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Anatomy of Herpes Simplex Virus DNA: Strain Differences and Heterogeneity in the Locations of Restriction Endonuclease Cleavage Sites

(agarose gel electrophoresis/minor fragments/defective DNA)

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ABSTRACT **Digestion of herpes simplex virus DNA by the *Hin*III or *Eco*RI restriction endonucleases yielded 11 to 15 fragments with molecular weights between 1×10^6 and 28×10^6. The electrophoretic profiles obtained in 0.3% agarose gels with DNA fragments from nine different strains of herpes simplex virus type 1 could be readily differentiated from the patterns exhibited by the corresponding fragments from four separate strains of type 2 virus; however, within each serotype, the laboratory strains differed significantly among themselves and also from isolates passaged a minimum number of times outside the human host. Digestion of all DNAs of herpes simplex virus with either enzyme reproducibly generated two classes of fragments (major and minor) which differed in molar concentration. Moreover, although the molecular weight of an intact herpes simplex 1(F1) DNA molecule is approximately 98×10^6, the summed molecular weights of all major and minor *Hin*III fragments totalled 160×10^6, and the seven major fragments alone accounted for only 60×10^6. These unusual features indicate the existence of limited heterogeneity in the positions of cleavage sites along individual molecules. We have eliminated the possibility that minor fragments arose from contamination with the defective DNA of high buoyant density which appears on serial undiluted passage of the virus. In fact, this latter type of DNA was resistant to cleavage by *Hin*III and gave large amounts of only two species of *Eco*RI fragments, suggesting that the defective molecules consist of many tandem repeats of a small segment of viral DNA. The heterogeneity in the viral DNA of normal density appears to be related to the structural organization of the molecules and does not necessarily imply differences in genetic content.**

We are attempting to construct maps of restriction enzyme fragments from the DNA of herpes simplex virus (HSV), and wish to report on some unusual observations relating to the structure of HSV DNA molecules that emerged from these studies. We have also been concerned about possible genetic differences between the various strains and isolates of HSV-1 and HSV-2. Two clinical isolates, designated HSV-1(F1) and HSV-2(G1), have been used in this laboratory as prototypes of naturally occurring strains and have been passaged at low multiplicity a maximum of four times in HEp-2 (human epidermoid carcinoma) cells to avoid selection of variants in culture. However, established "laboratory" strains characterized by histories of numerous passages outside the human host have been reported to differ from such isolates and among themselves with respect to certain structural and biological properties (1–3).

Relevant information about the HSV genome can be summarized as follows: (*i*) The DNA molecules isolated from virions are linear and double-stranded with a molecular weight of 95 to 100×10^6 (4, 5). The kinetic complexity of the viral DNA is also approximately 95×10^6 (6). (*ii*) A single viral particle or isolated DNA molecule (7) is sufficient to initiate productive infection. (*iii*) HSV-1 and HSV-2 DNAs have characteristic buoyant densities of 1.727 and 1.729 g/cm^3, respectively, corresponding to 67 and 69 mol % G+C (5), and show approximately 50% homology with 85% matching of base pairs (8). (*iv*) An additional DNA species with unusually high buoyant density (>1.731 g/cm^3) may also be present in viral preparations after serial passages at high multiplicity (9).

MATERIALS AND METHODS

Virus Strains. HSV-1 strains, designated F1, F5, and F9, and HSV-2(G1) are independent isolates from patients at the University of Chicago Hospitals (1). HSV-1(A428) was isolated from human trigeminal ganglion by Dr. A. Nahmias, Atlanta, Ga. All isolates were passaged a maximum of four times at low multiplicity in HEp-2 cells. The sources of the laboratory strains were as follows: HSV-1(MP), Hoggan and Roizman (ref. 3); HSV-1(CL101), Dr. S. Kit, Houston, Texas; HSV-1(35), HSV-1(13), and several variants thereof, Dr. M. Terni, Ferrara, Italy; HSV-1(Justin)*, Dr. A. Sabin, Bethesda Md.; HSV-1(72-43), HSV-2(333), and HSV-2(316), Dr. F.

Abbreviations: HSV-1, herpes simplex virus type 1, human herpesvirus 1; HSV-2, herpes simplex virus type 2, human herpesvirus 2; HEp-2, human epidermoid carcinoma, no. 2; *Hin*III, endo R, *Hin*dIII restriction endonuclease from *Hemophilus influenzae* strain Rd; *Eco*RI, endo R, *Eco*R$_1$I restriction endonuclease from *Escherichia coli*.

