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Genome Structure and Virion Polypeptides of the Primate Herpesviruses *Herpesvirus aotus* Types 1 and 3: Comparison with Human Cytomegalovirus

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Two serologically distinguishable primate herpesviruses, *Herpesvirus aotus* type 1 and type 3, were examined with regard to their genomes and structural polypeptides. The duplex DNA genomes of these two viruses were found to be essentially identical in molecular weight ($M_r \cong 145 \times 10^6$) and guanine plus cytosine composition (55%). Both contained unique and inverted repeat nucleotide sequences of the same size and arrangement, which, as judged by DNA-DNA hybridization and restriction enzyme analyses, were at least 95% homologous. In addition, no differences were observed in electrophoretic profiles of virion polypeptides. Because of their great similarity with respect to these criteria, the two viruses ought to be considered independent isolates (or strains) of a single virus, which should be designated *H. aotus* type 1. The elevated molecular weight and presence of two sets of inverted repeat sequences closely resemble the structure of the human cytomegalovirus genome. However, no sequence homology (<5%) nor similarity in virion polypeptides was detected between *H. aotus* type 1 and human cytomegalovirus.

Independent isolates of herpesviruses have been obtained from owl monkey (*Aotus trivirgatus*) kidney cell cultures (3, 10, 11). Three such isolates were found to be serologically distinguishable by cross-neutralization tests and were accordingly designated *Herpesvirus aotus* types 1, 2, and 3 (10). Although preliminary seroepidemiological data suggested that owl monkeys are the principal host in nature, no pathogenic properties have yet been described for these viruses. A fourth herpesvirus isolate from oral and anal swabs of an owl monkey was reported (1), but was not compared with *H. aotus* types 1 to 3. In some biological characteristics, such as cytopathology and host range in nonhuman primate cell culture, *H. aotus* type 2 clearly differs from *H. aotus* types 1 and 3; the latter two serotypes, however, are not easily distinguishable by the above criteria, nor are they easily distinguishable by their common lack of infectivity in hamster and rabbit cells, as well as embryonated eggs (10).

Information concerning the extent to which herpesviruses sharing a natural host are related at the molecular level will be necessary for an eventual understanding of their epidemiology and evolution. We have, therefore, initiated a study of the virion components of the three *H. aotus* serotypes. We have found (46) that the structure of *H. aotus* type 2 DNA closely resembles that previously reported for the DNAs of the highly oncogenic New World primate viruses *Herpesvirus saimiri* (4) and *Herpesvirus ateles* (16). In this communication, we describe studies on the DNAs and virion polypeptides of *H. aotus* types 1 and 3. Our results show that, in spite of the absence of cross-neutralization, these two serotypes are, in fact, highly related. Moreover, inverted repeat nucleotide sequences occur in both viral DNAs, which, when taken together with the large size of these genomes, present strong structural similarities to the genome of human cytomegalovirus (HCMV). On the other hand, DNA-DNA cross-hybridization between *H. aotus* type 3 and HCMV failed to reveal significant homology.

(Preliminary portions of this work were presented at the 1978 Herpesvirus Workshop, Cambridge, England [P. Sheldrick, N. Berthelot, and B. Fleckenstein].)

MATERIALS AND METHODS

Viruses and cell cultures. *H. aotus* types 1 and 3 were propagated on OMK strain 637 cells, an estab-

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lished line of owl monkey kidney cells, and grown in monolayer cultures in minimum essential medium with 10% heat-inactivated fetal calf serum. Both viruses and cells were provided by M. D. Daniel. About 8 to 10 days after the infection of cell cultures, when pronounced cytopathic changes with rounded and detached refractile cells were visible, the viruses were harvested from culture fluids and floating cells. HCMV Ad169 and Towne were propagated in human embryonic lung cells or foreskin fibroblasts. The cells were grown in stationary cultures by standard procedures. Virus stock and cells were checked for mycoplasma by isolation procedures on agar plates under anaerobic conditions as described elsewhere (18) and found to be free of contamination.

Purifications of virions and DNA. Herpesvirus particles (*H. aotus* types 1 and 3, HCMV) were extracted from the cytoplasm of infected cells by Dounce homogenization or concentrated from culture fluids by pellet sedimentation at 17,000 rpm for 90 min in Spinco SW27/28 rotors. The viruses were partially purified by sedimentation through 15 to 30% (wt/wt) sucrose gradients at 20,000 rpm and 4°C for 30 min in SW27/28 rotors and concentrated again by pellet centrifugation. For DNA purifications, the viruses were lysed with 2% (wt/vol) sodium lauroyl sarcosinate at 60°C for 60 min; the DNA was centrifuged to equilibrium in CsCl at 33,000 rpm in a Spinco 50 Ti rotor and dialyzed against 20 mM Tris-hydrochloride (pH 8.5).

*Hin*dIII fragments of HCMV Ad169 cloned in cosmid pHC79 (17) were isolated by a quick lysis procedure with Triton X-100 or through a slight modification of the rapid boiling extraction method of Holmes and Quigley (22).

To isolate DNA from lytically infected cultures, we suspended cells in 50 mM Tris-hydrochloride (pH 7.5) with 10 mM EDTA and 2% (wt/vol) sodium lauroyl sarcosinate; the cells were then digested with 100 μg of preincubated (10 min, 37°C) proteinase K (E. Merck AG, Darmstadt, West Germany) per ml. Nucleic acids were extracted with phenol–chloroform-isoamyl alcohol (24:1) and fractionated by isopycnic centrifugation in potassium iodide gradients (18, 62).

Analytical isopycnic centrifugation. The determination of the buoyant densities of intact or fragmented viral DNA in CsCl was carried out in a model E analytical ultracentrifuge. CsCl (500 mg) was dissolved in 400 μl of TNE buffer (10 mM Tris-hydrochloride [pH 8.5], 0.1 M NaCl, 1 mM EDTA) containing 0.8 to 1.2 μg of viral DNA. To avoid the fragmentation of intact virion DNA molecules, the solution was entered slowly into the centrifuge cells of a Spinco Ti ANF 4-place rotor. DNA was spun to equilibrium at 44,000 rpm for 24 h at 25°C, and UV absorbance was scanned at 262-nm wavelength. DNA of *Mycoplasma orale* type 1 (1.685_6 g/ml) (27), purified from liquid cultures, and *Micrococcus lysodeikticus* DNA (1.730_6 g/ml) (58) were included as internal density markers in analytical runs. The buoyant density values were calculated by the equation of Mandel et al. (36): $\rho = \rho_0 + 4.2\, w^2 (r^2 - r_0^2) \times 10^{-10}$ g/ml.

