JOURNAL OF VIROLOGY, May 1972, p. 738-745
Vol. 9, No. 5

Printed in U.S.A

Genetic Relatedness of Type 1 and Type 2 Herpes Simplex Viruses

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Received for publication 3 February 1972

The extent of homology between herpes simplex virus $_1$ and $_2$ (HSV-1 and HSV-2) deoxyribonucleic acid (DNA) was measured in two ways: (i) by determination of the relative rate of hybridization of labeled HSV-1 and HSV-2 DNA to excess unlabeled HSV-1 or HSV-2 DNA immobilized on filters and (ii) by determination of the rate of hybridization of labeled HSV-1 and HSV-2 DNA to excess unlabeled HSV-1 or HSV-2 DNA in solution. Approximately 40% of HSV-1 and HSV-2 DNA is homologous at hybridization temperatures 25 C below the melting temperature (T_m) of HSV DNA (liquid-filter annealing). Lowering the temperature to 34 C below the T_m increased the extent of homology to 46% (liquid annealing). The extent of base-pairing in HSV-1-HSV-2 heteroduplex DNA was determined by thermal chromatography on hydroxyapatite. Heteroduplexes of HSV-1 and HSV-2 DNA eluted in a single peak whose midpoint (Te_{50}) was 10 C below that of the homoduplex. Conspicuously absent were heteroduplexes that eluted at more than 15 C below the Te_{50} of the homoduplex. The data indicate the existence of a variable region of DNA (54%) with very little, if any, homology and an invariable region (46%) with relatively good (85%) matching of base pairs.

Viruses of the herpes group are morphologically indistinguishable, share many common features of intracellular development, but differ widely in biologic properties (21). The base composition of the deoxyribonucleic acid (DNA) species of the herpes viruses ranges from 43 to 74 moles per cent (%) guanine plus cytosine (GC). Little or no genetic homology has been found between antigenically unrelated and biologically dissimilar herpes viruses (1, 28, 29).

The naturally occurring herpes simplex viruses (HSV) form a group of serologically related viruses which can be segregated on the basis of their effects on cells (6) and immunologic properties (4, 24) into two major subtypes, designated HSV-1 and HSV-2.

The significant phenotypic differences between prototype HSV-1 and HSV-2 strains can be summarized briefly as follows: (i) HSV-1 strains have usually been isolated from primary and recurrent lesions of the face and cornea; HSV-2 have usually been isolated from recurrent infection of the genitalia (4, 6, 25). Less certain is the association of HSV-1 with carcinoma of the lip (27) and that of HSV-2 with carcinoma of the cervix (20). (ii) In cell culture, prototype HSV-1 and HSV-2 strains have been found to differ with respect to ability to multiply in cells of certain species (7), morphologic aspects of intracellular development (26), effect on social behavior of infected cells (6), and ability to transform cells of certain lines (5). (iii) Quantitative neutralization tests indicate that HSV-1 and HSV-2 are related but not identical. The molecular basis for the neutralization of HSV-1 by heterologous serum is not entirely clear. Although Schneweiss and Nahmias (25) and Geder and Skinner (9) reported both common and type-specific antigens, Savage et al. (J. Gen. Virol., *in press*) found that all of the glycoproteins making up the envelope of HSV-1 virions react on immunoabsorbent columns to a greater or lesser extent with anti-HSV-2 sera and vice versa. (iv) Differences in surface properties of the virions have been demonstrated (6). Analysis of the structural proteins of prototype strains revealed differences in the electrophoretic mobility of virion structural proteins (Roizman and Terlizzi, *manuscript in preparation*).

The purpose of the studies described in the paper was to measure the extent of genetic relatedness between prototype HSV-1 and HSV-2 strains. Two series of experiments were done. In the first, we estimated the fraction of HSV-1 and HSV-2 DNA species that were homologous by two-phase (liquid-filter) (17) and single-phase

(liquid) hybridization. In the second series of experiments, we measured the extent of base-pairing between homologous regions of the DNA (HSV-1-HSV-2 DNA heteroduplex) by comparing the thermal elution of the homo- and heteroduplex DNA species from hydroxyapatite columns.