* Shortly after Dr. Sabin isolated the Justin strain of HSV from a genital lesion in 1966, it was identified in Dr. J. L. Melnick's laboratory as HSV-2 by the neutralization test in use at the time. On this basis it has been referred to as HSV-2 in publications from Dr. Sabin's laboratory. Recently, both early and late passage levels of this strain were identified as HSV-1 in two other laboratories by immunofluorescence (A. Nahmias) and quantitative serum dilution neutralization tests (F. Rapp) in current use. Despite these immunological tests, Dr. F. Rapp found in 1973 that the DNA of the Justin strain had a buoyant density pattern similar to that of HSV-2 (personal communication, A. Sabin). We believe that this was probably due to the fact that most of the Justin strain viral DNA is defective—as much as 80% in some of our preparations. In our laboratory, the Justin strain was identified as HSV-1 on the basis of structural polypeptide content and by its DNA buoyant density and restriction endonuclease cleavage pattern.

TABLE 1. *Action of restriction endonucleases on HSV DNA*

Enzyme	No. of fragments and mol. wt. range ($\times 10^{-6}$) HSV-1(F1) 67% G+C	HSV-2(G1) 69% G+C	T5+ 40% G+C
*Hin*III ↓ A-A-G-C-T-T	7 Major (27.5–2.1)* 8 Minor (27.5–5.0)	7 Major (24.5–1.1) 3 or 4 Minor (13.5–5.5)	16 (11–0.5)
*Eco*RI ↓ G-A-A-T-T-C	10 Major (17.5–1.2) 4 Minor (14.3–8.2)	7 Major (24–2.2) 7 or 8 Minor (21–4.7)	7 (29–0.4)
*Hpa*I ↓ G-T-T-A-A-C	18 Major (13.2–1.0) 4 Minor (14.5–4.1)		23 (12–0.6)
*Sal*I	35 ± 3 (7.0–0.6)		4 (36–8)
*Sma*I	>100 (<3.0)		3 (44–8)

The enzyme nomenclatures and palindrome sequences are essentially as described by Smith and Nathans (14) and in references therein, or from information compiled by R. J. Roberts, Cold Spring Harbor, N.Y. Activities on phage T5+ DNA are included for comparative purposes (Hayward, Gabain, and Bujard, in preparation).

* Molecular weights ($\times 10^{-6}$) of the largest and smallest fragments observed. Additional fragments below 0.5 to 1.0 $\times 10^6$ in molecular weight would not have been detected.

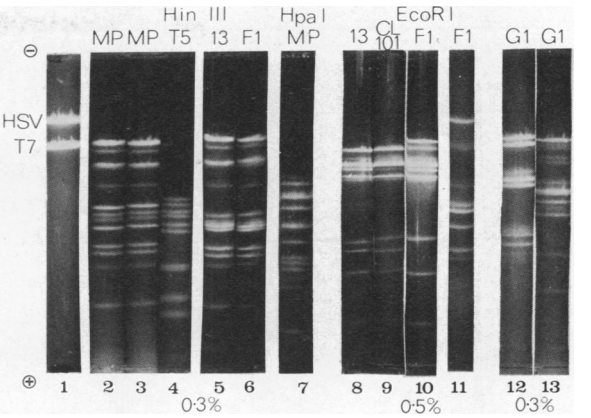

FIG. 1. Examples of electrophoretic separations of HSV DNA fragments stained with ethidium bromide. Except where stated below, the incubations and electrophoresis were performed as described in *Materials and Methods*. Gels *1–7*, 0.3% agarose. Gel *1*, uncleaved HSV-1(F1) and phage T7 DNAs. Gels *2–6*, limit digests of 3 μg of DNA with 2 μl of *Hin*III enzyme: *2*, HSV-1(MP); *3*, HSV-1(MP), 40 μl of enzyme; *4*, phage T5+; *5*, HSV-1(13); *6*, HSV-1(F1). Gel *7*, limit digest of HSV-1(MP) DNA with *Hpa*I enzyme. Gels *8–10*, 0.5% agarose, limit digests of 4 μg of DNA with *Eco*RI enzyme: *8*, HSV-1(13); *9*, HSV-1(CL-101); *10*, HSV-1(F1). Gel *11*, *Eco*RI-digested HSV-1(F1) DNA (8 μg). Electrophoresis carried out at 1 V/cm for 120 hr through a 30 cm long gel of 0.5% agarose. Only that portion of the gel containing fragments in the 8 $\times$ 10^6 to 17.5 $\times$ 10^6 molecular weight range is shown. Gels *12* and *13*, 0.3% agarose: *12*, HSV-2(G1), *Hin*III; *13*, HSV-2(G1), *Eco*RI.