Sucrose gradient centrifugation. Velocity sedimentation was performed with 0.6-μg samples of [^{3}H]thymidine-labeled *H. aotus* type 1 DNA (6 μg/ml; 6,500 cpm/μg) or 1.5 μg of *H. aotus* type 3 DNA (15 μg/ml; 920 cpm/μg) in 0.01 mM Tris-hydrochloride (pH 7.5)–10^{-3} M EDTA. One microgram of [^{14}C]thymidine-labeled channel catfish virus (CCV) DNA was included (10 μg/ml; 300 cpm of ^{14}C per μg) as an internal size standard. The samples were layered onto 4.6-ml linear 5 to 20% (wt/vol) sucrose gradients in 0.01 M sodium-potassium phosphate (pH 7.5)–1 M NaCl–10^{-3} M EDTA and centrifuged in a Beckman SW50.1 rotor at 35,000 rpm for 120 min at 20°C. Fractions were collected dropwise onto Whatman GF/C filters, which were sequentially soaked in 10% trichloroacetic acid and 70% ethanol before drying for scintillation counting.

Electron microscopy. Single DNA strands were prepared by sedimentation through high-salt 5 to 20% sucrose gradients as previously described (51, 52). DNA was spread from a 50% formamide hyperphase onto a 17% formamide hypophase by published procedures (12). Internal size standards were single-stranded ϕX174 DNA ($M_r = 1.7 \times 10^6$) (47) and double-stranded PM2 DNA ($M_r = 6.4 \times 10^6$) (42). Electron microscopy was carried out with a Siemens model 1A or Zeiss EM 10, and contour lengths were measured from enlarged positive prints by means of a digitizer board interfaced to a Hewlett-Packard model 9820 A calculator.

Reassociation kinetics and T_m determination of DNA hybrids. DNA sequence homologies were determined by C_0t-hybridizations and the measurement of the melting temperatures (T_ms) of homoduplex and heteroduplex molecules. *H. aotus* type 3 DNA was radioactively labeled to a final specific activity of 5,000 cpm/μg by adding 1 μCi of [^{3}H]thymidine per ml (15 to 25 Ci/mmol; Amersham-Buchler, Braunschweig, West Germany) to infected OMK cell cultures. Further details of the methods, such as the denaturation of DNA, hybridization conditions, separation of single-stranded and duplex DNA by hydroxyapatite chromatography, melting of heteroduplex molecules in 0.14 M sodium phosphate buffer (pH 6.8), and counting of DNA fractions, have been described before (16).

Cleavage of DNA with restriction endonucleases, gel electrophoresis, and blot hybridization. Cleavages with restriction endonucleases (endo R) *Bam*HI, *Hin*dIII, *Kpn*I, *Sac*II, *Xba*I, and *Xho*I, were carried out by incubation in a 20 mM Tris-hydrochloride buffer (pH 7.5) with 10 mM $MgCl_2$, 7 mM mercaptoethanol, and 15 mM KCl at 37°C with at least a threefold excess of enzyme activity. The buffers for *Eco*RI and *Pst*I contained 10 mM Tris-hydrochloride (pH 7.5), 10 mM $MgCl_2$, 10 mM mercaptoethanol, and 50 mM NaCl; *Sal*I cleavage was done in a 8 mM Tris-hydrochloride buffer (pH 7.6) with 6 mM $MgCl_2$ and 150 mM NaCl. Digestions with *Sma*I were performed in 20 mM Tris-hydrochloride buffer (pH 9.0) with 10 mM $MgCl_2$–15 mM KCl at 30°C. Endo R · *Bam*HI was isolated from *Bacillus amyloliquefaciens* by the method of Wilson and Young (61). Endo R · *Sal*I was isolated from extracts of *Streptomyces albus* G by Bio-Gel A-0.5 M chromatography. Endo R · *Eco*RI and endo R · *Pst*I were kindly supplied by A. Rösch; endo R · *Kpn*I, endo R · *Sac*II, and endo R · *Xho*I were supplied by C. Mulder; and endo R · *Eca*I was supplied by H. Mayer. Endo R · *Hin*dIII and endo R · *Cla*I were purchased from Boehringer, Mannheim, West Germany. The electrophoresis of DNA fragments in polyacrylamide gels was done as described elsewhere (4); agarose gel electrophoresis (0.6%) was performed in 40 mM Tris-hydrochloride (pH 7.9) buffer with 5 mM sodium acetate and 1 mM EDTA, using horizontal slab gel

chambers. The transfer of DNA to nitrocellulose filters, nick-repair labeling of virion DNA with ^{32}P, and blot hybridizations were essentially performed as described by Desrosiers et al. (14).

Protein gel electrophoresis. Viral structural polypeptides were labeled in cell culture with 10 μCi of [^{35}S]methionine per ml in minimum essential medium containing 5% fetal calf serum and a reduced methionine concentration (50 mg/ml). Nucleocapsid and envelope proteins were prepared from purified virus particles by the procedure of Sarmiento and Spear (48). Virions labeled with [^{35}S]methionine 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 in the electron microscope. Polypeptides of purified virus particles or protein fractions were denatured in 62.5 mM Tris-hydrochloride, (pH 6.8) buffer with 10% (wt/vol) sucrose, 5% β-mercaptoethanol, 0.3% sodium dodecyl sulfate, and 0.01% bromophenol blue by heating to 96°C for 5 min. The proteins were separated in a 7.5 to 20% (wt/vol) bis-linked polyacrylamide gradient gel system with 25 mM Tris-hydrochloride (pH 8.8), 198 mM glycine, and 0.1% sodium dodecyl sulfate (32), and visualized by fluorography with En^{3}Hance (New England Nuclear Corp., Boston, Mass.) and ultrafilm (LKB, Bromma, Sweden) or Kodak X-Omat R.

