TERMINAL FRAGMENTS OF HERPES SIMPLEX VIRUS DNA PRODUCED BY RESTRICTION ENDONUCLEASE

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SUMMARY: Digestion of HSV-1 DNA with λ 5'-exonuclease prior to digesting the DNA with the Eco R I restriction endonuclease specifically affects two of the fragments normally obtained after restriction endonuclease digestion. Therefore these two fragments contain the sequences which occur at the termini of HSV-1 DNA. One of the fragments affected is a "minor" fragment which is always present in less than molar yield. The possible relationship between the occurrence of minor Eco R I fragments and the partial refractoriness of HSV-1 DNA to λ 5'-exonuclease digestion is discussed.

INTRODUCTION: The DNA of herpes simplex virus type 1 (HSV-1) has been the subject of a number of recent investigations. HSV-1 DNA, which is a linear molecule with a molecular weight between 95 and 100 x 10^6 daltons, possesses two types of repetitious sequences: terminal repetitions of 0.5 x 10^6 daltons (1,2) as well as internal sequences which are inverted repetitions of larger portions of each terminus (3,4). The pattern of fragments produced by digestion of a variety of strains of HSV-1 DNA with the Eco R I restriction nuclease has been reported by a number of workers (5-7). The patterns from different strains are largely similar, although distinct strain-specific differences have been noted (6,7). A common feature of all reported patterns is the occurrence of minor fragments present in distinctly less than molar yields. Usually

two such fragments are present, but sometimes more are observed.

We report here that λ 5'-exonuclease digestion of HSV-1 (Patton) DNA, prior to Eco R I restriction nuclease digestion, affects one of the major and one of the minor fragments, demonstrating that these sequences occur at the termini of HSV-1 DNA. The importance of this information is twofold: (1) it aids in determining the genetic organization of HSV-1 DNA and (2) it suggests an explanation for the appearance of fragments in less than molar yield.

EXPERIMENTAL: Procedures for DNA preparation from plaque purified HSV-1 (Patton), λ 5'-exonuclease digestion and electron microscopy have been described in detail elsewhere (2). The DNA preparation involved detergent lysis of partially purified virions followed by CsCl banding of the released DNA. Following λ 5'-exonuclease digestion, the reaction was stopped by adjusting the solution to 2 x SSC (0.3 M NaCl, 0.03 M sodium citrate). To 3.63 ml of this solution, containing 10 μg of DNA, was added 1 ml of 1 mg/ml pronase in 0.1 M sodium-EDTA, pH 8.5. This mixture was incubated at 37° for 20 hrs. The DNA was then precipitated by the addition of two volumes of cold ethanol. Controls (Fig. 1a and 1c) went through all steps except that the λ 5'-exonuclease was omitted. Eco R I restriction enzyme preparation and digestion, as well as electrophoresis of the digestion products on 0.5% agarose disc gels were as described by Helling et al. (5), except that phenol extraction of the DNA fragments before electrophoresis was omitted. After electrophoresis, the gels were stained with ethidium bromide and photographed under a short wave uv light using a Wratten G (orange) filter.

RESULTS AND DISCUSSION: Intact HSV-1 DNA was digested with Eco R I restriction endonuclease and the products separated by disc gel electrophoresis in 0.5% agarose. The pattern obtained (Fig. 1a) is quite similar to that reported for other strains of HSV-1 such as the KOS strain studied by Skare et al. (6). In particular, it is seen that two relatively high molecular weight fragments, B and H, are present in clearly less than molar yields.

The exonuclease produced by λ bacteriophage is highly specific for the 5' ends of double stranded DNA (8). Therefore, limited digestion of HSV-1 DNA by this enzyme should affect only those sequences occurring at the extreme ends of the linear molecule.

