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Size, Composition, and Structure of the Deoxyribonucleic Acid of Herpes Simplex Virus Subtypes 1 and 2

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Studies of the size, composition, and structure of the deoxyribonucleic acid (DNA) of the F and G prototypes of herpes simplex virus (HSV) subtypes 1 and 2 (HSV-1 and HSV-2) showed the following. (i) As previously reported by Goodheart et al. HSV-1 and HSV-2 DNA have a buoyant density of 1.726 and 1.728 g/cm^3, corresponding to 67 and 69 guanine ± cytosine moles per cent, respectively. The difference in guanine plus cytosine content of the DNA species was confirmed by the finding of a 1 C difference in T_m. (ii) The DNA from purified virus on cocentrifugation with T4 DNA in neutral sucrose density gradients sedimented at 55*S*, corresponding to 99 ± 5 million daltons in molecular weight. HSV-1 and HSV-2 DNA could not be differentiated with respect to size. (iii) Cosedimentation of alkali-denatured DNA from purified virus with T4 DNA on alkaline sucrose density gradients consistently yielded several bands of single-stranded HSV DNA ranging from fragments 7×10^6 daltons to intact strands 48×10^6 daltons in molecular weight.

Naturally occurring herpes simplex viruses (HSV) differ in a number of biological, physical, and immunological properties (5, 7, 8, 10, 17-19, 20, 27). As determined in neutralization tests, most [but not all (31)] fresh isolates fall into two subtype groups designated as 1 (HSV-1) and 2 (HSV-2).

This report concerns the properties of HSV deoxyribonucleic acid (DNA). Previous reports have estimated the molecular weight of HSV-1 DNA to be 68×10^6 (24) to 100×10^6 daltons (2) and to contain 65 to 68 per cent guanine plus cytosine (10, 11, 20, 22, 23). HSV-2 DNA was reported (10) to contain 70.4 moles per cent guanine plus cytosine, and nothing was known of its size or relatedness to HSV-1 DNA. In the experiments described in this paper, we have directly compared the size, composition, and structure of HSV-1 and HSV-2 DNA.

MATERIALS AND METHODS

Solutions. Virus buffer consisted of 0.15 M NaCl and 0.02 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, *p*H 7.5. Neutral DNA buffer consisted of 1 M NaCl, 0.001 M ethylenediaminetetraacetic acid (EDTA), and 0.05 M Tris-hydrochloride, *p*H 7.5. Alkaline DNA buffer consisted of 0.8 M NaCl, 0.3 M NaOH, and 0.001 M EDTA. Reticulocyte standard buffer (RSB) consisted of 0.01 M sodium chloride, 0.001 M magnesium chloride, 0.01 M Tris, *p*H 7.5. Standard saline citrate (1 × SSC) consisted of 0.15 M sodium chloride, 0.015 M sodium citrate. SDS buffer consisted of 0.1 M NaCl, 0.001 M EDTA, 0.01 M Tris, *p*H 7.4.

Cells. HEp-2 and Vero cells were originally obtained from Flow Laboratories, Rockville, Md., and were grown in minimal essential medium supplemented with 10% calf serum.

Virus and infection of cells. The properties of the F and G prototypes of HSV-1 and HSV-2 have been previously described (7). The procedures used for infection of HEp-2 and Vero cells with HSV-1 and HSV-2 were those described previously for infection of HEp-2 cells with HSV-1 (7).

Labeling of cells with radioactive isotopes. HEp-2 cells infected with 20 plaque-forming units (PFU) of HSV-1 or HSV-2 per cell were labeled between 4 and 20 hr postinfection with 10 μCi of ^{3}H-thymidine or 0.1 μCi of ^{14}C-thymidine (Schwarz-Mann, Orangeburg, N.Y.) per ml of medium of mixture 199 lacking thymidine and supplemented with 1% dialyzed calf serum.