RESULTS

Buoyant density, molecular size, and inverted repeat nucleotide sequences of *H. aotus* types 1 and 3 DNAs. Equilibrium sedimentation in analytical CsCl density gradients of DNA preparations from purified *H. aotus* types 1 and 3 virions gave sharp bands at $\rho = 1.714$ g/cm^3 (Fig. 1a, b, d, and e). For purposes of comparison, the buoyant density of HCMV DNA was determined in the same experiment (Fig. 1g and h) to

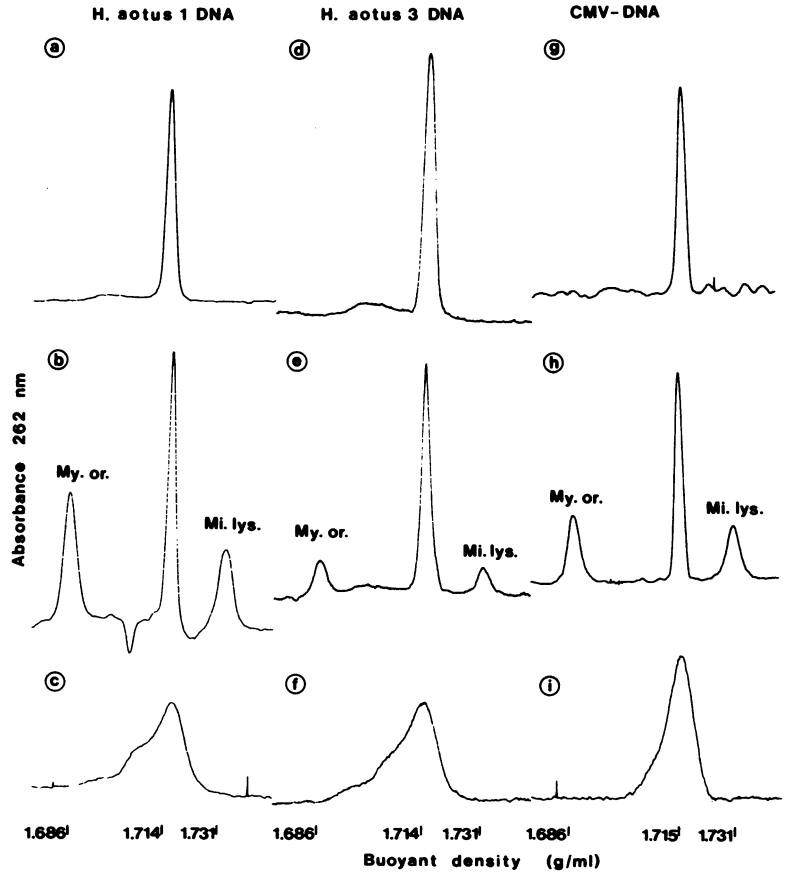


FIG. 1. Photoelectric scannings of purified virion DNA from *H. aotus* types 1 and 3 and HCMV after centrifugation to equilibrium in CsCl in a Spinco model E analytical centrifuge. (a) Intact *H. aotus* type 1 DNA; (b) *H. aotus* type 1 DNA with *M. orale* 1 and *M. lysodeikticus* DNA as internal density markers; (c) same as in (a), but mechanically sheared before centrifugation; (d) intact *H. aotus* type 3 DNA; (e) *H. aotus* type 3 DNA with density markers; (f) sheared DNA of *H. aotus* type 3; (g) intact HCMV DNA; (h) HCMV DNA with density markers; (i) sheared HCMV DNA.

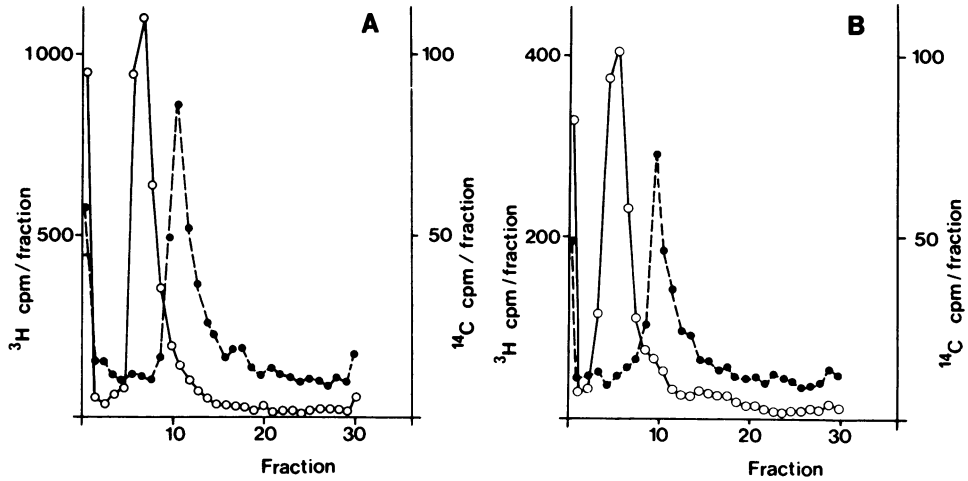


FIG. 2. Velocity sedimentation of *H. aotus* types 1 and 3 double-stranded DNAs. Sedimentation was performed in 5 to 20% sucrose gradients as described in the text. (A) ^{3}H-labeled *H. aotus* type 1 DNA, (B) ^{3}H-labeled *H. aotus* type 3 DNA. Double-stranded CCV [^{14}C]DNA (●) was included in both gradients as a molecular weight standard (M_r = 85 × 10^6) (9).

be ρ = 1.715 g/cm^3, agreeing with previous estimates (25). With the usual assumption that modified bases are absent, the guanine plus cytosine (G+C) contents of the above DNAs are, thus, 55 and 56%, respectively (49).

