JOURNAL OF VIROLOGY, Nov. 1979, p. 449-457
0022-538X/79/11-0449/09$02.00/0

Vol. 32, No. 2

DNase Induced After Infection of KB Cells by Herpes Simplex Virus Type 1 or Type 2

II. Characterization of an Associated Endonuclease Activity

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Received for publication 22 March 1979

Purified preparations of the "exonuclease" specified by herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) possess an endonuclease activity. The exonuclease and endonuclease activities copurify and cosediment in a sucrose density gradient. Endonuclease activity is only observed in the presence of a divalent cation, and Mg^{2+} or Mn^{2+} is equally effective as a cofactor with an optimal concentration of 2 mM. A slight amount of endonuclease activity is observed in the presence of Ca^{2+}, whereas no activity occurs in the presence of Zn^{2+}. In the presence of Mg^{2+}, Ca^{2+} and Zn^{2+} are inhibitory. Comparison of exonuclease and endonuclease activity in the presence of various divalent cations revealed that, at concentrations of Mn^{2+} greater than 1 mM, only endonuclease activity occurs whereas endonuclease and exonuclease activity occur at all concentrations of Mg^{2+}. The endonuclease was affected by putrescine and spermidine to the same extent as the exonuclease activity, but in marked contrast the endonuclease was inhibited by a 10-fold-lower concentration of spermine compared to the exonuclease. The activity specified by HSV-1 and HSV-2 has very similar properties. HSV-1 and HSV-2 endonuclease cleave covalently closed circular DNA to yield, firstly, nicked circles and then linear DNA which is subsequently hydrolyzed to small oligonucleotides. Cleavage does not appear to be base sequence specific. Conversion of nicked circles to linear DNA and subsequent degradation of linear DNA occurs more rapidly in the presence of Mg^{2+} than Mn^{2+} presumably by virtue of the presence of the exonuclease activity. Nonsuperhelical covalently closed circular duplex DNA is cleaved by the endonucleases at a rate 60 times slower than the rate observed on the supercoiled form. These data indicate that the HSV-1 and HSV-2 endonuclease preferentially recognize single-stranded DNA regions.

The DNase induced in an animal cell after infection by herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) has been described as an alkaline exonuclease by virtue of the fact that 5'-monophosphate nucleosides are produced by the action of these enzymes on DNA (23; J. M. Morrison and H. M. Keir, Biochem. J. **103**:70P, 1967). However, we found that purified preparations of DNase from HSV-1- and HSV-2-infected human cells were able to cleave covalently closed circular DNA (9) and thus these "exonucleases" have an associated endonuclease activity. This report describes the characteristics of the endonuclease activity.

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MATERIALS AND METHODS

Organisms. HSV-1 (KOS strain) and HSV-2 (333 strain) were grown and titered as described previously (9). KB cells were infected with HSV-1 or HSV-2 as described previously (9), and the infected cells were harvested 15 to 18 h postinfection.

Escherichia coli strain 1411 carrying the plasmid pMB9 (4) was kindly provided by John E. Donelson. This strain of *E. coli* was grown in L-broth (1% tryptone-0.5% yeast extract-0.5% NaCl [pH 7.5]) containing 20 μg of tetracycline per ml.

Bacteriophage PM2 and its host pseudomonas BAL-31 were kindly provided by David Kowalski. PM2 was grown and purified as described by Salditt et al. (30).

Enzymes. HSV-1- and HSV-2-specified DNases were obtained from infected KB cells and purified, about 1,500-fold, as described previously (9). These purified enzyme preparations were used in the studies described in this report.

Pancreatic DNase I (DPFF) was purchased from Worthington Biochemical Corp.

Nicking-closing enzyme was isolated from HeLa cell nuclei by the method of Vosberg and Vinograd (33) and purified to fraction I before use.