Purification of virus: preparation of nucleocapsids. Cells infected with HSV-1 or HSV-2 were harvested by scraping and centrifuged at 800 × *g* in a PR-2 refrigerated centrifuge for 10 min at 4 C. The cell pellet was washed with phosphate-buffered saline (PBS) and resuspended in RSB containing 0.5% Nonidet P-40 (Shell Chemical Co., New York, N.Y.) for 10 min at 4 C. The cells were then homogenized with six

strokes of a tight fitting Dounce homogenizer, and the nuclei were removed by centrifugation of the homogenate at 800 × *g* for 10 min at 4 C in the PR-2 centrifuge. The cytoplasm was layered onto 37-ml linear 10 to 50% (w/w) sucrose density gradients, prepared in virus buffer and centrifuged for 1 hr at 25,000 rev/min in the SW27 rotor at 5 C.

Purification of virus: preparation of enveloped virus. The preparation of enveloped HSV-1 followed a procedure of Spear and Roizman (*manuscript in preparation*). Briefly, cells infected with HSV-1 were harvested and washed as described above. The cell pellet was resuspended in 0.01 M Tris-hydrochloride (*p*H 7.4) for 10 min at 4 C, homogenized with four strokes of a tight fitting Dounce homogenizer, and adjusted to 0.25 M sucrose by the addition of one-seventh volume of 2 M sucrose in 0.01 M Tris-hydrochloride (*p*H 7.4). The nuclei were removed by centrifugation for 10 min at 800 × *g* in a PR-2 refrigerated centrifuge. The cytoplasm was layered onto 17-ml linear dextran 10 gradients (1.04 to 1.09 g/cm^3) and centrifuged for 1 hr at 25,000 rev/min and 5 C in a SW 25.3 rotor.

Determination of relative sedimentation rates of HSV-1 and HSV-2 DNA: preparation of ^{14}C-labeled T4 DNA. T4 phage prepared in *E. coli* and labeled with ^{14}C-uridine was the kind gift of Robert Haselkorn. The phage was resuspended in 0.5% sodium dodecyl sulfate (Matheson Scientific Co., Elk Grove Village, Ill.) and 2% sarkosyl NL97 (Geigy Chemical Co., Ardsley, N.Y.) in neutral DNA buffer and gently rolled with phenol at 60 C for 2 min. The phenol phase was removed, and the DNA was gently rolled with chloroform-isoamyl alcohol (2%, v/v) until the aqueous phase was clear.

^{3}H-thymidine-labeled simian virus 40 form I DNA (obtained from S. Kit and D. M. Dubbs) and ^{3}H-thymidine-labeled *E. coli* plasmid DNA (obtained from N. Cozarelli) were used to determine the relative sedimentation constant of the purified T4 DNA in alkaline sucrose gradients.

Sedimentation in neutral sucrose gradients. ^{3}H-thymidine-labeled HSV-1 or HSV-2 nucleocapsids were disrupted by exposure to 0.5% sodium dodecyl sulfate, 2% sarkosyl in neutral DNA buffer at 60 C for 2 min; mixed with ^{14}C-labeled T4 DNA; poured onto 12-ml linear 10 to 30% (w/w) sucrose gradients prepared in neutral DNA buffer; and sedimented for 3.5 hr at 40,000 rev/min in an SW41 rotor at 20 C. Fractions (0.3 ml) were collected from the top of the gradients, and the DNA was precipitated onto filters (HAWP 25-mm; Millipore Filter Corp., Bedford, Mass.) with 5% trichloroacetic acid by using herring sperm DNA (Sigma Chemical Co., St. Louis, Mo.) as a carrier. The filters were dried and assayed for radioactivity in a Packard Tri-Carb liquid scintillation spectrometer.

Sedimentation in alkaline sucrose gradients. ^{3}H-thymidine-labeled HSV-1 or HSV-2 nucleocapsids were mixed with ^{14}C-labeled T4 phage and resuspended in 0.5% sodium dodecyl sulfate and 2% sarkosyl in alkaline DNA buffer. The DNA species were poured onto 12-ml linear 10 to 30% sucrose gradients prepared in alkaline DNA buffer and sedimented for 3.5 hr at 40,000 rev/min in an SW41 rotor at 20 C.

The procedures for the collection of sucrose density gradient fractions, precipitation of the DNA, and assay of radioactivity were as described above.