After molecular weight reduction by shearing, some herpesvirus DNAs exhibit altered buoyant density profiles as a result of an uneven distribution of guanine · cytosine and adenine · thymine base pairs (15). Such a result was obtained when *H. aotus* type 1 and three DNAs were sheared to an average molecular weight of <10 × 10^6 (Fig. 1c and f). Shoulders were formed in both profiles at low buoyant density (~1.708 g/cm^3) and accounted for roughly one fourth of the total DNA, whereas the remaining DNA banded at a buoyant density of about 1.716 g/cm^3. HCMV DNA sheared to the same molecular weight produced a profile with no apparent shoulder, but which was slightly skewed toward low buoyant densities (Fig. 1i).

Molecular weight estimates were obtained for *H. aotus* type 1 and three DNAs by velocity sedimentation in 5 to 20% sucrose gradients. with CCV DNA (M_r = 84 × 10^6) (9) as an internal size standard. Both DNAs sedimented more rapidly than did CCV DNA (Fig. 2) and, using the equation S1/S2 = $(M_r1/M_r2)^{0.38}$ (19) relating sedimentation distance to molecular weight, the values for serotypes 1 and 3 were M_r ~147 × 10^6 and M_r ~143 × 10^6, respectively—within the estimated limits (±4 × 10^6) of experimental error. An independent size determination of *H. aotus* type 3 DNA was carried out by electron microscopy (Table 1). The contour length measurements of 14 molecules provided a mean M_r = 143 × 10^6 (±6 × 10^6), in good agreement with the value obtained from velocity sedimentation. Under similar spreading conditions, the molecular weight obtained for HCMV (strain Ad169) DNA was M_r = 152 × 10^6 (±6 × 10^6) (P. Sheldrick and N. Berthelot, unpublished

TABLE 1. Contour length measurements of DNA from *H. aotus* types 1 and 3

DNA	*H. aotus* type 1[a]				*H. aotus* type 3			
	n	ϕX174 (units)	PM2 (units)	M_r (×10^6)	*n*	ϕX174 (units)	PM2 (units)	M_r (×10^6)
Native DNA	—[b]	—	—	—	14	—	22.3 ± 0.99	143 ± 6
Denatured DNA								
U_L	8	30.5 ± 1.6	—	51.8 ± 2.7[c]	8	30.2 ± 1.1	—	51.3 ± 1.9[c]
U_S	23	5.62 ± 0.25	—	9.55 ±·42[c]	22	5.56 ± 0.24	—	9.45 ± 0.41[c]
IR_S/TR_S	15	—	0.32 ± 0.04	2.05 ± 0.26[d]	13	—	0.36 ± 0.02	2.30 ± 0.13[d]
$IR_L/TR_L+IR_S/TR_S$	8	—	0.57 ± 0.05	3.65 ± 0.32[d]	6	—	0.59 ± 0.09	3.78 ± 0.58[d]

[a] ± indicates the standard deviation of the measurements.
[b] —, Not done.
[c] Calculated for single-stranded DNA from 1 ϕX174 unit = 1.7 × 10^6 (47).
[d] Calculated for double-stranded DNA from 1 PM2 unit = 6.4 × 10^6 (42).

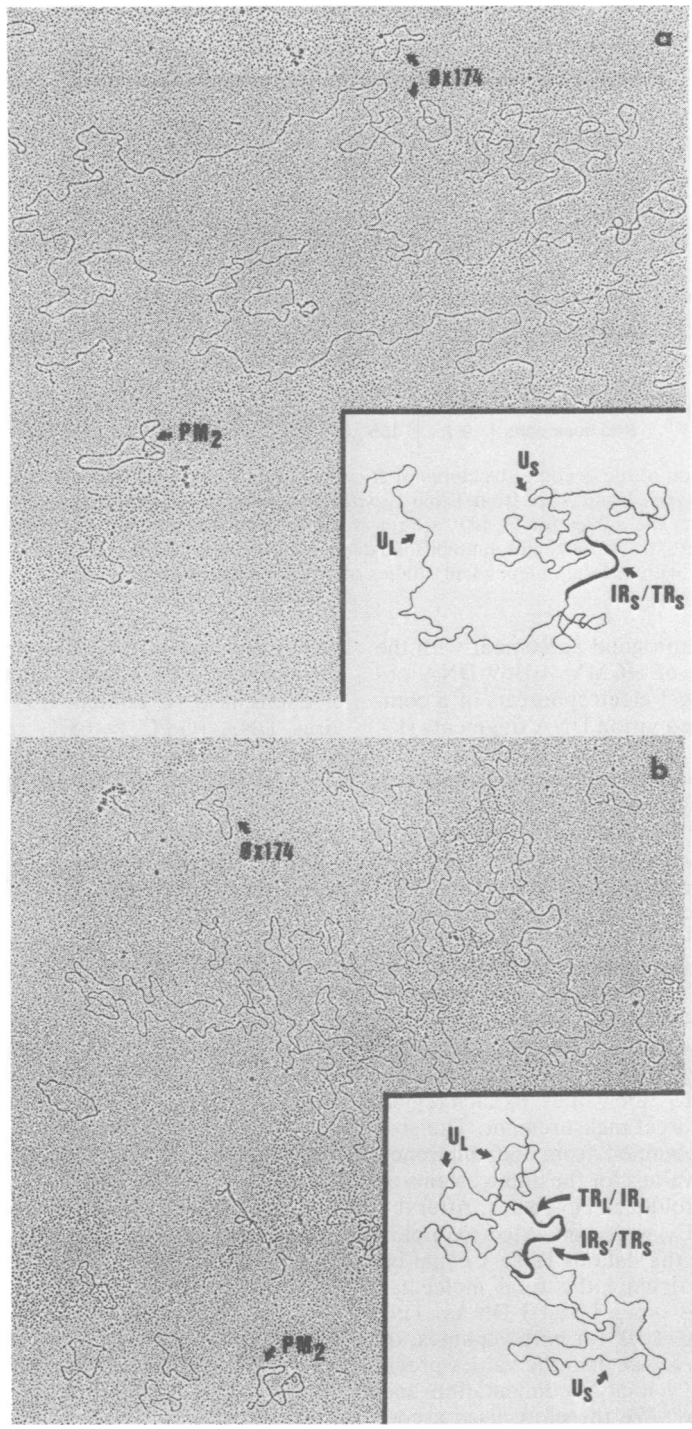
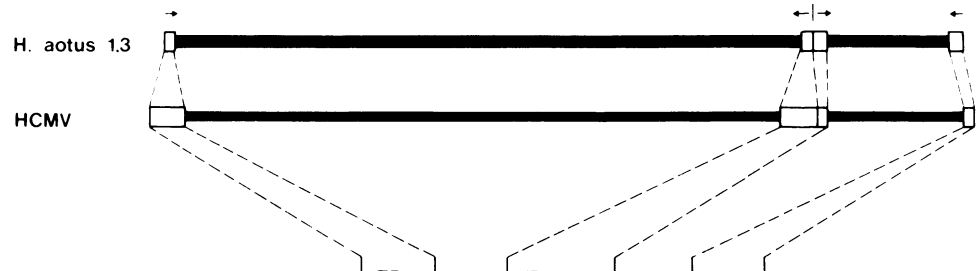