Rapp, Hershey, Pa.; HSV-1(VR3) and HSV-2(MS), Dr. A. Nahmias. Except as indicated in the *text*, virus pools were prepared by infection at low multiplicity (<0.001 plaque-forming units per cell). Plaque purifications were performed by terminal dilution.

DNA Purification. Nucleocapsids were purified from cytoplasmic extracts of Nonidet-P40-treated, infected HEp-2 cells by velocity sedimentation in sucrose gradients (10), and disrupted with 0.5% sodium dodecyl sulfate/0.5% sarcosyl/10 mM EDTA. After two extractions with phenol and chloroform/isoamyl alcohol, the DNA was precipitated with ethanol, dissolved in a small volume of 10 mM Tris·HCl/1 mM EDTA (pH 8.4), and dialyzed extensively. [^{3}H]DNA was prepared by adding 5 μCi/ml of [^{3}H]thymidine to the growth medium.

*Enzymes. Hin*III and *Eco*RI restriction endonucleases (from *H. influenzae* strain Rd and *E. coli* strain RY13) were purified through two phosphocellulose steps by procedures adapted from Smith and Wilcox (11) and Sharp *et al.* (12). *Hin*III incubation mixtures (200 μl) contained 3 μg of DNA, 50 mM NaCl, 10 mM Tris·HCl (pH 7.6), 5 mM $MgCl_2$, and 2 μl of enzyme. *Eco*RI incubation mixtures (200 μl) contained 4 μg of DNA, 30 mM Tris·HCl (pH 7.6), 10 mM MgCl, and 0.5 μl of enzyme. Reactions were stopped after 2.5 hr at 37° by addition of EDTA. Initial enzyme samples were obtained from the laboratories of Dr. D. Nathans (*Hin*III, *Eco*RI, and *Hpa*I), Dr. C. Mulder (*Sma*I, from *Serratia marcescens*), and Dr. R. Roberts (*Sal*I, from *Streptomyces albus* strain G).

Agarose Gel Electrophoresis. The DNA samples were layered onto cylindrical columns of 0.3% or 0.5% agarose gel (Sea-Kem) and subjected to electrophoresis in Tris/phosphate buffer for 18 hr at 4° (13). After electrophoresis the gels were stained with ethidium bromide and photographed in shortwave UV light (12). For ^{3}H or double ^{3}H and ^{32}P radioactivity profiles, the gels were cut into 1-mm slices. These were dissolved by autoclaving in 1 ml of H_2O and dried onto glass fiber paper for determination of radioactivity in toluene-based scintillation fluid.

RESULTS

Digestion of HSV DNA with Restriction Endonucleases. Preliminary surveys of five enzymes known to produce a relatively small number of fragments with phage DNA of similar size (summarized in Table 1) indicated that the most useful enzymes for analysis of HSV DNA would be *Hin*III, *Eco*RI, and *Hpa*I. Fig. 1 shows representative photographs of agarose gels, stained with ethidium bromide, containing electrophoretically separated DNA fragments. Some typical radioactivity profiles from similar separations of cleaved HSV DNAs are given in Fig. 2. The diagram in Fig. 3 summarizes our data on the relative positions and intensities in 0.3% agarose gels of the *Hin*III and *Eco*RI fragments from DNAs of 13 representative strains of HSV-1 and HSV-2.

Evidence for Heterogeneity in the Viral DNA. The electrophoretic profiles of limit digests of both *Eco*RI- and *Hin*III-cleaved DNAs contained relatively faint "minor" bands (designated by numbers in Fig. 2 and Table 2) interspersed among the more intense "major" bands (designated by letters). The major fragments have been determined to be present in amounts equivalent to one copy per genome, whereas the minor fragments occur in sub-molar concentrations (Hayward, Jacob, Wadsworth, and Roizman, in preparation).