Extraction and sedimentation of viral DNA from whole cytoplasmic lysate and from purified enveloped virus. HEp-2 cells were infected with 40 PFU of HSV-1 per cell and labeled from 4 hr postinfection with 10 μCi of ^{3}H-thymidine per ml of medium. At 16 hr postinfection, the cells were harvested, allowed to swell in 0.01 M Tris (pH 7.4) and 2.5 mM EDTA, and then Dounce-homogenized as previously described. A sample of the cytoplasm was layered on a dextran 10 gradient and centrifuged to obtain enveloped virus. The DNA was extracted from enveloped virions and cosedimented with T4 DNA as described above. Another sample of the cytoplasm was dissolved in 0.5% sodium dodecyl sulfate, 2% sarkosyl in neutral DNA buffer at 60 C for 2 min; mixed with ^{14}C-labeled T4 DNA; and cosedimented in neutral sucrose density gradients. Finally, a sample of cytoplasm and a sample of purified enveloped virus harvested from the dextran 10 gradient were each mixed with T4 phage labeled with ^{14}C in the DNA; solubilized in 0.5% sodium dodecyl sulfate, 2% sarkosyl in alkaline DNA buffer; and centrifuged in alkaline sucrose density gradients. All centrifugations were done as described above.

Purification of viral DNA for isopycnic banding and thermal denaturation. Purified herpes simplex nucleocapsids were resuspended at 60 C in neutral DNA buffer containing 0.5% sodium dodecyl sulfate and 2% sarkosyl, gently poured onto 12-ml linear 10 to 30% sucrose gradients, and centrifuged in an SW41 rotor for 3.5 hr at 40,000 rev/min and 20 C. Fractions (0.3 ml) were collected through a flow cell continuously monitored for absorbance at 260 nm. The fractions containing intact HSV DNA were dialyzed against 0.1× SSC, digested with 50 μg of heat inactivated pancreatic ribonuclease (Schwarz-Mann, Orangeburg, N.Y.) per ml, adjusted to 1.5× SSC, and further digested with 50 μg of ribonuclease per ml. Sodium dodecyl sulfate was added to make a final concentration of 0.5%, and the protein was extracted by rolling the DNA with phenol and chloroform-isoamyl alcohol. The DNA was then dialyzed four times for 24 hr against 100 volumes of 0.1× SSC to remove traces of phenol.

Isopycnic banding of HSV DNA in cesium chloride. Purified HSV-1 or HSV-2 DNA was mixed with 2 μg of SP01 DNA (gift of K. Bott), dialyzed against cesium chloride at a density of 1.715 g/cm^3 in 0.01 M Tris-hydrochloride (*p*H 7.4), and centrifuged in the Spinco model E analytical centrifuge at 44,700 rev/min and 25 C for 20 hr. Ultraviolet (UV) absorption photographs were scanned with the Joyce Loebel microdensitometer.

Melting temperature of HSV DNA. Purified HSV-1 DNA and HSV-2 DNA in 0.1× SSC were dialyzed against 0.1× SSC in the same flask to eliminate possible artifacts atributable to differences in salt concentration. The thermal denaturation profiles of the two DNA species were determined simultaneously in 0.5-ml Teflon-stoppered quartz cuvettes with a 1-cm light path (Hellma Cells Inc., Jamaica, N.Y.) by using a Gilford recording spectrophotometer equipped with

a linear temperature programmer and internal thermistor (16).

RESULTS

Viral preparations: naked nucleocapsids. Nonidet P-40-extracted cytoplasm of 20 hr-infected HEp-2 or Vero cells yielded (2) prominent bands on centrifugation through a 10 to 50% (w/w) sucrose density gradient. Electron microscopy of negatively stained preparations showed that the top band contained a mixed population of partially disrupted, intact, empty, and full nucleocapsids. The bottom band contained predominantly full nucleocapsids contaminated with small amounts of membrane debris.

The bottom band from the sucrose gradients was used as the source of DNA from nucleocapsid preparations.

Enveloped virus preparations. The cytoplasm of HSV-1-infected cells on centrifugation through a dextran 10 density gradient as described above yielded a single band in the middle of the tube. Electron microscopic analysis indicated that the band contained fully enveloped virus impermeable to phosphotungstic acid and was free from partially disaggregated virions. This finding confirms numerous other observations that the cytoplasm of cells infected with HSV-1 virus contains predominantly enveloped nucleocapsids (28).