FIG. 3. Foldback forms of single-stranded DNA from *H. aotus* type 3. (a) Single-looped foldback of the inverted repeats flanking U_S. (b) Double-looped foldback of the inverted repeats flanking both U_S and U_L. The interpretative drawings refer to features of the molecules located directly above in the photos. Magnification, ×6,000.



		TR_L	U_L	$IR_L + IR_S$	U_S	TR_S	TOTAL
H. aotus 1.3	Mol. wt ($\times 10^{-6}$)	1.5	117	3.7	22	2.2	146
	Kilo base pairs	2.2	175	5.5	33	3.3	219
HCMV	Mol. wt ($\times 10^{-6}$)	6.5	110	8.1	25	1.6	151
	Kilo base pairs	9.7	165	12.1	37	2.4	226

FIG. 4. Comparison of the genome structures of *H. aotus* types 1 and 3 and HCMV. The molecular weight values for *H. aotus* types 1 and 3 are from Table 1, with conversion from single-stranded values to duplex as discussed in the text. The values for HCMV are for strain Ad169 as determined by electron microscopy (P. Sheldrick, M. Laithier, and N. Berthelot, unpublished data) in parallel with the two *H. aotus* serotypes. They are in general agreement with several independent studies of HCMV genome structure (13, 17, 33, 43, 54, 60).

data). This value is in good agreement with the size determination of HCMV Ad169 DNA obtained by agarose gel electrophoresis of a complete series of cloned virion DNA fragments (17, 20, 55).

To test for inverted repeat nucleotide sequences, intact single strands of *H. aotus* types 1 and 3 DNAs were isolated from high-salt 5 to 20% neutral sucrose gradients (51) and examined by electron microscopy. Single-looped and double-looped fold-back molecules were found for both DNAs (Fig. 3). The mean contour lengths of the loops (unique sequence regions) and the duplex stems (inverted repeats) are given in terms of the ϕX174 and PM2 internal standards in Table 1, along with the calculated molecular weights of each region of the genome. With the exception of TR_L/IR_L, the values for each region were obtained by direct measurement. The size of TR_L/IR_L was obtained from the difference between the mean values for the duplex stems of single- and double-foldback molecules. After the values for U_S and U_L were converted to duplex molecular weights, the data in Table 1 could be used directly to calculate the total molecular weight for *H. aotus* types 1 and 3 DNAs. This value was $M_r \cong 130 \times 10^6$ for both genomes, or approximately 10% lower than the values previously obtained by velocity sedimentation and electron microscopy. We therefore used a correction factor of +13% for converting single-stranded M_rs to duplex M_rs; when this was done, the values were $M_r \cong 146 \times 10^6$ for both genomes. The correction factor is necessary, presumably because of the differential contraction of the single-stranded sample and the internal standard (ϕX174 DNA, G+C $\cong$ 45%) DNAs of different G+C content (51); herpes simplex virus DNA (G+C $\cong$ 68%) requires a +25% correction factor (51), whereas Marek's disease virus DNA (G+C $\cong$ 45%) requires none (6). The structure of *H. aotus* types 1 and 3 DNAs is compared with that of HCMV DNA in Fig. 4.

DNA sequence homology between *H. aotus* types 1 and 3. An estimation of genetic related-

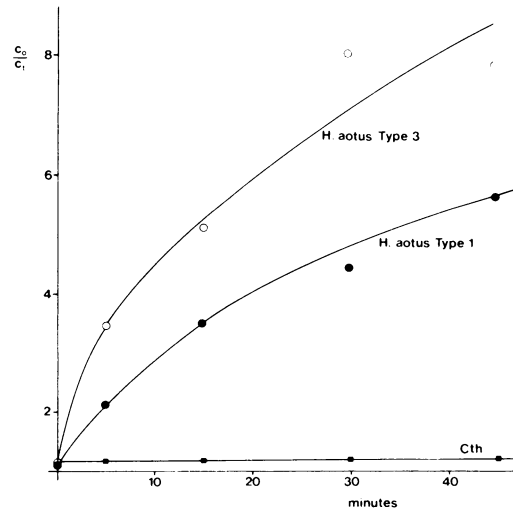


FIG. 5. Reassociation of ^{3}H-labeled *H. aotus* type 3 DNA (1 μg/ml, 5,000 cpm/μg) with DNA (100 μg/ml) from cells infected by *H. aotus* type 3 (○) or *H. aotus* type 1 (●) and calf thymus (■).

ness between genomes can be obtained by DNA-DNA cross-hybridization and, when the relatedness is high, by restriction endonuclease analyses. These two were, therefore, applied to the *H. aotus* types 1 and 3 genomes. Radiolabeled *H. aotus* type 3 DNA was reannealed in the presence of unlabeled DNA from OMK cells infected with either *H. aotus* type 1 or type 3. As a control, self-hybridization was measured in the presence of calf thymus DNA at a concentration equal to that of the total DNA from infected cells. As shown in Fig. 5, DNA from *H. aotus* type 3-infected cells drove at least 87% (C_0/C_t = 8) of the probe into duplex structures. Similarly, DNA from *H. aotus* type 1-infected cells drove self-association to at least 80% (C_0/C_t = 5) completion.