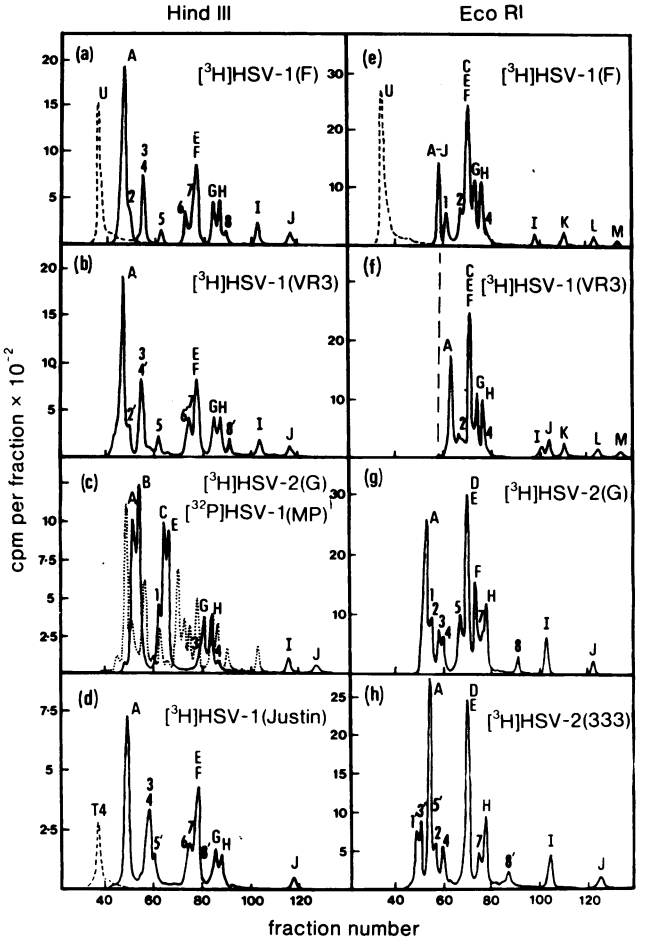

FIG. 2. Radioactivity profiles of the fragments that resulted from cleavage of HSV [³H]DNAs with *Hin*III or *Eco*RI enzymes. Electrophoresis carried out in 0.3% agarose gels. *Solid curves:* Panels a–d, limit digests of [³H]DNA with *Hin*III: (a) HSV-1(F1); (b) HSV-1(VR3); (c) HSV-2(G1); (d) HSV-1(Justin). Panels e–h, limit digests of [³H]DNA with *Eco*RI: (e), HSV-1(F1); (f) HSV-1(VR-3); (g) HSV-2(G1); (h) HSV-2(333). *Broken curves:* Panels a and e, uncleaved samples of the same HSV-1(F1) [³H]DNA (designated U) run in a parallel gel. Panel c, HSV-1(MP) [³²P]DNA mixed with the [³H]HSV-2(G1) sample before digestion and analyzed on the same gel. Panel d, uncleaved T4 [¹⁴C]DNA in the same gel. Those HSV DNA fragments designated as "major" are identified by letters, and the "minor" fragments by numbers. Primed numbers indicate alterations relative to the prototype HSV-1(F1) or HSV-2(G1) strains. Fraction numbers are approximately equivalent to distance migrated in mm.

The molecular weights of the HSV DNA fragments were estimated by coelectrophoresis with the *Eco*RI fragments from λ_{c1857} DNA in the same gel (not shown). For each strain, the summed molecular weights of all fragments were greatly in excess of that for the intact parental DNA molecule, but the major fragments alone accounted for much less than the total genetic information (Table 2).