Isopycnic banding of HSV-1 and HSV-2 DNA in neutral CsCl. The density of HSV-1 and HSV-2 DNA in neutral CsCl was determined in the Spinco model E centrifuge with SP01 as an internal marker as described above. The results are shown in Fig. 1. The densities of HSV-1 DNA and HSV-2 DNA calculated according to Szybalski (30) with the value of 1.742 g/cm^3 for the density of SP01 DNA are 1.726 $\pm$ 0.0005 and 1.728 $\pm$ 0.0005 g/cm^3, respectively.

Thermal denaturation of HSV-1 and HSV-2 DNA. The UV absorption-thermal denaturation profile of HSV-1 and HSV-2 DNA was determined in 0.1$\times$ SSC as described above. The results shown in Fig. 2 indicate that the melting temperatures of HSV-1 and HSV-2 DNA in 0.1$\times$ SSC are 82 and 83 C, respectively.

Determination of relative sedimentation rates and molecular weight of HSV-1 and HSV-2 DNA: sedimentation of native DNA. The sedimentation rate of HSV-1 and HSV-2 DNA in neutral sucrose density gradients was compared with that of T4 DNA as described above. The results shown in Fig. 3 indicate the following.

(i) Both HSV-1 and HSV-2 DNA extracted from nucleocapsids with sodium dodecyl sulfate and sarkosyl and centrifuged in neutral sucrose density gradients form a single sharp band slightly above that of T4 DNA (Fig. 3A, B). Identical

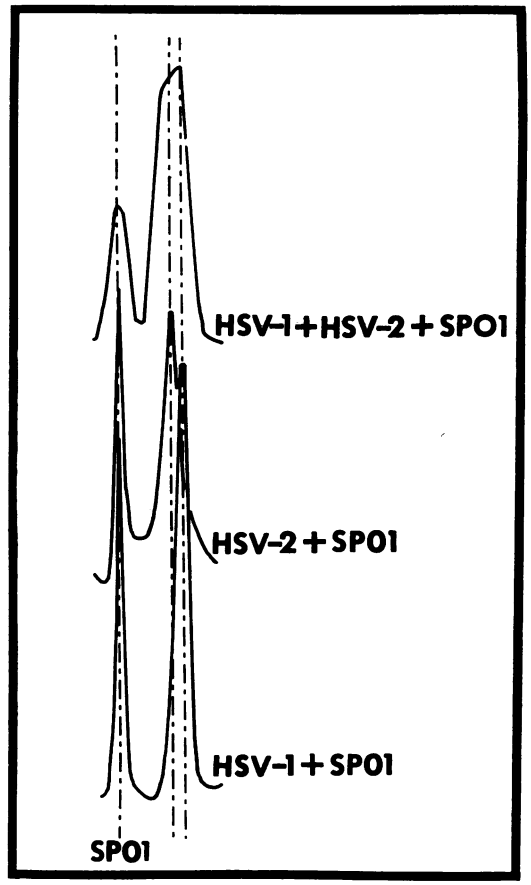

FIG. 1. *Buoyant density determination of HSV-1 DNA, HSV-2 DNA, and of an artificial mixture of HSV-1 and HSV-2 DNA determined by centrifugation with SP01 DNA in a model E analytical centrifuge as described in Materials and Methods. The UV absorption photograph was scanned with a Joyce Loebel microdensitometer.*

results were obtained when HSV nucleocapsids were mixed with T4 phage and coextracted by gently rolling with phenol and chloroform-isoamyl alcohol.

(ii) T4 DNA used in this study was intact double-stranded DNA by the following criteria. T4 DNA formed a single discrete band in neutral sucrose gradients. Alkali denaturation of the T4 DNA yielded a single discrete band in alkaline sucrose gradients. Cosedimentation of T4 DNA with simian virsus 40 form I DNA and *Escherchia coli* plasmid DNA in alkaline sucrose gradients demonstrated that the sedimentation rate of the denaturated T4 DNA was identical to that previously reported (3, 14).