To test the extent of homology in the *H. aotus* type 3-*H. aotus* type 1 heteroduplexes, we compared their melting behavior with that of *H. aotus* type 3 homoduplexes by heating to increasing temperatures (5°C increments), followed by rapid quenching and hydroxyapatite

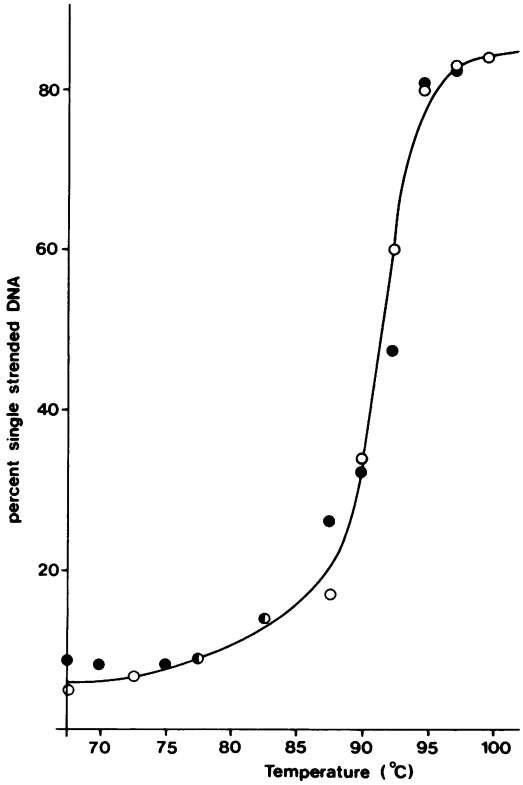


FIG. 6. Determination of T_ms of heteroduplex DNA molecules (^{3}H-labeled *H. aotus* type 3 DNA/unlabeled *H. aotus* type 1 virion DNA) (○) and homoduplex molecules (^{3}H-labeled *H. aotus* type 3 DNA/unlabeled *H. aotus* type 3 DNA) (●).

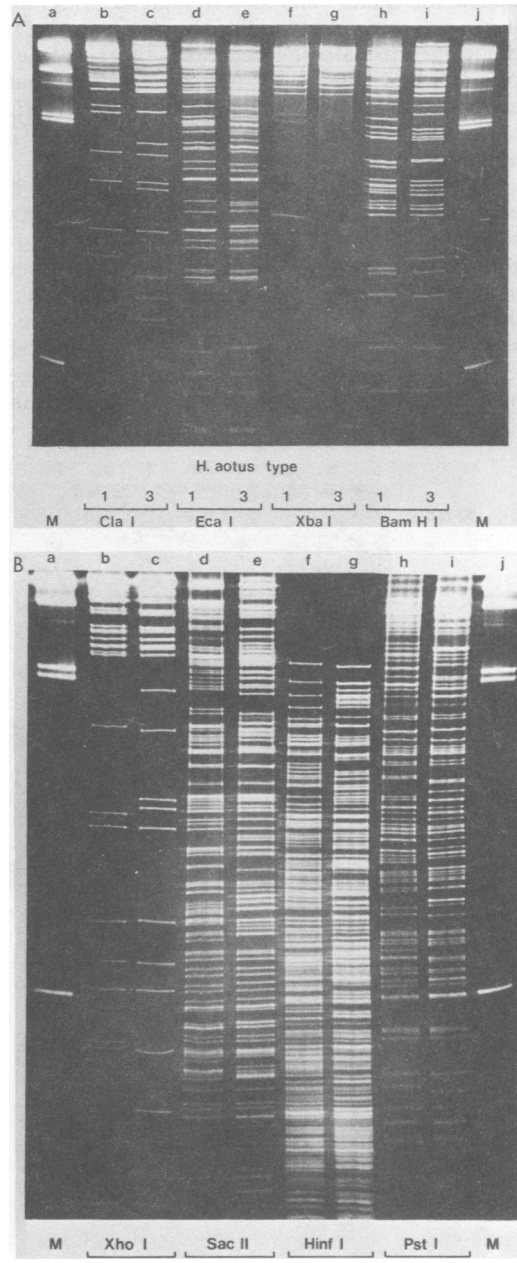


FIG. 7. Comparative cleavage of *H. aotus* type 1 and type 3 DNA with restriction endonucleases (A) *Cla*I, *Eca*I, *Xba*I, and *Bam*HI and (B) *Xho*I, *Sac*II, *Hin*fI, and *Pst*I. The fragments were separated by vertical electrophoresis in 5 to 15% (wt/wt) polyacrylamide, stained with 0.5 μg of ethidium bromide per ml, and visualized under short-wavelength UV light. DNA fragments of bacteriophage lambda generated by cleavage with endo R · *Hin*dIII were used as size markers (M) (M_rs [× 10^6] = 15.58, 6.36, 4.38, 2.86, 1.49, 1.37, 0.37).

chromatography. The denaturation curves obtained for mono- and heteroduplexes (Fig. 6) were not measurably different ($\Delta T_m \leq 3°C$). Since 1% mismatching decreases the T_m of duplex DNA by about 1.5°C (56), we conclude that at least 80% of the base sequences in the *H. aotus* types 1 and 3 genomes are at least 98% homologous.

On the basis of the above experiments, it appeared that *H. aotus* types 1 and 3 DNAs share a minimum of 78% (80% × 98%) homologous nucleotide sequences. If the maximum homology is 78%, by statistical considerations, there would be little probability of having restriction endonuclease cleavage sites in common (39, 57). *H. aotus* types 1 and 3 DNAs do, however, share many restriction sites, since in electrophoretic profiles of digests with 10 restriction enzymes (Fig. 7), approximately 70% of the fragments from the two serotypes comigrated. This level of fragment comigration indicates that the overall sequence homology for the two DNAs is ≥95% (39, 57).