Additional experiments (not detailed here) showed that the minor bands were not caused by (*i*) impurities in the enzyme preparations, (*ii*) annealing interactions between the short complementary single-stranded ends on the DNA fragments (16), or (*iii*) contamination with host cell DNA (average density 1.698 g/cm³). Similarly, the minor components cannot

TABLE 2. *Classification and molecular weights of HSV DNA fragments*

HSV-1(F1)		HSV-2(G1)	
*Eco*RI	*Hin*III	*Eco*RI	*Hin*III
(a) Individual fragments (molecular weights × 10^{-6})			
A-J 17.5	A 27.5	A 24	A 24.5
1 14.3	*1* 27.5[a]	*1* 21	B 21
2 12.0	*2* 25	*2* 19.5	*1* 13.5
C 10.5	*3* 19	*3* 17.5	D 12.5
3 10.5[b]	*4* 17	*4* 15.5	(*2* ?)[c]
E 10.3	*5* 13.5	*5* 12.5	E 11.5
F 10.3	*6* 9.2	D 10.4	*3* 7.0
G 9.8	*7* 8.3	(*6* ?)[c]	G 6.6
H 8.5	E 8.0	E 10.1	H 6.0
4 8.2	F 7.6	F 9.2	*4* 5.5
I 3.7	G 6.0	*7* 8.5	I 2.2
K 2.7	H 5.5	H 8.0	J 1.1
L 1.8	*8* 5.0	*8* 4.7	
M 1.2	I 3.2	I 3.4	
	J 2.1	J 2.0	
(b) Summations (molecular weights × 10^{-6})			
Majors 77	60	67	85
Minors 44	100	94	26
Total 121	160	161	111

The distinction between major fragments (designated by letters) and minor fragments (numbers and *italics*) is based on the relative intensities of bands stained with ethidium bromide and on quantitative measurements of the amount of radioactivity in each band with ³²P-labeled DNA (details to be published elsewhere). Molecular weights of the HSV fragments were estimated from standard curves of electrophoretic mobility in agarose gels plotted against molecular weight on a logarithmic scale using the six *Eco*RI fragments of λ_{c1857} DNA as reference species (15).

[a] Evidence for the existence of this fragment was deduced from the results of *Eco*RI cleavage of the isolated 27.5 × 10^6 molecular weight band from a *Hin*III digest.

[b] Detected in double cut digests (*Hin*III and *Eco*RI).

[c] Possible existence of an additional minor fragment indicated from molar ratio analysis.

be explained by blocked or modified cleavage sites in some molecules because most of the minor fragments do not have the appropriate molecular weights nor do they comigrate with authentic partial digestion products. Endogenous contaminating viruses were also excluded because they would necessarily contain DNA of the same size and buoyant density as HSV DNA and be present in the same proportion in all cell lines and virus stocks tested. Furthermore, there would have to be entirely different viruses contaminating HSV-1 strains compared to those in HSV-2 to explain the large differences in the minor band patterns between the two serotypes. These arguments eliminate most trivial explanations for the appearance of minor bands and indicate that the heterogeneity must reside within the HSV DNA molecules themselves.

Heterogeneity Is Not Caused by Defective DNA of High Density. Several virus stocks that had been passaged at high multiplicity prior to their receipt in this laboratory contained significant proportions of DNA with high buoyant density ($\rho > 1.731$ g/cm³). These preparations, which included HSV-1-(Justin) and variants of HSV-1(13), yielded several new bands with the *Eco*RI enzyme in addition to the major and minor

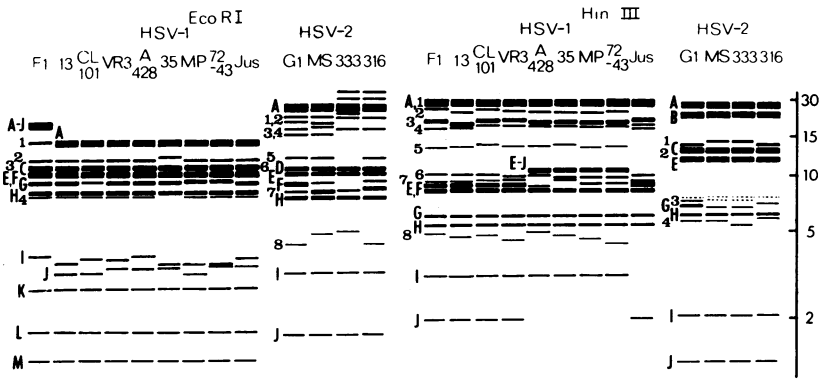

FIG. 3. Comparison of the mobilities and relative intensities of the bands obtained by *Eco*RI and *Hin*III cleavage of DNA from representative strains and isolates of HSV-1 and HSV-2. The diagram is a summary of information from many different experiments involving electrophoresis of digested DNA samples through 0.3% agarose gels. In some cases ^{3}H- and ^{32}P-labeled DNAs were directly compared by coelectrophoresis, and in others the different samples were run in parallel gels and compared after ^{32}P autoradiography or after staining with ethidium bromide. The individual major fragments from prototype strains HSV-1(F1) and HSV-2(G1) are identified by letters (A, B, C, etc.), using a nomenclature to be explained in detail in a subsequent publication. For the present purposes, the minor fragments are labeled by numbers (*1*, *2*, *3*, etc.) in order of decreasing size. Direction of migration is from top to bottom in the diagram, and an approximate molecular weight scale with values to be multiplied by 10^6 is indicated.