(iii) The ratio of the distances sedimented by HSV-1 and HSV-2 DNA to that sedimented by

T4 DNA was 0.96 ± 0.02. The sedimentation coefficient of HSV-1 DNA and HSV-2 DNA in neutral sucrose density gradients derived from the relationship of Burgi and Hershey (4), $(d_1/d_2) = (s_1/s_2)$, was 55S. The molecular weight of HSV DNA was determined from the equation $s_1/s_2 = (M_1/M_2)^{0.38}$ (4, 9). With the value of 110 × 10^6 daltons for the molecular weight of T4 DNA (1, 6, 26), the molecular weight of HSV DNA was calculated to be 99 ± 5 × 10^6 daltons.

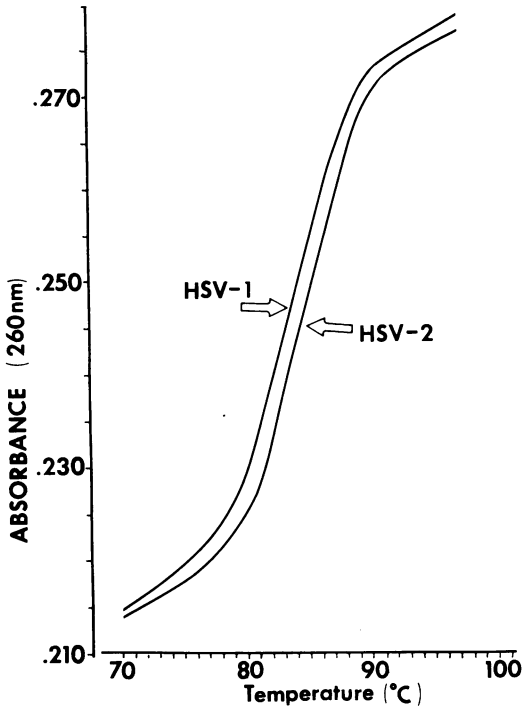

FIG. 2. *UV absorbance-thermal denaturation profile of HSV-1 and HSV-2 DNA in 0.1× SSC, determined as described in Materials and Methods.*

(iv) Cosedimentation of HSV-1 and HSV-2 DNA in neutral sucrose gradients (Fig. 3C) indicated that the sedimentation coefficients of these DNA species cannot be differentiated by zone centrifugation.

Sedimentation of denatured DNA. The sedimentation rate of denatured HSV-1 and HSV-2 DNA was compared to that of denatured T4 DNA in alkaline sucrose density gradients as described above. Figure 4 shows the distribution of DNA in the gradients. The results indicate the following.

(i) HSV-1 and HSV-2 DNA formed multiple bands in alkaline sucrose density gradients. The formation of multiple bands is not an artifact of the procedures used in the purification of the virus or in the extraction and the sedimentation of DNA. The DNA extracted from enveloped HSV-1 showed a similar distribution in alkaline sucrose gradients. The distribution of DNA bands was unaffected by the presence of 2.5 mM EDTA during all stages of viral purification. T4 DNA coextracted with HSV-DNA from mixtures of HSV nucleocapsids and T4 phage yielded a single band on sedimentation in alkaline sucrose. Mixtures of T4 phage and HSV nucleocapsids lysed directly on the top of alkaline gradients yielded similar results to those shown in Fig. 4A and B.

(ii) The most prominent and most rapidly sedimenting band of HSV-1 and HSV-2 DNA localized slightly above T4 DNA (Fig. 4A, B). The ratio of the distances of the sedimentation of HSV DNA to that of T4 DNA was 0.93. On the basis of this ratio, the most promient HSV band was estimated to be 68S. With the equation and coefficients of Studier (29), we estimate the molecular weight of HSV DNA in the rapidly sedimenting band to be 49 × 10^6 daltons. Co-centrifugation of HSV-1 and HSV-2 indicated that the sedimentation coefficients of the most prominent

