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Characterization of *Herpesvirus saimiri* and *Herpesvirus ateles* Structural Proteins

SUSANNE MODROW AND HANS WOLF[1]

Max von Pettenkofer-Institute, Munich, Pettenkoferstr. 9a, D-8000 Munich 2, West Germany

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The structural proteins of *Herpesvirus saimiri* strains 11 and 11 att and of *Herpesvirus ateles* strains 73 and 810 were characterized by electrophoresis in SDS-polyacrylamide gels. For *H. saimiri* 21 virus structural proteins could be identified with molecular weights ranging from 28,000 to 210,000 Da. For *H. ateles* 810 and *H. ateles* 73, 20 polypeptides were characterized. Using lactoperoxidase for iodination of surface proteins and immunoprecipitation, 5 polypeptides could be identified as envelope and 4 as capsid surface proteins.

Herpesvirus saimiri is ubiquitous and horizontally transmitted in squirrel monkey (*Saimiri sciureus*) populations without causing overt disease (*1*). The virus is, however, highly oncogenic in various experimental hosts, especially in different marmoset species (*Saguinus oedipus, S. nigricollis, S. fuscicollis*) (*2, 3*).

The attenuated nononcogenic strain *H. saimiri* 11 att, which was derived from the oncogenic wild-type *H. saimiri* 11 (*4*), induces a latent or perhaps persistent infection in marmosets (*5, 6*) and protects animals against challenge inoculation with *H. saimiri* 11 (*7*).

Herpesvirus ateles, an endemic virus of spider monkeys (*Ateles sp.*), resembles *H. saimiri* rather closely in its potential to induce malignant tumors of the lymphatic system in New World Monkeys (*8, 9*). The virus strains *H. ateles* 810 (originally isolated from kidney cell cultures of *Ateles geoffroyii*) (*8*) and *H. ateles* 73 (isolated from leukocytes of another spider monkey *Ateles paniscus*) (*10*) have identical biological properties; their DNAs have a low degree (2.4%) of mismatching (*11*).

So far, the structural proteins of *H. saimiri* and *H. ateles* have not been described. The characterization of virion proteins is, however, of importance for a detailed analysis of the regulation of synthesis of viral gene products (Modrow and Wolf, in press), their correlation to virus-specific antigens, and their localization on the genome. The identification of the surface proteins of virions and capsids should also allow a further characterization of a subset of those viral polypeptides which supposedly have first contact with the host cell. A comparison of the structural proteins of the various *H. saimiri* and *H. ateles* strains may permit conclusions about their different biological behavior. In addition, the protein profiles should enable us to select strains suitable for the construction of recombinants, which can be used for detailed genetic analysis.

The protein profiles of the various *H. saimiri* and *H. ateles* strains are shown in Fig. 1. After labeling with [^{35}S]methionine, 21 polypeptides could be identified for the *H. saimiri* strains; their molecular weights ranged from 28 to 210 kDa. Two proteins were labeled with [^{32}P]phosphate: pp 135 and pp 57. A comparison of the protein pattern of *H. saimiri* 11 and *H. saimiri* 11 att showed no differences in the molecular weight of the single protein bands.

For *H. ateles* 73 and *H. ateles* 810, 20 different virion proteins could be identified by [^{35}S]methionine labeling. Three of them were found to be phosphorylated (pp 128, pp 57, pp 56). The molecular weights of *H. ateles* 73 proteins differed slightly from *H. ateles* 810 polypeptides and were distinguishable from the protein patterns ob-

[1] To whom reprint requests should be addressed.

