum (New Zealand white rabbit). [^{35}S]methionine-labeled phage T7 proteins were used as molecular weight standards.

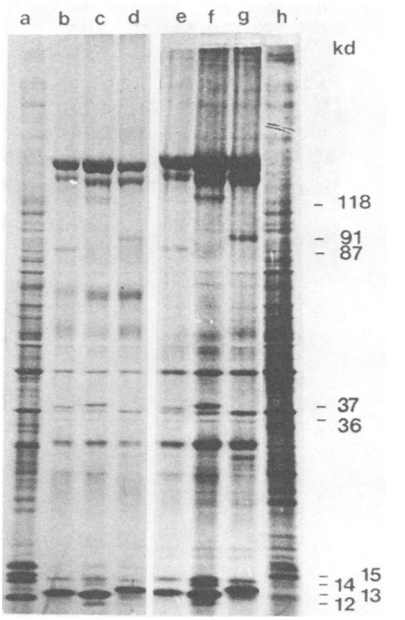

FIG. 5. Separation of virion proteins from three *H. saimiri* strains in a 7.5 to 20% polyacrylamide gel. Lanes: a to d, Polypeptides stained with Coomassie blue; and e to h, ^{35}S-labeled polypeptides visualized by autoradiography. Lanes: a and h, Proteins of uninfected OMK cells; b and e, structural proteins of strain OMI; c and f, strain 11; and d and g, strain S295C.

A clear discrete band of a glycosylated compound was seen at the position where a 220-K polypeptide would be expected in a polyacrylamide gel (Fig. 7). Sometimes, however not consistently, a band was observed at this position after PAGE of ^{35}S-labeled proteins from virions and infected cells. There was no protein of this size that could be precipitated by immune sera. Thus, we believed that it should not be included in the list of structural *H. saimiri* virion proteins. It may be a minor structural component that is very heavily glycosylated, or it may be an early viral protein or a glycosylated cellular protein; it could also represent the aggregation of another structural glycoprotein.

Phosphoproteins. Four structural phosphoproteins became apparent upon labeling with inorganic ^{32}P in permissively infected cell cultures (Fig. 8). One of these proteins (pp 135) had an

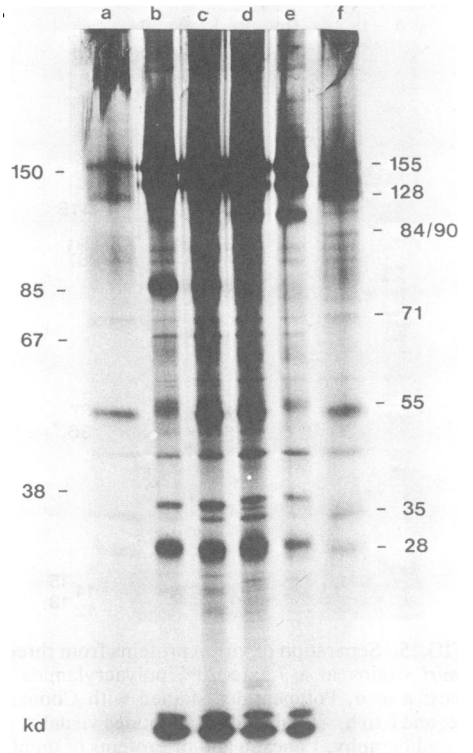

FIG. 6. Nucleocapsid and envelope proteins of *H. saimiri* OMI 11 after separation in a 7.5 to 20% polyacrylamide gel. Lanes: a to c, Proteins of strain OMI; d to f, proteins of strain 11. Lanes: a and f, Proteins solubilized by Nonidet P-40; b and e, nucleocapsid proteins after Nonidet P-40 treatment; and c and d, complete virus particles before detergent treatment.

identical size in the three *H. saimiri* strains included in this study. The other three phosphoproteins, all belonging to the nucleocapsid fraction, revealed a strain-related size variability. The 118-K protein of *H. saimiri* 11 appeared phosphorylated, as did the putative homologous proteins from strain prime (87 K) and OMI (91 K). This gives additional evidence that these proteins are equivalent viral proteins in the different strains. A very heavily labeled phosphoprotein different in size between the three virus strains appeared at the 55- to 57-K range (Fig. 8). This polypeptide became visible as a weakly labeled band upon [^{35}S]methionine labeling, and it comigrated with strongly labeled proteins from extracts of infected cells (Fig. 1). However, Coomassie blue staining of virion proteins revealed a polypeptide of this size for each virus strain, and the size heterogeneity corresponded to the migration patterns of the ^{32}P-labeled proteins (Fig. 5 and 8). The fourth phosphorylated protein, slightly variable in size between the strains, appeared in the group of histone-like virion proteins. An additional phosphoprotein became visible around the 18-K position; however, this did not correspond to any of the structural components of *H. saimiri* clearly detectable by [^{35}S]methionine labeling and Coomassie blue staining.