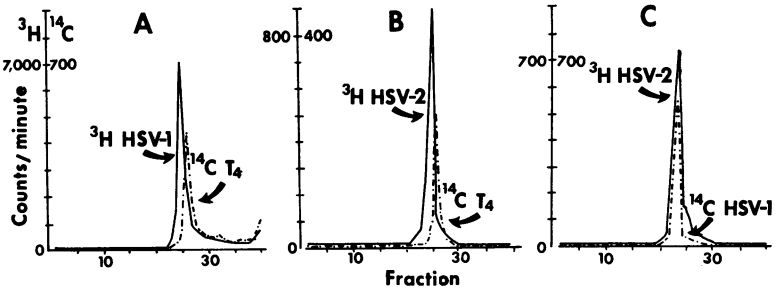

FIG. 3. *Zone sedimentation of HSV DNA in neutral sucrose density gradients. (A) Co-centrifugation of ^{3}H-HSV-1 DNA with ^{14}C-T4 DNA. (B) Co-centrifugation of ^{3}H-HSV-2 DNA with ^{14}C-T4 DNA. (C) Co-centrifugation of ^{3}H-HSV-2 DNA with ^{14}C-HSV-1 DNA. The DNA species were centrifuged for 3.5 hr in an SW41 rotor at 40,000 rev/min and 20 C. Symbols: dashed line, ^{14}C-labeled DNA; solid line, ^{3}H-labeled DNA. Direction of sedimentation is to the right.*

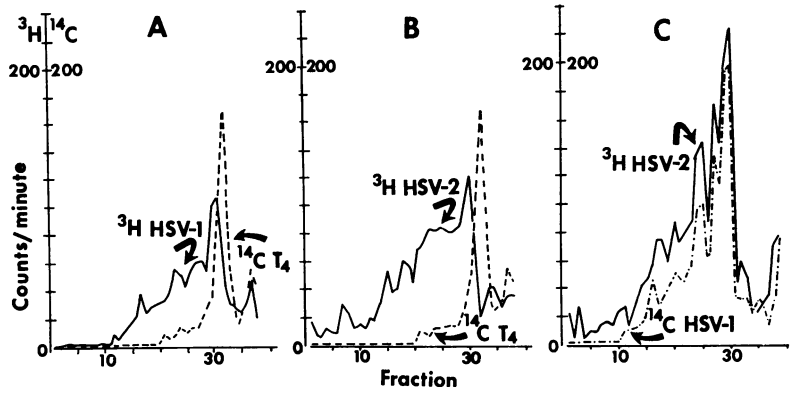

FIG. 4. *Zone sedimentation of alkali denatured HSV DNA and T4 DNA in alkaline sucrose density gradients. (A) Co-centrifugation of ^3H-HSV-1 DNA with ^{14}C-T4 DNA. (B) Co-centrifugation of ^3H-HSV-2 DNA with ^{14}C-T4 DNA. (C) Co-centrifugation of ^3H-HSV-2 DNA with ^{14}C-HSV-1 DNA. The DNA species were centrifuged for 3.5 hr in an SW41 rotor at 40,000 rev/min and 20 C. Symbols: dashed line, ^{14}C-labeled DNA; solid line, ^3H-labeled DNA. Direction of sedimentation is to the right.*

band of the two DNA species cannot be differentiated by zone centrifugation in alkaline sucrose density gradients.

(iii) With respect to the slower sedimenting species of HSV-1 and HSV-2 DNA the data are as follows. At least three discrete bands are usually found, ranging in ratio of distances sedimented relative to T4 DNA of 0.51 to 0.84, corresponding to a range in molecular weights of 7×10^6 to 30×10^6 daltons. Some variability in the distribution of the DNA in alkaline sucrose gradients was found between different preparations of the same virus. The more slowly sedimenting species constitute at least half the total viral DNA.

(iv) Cosedimentation of HSV-1 or HSV-2 DNA extracted from nucleocapsids grown in Vero cells with T4 DNA yielded a similar distribution to that seen in Fig. 4A and B.

Structural identity of viral DNA in crude cytoplasmic lysate and in purified enveloped virus. These experiments were designed to determine whether the single-stranded breaks in viral DNA were a constant feature of purified virions or whether similar breaks were present in crude cellular extracts containing virions. The size of the labeled native and denatured DNA in the whole cytoplasm and in the enveloped virus band obtained from HEp-2 cells infected for 16 hr with HSV-1 was determined in neutral and alkaline sucrose density gradients as described above. The results shown in Fig. 5 indicate the following.