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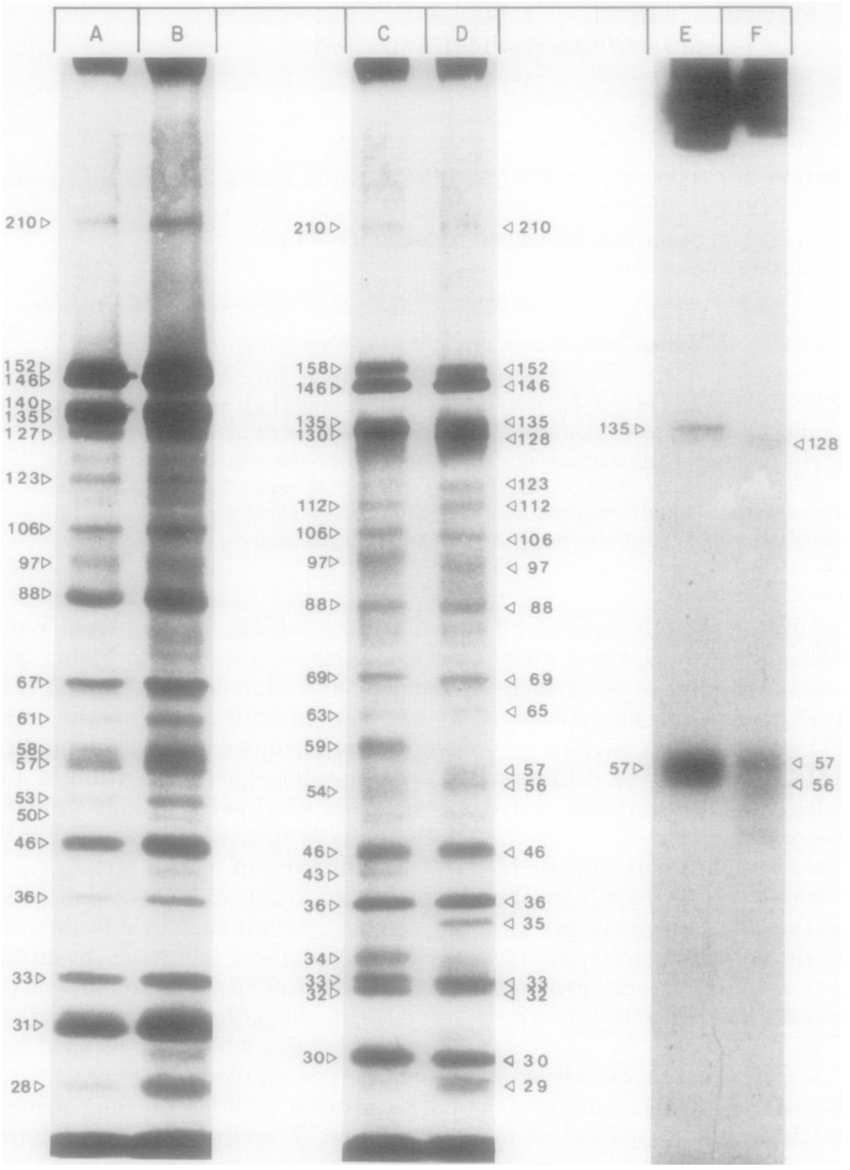

FIG. 1. SDS-polyacrylamide gel of virion proteins. All virus strains were propagated in owl monkey kidney cells (OMK-cells) in minimum essential medium (MEM, Earl's salts, Gibco) supplemented with 2% heat-inactivated fetal calf serum (Seromed) and 20 μg/ml TPA (phorbol-12-myristate-13-acetate (*12*)). Cells were infected with either virus strain using 1-2 PFU/cell; after an adsorption period of 2 hr at room temperature, medium was added and cells were labeled with 2 μCi/ml [^{35}S]methionine (NEN) in MEM with 20% of the normal amount of methionine or 10 μCi/ml [^{32}P]orthophosphate (NEN) in phosphate-free MEM. Virions were purified from the cell culture supernatant fluids in 10-30% (w/w) sucrose sedimentation velocity gradients. The final virus pellets were resuspended in solubilization buffer (50 m*M* Tris-HCl, pH 7.0, 2% SDS, 5% mercaptoethanol, 3% sucrose, bromephenol blue) and heated 5 min at 100°. 10,000 cpm per 50-μl sample per slot were applied for [^{35}S]methionine and 5,000 cpm for [^{32}P]phosphate-labeled samples. Electrophoresis was done in 10% acrylamide, 0.26% diallyltartardiamide SDS-gels as described earlier (*12*). Dried gels were exposed to LKB ^{3}H Ultrofilm. The molecular weights of the protein bands were calculated using a program for the TI-59 calculator (unpublished), which allows the calculation of the best-fitting curve from the marker proteins and then derives the molecular weights of the protein bands according to their distance of migration. Lanes A-D labeled with [^{35}S]methionine; lanes E-F labeled with [^{32}P]phosphate. A, *H. saimiri* 11; B, *H. saimiri* 11 att; C, *H. ateles* 810; D, *H. ateles* 73; E, *H. saimiri* 11; F, *H. ateles* 73.