Preparation of DNA. Double- and single-stranded *E. coli* [^{14}C]DNAs were prepared as described previously (9). PM2 DNA was isolated from purified phage (6, 7) and further purified by CsCl-ethidium banding (27). The ethidium bromide was removed by extraction with 1-butanol, and the DNA was dialyzed against 0.1 M Tris-hydrochloride (pH 8.0) containing 5 mM EDTA. Plasmid pMB9 DNA was isolated and purified as described by Wensink et al. (35). Nonsuperhelical, convalently closed circular (form I') DNA was prepared by treating superhelical, covalently closed circular (form I) PM2 DNA with nicking-closing enzyme. PM2 DNA (250 μg/ml) was incubated at 37°C in 0.02 M potassium phosphate buffer (pH 7.0), 0.2 mM EDTA, 60 μg of bovine serum albumin per ml, and 0.1 ml of fraction I nicking-closing enzyme per ml of reaction mixture (33). Conversion of form I DNA to form I' DNA was monitored by agarose gel electrophoresis. With our preparation of nicking-closing enzyme and under the above reaction conditions, complete relaxation of form I PM2 DNA had occurred by 30 to 60 min. The mixture was then heated to 65°C for 15 min (to inactivate the nicking-closing enzyme) and then dialyzed against 0.05 M Tris-hydrochloride (pH 8.0) containing 1 mM EDTA. Agarose gel electrophoresis of a sample of relaxed PM2 DNA before and after heating to 90°C in the presence of alkali verified that the relaxed DNA was covalently closed circular.

Enzyme assays. Exonuclease activity was measured as described previously (9). Endonuclease activity was measured by two methods: semiquantitatively by a sensitive ethidium bromide fluorescence assay (22) and qualitatively by agarose gel electrophoresis. The fluorescence assay measured the rate of conversion of form I DNA to form II DNA (circular duplex DNA with one or more nicks; a nick refers to a single phosphodiester interruption in one strand of a duplex DNA molecule). The standard reaction mixture contained 0.05 M Tris-hydrochloride (pH 8.0), 4 mM β-mercaptoethanol, 10 mM $MgCl_2$, 1 μg of form I PM2 DNA, and enzyme in a total volume of 0.1 ml. The reaction was initiated by addition of enzyme, and the mixture was incubated at 37°C for various periods of time. Reaction times were kept as short as possible, generally 5 to 10 min, to minimize the effect of nonspecific "nicking" of the DNA by the reducing agent (3, 16). The reaction was terminated by the addition of 1 ml of fluorescence buffer (0.05 M potassium phosphate, 0.5 mM EDTA, 0.5 μg of ethidium bromide per ml, pH 11.6 [26]), and the mixture was heated at 90°C for 2 min and then cooled in water at 24°C. The relative fluorescence was measured at ambient temperature in a Turner fluorocolorimeter equipped with a Corning 7-60 excitation filter (366 nm peak) and a Wratten 23A emission filter, with the instrument set on the 10× scale. Endonuclease activity of a particular enzyme sample was determined as the decrease in relative fluorescence units compared to a control sample which contained no enzyme. The reaction was linear with time until 20 to 30% of form I DNA had been cleaved.

Samples for agarose gel electrophoresis after enzyme treatment were prepared in a manner similar to that of Rogers and Rhoades (29). DNA was denatured with 0.15 M NaOH. For agarose gel electrophoresis the standard reaction mixture contained 0.05 M Tris-hydrochloride (pH 8.0), 4 mM β-mercaptoethanol, 10 mM $MgCl_2$, 0.6 μg of form I PM2 DNA, and enzyme in a final volume of 0.025 ml. The reaction was initiated by addition of enzyme, and mixtures were incubated at 37°C. The reaction was terminated by the addition of 5 μl of 5% sodium dodecyl sulfate, 0.14 M EDTA, and 0.1% bromophenol blue followed by 5 μl of 70% sucrose, and 25 μl of the mixture was layered into a slot in the gel. When denatured DNA was desired, the reaction was terminated by the addition of 5 μl of 0.125 M EDTA, 0.875 M KOH, and 0.1% bromophenol blue followed by 5 μl of 70% sucrose. Samples were heated at 90°C for 1 min and rapidly chilled in an ice bath, and 25 μl of the mixture was layered into a slot in the gel.

The reaction mixture for measurement of DNase I activity contained 0.05 M Tris-hydrochloride (pH 8.0), 10 mM $MgCl_2$, and 0.1 mg of bovine serum albumin per ml and the same amount of PM2 DNA in the same final volumes as described above for assay of the HSV endonucleases.

Agarose gel electrophoresis. Electrophoresis of DNA in horizontal agarose slab gels was performed essentially as described by Johnston et al. (15) with some minor modifications. The concentration of agarose (Sigma type II, medium EEO) was 0.7% (wt/vol), and ethidium bromide was not included in the gel or electrode buffer. Electrophoresis of samples in an agarose slab gel (16 by 16 cm) was carried out at 4°C at 200 V for 5 h. Gels and electrode buffer were chilled to 4°C before electrophoresis. After electrophoresis, gels were stained in buffer containing 2 μg of ethidium bromide per ml for 30 min.