species (see Fig. 4, gels 2 and 3). However, one or two passages at low multiplicity were sufficient to eliminate both the high density DNA and the additional *Eco*RI bands without altering the minor band patterns. Serial undiluted passaging of plaque-purified HSV-1 (Justin) regenerated particles containing defective DNA (ρ = 1.732 g/cm^3) in a one to one ratio with particles containing normal density DNA (Frenkel, Jacob, Honess, Hayward, Locker, and Roizman, in preparation). These high density DNA molecules had molecular weights of at least 90 $\times$ 10^6 but lacked *Hin*III cleavage sites and yielded only two types of *Eco*RI fragments with molecular weights of approximately 5.1 and 5.3 $\times$ 10^6 (Fig. 4, gels 5 and 7). Plaque purification of strains HSV-1(VR-3), HSV-2(G1), and HSV-2(333) did not alter the electrophoretic profiles of their DNA fragments in any way.

Strain Differences. The molecular weights and major or minor classification of all observed fragments of our two representative strains HSV-1(F1) and HSV-2(G1) are listed for comparison in Table 2. Minor but reproducible differences were also evident among the fragment patterns from DNAs of strains within each serotype (Fig. 3). It is convenient to compare the electrophoretic profiles of other HSV-1 strains with that of HSV-1(VR-3) because the observed differences can then be interpreted as one-step alterations of two types, i.e., (*a*) replacement of two smaller fragments by a single larger one, caused by the absence of one cleavage site, and (*b*) fluctuations in the positions, and presumably therefore in the size, of certain fragments.

Three alterations of the first type were observed: (*i*) In HSV-1(F1) DNA, the largest *Eco*RI fragment, designated A-J (17 $\times$ 10^6 molecular weight), appears to correspond to fragments A (14 $\times$ 10^6) and J (3.2 $\times$ 10^6) of HSV-1(VR-3) and all other strains, including two other low passage isolates, HSV-1(F9) and HSV-1(A428) (Fig. 2, e and f). (*ii*) In the *Hin*III digests of strains HSV-1(MP), HSV-1(35), HSV-1-(72-43), and HSV-1(A428) [and also HSV-1(Kos), not shown], the major fragments E (8.0 $\times$ 10^6) and J (2.1 $\times$ 10^6) appear to be joined into a single new fragment (designated E-J) of 10.0 $\times$ 10^6 molecular weight. (*iii*) HSV-1(Justin) provides the only example we have observed of another *Hin*III alteration, namely, an apparent fusion of major fragment I (3.2 $\times$ 10^6) with the 4.8 $\times$ 10^6 minor fragment (band *8*) (Fig. 2, b and d).

HSV-1 DNA fragments generated by each enzyme also displayed variability of the second type: (*i*) The relative mobilities and positions of the two major *Eco*RI fragments (I and J) in the 3.0 to 3.5 $\times$ 10^6 molecular weight range fluctuate considerably. Quite possibly no two strains are exactly alike in this respect, and in some, the positions of the two fragments might be reversed relative to that in HSV-1(VR-3).