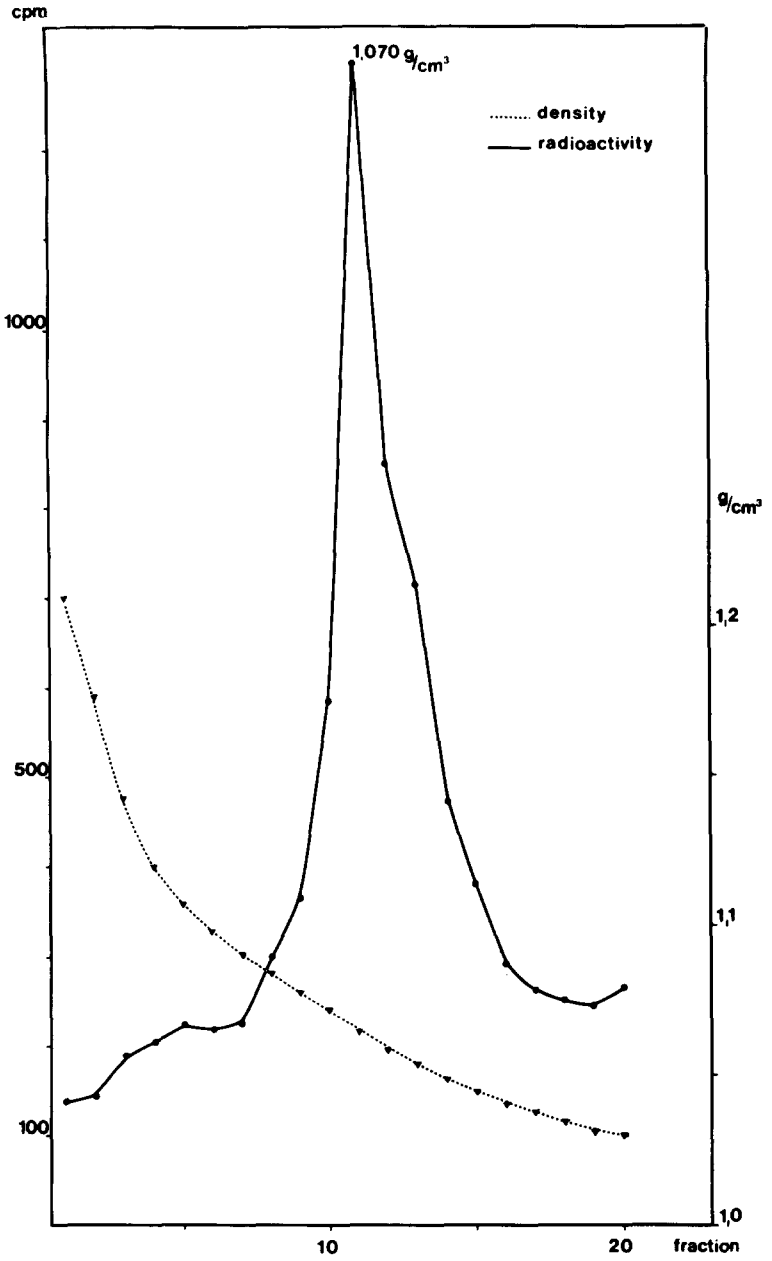

FIG. 2. Purification of virions. Infected cell cultures were centrifuged at 4000 *g* and 4° for 20 min to remove cells and cellular debris. Supernatant fluids were centrifuged at 27,000 *g* at 4° for 2 hr. The resulting virus pellet was resuspended in a small volume of tissue culture medium and homogenized with 10 strokes in a Dounce homogenizer with a close-fitting pestle. Percoll (Pharmacia) was mixed with 9% NaCl (9:1) and the homogenized virus suspension diluted with VSB (10 m*M* Tris-HCl, pH 7.4, 10 m*M* KCl, 1 m*M* EDTA) to a density of 1.06 g/cm³ and centrifuged for 45 min with 42,000 *g* at 4°. The gradients were harvested in 20 fractions and 50-μl aliquots were counted in Aquasol 2. The refractive index of every second fraction was determined. To remove the Percoll, peak fractions were pooled, loaded on top of a 25–50% (w/w) sucrose gradient in VSB, and centrifuged for 3 hr at 60,000 *g* and 4°. The gradients were harvested in 20 fractions, and 50-μl aliquots were counted in Aquasol 2. Virus was removed from the sucrose solution by dilution with VSB and pelleting (60,000 *g*, 1 hr, 4°).

served with *H. saimiri* isolates. The overall pattern of the protein profiles of the various *H. saimiri* and *H. ateles* strains, however, was fairly similar, eight proteins being conserved according to their molecular weights (p 210, p 146 p 106, p 97, p 88, p 46, p 36, p 33). The close relationship of proteins from *H. saimiri* and *H. ateles* reflects the relatedness of their DNAs which show 35% homology (*11*).