Pertinent to these studies is the following information concerning HSV DNA: (i) HSV-1 and HSV-2 DNA species have buoyant densities of 1.726 and 1.728 g/cm^3 corresponding to 67 and 69 moles % GC, respectively (10, 13). The difference in their base composition was substantiated by the finding of a 1 C difference in melting temperature (T_m) (13). (ii) HSV-1 and HSV-2 DNA appear to be linear molecules with an identical molecular weight of $99 \pm 5 \times 10^6$ daltons (2, 13). (iii) Reassociation kinetics of HSV-1 DNA indicate that the size of its unique sequences is $95 \pm 5 \times 10^6$ daltons; appreciable amounts of repetitive sequences have not been found (8). (iv) Sedimentation in alkaline sucrose velocity gradients demonstrated that both DNA species contained intact strands and nonrandom fragments (13).

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. Reticulocyte standard buffer (RSB) consisted of 0.01 M sodium chloride, 0.0015 M magnesium chloride, and 0.01 M Tris-hydrochloride, *p*H 7.5. Neutral DNA buffer consisted of 1.0 M sodium chloride, 0.001 M ethylenediaminetetraacetic acid (EDTA), and 0.05 M Tris-hydrochloride, *p*H 7.5. Standard saline citrate (1 × SSC) consisted of 0.15 M sodium chloride and 0.015 M sodium citrate. Phosphate buffer (PB), *p*H 6.8, was made as a 4.8 M stock solution by combining an equal volume of 4.8 M monobasic sodium phosphate with 4.8 M dibasic sodium phosphate (Baker Chemical Company, Phillipsburg, N.J.).

Cells. HEp-2 cells 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, respectively, and the procedures used for the infection of HEp-2 cells with HSV-1 and HSV-2 have been described previously (6).

Labeling of cells with radioactive isotopes. HEp-2 cells infected with 20 plaque-forming units of HSV-1 or of HSV-2 per cell were labeled between 4 and 20 hr postinfection with 10 μCi of ^{3}H-thymidine or with 0.1 μCi of ^{14}C-thymidine (New England Nuclear Corp., Boston, Mass.) per ml of medium 199 lacking thymidine but supplemented with 1% dialyzed calf serum (13).

Purification of viral DNA. Viral DNA was purified from HSV nucleocapsids. The preparation of HSV nucleocapsids has been described previously (13). Briefly, cells infected with HSV-1 or with HSV-2 were harvested 20 hr after infection. The cell pellet was suspended in RSB containing 0.5% Nonidet P-40 (Shell Chemical Co., New York, N.Y.) for 10 min at 4 C and was homogenized with four strokes of a tight-fitting Dounce homogenizer. The nuclei were removed by centrifugation of the homogenate at 800 × *g* for 10 min at 4 C in a PR-2 centrifuge. The cytoplasm was layered on 37-ml linear 10 to 50% (w/w) sucrose gradients prepared in virus buffer and centrifuged for 1 hr at 25,000 rev/min in an SW27 rotor at 5 C. The bottom band which contains mostly full viral nucleocapsids (13), was collected, diluted in virus buffer at 4 C, and centrifuged at 25,000 rev/min in an SW27 rotor for 1 hr at 5 C. The pellet was dissolved in neutral DNA buffer containing 1% (w/v) sodium dodecyl sulfate (SDS; Matheson Scientific Co., Elk Grove Village, Ill.) and 2% Sarkosyl NL97 (Geigy Chemical Co., Ardsley, N.Y.). The solubilized viral preparation was gently poured onto 12-ml linear 10 to 30% (w/w) sucrose gradients and centrifuged for 3.5 hr at 40,000 rev/min in a SW41 rotor at 20 C. Fractions (0.3 ml) were collected through a flow cell continuously monitored for absorbance at 260 nm. Fractions containing intact HSV DNA were dialyzed against 0.1× SSC, digested for 30 min at 37 C with 50 μg of heat-inactivated ribonuclease (Schwarz-Mann, Orangeburg, N.Y.) per ml, adjusted to 1× SSC, and further digested with 50 μg of ribonuclease per ml. SDS was added to make a final concentration of 0.5% (w/v), and proteins were extracted by rolling the DNA with phenol and chloroform-isoamyl alcohol (13). The DNA was then dialyzed for 24 hr against 100 volumes of 0.1× SSC to remove traces of phenol.

A sample of each viral DNA preparation was dialyzed against CsCl and centrifuged to equilibrium in a Spinco model E centrifuge (13). HSV-1 and HSV-2 DNA species formed a single band at a density of 1.726 and 1.728 g/cm^3, respectively (13).

Purification of HEp-2 cell DNA. The extraction and purification of HEp-2 cell DNA followed the procedures of Marmur (19) for the purification of bacterial DNA species.