Absence of DNA sequence homology between *H. aotus* type 3 and HCMV. In view of the structural similarity of *H. aotus* types 1 and 3 DNAs to the DNA of HCMV (Fig. 4), we examined the possibility that they might also exhibit base sequence homology. Accordingly, ^{32}P-labeled *H. aotus* type 3 DNA was hybridized to nitrocellulose filters onto which unlabeled *Hin*dIII and *Eco*RI fragments from a series of cosmid clones, representing the entire genome of HCMV (17), had been transferred by the

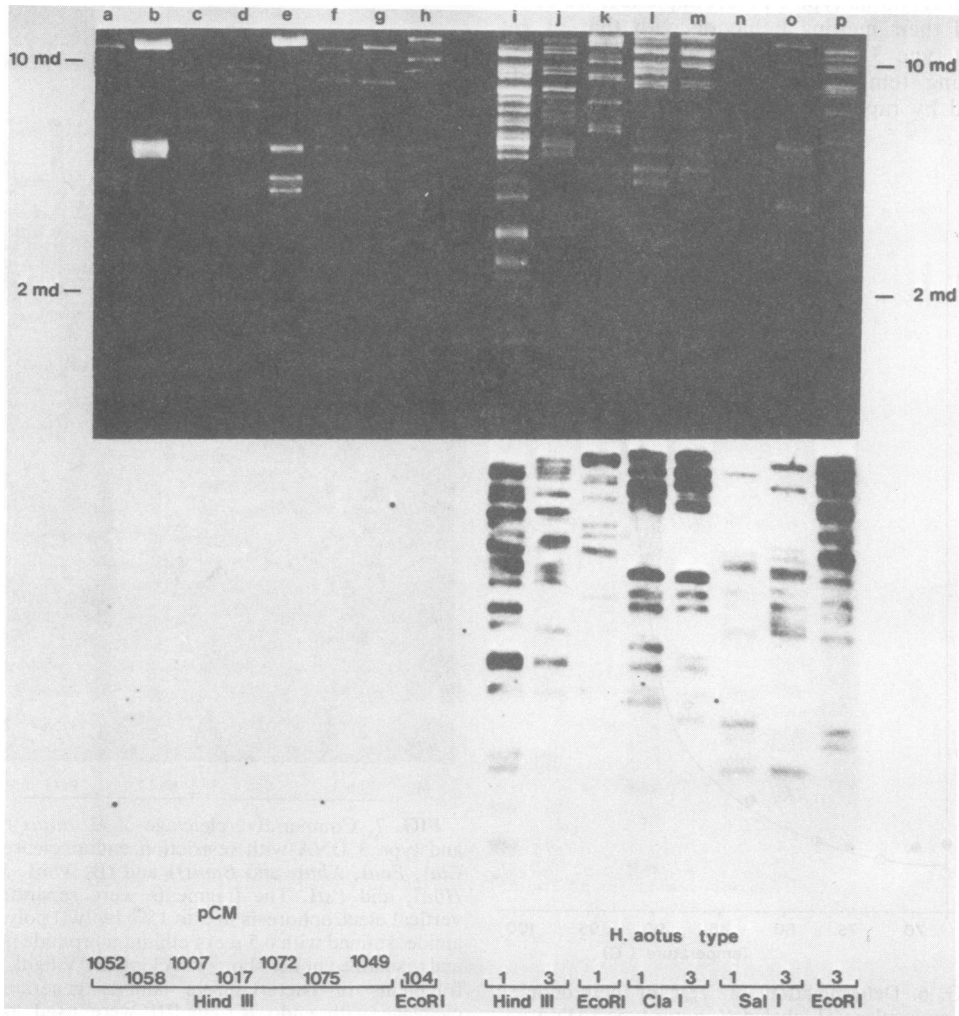


FIG. 8. Hybridization of ^{32}P-labeled (6.8 × 10^7 cpm/μg) DNA of *H. aotus* type 3 with blotted restriction fragments of *H. aotus* types 1 and 3 and a series of cosmid clones (pCM104 and -1007 to -1075) representing the genome of HCMV Ad169. md, Megadalton.

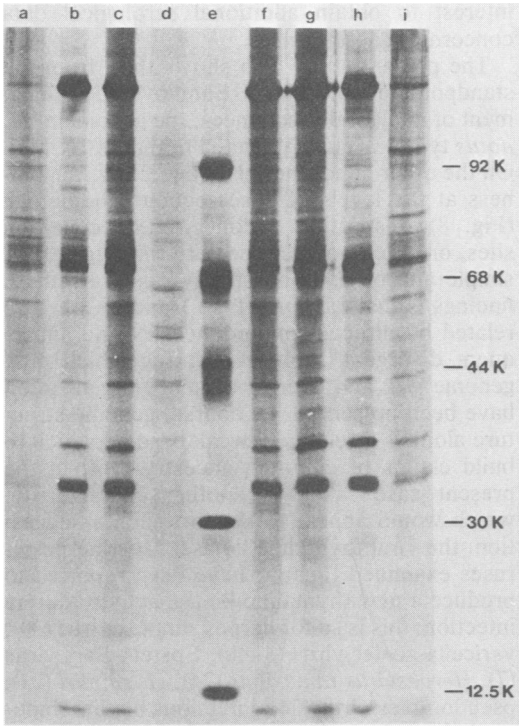


FIG. 9. Separation of [^{35}S]methionine-labeled structural proteins of *H. aotus* types 1 and 3 by electrophoresis in a 7.5 to 20% (wt/vol) polyacrylamide gradient gel. Lane a, Envelope proteins of *H. aotus* type 3 isolated after lysis of virus particles with 0.5% Nonidet P-40; lane b, nucleocapsid proteins of *H. aotus* type 3; lane c, total virion proteins of *H. aotus* type 3; lane d, proteins from supernatants of mock-infected cultures; lane e, size markers; lanes f and g, total virion proteins of *H. aotus* types 1 and 3, respectively; lane h, nucleocapsid proteins of *H. aotus* type 1; lane i, envelope polypeptides of *H. aotus* type 3.

Southern procedure. None of the HCMV *Hin*dIII fragments showed detectable hybridization to *H. aotus* type 3 DNA (Fig. 8), in agreement with experiments in which unlabeled DNA from HCMV-infected human fibroblasts failed to drive the same ^{32}P probe into duplex structures (data not shown). Thus, HCMV does not share (<5%) genetic information with *H. aotus* type 3 (or type 1).