For surface iodination studies, virus labeled with [^{3}H]thymidine to enable tracing was purified from the cell culture supernatant fluid using combined Percoll (Pharmacia, Fig. 2) and isopycnic sucrose gradients (20–50% w/w). Capsids were prepared from purified virions by incubation in 0.5% NP40 for 15 min at 0° and subsequent pelleting (1 hr, 60,000 *g*, 4°) from the diluted mixture. The density of virions could be determined without influences arising from the osmotic environment (*13*) using Percoll equilibrium density gradients. This method gave a true density of 1.070 ± 0.005 g/cm^3 for enveloped particles, whereas the comparable value in sucrose was found to be 1.22 g/cm^3. By the use of two consecutive isopycnic centrifugation steps on different gradient materials, fairly pure virus particles could be obtained; furthermore, the second centrifugation on a sucrose gradient resulted in the complete removal of Percoll. Virion and capsid polypeptides were labeled with ^{125}I using lactoperoxidase immobilized on carrier beads (NEN). The ^{125}I-labeled proteins were immunoprecipitated with a serum raised against virus particles (produced by multiple intracutaneous inoculations in rabbits) and electrophoresed in 10% SDS–polyacrylamide gels (Fig. 3).

Iodination with the lactoperoxidase system is a very gentle method, which allows the selective iodination of surface proteins. Since the viral envelope is partially destroyed during the purification procedure, capsid polypeptides are exposed to the surface; therefore when iodinating whole virions, capsid proteins are labeled with ^{125}I as well as envelope polypeptides. Comparing the different patterns of *H. saimiri* 11- and *H. saimiri* 11 att-derived

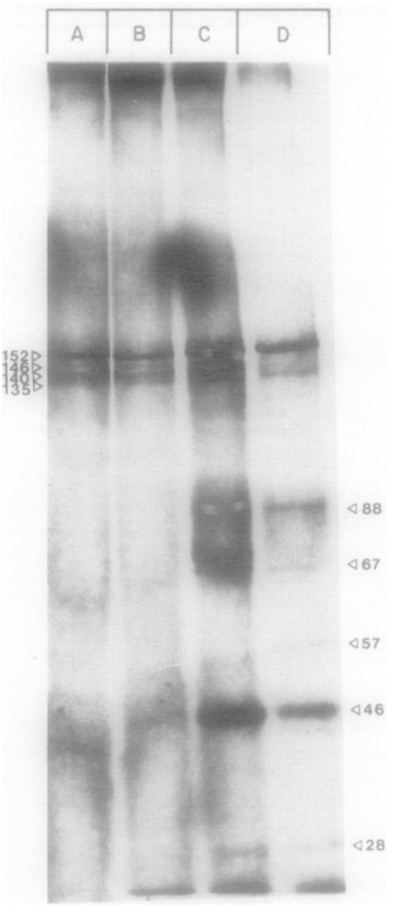

FIG. 3. ^{125}I-Labeled surface proteins. Virions and capsid polypeptides were labeled with ^{125}I using lactoperoxidase immobilized on carrier beads following the instructions given by the manufacturer (NEN). The ^{125}I-labeled proteins were immunoprecipitated with a rabbit serum against virus particles. 10 μl serum was preadsorbed with an extract of 5×10^6 uninfected, unlabeled OMK-cells for several hours. 3 mg protein A-Sepharose beads (Pharmacia), preswollen in immunoprecipitation buffer (1% Triton X-100, 0.1% SDS, 137 m*M* NaCl, 1 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 10% glycerin, 20 m*M* Tris-HCl, pH 9.0) were added and incubated 2–3 hr at 4°. The ^{125}I-labeled protein suspension (1×10^6 cpm) was added and incubated at room temperature for 3 hr. The Sepharose beads with the bound immunocomplexes were washed with immunoprecipitation buffer and suspended in 50 μl solubilization buffer and electrophoresed in 10% SDS-polyacrylamide gels. The fixed, stained, and dried gels were exposed to Kodak XR5 films with Lightning Plus intensifying screens (Du Pont). A, *H. saimiri* 11 att capsids; B, *H. saimiri* 11 capsids; C, *H. saimiri* 11 att virions; D, *H. saimiri* 11 virons.

virions and capsids, four proteins could be identified as capsid polypeptides (p 152, p 146, p 140, pp 135) and five as parts of the virus envelope (p 88, p 67, p 57, p 46, p 28). No difference was found between the two *H. saimiri* strains.

Our experiments suggest that recombinants between *H. ateles* 73 and *H. ateles* 810 could be useful for the localization of certain gene products by comparing protein profiles and restriction enzyme profiles of the parental strains and the recombinants. In contrast, *H. saimiri* 11 and *H. saimiri* 11 att do not carry enough phenotypic markers and may require a detailed analysis of virus-induced proteins, if similar attempts of genetic analysis are to be made.

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