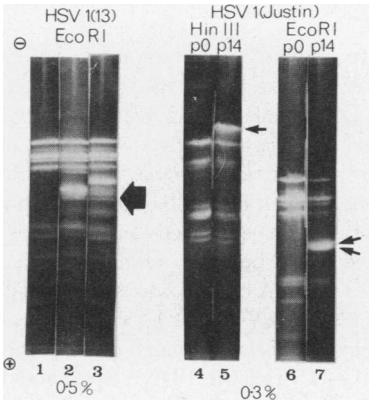

FIG. 4. Electrophoretic separations of DNA fragments from HSV DNA preparations that contained "defective" DNA molecules of high buoyant density. Gels *1–3*, *Eco*RI digests of DNA from three variants of HSV-1(13) analyzed in 0.5% agarose gels: *1*, HSV-1(13B4), less than 2% heavy density DNA; *2*, HSV-1(13D10), 30% heavy density DNA; *3*, HSV-1(13F3), 35% heavy density DNA. Gels *4–7*, 0.3% agarose: *4* and *6*, HSV-1(Justin) DNA from plaque-purified virus (p0) with less than 2% heavy density DNA; *4*, *Hin*III digest; *6*, *Eco*RI digest. Gels *5* and *7*, HSV-1(Justin) DNA containing 50% heavy density DNA from the fourteenth serial undiluted passage of the virus (p14): *5*, *Hin*III digest; *7*, *Eco*RI digest. The amounts of heavy density DNA (ρ = 1.729–1.733) in the preparations were monitored by analytical equilibrium sedimentation in CsCl. *Arrows* indicate the positions of cleavage products from heavy density DNA molecules.

(*ii*) The relative positions of most of the *Hin*III minor fragments also vary slightly among all the different HSV-1 strains and isolates. Minor bands *6* and *7* provide obvious examples, and the apparent molecular weight of the smallest minor fragment (band *8*) ranges from 5.0×10^6 in HSV-1(F1) through 4.8×10^6 in HSV-1(VR-3) to 4.6×10^6 in HSV-1(72-43). (*iii*) Other alterations yielding displaced fragments involve the major *Eco*RI fragment G (9.7×10^6) and minor band *3* of HSV-1(CL101) and the two minor *Eco*RI bands *2* and *4* of HSV-1(35).

Similar differences in both major and minor fragments were also observed among the DNA molecules from four HSV-2 strains (Fig. 2 g and h and Fig. 3).

DISCUSSION

Nonpermuted DNA molecules with identical base sequences would be expected to yield restriction enzyme fragments in equimolar concentrations and with summed molecular weights equal to that of the parent DNA molecules. However, for HSV-1 and -2 DNAs, the fragments fall into two or more sets with different molar concentrations. The summed molecular weights of the set of fragments with the highest molar concentrations (majors) constitute only 60–85% of the expected size for a single molecule, and the summed molecular weights of all fragments exceed that of the intact viral DNA molecule (and the kinetic complexity of HSV DNAs). These results indicate that several different forms of HSV DNA molecules must exist in the virions. They could either contain different sets of genetic information or have permutations in the arrangement of otherwise identical base sequences. Current evidence favors the second alternative, and in a subsequent publication we will present a model describing four different inverted rearrangements of the base sequences in HSV DNA molecules (Hayward, Jacob, Wadsworth, and Roizman, in preparation).

We wish to emphasize that minor fragments observed in digests of HSV DNAs cannot be attributed to the presence of defective DNA molecules of high buoyant density of the type reported by Bronson *et al.* (9). The minor fragment patterns were highly reproducible and invariably present in all strains tested even after plaque purification, whereas the heavy density DNA appeared only after serial undiluted passaging and could be easily eliminated. Studies on a particular species of DNA of high buoyant density present in high passage stocks of HSV-1(Justin) virus indicate that the defective molecules consist of many tandem repeats of a small segment of (G+C)-rich viral DNA sequences from one end of the normal density molecules (Frenkel, Jacob, Honess, Hayward, Locker, and Roizman, in preparation.)

Comparison of the restriction enzyme digests of various HSV DNAs revealed large and characteristic differences between the HSV-1 and HSV-2 groups. More subtle differences of two kinds occurred among strains of the same serotype. One involved variations in the sizes of certain fragments, in particular for HSV-1 DNAs the *Eco*RI fragment "I" and some of the *Hin*III minor fragments. Since these fragments have been tentatively identified as coming from the ends of the molecule, the size fluctuations might reflect differences in the extent of terminal redundancy (17, 18). The other differences can all be interpreted as simple losses of cleavage sites in certain strains, such as HSV-1(MP), HSV-1(Justin), HSV-1(F1), and HSV-2(333), relative to other strains of the same serotype. We cannot ascribe these changes solely to selection of variants in culture because preliminary studies show that low passage isolates from the same and different geographic locations also differ among themselves. Further studies with additional enzymes will be needed to clarify the extent of natural diversity in the DNA of herpes simplex viruses in human populations.

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