

Herpes Simplex Virus: Genome Size and Redundancy Studied by Renaturation Kinetics

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Herpes simplex virus subtype 1 deoxyribonucleic acid (DNA) was sheared in a French press to uniform fragments, denatured by heating, then allowed to reassociate. The renaturation reaction followed second-order kinetics with a single rate constant indicating that at least 95% of the genome was unique and that repetitive sequences, if present, were not detectable by this technique. The kinetic complexity of the herpes simplex genome was determined by DNA renaturation kinetics to be $(95 \pm 1) \times 10^6$ daltons. Since this value is in excellent agreement with the molecular weight of viral DNA $[(99 \pm 5) \times 10^6$ daltons] obtained from velocity sedimentation studies, it is concluded that virions contain only one species of double-stranded DNA molecules 95×10^6 to 99×10^6 daltons in molecular weight.

Repetitive nucleotide sequences appear to be a characteristic feature of the deoxyribonucleic acid (DNA) of eukaryotic cells; they appear to be absent or less prominent in prokaryotic cells or their viruses (2, 3, 5). There have been no reports of analyses of the degree of redundancy of the genomes of large DNA viruses (poxviruses, herpesviruses) to determine whether these resemble the eukaryotic cells in which they multiply or the viruses of prokaryotic cells. This paper deals with the genome of herpes simplex virus. Previous determinations of the size of the DNA of herpes simplex subtype 1 and subtype 2 (HSV-1 and HSV-2) by velocity cosedimentation with T4 DNA in linear neutral sucrose density gradients yielded a molecular weight of $(99 \pm 5) \times 10^6$ daltons (6). This is in good agreement with the size of the DNA obtained for HSV-1 DNA alone by the Kleinschmidt technique (1). Questions arise as to whether the DNA isolated from virus represents one or more species of the same size and whether repetitive sequences can be detected in the viral genome.

In these experiments, HSV-1 DNA was prepared by procedures described in detail previously (6). Briefly, HEp-2 cells were infected at a multiplicity of 10 plaque-forming units per cell. At 18 hr after infection, the cells were harvested; the cytoplasm was extracted with the nonionic detergent NP-40 (Shell Oil Co., New York N.Y.) and then sedimented in neutral sucrose density gradients. The DNA was then extracted from the nucleocapsid band with 0.5% sodium dodecyl sulfate, 2% Sarkosyl and banded in

neutral sucrose density gradients. The band containing intact viral DNA was then extracted with phenol and chloroform-isoamyl alcohol, digested with pancreatic ribonuclease rendered free of deoxyribonuclease activity by heating, and then reextracted with phenol and chloroform-isoamyl alcohol. The DNA was then extensively dialyzed, concentrated by ethanol precipitation, and sheared in a French press at 20,000 p.s.i. The alkaline sedimentation constant of the sheared DNA was determined by sedimentation in 0.1 M NaOH, 0.9 M NaCl (pH 13) in a Beckman model E centrifuge as described by Studier (8). Prior to the onset of renaturation, the sheared DNA was denatured by heating in $1 \times$ SSC (0.15 M sodium chloride, 0.015 M sodium citrate) to 105 C for 10 min. Renaturation was followed in a Gilford model 2000 spectrophotometer equipped with an automatic absorbance and temperature recorder. The temperature equilibration interval in this instrument was previously reported to be 7 min (5). Renaturation was performed in $1 \times$ SSC at 70 C. The melting temperature of HSV DNA in this solvent was reported to be 97 C (7). We used $1 \times$ SSC rather than a higher salt concentration to decrease the rate of association. After the reassociation was completed, the temperature in the cuvette chamber was raised to 105 C to determine the maximum absorbance of the denatured DNA. Figure 1 gives the plot of the initial stages of the renaturation reaction for HSV-1 DNA. Figure 2 shows the association kinetics plotted in terms of C_0t (3). The ideal renaturation reaction is expected to follow second-order kinetics with

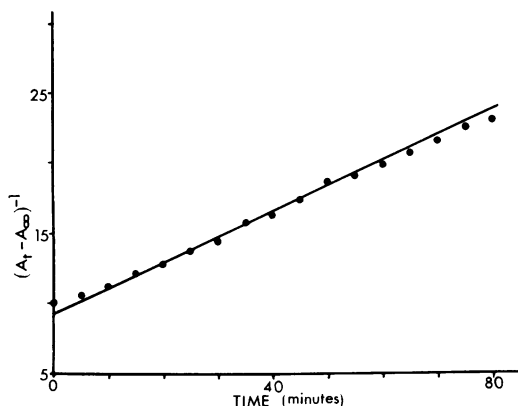


FIG. 1. Second-order rate plot for the renaturation of HSV-1 DNA in $1\times$ SSC at 70 C. The alkaline sedimentation coefficient $S_{20,w}^{pH13}$ of the DNA fragments is 9.1S. The slope of this plot is $(3.03 \pm 0.04) \times 10^{-3}$ per sec. The rate constant k_2 under these conditions is 11.1 ± 0.1 liter per mole per sec.

the empirical rate constant inversely proportional to the complexity of the annealing sequences (2, 9). Extensive degree of redundancy in the DNA sequences should result in different apparent renaturation rates for the repeating and the unique sequences (3, 9). The renaturation of HSV-1 DNA followed second-order kinetics with a single rate constant up to the point where 80% of the DNA was in hybrid form. We therefore conclude that the viral genome does not contain a detectable degree of sequence repetition. Small degrees of redundancy cannot be ruled out, however, by this method since the temperature equilibration interval of the optical absorbance system is on the order of 7 min (5), by which time 5% of the DNA has already annealed.