Isolation and purification of bacterial DNA species. *Escherichia coli* strain W3110 and *Hydrogenomonas eutropa* were grown in media containing $^{32}PO_4$ or $^{14}CO_2$ (both obtained from Schwarz-Mann, Orangeburg, N.Y.). The DNA fractions were extracted as previously described (11) and further purified by centrifugation through cesium chloride (ρ = 1.4 g/ml).

Liquid filter DNA-DNA hybridization: (i) preparation of DNA discs. DNA in 1× SSC was sheared by passage 10 times through a 30-gauge needle at full thumb pressure and by sonic treatment for 30 sec at 4 C. The DNA was alkali-denatured, diluted to a concentration of less than 0.20 μg/ml with 1× SSC, neutralized with 1 N HCl, and filtered slowly through nitrocellulose filters (type HAWP, 25 mm, 45-nm pore size; Millipore Corp., Bedford, Mass.), which had been presoaked in 1× SSC. The filters were dried at 37 C for 1 hr and baked at 80 C for 5 hr. Microhybridization discs, 5 mm in diameter,

containing approximately 2 μg of DNA, were cut from the larger discs with a leather punch.

(ii) **Conditions of hybridization.** Microhybridization discs loaded with 2 μg of HSV-1 or HSV-2 DNA or with HEp-2 DNA were incubated with sheared, alkali-denatured, and labeled HSV-1 or HSV-2 DNA at 61 C in 0.3 ml of a solution containing 5× SSC and 30% (v/v) formamide. After incubation, the discs were removed, washed five times for a total of 2 hr in 2× SSC, soaked in 70% (v/v) ethanol, dried at 60 C for 1 hr, and assayed for radioactivity in a liquid scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.).

(iii) **Exonuclease-1 assay.** Exonuclease-1 (16) was used to determine the fraction of unrenatured DNA in solution at the end of incubation. DNA solutions were dialyzed two times against a solution containing 66 mM glycine, *p*H 9.5, 6.6 mM $MgCl_2$, 1.5 mM 2-mercaptoethanol for 4 hr. Two units of *E. coli* alkaline phosphatase and one unit of exonuclease-1 (both gifts of Nicholas Cozarelli) were added, and the mixture was incubated at 37 C for 30 min. The reaction was terminated by the addition of trichloroacetic acid. Herring sperm DNA was then added as a carrier. Acid-precipitable DNA was collected on membrane filters (Millipore Corp.) and assayed for radioactivity.

Liquid DNA-DNA hybridization. Unlabeled HSV-1 or HSV-2 DNA in 2- to 4-μg amounts (or HEp-2 DNA in 40-μg amounts) was mixed with 0.04 μg of tritium-labeled HSV-1 or HSV-2 DNA (250,000 counts per min per μg) and 0.3 μg of carbon 14-labeled HSV-1 or HSV-2 DNA (8,000 counts per min per μg) in a solution containing 10 mM Na_3 EDTA. The DNA mixtures were denatured in a solution containing 0.2 N NaOH, then sonically treated at 50 w for 2 min at 4 C with the standard microprobe (Heat Systems Co., Melville, N.Y.). PB (4.8 M) was added to yield a final concentration of 0.24 M PB, and sufficient 1 N HCl was added to neutralize the NaOH. The final sodium ion concentration was 0.5 N. After incubation at 68 C for 4 to 10 hr, the mixtures were rapidly cooled and diluted 10 volumes with water and PB to a concentration of 0.14 M PB. Samples were stored at −20 C prior to chromatography.

Hydroxyapatite chromatography. The procedures for chromatography and thermal elution from hydroxyapatite have been described in detail previously (12). For chromatography of reassociated viral DNA, the DNA in 0.14 M PB was poured into a jacketed column containing 2 g (5 ml) of hydroxyapatite (Bio-Gel Corp., Richmond, Calif.) at 50 C. Single-stranded DNA was washed through the column with 0.14 M PB at 50 C and collected in 8-ml fractions. A solution consisting of 0.14 M PB and 8 M urea was pumped through the column at 2 ml/min, and the column temperature was raised 0.25 C/min with a linear temperature programmer. Column fractions (8 ml) were mixed with Instagel (Packard Instrument Corp., Downers Grove, Ill.) and assayed for radioactivity.