(i) Labeled DNA released by sodium dodecyl sulfate and sarkosyl from virions in the cytoplasm of cells infected for 16 hr with HSV-1 (Fig. 5A) sedimented in neutral sucrose density gradients exactly like the DNA extracted from purified virions. The ratio of the distances sedimented by the cytoplasmic DNA relative to that of T4 DNA was 0.96%, i.e., the same as found in the results of the experiment summarized in Fig. 3.

(ii) Cosedimentation of denatured DNA extracted from the cytoplasm (Fig. 5B) or from enveloped virus (Fig. 5C) with T4 DNA in alkaline sucrose density gradients yielded identical results, indicating that enveloped virus accumulating in the cytoplasm contains DNA with single-stranded nicks and that the breaks are not the consequence of manipulations involved in virus purification.

DISCUSSION

These studies were undertaken to compare the size and structure of the DNA of the two major subtypes of herpes simplex virus. The data obtained in these studies are summarized in Table 1.

Composition of HSV-1 and HSV-2 DNA. Previous estimates of the buoyant density of HSV-1 and HSV-2 DNA were based on comparative distances between cellular and viral DNA bands of HSV-1 and HSV-2 infected cells (10). These studies indicated that HSV-2 DNA had a higher buoyant density than HSV-1 DNA. With SP01 DNA as an internal marker, we obtained values of 1.726 and 1.728 g/cm^3 for the buoyant density of HSV-1 and HSV-2, respectively (Table 1). Based on the assumption that there are no unusual bases in HSV-1 and HSV-2 DNA, the mole percentages of guanine plus cytosine bases are estimated to be 67 and 69% (25). UV absorbancy-thermal denaturation studies indicated a 1 C difference in the T_m of HSV-1 and HSV-2 DNA as expected for DNA species differing by 2 moles per cent guanine plus cytosine.

Size of HSV-1 and HSV-2 DNA. Previous estimates of the molecular weight of HSV-1 DNA

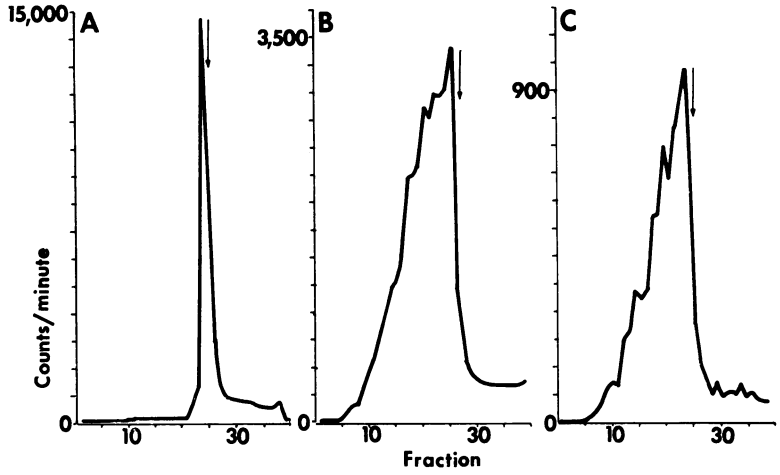

FIG. 5. *Zone sedimentation in neutral and alkaline sucrose density gradients of HSV DNA extracted from crude cytoplasm of infected cells and from purified enveloped virus in that cytoplasm. (A) Native ^{3}H-HSV-1 DNA in the crude cytoplasm sedimented in neutral sucrose gradients. (B) Denatured ^{3}H-HSV-1 DNA in alkaline sucrose gradients. (C) Denatured ^{3}H-HSV-1 DNA extracted from enveloped virions purified from the crude cytoplasmic lysate by sedimentation through dextran 10 density gradient. Centrifugation was for 3 hr in an SW41 rotor and at 40,000 rev/min and 20 C. The position of ^{14}C-T4 DNA cosedimented with ^{3}H-HSV-1 DNA is indicated by arrow. Direction of sedimentation is to the right.*

TABLE 1. *Comparison of size and structure of the two major subtypes of herpes simplex virus (HSV-1 and HSV-2)*

Virus	Native DNA				Single-strand DNA		
					Intact strand		Overall range of fragments[c]
	S[a]	Mol wt (daltons)	Buoyant density (g/cm^3)	Guanine cytosine moles per cent	S[b]	Mol wt (daltons)	Mol wt (daltons)
HSV-1 (F)	55	99 ± 5[d]	1.726 ± 0.0005	67	68	48[d]	7–48[d]
HSV-2 (G)	55	99 ± 5	1.728 ± 0.0005	69	68	48	7–48