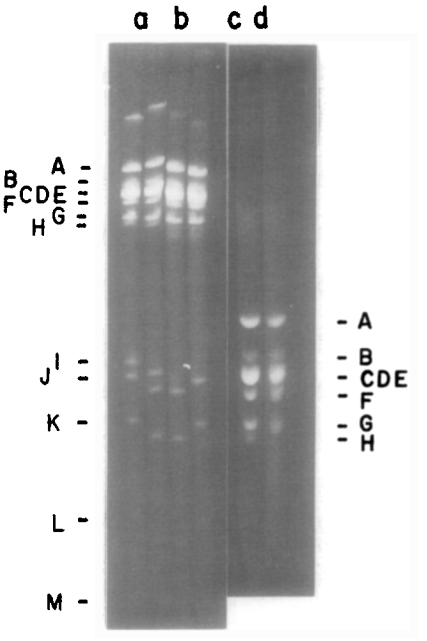

Fig. 1. Effect of λ 5'-exonuclease digestion on the pattern of Eco R I fragments obtained from HSV-1 DNA. In all cases, approximately 1 μg of DNA was digested with Eco R I restriction nuclease, and the products were applied directly to 0.5% agarose gels (Experimental). Electrophoresis was at 1.5 volts/cm gel length with constant cooling of the lower buffer reservoir. a) Without prior exonuclease digestion, electrophoresis for 20 hrs; b) with λ 5'-exonuclease digestion, electrophoresis for 20 hrs; c) same as (a) but electrophoresis was carried out 44 hrs; d) same as (b) but electrophoresis was carried out 44 hrs. The fragment designations are similar to those used by Hayward et al. (7). The smallest fragment, M, is barely perceptible in (a) and (b); however, longer photographic exposure clearly revealed its presence in both cases.

When the HSV-1 DNA was digested with an excess of λ 5'-exonuclease for a length of time sufficient to produce between 1% and 1.5% solubilization of acid precipitable nucleotides (2) and then digested with the restriction nuclease, the results shown in Fig. 1b and 1d were obtained. The only fragments noticeably affected by exonuclease digestion are the minor fragment H and the major fragment I. These two fragments are missing, although there is diffuse, stained material migrating just ahead of each of the expected positions of these fragments. This diffuse, stained ma-

terial presumably corresponds to H and I fragments with varying portions of their terminal sequences converted to single strands. Since the extent of digestion was small compared to the size of all the fragments, except possibly fragment M [which represents just under 1% of the total molecule according to Skare et al. (6)], we conclude that fragments H and I occur at the extremes of the whole molecule. Another conclusion that can be drawn from these results is that HSV-1 DNA does not represent a population of circularly permuted sequences, since all Eco R I fragments would be affected by prior exonuclease digestion in a circularly permuted population. Lack of circular permutation in HSV-1 DNA was recently concluded by Hirsch et al. (9).

Since fragment I is present in approximately molar yield before exonuclease digestion, it obviously can account for all of one end of the whole molecule. However, fragment H is present in substantially less than a molar yield, and cannot account for all of the other end. Quite possibly related to this problem is our observation that some ends in our HSV-1 DNA seem to be refractory to λ 5'-exonuclease digestion (2). Of 757 molecules examined under the electron microscope after limited exonuclease digestion, 406 clearly had single strand material at both ends, 312 had single strand material at one end only while the other end appeared completely double stranded, and 39 appeared completely double stranded at both ends. The following hypothesis might reconcile the partial refractoriness of HSV-1 DNA to exonuclease digestion and the occurrence of minor Eco R I fragments. Purified HSV-1 DNA may have some modification of one specific end of approximately half of the molecules. The effects of this modification would be twofold: first, the end involved would become refractory to λ 5'-exonuclease; second, when the modified

end is released as an Eco R I fragment, its electrophoresis mobility is substantially different from the corresponding unmodified fragment. In other words, all HSV-1 DNA molecules have exonuclease-sensitive fragment I sequences at one end, while a portion have exonuclease-sensitive fragment H sequences at the other; the remainder have a modified, exonuclease-refractory sequence at this end. Perhaps minor fragment B is in fact the modified fragment H, although the differences in electrophoretic mobility between B and H are substantial. Preliminary efforts to remove the proposed modification by deproteinization have had negative results. Following Eco R I restriction endonuclease digestion of the DNA, four deproteinization procedures were applied to the fragment mixtures prior to electrophoresis. The procedures included extraction with phenol or chloroform-octanol, digestion with pronase in the presence of sodium dodecyl sulfate and treatment with urea. In all cases the disc gel patterns of the fragments remained the same; in particular minor fragments B and H were still observed with no change in their relative intensities or mobilities. It is hoped that current efforts will lead to the elucidation of the source of minor fragments and refractoriness to exonuclease digestion.

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