RESULTS

Liquid-filter (two-phase) DNA-DNA hybridization. The extent of homology of HSV-1 and HSV-2 DNA was determined initially by liquid-filter DNA-DNA hybridization at 25 C below the melting temperature (T_m) of native HSV DNA (19). The molecular weight of the sheared labeled DNA was determined to be 200,000 daltons by sedimentation in alkaline sucrose gradients, using *E. coli* plasmid DNA as a standard (13). The rates of binding of labeled HSV-1 and HSV-2 DNA species to unlabeled DNA in 50-fold excess and immobilized on replicate discs cut from the same filters are shown in Table 1. Self-association of the denatured labeled DNA species in solution was calculated to be less than 25%

TABLE 1. *Liquid-filter DNA-DNA hybridization*[a]

DNA fixed to nitrocellulose filter	Expt. no.	^{3}H-HSV-1 DNA per cent input bound			^{14}C-HSV-2 DNA per cent input bound			Ratio of heterologous to homologous hybridization		
		1[b]	2	3	1	2	3	1	2	3
HSV-1	1a	4.1	7.7		1.4	3.1		0.35	0.41	
	1b	5.3		10.8	3.0		6.9	0.57		0.64
HSV-2	2a	0.42	0.7		1.4	2.9		0.29	0.24	
	2b	1.7		3.4	6.0		8.7	0.29		0.4
HEp-2		0–0.01	0.05	0.05	0.1–0.2	0.2	0.15			

[a] Denatured ^{3}H-labeled type 1 herpes simplex virus (^{3}H-HSV-1) and ^{3}H-HSV-2 DNA were incubated as described in Materials and Methods with identical discs containing an excess of denatured HSV-1 DNA (experiments 1a and 1b) or in the reciprocal experiment with discs containing denatured HSV-2 DNA (experiments 2a and 2b). The percentage of homologous nucleotide sequences was determined from the relative rate at which heterologous and homologous DNA bound to microhybridization discs cut from the same filter.

[b] Hours.

at 3 hr [$C_0t < 0.007$ moles-sec/liter (3)] and was determined to be less than 20% in 2 hr by exonuclease susceptibility, eliminating the possibility of significant liquid-liquid, liquid-filter competition. The results of liquid-filter hybridization shown in Table 1 indicate the following: (i) approximately 40% (an average of eight determinations ranging from 24 to 64%) of the sequences of the two DNA species are capable of annealing with each other under the conditions employed. (ii) The relative rates at which heterologous and homologous DNA species bound to replicate filters did not vary significantly over the 3 hr of the test. (iii) There was significant variation in the extent of hybridization of the homologous as well as heterologous DNA species when microhybridization discs not cut from the same filter were employed (Table 1 comparison of experiment 1a with 1b and 2a with 2b). Since the same labeled DNA species were used in each series of hybridizations, we interpret this variation to be due to differences in effective concentration of DNA in nonreplicate discs.

Liquid (single-phase) DNA-DNA hybridization. To directly control the reassociation reaction and to facilitate analysis of the reassociated DNA species, small amounts of HSV-1 and HSV-2 DNA species labeled with ^{14}C and ^{3}H, respectively, were reassociated with a 50-fold excess of unlabeled HSV-1 DNA. In another series of experiments, the labels on the DNA were reversed, and unlabeled HSV-2 DNA was used in place of HSV-1 DNA. The DNA species were alkali-denatured and sonically treated as described above to produce single-stranded fragments of about 10^5 daltons as determined by sedimentation in alkaline sucrose gradients with DNA sheared at 50,000 psi as a marker. Hybridization was continued for sufficient time to permit 77 to 94% of the homologous DNA (unlabeled and ^{14}C-labeled) to renature [C_0t approximately 0.8 moles-sec/liter (3)]. The extent of reassociation was determined by hydroxyapatite chromatography as described above. The results shown in Table 2 indicate the following: (i) under conditions which should permit formation of heteroduplex DNA species with a considerable amount of mismatching of base pairs [hybridization carried out 34 C below the T_m of native HSV DNA (17)], 46% of the sequences of HSV-1 and HSV-2 DNA species was able to anneal. (ii) The hybridization was specific. Labeled HSV-1 or HSV-2 DNA species did not hybridize to a 500-fold excess of HEp-2 cell DNA. (iii) The extent of hybridization was not affected by the nature of the label on the DNA. This emerged from experiments in which an excess of HSV-1 (or of HSV-2) DNA was denatured, fragmented, and allowed to reassociate with its homologous tritium- and carbon-14-labeled HSV-1 (or HSV-2) DNA species (Fig. 2A and B).