DISCUSSION

Only a few reports so far have dealt with proteins of *H. saimiri*; for an eventual understanding of viral replication and transformation, whether a polypeptide is a regulatory early protein or a structural component of the virus must be known. In this study, we assigned 21 polypeptides to the list of structural proteins. For the polypeptides, the following criteria were applied: (i) presence of the protein in the purified virion preparation, (ii) absence of a polypeptide that could be labeled with radioactive methionine in mock-infected cells and would be found at an equivalent position in polyacrylamide gels, and (iii) precipitation by immune sera of naturally infected monkeys (squirrel monkeys) and experimentally immunized animals. Certainly,

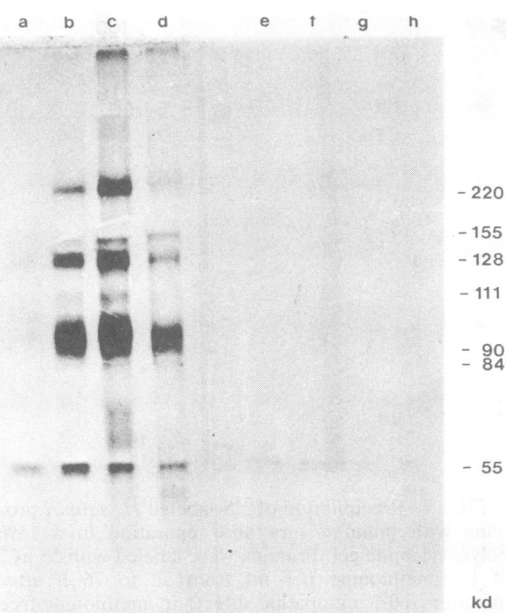

FIG. 7. Glycoproteins of virus particles from three *H. saimiri* strains, detected by in situ labeling after separation in a 7.5 to 20% polyacrylamide gel with ^{125}I-labeled ConA. Lanes: a, Strain 11 (20 μg of protein); b, strain 11 (100 μg of protein); c, strain OMI; d, strain S295C. Proteins in lanes e to h were incubated in the presence of D-mannose or D-glucose which inhibits binding of ^{125}I-labeled ConA.

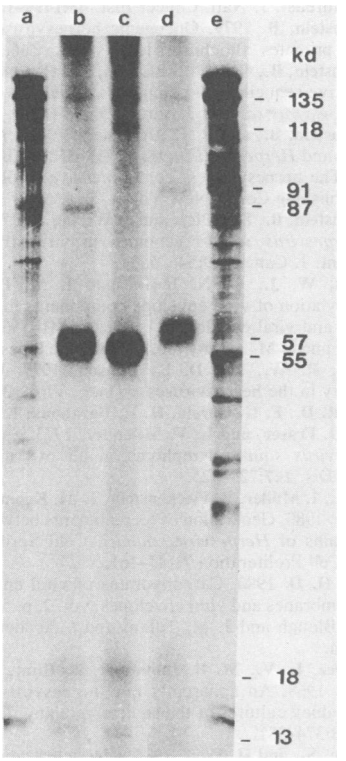

FIG. 8. Phosphoproteins of virus particles from three *H. saimiri* strains detected by labeling replicating virus with $^{32}P_i$. Proteins were separated in a 7.5 to 20% polyacrylamide gel. Lanes: a and e, [^{35}S]methionine-labeled proteins of strains OMI and S295C, respectively; b, $^{32}P_i$-labeled proteins of strain OMI; c, $^{32}P_i$-labeled proteins of strain 11; d, $^{32}P_i$-labeled proteins of strain S295C.

this list represents a minimum estimation, since superposition of virion proteins in the denaturing polyacrylamide gels is possible: one or several viral proteins may be hidden behind the cellular 46-K protein, and virion proteins of minor abundancy or low methionine content may not be detectable by ^{35}S-labeling and immunoprecipitation.