Photographs of stained gels were made with a Nikkormat FTN 35-mm camera, equipped with a wide-angle 35-mm lens and a Nikkor R60 red filter, using Kodak (5069) high-contrast copy film. Gels were illuminated from above with a short wavelength (254 nm) UV lamp (U.V. Products, Inc.).

Sedimentation. Centrifugation of HSV-DNase in 5 to 20% sucrose gradients was as described previously (9). Samples were centrifuged at 49,000 rpm for 18 h at 4°C in a Beckman SW50.1 rotor.

RESULTS

Comparison of the properties of purified HSV endonuclease and HSV exonuclease. Endonuclease activity was measured during chromatographic purification of HSV exonuclease on phosphocellulose, DEAE-cellulose, and DNA-cellulose, and it was found that the endonuclease activity copurified on all three chromatographic steps (data not shown). The ratios of endonuclease activity to exonuclease in fractions across the peak eluted from the final DNA-cellulose column were almost identical, as were the ratios of the activities in pooled fractions from the two preceding chromatographic steps.

The purified endonuclease activity had iden-

tical distribution and sedimentation coefficients as the purified HSV exonuclease when centrifuged on a sucrose density gradient in the presence of 0.5 M KCl (Fig. 1B). Symmetrical, coincident peaks of exonuclease and endonuclease activities were observed, and the ratio of endonuclease activity to exonuclease activity across the peaks was constant. In the absence of 0.5 M KCl (Fig. 1A), a non-symmetrical peak of endonuclease was obtained, similar to that which we had previously described for the exonuclease (9). The non-symmetrical peaks of activity obtained in the absence of 0.5 M KCl probably reflect either protein-protein interactions (with bovine serum albumin present in the sample to help stabilize the enzyme) or the presence of different conformational states of the enzyme which are altered in the presence of higher ionic strength. The fact that the exonuclease and endonuclease both show a change from non-symmetrical to symmetrical peaks of activity as well as a slight decrease in sedimentation coefficient in high-ionic-strength solutions compared to low-ionic-strength solutions, suggests that both activities are strongly associated. Data, similar to that shown in Fig. 1 for HSV-2 DNase, were also obtained for HSV-1 DNase.

Like the exonuclease activity (9), the endonuclease activity was rapidly lost in the absence of a reducing agent and the endonuclease was irreversibly inactivated by *p*-hydroxymercuribenzoate. The concentration of mercaptoethanol necessary to achieve linear reaction rates was 4 to 5 mM.

The response of HSV-1 and HSV-2 endonuclease to increasing concentrations of monovalent cations (Fig. 2) was similar to that observed with the HSV-1 and HSV-2 exonuclease (9). Endonuclease activity was inhibited by increasing concentrations of NaCl, with an 85 to 90% loss of activity at a concentration of 0.2 M (Fig. 2). Substitution of KCl for NaCl gave the same result.

Like the HSV exonuclease (9), the HSV endonuclease also had an absolute requirement of a divalent metal ion for activity.

Effect of divalent metal ions. Of the divalent cations tested, Mg^{2+} and Mn^{2+} gave virtually the same response, with maximal activity being obtained at a cation concentration of about 2 mM (Fig. 3). The effect of higher concentrations of Mn^{2+} could not be determined with the fluorescence assay because of quenching of the fluorescence at Mn^{2+} concentrations greater than 2 mM. However, qualitative assays by agarose gel electrophoresis indicated that there was little, if any, difference in the rate of cleavage of form I DNA by HSV-1 or HSV-2 endonuclease in the presence of 2 mM or 5 mM Mn^{2+}. The rate of endonuclease activity in the presence of Ca^{2+} was 4 to 7% of that obtained with Mg^{2+}, and no activity was observed in the presence of Zn^{2+}. Qualitative endonuclease assays by agarose gel electrophoresis were consistent with these observations. The effect of Mn^{2+}, Ca^{2+}, or Zn^{2+} on endonuclease activity was also deter-