Thermal stability of HSV-1-HSV-2 heteroduplex DNA. The T_m of HSV-1 and HSV-2 DNA has been determined to be 82 and 83 C, respectively, in 0.1× SSC (13). With the equation of Schildkraut and Lifson (23), the corresponding T_m in 0.14 M PB would be 98 and 100 C, respectively. We added 8 M urea to the elution solvent (0.14 M PB) to lower the T_m of HSV DNA sufficiently to allow elution of the DNA before 95 C. A mixture of $^{32}PO_4$-labeled *E. coli* (50 moles % GC) DNA and *H. eutropa* (68 moles

TABLE 2. *Liquid DNA-DNA hybridization*[a]

Test system			C_0t of total DNA (mole-sec/liter)	Per cent input of labeled DNA in duplexes (bound to HAP in 0.14 M PB)		Homology
DNA in excess	Labeled DNA			(^{3}H-label DNA)	(^{14}C-label DNA)	Normalized per cent input of heterologous DNA in duplexes[b]
	Heterologous	Homologous				
HSV-1	^{3}H-HSV-2	^{14}C-HSV-1	0.29(1.2)[c]	47	94	50
HSV-1	^{3}H-HSV-2	^{14}C-HSV-1	0.22(.92)	41	86	47
HSV-2	^{3}H-HSV-1	^{14}C-HSV-2	0.14(.59)	36	77	47
HSV-2	^{3}H-HSV-1	^{14}C-HSV-2	0.18(.76)	32	83	40
HEp-2	^{3}H-HSV-1-^{14}C-HSV-2		2.50(11)	1	1	0

[a] Unlabeled herpes simplex virus (HSV) DNA in 50-fold excess was hybridized to the heterologous ^{3}H-labeled HSV DNA in the presence of the homologous ^{14}C-labeled DNA as described in Materials and Methods. Residual single-stranded DNA was separated from DNA in duplexes by chromatography on hydroxyapatite. The percentage of homologous nucleotide sequences was determined from the relative rate of hybridization of the heterologous and homologous DNA species.

[b] Normalized by dividing per cent of input heterologous DNA bound by per cent of input homologous DNA bound times 100.

[c] Numbers in parenthesis are the "equivalent C_0t" obtained by applying the correction for increased reaction rate (4.18-fold) in 0.5 N Na^+.

% G + C) DNA which had been sheared at 50,000 psi and reassociated was chromatographed by thermal elution from hydroxyapatite in 0.14 M PB-8 M urea. Data shown in Fig. 1 indicated that the *E. coli* DNA eluted with the midpoint of its thermal elution curve (Te_{50}) at 77 C, whereas *H. eutropa* eluted at 84 C. These data serve to calibrate the elution conditions employed with reassociated HSV DNA.

The thermal elution profiles of HSV-1 and HSV-2 homoduplex DNA species and of HSV-1–HSV-2 heteroduplex DNA are shown in Fig. 2 and indicate the following: (i) renatured HSV-1 or HSV-2 (homoduplex) DNA species elute from hydroxyapatite in 0.14 M PB–8 M urea at an average Te_{50} of 85 C as expected of DNA species of 67 to 69 moles % G + C (Table 3 and Fig. 2). (ii) Tritium-labeled HSV-1–HSV-2 heteroduplex DNA species elute at a Te_{50} 10 C below that of the reassociated HSV-1 or HSV-2 DNA (Table 3 and Fig. 2C and D). Less than 4% of the tritium-labeled DNA in duplexes can be attributed to self-reassociation. (iii) In principle, the slope of the cumulative elution plot for the homoduplex reflects the degree of heterogeneity of DNA fragments with respect to base composition. If the mismatching of base pairs occurs at random in the heteroduplex DNA species and if these DNA species have similar heterogeneity with respect to base composition as the homoduplex DNA, it would be expected that the cumulative slope of the elutions of the heteroduplex DNA and of the homoduplex DNA would be similar if not identical. The data shown in the right panel of Fig. 2 indicate that the cumulative slope for the heteroduplex is only slightly smaller than that of the homoduplex. The data indicate that the mismatched base pairs are distributed randomly in the heteroduplex DNA. (iv) The data suggest that there are two classes of HSV DNA fragments, i.e., one class with Te_{50} 10 C below that of the homoduplex DNA and one class with either no homology or with a Te_{50} more than 25 C below that of the homoduplex DNA. In support of this conclusion are two facts. First, the Te_{50} of the heteroduplex DNA is clustered at about 10 C below that of the homoduplex, with very little scatter either less than 10 C or more than that value. Secondly, it is particularly significant that very little heteroduplex DNA eluted between 15 and 25 C below the Te_{50} of the homoduplex.