The viral polypeptide patterns from infected cells described in this study are in good agreement with the profiles of infected cell specific proteins given by Randall and Honess (21). However, we found a few discrepancies with a recent study on *H. saimiri* virion proteins (18a): (i) The 46-K protein was not included in the list of virion proteins; it comigrated with cellular actin and could not be immunoprecipitated with immune sera. Thus, it appeared rather unlikely that this protein is a virus-coded structural polypeptide. If there exists a 46-K virion constituent in low abundancy, this would be difficult to prove. (ii) As stated above, the 220-K glycoprotein, variable in intensity, is not necessarily a structural component. At least four virus-specific proteins were identified unambiguously as being in the size range of cellular histones. Phosphorylation was not only detected in the 135- and 57-K protein but also in the large polypeptides of strain-specific variation (87, 91, and 118 K) and in the histone-like 13- to 14-K proteins.

Four of seven envelope proteins of *H. saimiri* were found to be glycosylated by labeling with ^{125}I-coupled ConA. Herpesvirus glycoproteins analyzed so far contain internal mannose and glucosamine residues (13, 17, 20); thus, they are expected to be recognized by ConA as well as by incorporation of radioactive mannose and glucosamine. However, it should be borne in mind that herpesvirus envelopes may contain unusual carbohydrate moieties that are not detected by either of these methods. For instance, the 35-K *H. saimiri* envelope protein did not appear appreciably glycosylated; Randall and Honess (21) also did not find a glycoprotein of this size range in extracts of *H. saimiri*-infected cells. Nevertheless, the heterogeneous migration behavior of this polypeptide seems to suggest secondary modification. The nonglycosylated polypeptides that could be assigned to the virion envelope by solubilization with weak detergents may represent, at least in part, internal membrane proteins forming the matrix between surface glycoproteins and nucleocapsid. Similarly, nonglycosylated proteins were found to be released from herpes simplex envelopes by a mild detergent treatment (22).

The majority of structural virion proteins from three different *H. saimiri* strains appeared identical in PAGE. There were only three phosphorylated nucleocapsid proteins and a single unmodified internal virion protein that revealed a strain-specific variability. Thus, the size differences of polypeptides will not be sufficient criteria to localize the coding sequences by comparative analysis of polypeptide patterns from recombinants between different virus strains. Even tryptic peptide analysis of 155- to 160-K proteins from *H. saimiri* virions did not reveal an unambiguous strain-specific pattern (B. Fleckenstein, M. Ponce de Leon, R. Eisenberg, and G. Cohen, unpublished data). It was found that the leftmost 7 to 10 kbp L-DNA regions of some *H. saimiri* strains (group A) are highly homologous in DNA sequences to each other but do not share any detectable homology with the left region and L-DNA of a second group (B) of virus strains (C. Mulder, E. Szomolanyi, P. Medvecsky, R. C. Desrosiers, J. M. Koomey, L. A. Falk, and B. Fleckenstein. Abstract of the 5th International Congress of Virology, Strasbourg, France, 1981). According to these obser-

vations, *H. saimiri* strains OMI and 11 fall into one group and are distinct from the prime strain. Those patterns of phylogenetic relatedness are not appreciably reflected in the size variations of nucleocapsid proteins described in this study. All in all, DNA sequence variations appear to be superior to polypeptide size variabilities in defining phylogenetic relationships of *H. saimiri* strains.

The size patterns of *H. saimiri* virion proteins give an additional example for the evolutionary diversity of the herpesvirus group, as no obvious equivalence is apparent for any of the *H. saimiri* late proteins in comparison with other members of the herpes group (14). The protein diversities, since too pronounced, will hardly be useful in the difficult search for criteria in herpesvirus taxonomy.