The slope of the plot in Fig. 1 is calculated by the least-square deviation method to be $(3.03 \pm 0.04) \times 10^{-3}$ per sec, at the 95% confidence level.

The value of k_2 is obtained from the following relationship:

$$1/(A_t - A_\infty) = 2.72 \times 10^{-4} k_2 t + 1/0.27 A_\infty$$

Where A_t is the 260 nm absorbance at time t , A_∞ is the absorbance of native DNA, k_2 is the second-order rate constant, and t is the time of renaturation. This equation was derived from the relationship given by Wetmur and Davidson (9) with the correction for the effect of the base composition on the hyperchromicity (5). K_2 derived from the slope of Fig. 1 for $1\times$ SSC is 11.1 ± 0.1 liter per mole per sec. The kinetic complexity, N_d , is next calculated from the relationship given by Wetmur and Davidson (9): $N_d = [5.5 \cdot 10^8 (S_{20,w}^{pH13})^{1.25}] / k_2$ where $S_{20,w}^{pH13} = 9.1S$

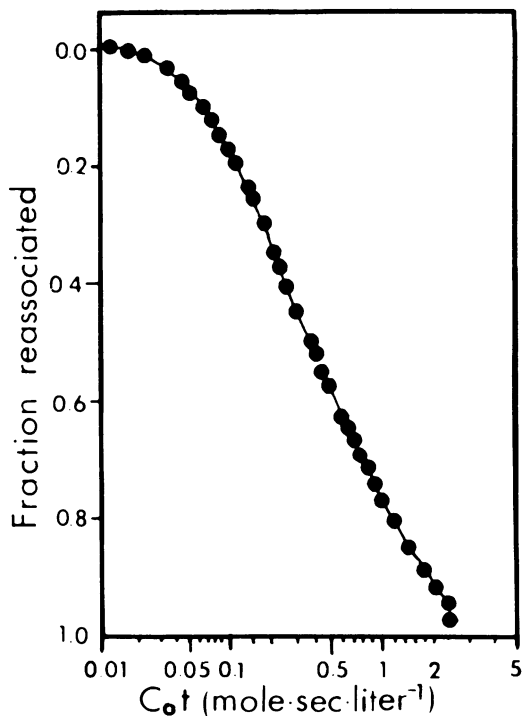


FIG. 2. Plot for the reassociation of HSV-1 DNA. The data obtained originally for $1\times$ SSC and for DNA fragments about 1,100 nucleotides long were corrected for 0.180 M cation concentration (3) and 400 nucleotide fragment size (9) to enable comparison with the data of Britten and Kohne (3).

as determined by the alkaline band sedimentation and 92 ± 1 liter per mole per sec, is the corrected value of k_2 for 1.0 M monovalent cation concentration (4). The kinetic complexity, N_d , for HSV-1 genome is found to be $(95 \pm 1) \times 10^6$ daltons.

We conclude the following. (i) Repetitive sequences were not detected by the technique applied. We cannot exclude the possibility that a few genes are reiterated several times. However, it is evident that the bulk of the genetic information is unique. (ii) The kinetic complexity of HSV-1 is $(95 \pm 1) \times 10^6$ daltons, in agreement with the value of $(99 \pm 5) \times 10^6$ daltons for the molecular weight of the DNA previously determined in this laboratory (6) by velocity cosedimentation with T4 DNA. (iii) From the close agreement between the value of the kinetic complexity of the DNA and its molecular weight, we conclude that the genetic information of HSV is encoded in a single type of DNA molecule of size approximately 99×10^6 daltons. Therefore, one molecule of DNA per virion should be suffi-

cient to permit it to produce infectious progeny in the susceptible cell.

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LITERATURE CITED

1. Becker, Y., H. Dym, and I. Sarov. 1968. Herpes simplex virus DNA. *Virology* 36:184-192.
2. Britten, R. J., and D. E. Kohne. 1967. Nucleotide sequence repetition in DNA. *Yearbook Carnegie Inst.* 65:78-106.
3. Britten, R. J., and D. E. Kohne. 1968. Repeated sequences in DNA. *Science* 161:529-540.
4. Britten, R. J., and J. Smith. 1970. A bovine genome. *Yearbook Carnegie Inst.* 68:378-386.
5. Gillis, M., J. DeLay, and M. De Clune. 1970. The determination of molecular weight of bacterial genome DNA from renaturation rates. *Eur. J. Biochem.* 12:143-153.
6. Kieff, E. D., S. L. Bachenheimer, and B. Roizman. 1971. Size, composition and structure of the DNA of subtypes 1 and 2 herpes simplex viruses. *J. Virol.* 8:125-132.
7. Russell, W. C., and L. V. Crawford. 1963. Some characteristics of the deoxyribonucleic acid from herpes simplex virus. *Virology* 21:353-361.
8. Studier, F. W. 1965. Sedimentation studies of the size and shape of DNA. *J. Mol. Biol.* 11:373-390.
9. Wetmur, J. G., and N. Davidson. 1968. Kinetics of renaturation of DNA. *J. Mol. Biol.* 31:349-370.