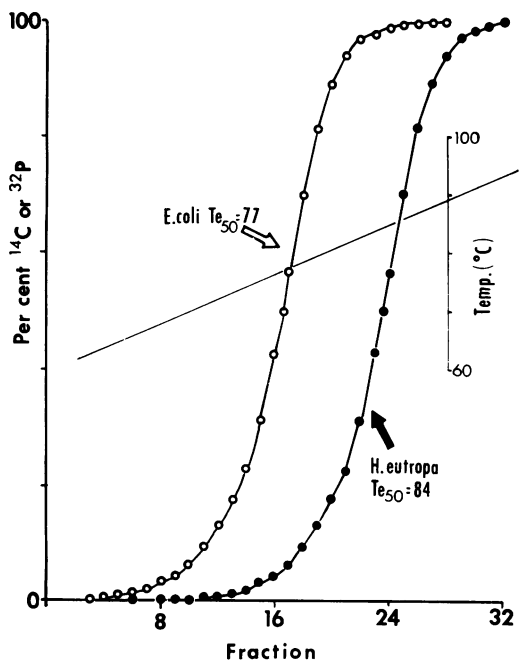

FIG. 1. *Thermal chromatography of H. eutropa and E. coli DNA. ^{14}C-labeled H. eutropa and ^{32}P-labeled E. coli DNA were sheared at 50,000 psi, alkali-denatured, neutralized, renatured (at 50 C), and chromatographed by thermal elution from hydroxyapatite in the presence of 8 M urea as described for viral DNA in Materials and Methods. Cumulative percentage of the total ^{14}C- (●) and ^{32}P- (○) labeled DNA, which eluted at each temperature.*

The significance of these data emerges from the fact that the hybridization reaction took place at 34 C below the Te_{50} of the homoduplex, and, hence, heteroduplexes with an elution temperature 15 to 25 C below the Te_{50} of the homoduplex should have formed if DNA species with such poor homology existed. Additional evidence in support of this conclusion emerges from the fact that a change in the temperature of hybridization from 25 to 34 C below the T_m of native HSV DNA increased the percentage of homologous sequences only 6%.

DISCUSSION

Analyses of viral evolution must consider both genetic and phenotypic properties. Mutation of a single base can markedly affect phenotype. Conversely, dissimilar genetic material could, because of redundancy in the genetic code, specify similar if not identical protein.

Although there are considerable biologic differences between HSV-1 and HSV-2 reflected in their structural components, the enzymes they specify, and their effects on multicellular and unicellular hosts, it is not possible to relate phenotypic manifestations to genetic relatedness largely because one structural viral gene may be responsible for more than one phenotypic factor.