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LITERATURE CITED

1. **Bodemer, W., W. C. Summers, and J. C. Niederman.** 1980. Detection of virus-specific antigens in EB-(P3HR-1) virus superinfected Raji cells by immunoprecipitation. Virology **103**:340–349.
2. **Bornkamm, G. W., H. Delius, B. Fleckenstein, F.-J. Werner, and C. Mulder.** 1976. Structure of *Herpesvirus saimiri* genomes: arrangement of heavy and light sequences within the M genome. J. Virol. **19**:154–161.
3. **Brugge, J., and R. L. Erikson.** 1977. Identification of a transformation-specific antigen induced by an avian sarcoma virus. Nature (London) **269**:346–348.
4. **Burridge, K.** 1978. Direct identification of specific glycoproteins and antigens in sodium dodecyl sulfate gels. Methods Enzymol. **50**:54–64.
5. **Daniel, M. D., D. Silva, and N. Ma.** 1976. Establishment of owl monkey kidney 210 cell line for virological studies. In Vitro **12**:290–294.
6. **Deinhardt, F. W., L. A. Falk, and L. G. Wolfe.** 1974. Simian herpesviruses and neoplasma. Adv. Cancer Res. **19**:167–205.
7. **Desrosiers, R. C., and L. A. Falk.** 1982. *Herpesvirus saimiri* strain variability. J. Virol. **43**:352–356.
8. **Falk, L. A., G. Wolfe, and F. Deinhardt.** 1972. Isolation of *Herpesvirus saimiri* from blood of squirrel monkeys (Saimiri sciureus). J. Natl. Cancer Inst. **48**:1499–1505.
9. **Fleckenstein, B.** 1979. Oncogenic herpesviruses of nonhuman primates. Biochim. Biophys. Acta **560**:301–342.
10. **Fleckenstein, B., G. W. Bornkamm, and H. Ludwig.** 1975. Repetitive sequences in complete and defective genomes of *Herpesvirus saimiri*. J. Virol, **15**:398–406.
11. **Fleckenstein, B., and R. C. Desrosiers.** 1982. *Herpesvirus saimiri* and *Herpesvirus ateles* p. 253–332. *In* B. Roizman (ed.), The herpesviruses, comprehensive virology. Plenum Publishing Corp., New York.
12. **Fleckenstein, B., I. Müller, and J. Werner.** 1977. Presence of *Herpesvirus saimiri* genomes in virus transformed cells. Int. J. Cancer **19**:546–554.
13. **Grimes, W. J., G. N. Irwin, and L. M. Patt.** 1980. Glycosylation of viral envelope components in cell membranes and viral envelopes, vol. 2, p. 541–556. *In* H. A. Blough and J. M. Tiffany (ed.). Academic Press, London.
14. **Honess, R. W., and D. H. Watson.** 1977. Unity and diversity in the herpesviruses. J. Gen. Virol. **37**:15–37.
15. **Hunt, R. D., F. G. Garcia, H. H. Barahona; K. W. King, C. E. O. Fraser, and L. V. Melendez.** 1973. Spontaneous *Herpesvirus saimiri* lymphoma in an owl monkey. J. Infect. Dis. **127**:723–725.
16. **Keil, G., I. Müller, B. Fleckenstein, J. M. Koomey, and C. Mulder.** 1980. Generation of recombinants between different strains of *Herpesvirus saimiri*. Cold Spring Harbor Conf. Cell Proliferation **7**:145–161.
17. **Klenk, H. D.** 1980. Carbohydrates of viral envelopes in cell membranes and viral envelopes, vol. 2, p. 519–539. *In* H. A. Blough and J. M. Tiffany (ed.). Academic Press, London.
18. **Melendez, L. V., M. D. Daniel, R. D. Hunt, and F. G. Garcia.** 1968. An apparently new herpesvirus from primary kidney cultures of the squirrel monkey. Lab. Anim. Care **18**:374–381.

18a. **Modron, S., and H. Wolf.** 1983. Characterization of *Herpesvirus saimiri* and *Herpesvirus ateles* structural proteins. Virology **125**:251–255.

19. **Morse, L. S., L. Pereira, B. Roizman, and P. A. Schaffer.** 1978. Anatomy of herpes simplex virus (HSV) DNA. X. Mapping of viral genes by analysis of polypeptides and functions specified by HSV-1 X HSV-2 recombinants. J. Virol. **26**:389–410.
20. **Olofsson, S., S. Jeansson, and E. Lycke.** 1981. Unusual lectin-binding properties of a herpes simplex virus type 1-specific glycoprotein. J. Virol. **38**:564–570.
21. **Randall, R. E., and R. W. Honess.** 1982. Proteins specified by *Herpesvirus saimiri*: purification and properties of a single polypeptide which elicits virus-neutralizing antibody. J. Gen. Virol. **58**:149–161.
22. **Sarmiento, M., and P. G. Spear.** 1979. Membrane proteins specified by herpes simplex viruses. IV. Conformation of the virion glycoprotein designated VP7(B_2). J. Virol. **29**:1159–1167.
23. **Todaro, G. I., C. I. Scherr, A. Sen, N. King, M. D. Daniel, and B. Fleckenstein.** 1978. Endogenous New World primate type C viruses isolated from owl monkey (*Aotus trivirgatus*) kidney cell line. Proc. Natl. Acad. Sci., U.S.A. **75**:1004–1008.