[a] In neutral sucrose relative to T4 DNA.
[b] In alkaline sucrose relative to T4 DNA.
[c] Viral DNA from 16-hr-old infected cells.
[d] Expressed $\times 10^6$.

were obtained by two techniques. One involving direct measurement of the sedimentation coefficient of the DNA yielded a molecular weight of 68×10^6 daltons (24) and is in all probability a low value. The second, using the Kleinschmidt technique, yielded an average value of 101×10^6 daltons with a range of 86×10^6 to 115×10^6 daltons (2). The size of HSV-2 DNA was unknown.

In principle, there are several techniques for determination of the absolute molecular weight of DNA molecules greater than 50×10^6 daltons: ^{32}P star enumeration (15), equilibrium sedimentation (26), and indirect determinations based on phosphate analysis and the molecular weight value of purified virions obtained by equilibrium sedimentation and sedimentation diffusion (1, 6). As reviewed by Friefelder (9), all of these techniques suffer from inherent errors. It is unlikely that DNA molecular weight determinations based on phosphate analysis and molecular weights of purified virions will ever be practical for the large enveloped DNA viruses. Other techniques, such as Kleinschmidt and sedimentation, depend on standardization against DNA species of known molecular weight. In regard to the Kleinschmidt technique, HSV presents an unusual problem. We are particularly concerned by recent observations (9, 12) that the mass per unit length for DNA of 65 to 70 guanine plus cytosine moles

per cent might be as much as 15% higher than that used in previous studies.

We chose to determine the molecular weight of HSV-1 and HSV-2 DNA by zone sedimentation in neutral sucrose density gradients by using T4 DNA as an internal standard for the following reasons. (i) Independent absolute determinations have been made for few DNA molecules, T4 being among them (1, 6, 26). (ii) T4 DNA is very close in size to HSV DNA. (iii) Recent data confirm that the Burgi and Hershey relationship is valid over small ranges of S and M (9).

The molecular weight of both HSV-1 and HSV-2 DNA was found to be $99 \pm 5 \times 10^6$ daltons.

Structure of HSV-1 and HSV-2 DNA. The molecular weight of denatured HSV-1 and HSV-2 DNA was obtained by zone centrifugation in alkaline sucrose gradients to determine whether the native DNA consisted of two continuous non-cross-linked single strands. The results indicated that, at most, only half of the single strands in any preparation of HSV-1 or HSV-2 DNA are intact; the remainder of the DNA is fragmented. Viral DNA in the cytoplasm of infected cells before isolation of purified virus had the same size distribution on alkali denaturation as the DNA of purified virus, effectively excluding the possibility that single-strand breaks arise during viral purification. The fragments of DNA, which range in size from 7×10^6 to 30×10^6 daltons, form several discrete bands in sucrose gradients, indicating that they are not products of random cleavage. No DNA was consistently found to be larger than intact single strands. Therefore, we conclude that native viral DNA contains single-strand breaks and that it is not cross-linked. Similar findings have recently been reported for Marek's disease virus DNA (13).

Significance of the single-strand fragments found in HSV DNA. Although the origin and biological significance of the single-strand fragments found in HSV DNA are not clear, several points should be made. (i) As noted previously, the fragments are not an artifact of the procedures used in viral purification or in extraction and analysis of the DNA. The alkaline denaturation experiments probably indicate the existence of breaks in the DNA, since alkali-labile linkages such as apurinic sites have not been found in viral DNA species. (ii) Both HSV-1 and HSV-2 DNA have been shown to have similar distributions of single-strand fragments. Furthermore, the finding is not a unique feature of virus growth in a particular cell line. (iii) The finding that DNA from purified virus yields on velocity centrifugation in alkaline sucrose gradients at least four bands ranging in size from 7×10^6 to 48×10^6 daltons suggests that the single-strand breaks are not random but are at specific sites. (iv) We do not know whether the fragments result from specific nicking of intact single strands or results from incomplete ligation of breaks necessary for control of transcription, as in T4 (21). These points are currently under investigation.

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