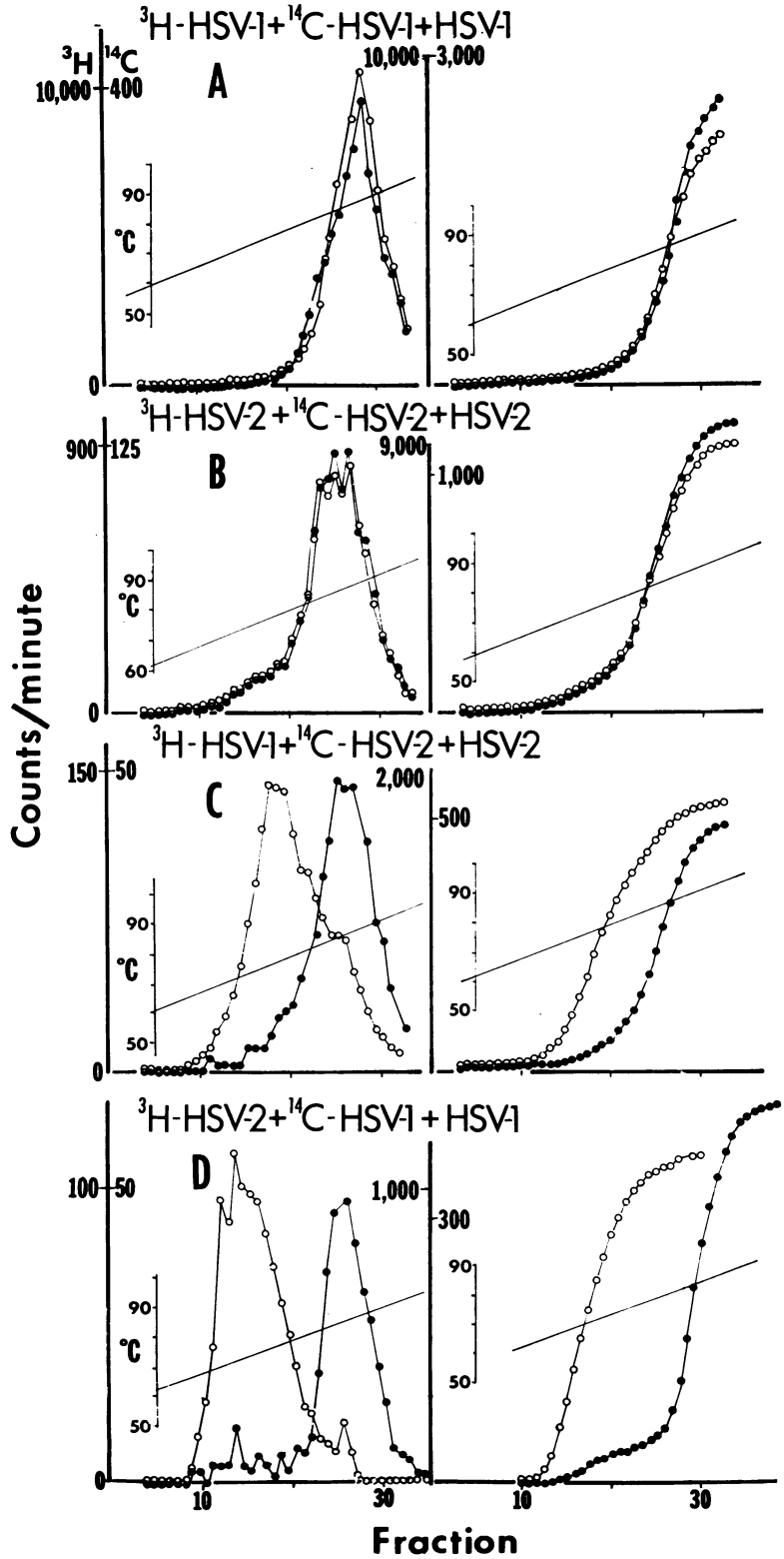

FIG. 2. *Thermal chromatography of renatured HSV-1 and HSV-2 DNA (homoduplex) and of HSV-1-HSV-2 (heteroduplex) DNA. HSV-1 DNA in 50-fold excess was hybridized to ^{3}H-labeled HSV-1 DNA (A) or ^{3}H-labeled HSV-2 DNA (D) in the presence of ^{14}C-labeled HSV-1 DNA. In reciprocal experiments, HSV-2 DNA in 50-fold excess was hybridized to ^{3}H-labeled HSV-2 DNA (B) or ^{3}H-labeled HSV-1 DNA (C) in the presence of ^{14}C-labeled HSV-2 DNA. The left panels indicate the net ^{3}H (○) and ^{14}C (●) counts/min eluted in each fraction. The cumulative data were plotted in the right panels. The procedures for hybridization, thermal chromatography, and assay of radioactivity are described in Materials and Methods.*

TABLE 3. *Midpoint of thermal elution (Te_{50}) of renatured HSV-1 and HSV-2 DNA and of HSV-1-HSV-2 heteroduplex DNA*

Test system		Homoduplex DNA(renatured HSV-1 and HSV-2 DNA)		Heteroduplex DNA (HSV-1-HSV-2 DNA)		Net difference in Te_{50}[a]
Cold DNA	Expt. no.	Labeled DNA	Te_{50}[a]	Labeled DNA	Te_{50}[a]	
HSV-1	1	^{3}H-HSV-1	85			
		^{14}C-HSV-1	85			
	2	^{14}C-HSV-1	84	^{3}H-HSV-2	71	13
	3	^{14}C-HSV-1	84	^{3}H-HSV-2	77	7
	Avg		84.5		74	10
HSV-2	4	^{3}H-HSV-2	85			
		^{14}C-HSV-2	85			
	5	^{14}C-HSV-2	86	^{3}H-HSV-1	76	10
	6	^{14}C-HSV-2	85	^{3}H-HSV-1	75	10
	Avg		85		75	10

[a] Units are degrees centigrade.