Structural polypeptides of *H. aotus* type 1 and type 3. A parallel analysis of [^{35}S]methionine-labeled proteins of *H. aotus* type 1 and type 3 by sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated a very high degree of similarity. From purified preparations of both viruses, 42 polypeptides could be identified in the size range between 155,000 and 10,500 daltons, probably each representing a virion constituent. No significant differences could be found in the abundancies or migration distances of *H. aotus* type 1 and type 3 proteins, regardless of whether nucleocapsids, envelope proteins, total virion polypeptides (Fig. 9), or phosphoproteins (data not shown) were analyzed. The patterns were dissimilar to the profiles of HCMV virion proteins (Fig. 10) (21, 29, 41, 50, 53) and to profiles of structural proteins from unrelated herpesviruses (24). The virtually identical migration patterns of *H. aotus* type 1 and type 3 proteins are remarkable, if one considers that the method has been used to distinguish structural polypeptides between different strains of one herpesvirus, such as *H. saimiri* (26).

In view of the size differences of structural viral proteins, it is not surprising that by immunofluorescence we could not find antigenic sites shared by HCMV and these *H. aotus* isolates. Indirect anti-complementary immunofluorescence (44) with lytically infected cells, using high-titer human anti-HCMV sera, did not produce any reactivity with *H. aotus* types 1 and 3,

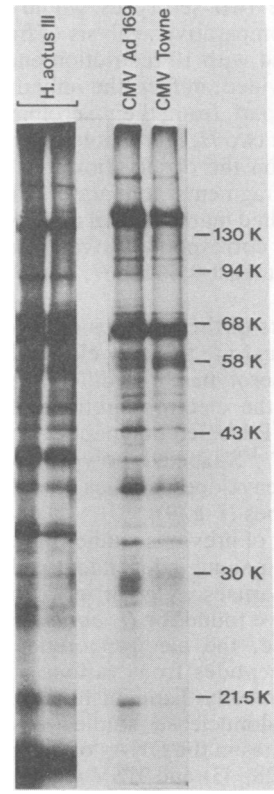


FIG. 10. Separation of structural proteins from *H. aotus* type 3 and HCMV strains Ad169 and Towne, labeled with ^{125}I-Bolton-Hunter reagent (New England Nuclear) and migrating in parallel 15% (wt/vol) polyacrylamide gels.

whereas HCMV-infected human fibroblasts showed brilliant staining of intranuclear inclusions (data not shown).

DISCUSSION

On the basis of negative cross-neutralization tests, *H. aotus* types 1 and 3 have been described as distinct serotypes (10), yet in the several physicochemical comparisons presented here, they clearly show a very high degree of similarity. Their genomes are, within the limits of experimental error, identical in size ($M_r \cong 145 \times 10^6$), G+C content (~55%), and the buoyant density distribution of fragments generated by shearing forces. Moreover, both genomes harbor inverted repeat sequences whose dimensions and locations are, as far as we have been able to determine, identical.

At a higher level of resolution, the heterologous DNA-DNA hybridization showed that at least 80% of the sequences in the two genomes can form stable duplex structures. The melting properties of the resulting heteroduplexes required that their sequence homology be at least 98%, so that the overall minimum homology between the two serotypes would have to be ~78%. A comparative analysis of fragment profiles obtained with 10 restriction endonucleases (Fig. 7) provided, in fact, the only unambiguous evidence (apart from their serological properties) that the two *H. aotus* isolates are nonidentical. Based on the observation that 70% of the restriction fragments comigrate, it is possible, using published mathematical models (39, 57), to estimate the corresponding overall sequence homology as at least 95% for *H. aotus* types 1 and 3.

The results of the analyses of virion polypeptides also illustrate the very close relatedness of these two serotypes. No differences were detectable in the electrophoretic profiles of ^{35}S-labeled or ^{32}P-labeled polypeptides, and the distribution of ^{35}S-labeled polypeptides between capsid and envelope moieties was the same for both serotypes (Fig. 9).

A number of previous studies have uncovered a variability among independent isolates of certain herpesviruses as great as or greater than that which we found for *H. aotus* types 1 and 3. For example, the electrophoretic patterns of virion polypeptides from various strains of *H. saimiri* are readily distinguishable (26), and restriction endonuclease studies have revealed heterogeneities in the DNAs of different isolates of HCMV (28, 43) and HSV (5, 35). We therefore suggest that *H. aotus* type 3 should not be accorded a taxonomically distinct status but, instead, should be considered as an independent isolate, or strain, of *H. aotus* type 1 (aotine herpesvirus 1) (45). It would be of obvious interest to obtain additional serological data concerning these viruses.

The present study also shows that, from the standpoint of molecular size and overall arrangement of nucleotide sequences, the genome of *H. aotus* type 1 is closely related to that of HCMV. On the other hand, there is little, if any, relatedness at the levels of base sequence homology (Fig. 8), restriction endonuclease recognition sites, or virion polypeptide patterns (Fig. 10). A simple and obvious possibility raised by these findings is that *H. aotus* type 1 and HCMV are related by direct common ancestry and subsequent divergent evolution, during which only genome size and overall sequence organization have been preserved. Of course, genome structure alone is a relatively weak base on which to build claims of common ancestry; yet, in the present case, there is another characteristic which would appear to support such a suggestion: the viral thymidine kinase. Most herpesviruses examined thus far have been reported to produce a new thymidine kinase activity during infection; this is so for herpes simplex virus (30), varicella-zoster virus (8, 40), Epstein-Barr virus (7), *Herpesvirus tamarinus* (34), *H. saimiri* (23), pseudorabies virus (34), infectious bovine rhinotracheitus virus (59), two equine herpesviruses (2, 37), two avian herpesviruses (30), and CCV (M. Laithier and P. Sheldrick, unpublished data). In contrast, infections by *H. aotus* type 1 (34) and HCMV (63), as well as a murine CMV (38) and an equine CMV (31), do not appear to induce a novel thymidine kinase activity. Honess and Watson (24) have signaled the potential importance of viral thymidine kinase in deciding relatedness among members of the herpesvirus group, and here we find that, at least for *H. aotus* type 1 and HCMV, genome structure points in the same direction as does the thymidine kinase-less phenotype of these two viruses.

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