Covariation of phenotypic traits in HSV has been reported (6, 22).

Homology between HSV-1 and HSV-2 DNA. Both liquid and liquid-filter hybridization tests indicate that HSV-1 and HSV-2 DNA species contain homologous regions. The extent of homology was calculated to be 40% in the two-phase system and 46% in the one-phase system. The difference in the two results may be due to the fact that the liquid hybridization was carried out at lower temperature and therefore the hybrid population was enriched by DNA species of relatively poor homology.

Degree of heterogeneity of the homologous sequences of HSV-1 and HSV-2 DNA. To test for the degree of homology of the DNA species in the heteroduplex, we compared the Te profile of renatured HSV-1 and HSV-2 DNA species and of the heteroduplex formed between HSV-1 and HSV-2 DNA. HSV-1–HSV-2 heteroduplexes eluted in a single peak 10 C below renatured HSV-1 or HSV-2 DNA. Less than 5% of renatured HSV-1 or HSV-2 homoduplexes eluted at the Te_{50} of the heteroduplex DNA. Since the heteroduplexes represent 46% of the DNA, and since the Te_{50} of the heteroduplex DNA corresponds to a temperature at which only 5% of the DNA of the homoduplexes elute, it necessarily follows that the low Te is due to unmatched bases and not to selective hybridization of adenine plus thymine-rich regions.

The data presented in this paper show that there are two classes of DNA sequences in HSV DNA. One class appears to consist of sequences unique to each subtype. The second class consists of sequences that are homologous and have relatively few (15%) unmatched bases (15). The data raise two questions, i.e., what is the significance of the DNA (about 54%) without homology and conversely what is the significance of the relatively narrow range of unmatched base pairs in the heteroduplex DNA. The answers are not entirely clear. Our interpretation of the data is as follows: (i) HSV DNA consists of two classes, that is, a class that evolved freely and is now subtype-specific, which we call variable DNA, and one which evolved under constraints and is largely (85%) shared in common by the two subtypes. We shall refer to this as the invariable DNA. (ii) One explanation of the minimal mismatching of base pairs observed in the invariant region of the DNA is that excessive changes in amino acid sequences specified by these DNA species may produce defective progeny, and in nature would constitute a lethal mutation. (iii) We have no information on the functions specified by the DNA in the variable and invariable regions.

Very little is known of the rate at which mutations occur naturally in animal virus DNA. HSV multiply in the nuclei of human cells and would be expected to be exposed to many of the same exogenous mutational factors as nuclear DNA. The extent of evolutionary divergence of higher organisms has been correlated with the thermal instability of heteroduplex DNA (14). If HSV-1 and HSV-2 DNA species were segments of a eukaryotic DNA, 10^{7} generations would need to pass to achieve the frequency of mismatched base pairs found in the heteroduplexes formed between the conserved or relatively invariant regions of HSV-1 and HSV-2 DNA.

ACKNOWLEDGMENTS

These investigations were supported by grant 6-3300-07-6094 from the L. B. Block Fund of the University of Chicago, by Public Health Service grant CA 08494A from the National Cancer Institute, by grant E 314F from the American Cancer Society, and by grant GB 27356 from the National Science Foundation. Steven Bachenheimer is a predoctoral trainee under Public Health Service grant AI-00238 from the National Institute of Allergy and Infectious Diseases.

We acknowledge N. Cozzarelli for the gift of exonuclease-1 and alkaline phosphatase and N. Frenkel for valuable discussions.

ADDENDUM IN PROOF

After this paper was submitted for publication, Bronson et al. (Biochim. Biophys. Acta **259**:24–34, 1972) reported that labeled RNA extracted from HSV-2-infected cells hybridized to HSV-1 DNA 40% as efficiently as to the homologous HSV-2 DNA. No data on the thermal stability were presented.

Current studies from our laboratory (Roizman and Frenkel, Proceedings Symposium of the M.D. Anderson Hospital and Tumor Institute, *in press*, 1972) show that (i) all deoxynucleotide sequences common to HSV-1 and HSV-2 are transcribed and represent 50% of all HSV-1 transcribed sequences but only 47% of the total nucleotide sequences in one strand and (ii) the sequences in common are distributed 55% among templates for viral RNA present in high abundance and 45% among templates for viral RNA present in low abundance.

LITERATURE CITED

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