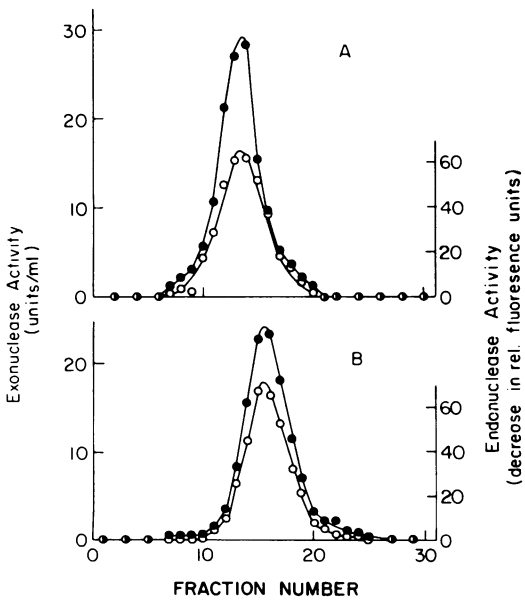

FIG. 1. *Sucrose density gradient centrifugation of HSV-DNase. A sample (0.1 ml) of purified HSV-2 DNase was centrifuged on a 5 to 20% sucrose gradient in the absence (A) or presence (B) of 0.5 M KCl. Fractions were assayed for exonuclease (●) and endonuclease activity (○). Centrifugation was from right to left.*

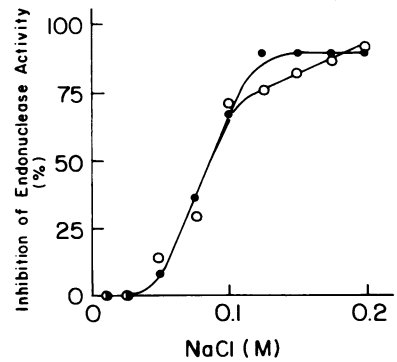

FIG. 2. *Effect of monovalent cations on HSV endonuclease. HSV-1 (●) and HSV-2 (○) endonuclease activity was determined in the presence of the additional concentrations of NaCl. The amount of activity inhibited was calculated by subtraction of the activity in the presence of NaCl from the activity in the absence of NaCl.*

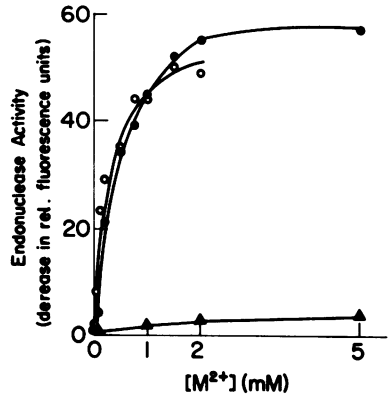

FIG. 3. *Effect of divalent cations on HSV endonuclease activity. Purified HSV-2 exonuclease (0.6 to 1.2 units) was assayed for endonuclease activity (5-min incubation) in the presence of the indicated concentrations of* Mg^{2+} (●), Mn^{2+} (○), *and* Ca^{2+} (▲).

mined when these cations were added to the reaction mixture in the presence of 1 mM Mg^{2+}. In the presence of 1 mM Mg^{2+}, 0.1 mM Ca^{2+} gave 16 to 21% inhibition of activity, 1 mM Ca^{2+} gave 65 to 67% inhibition, and 10 mM Ca^{2+} gave 96 to 98% inhibition. With 0.1 mM Zn^{2+}, 0 to 5% inhibition of activity was observed; 1 mM Zn^{2+} gave 62 to 65% inhibition and 10 mM Zn^{2+} gave 98 to 100% inhibition. In contrast, Mn^{2+} did not inhibit endonuclease activity in the presence of Mg^{2+}. Addition of Mn^{2+} to the suboptimal concentration of 1 mM Mg^{2+} resulted in an increase in endonuclease activity which followed the saturation curve shown in Fig. 3. Since Mg^{2+} and Mn^{2+} are equally effective in stimulating endonuclease activity (Fig. 3), the saturation curves for Mg^{2+} and Mn^{2+} shown in Fig. 3 also represent endonuclease activity as a function of total divalent cation concentration (Mg^{2+} + Mn^{2+}), irrespective of the combination of Mg^{2+} and Mn^{2+} used. The degree of endonuclease activity observed in the presence of Mg^{2+}, Ca^{2+}, or Zn^{2+} was similar to that seen with the exonuclease activity (9). In contrast, we previously reported that little or no exonuclease activity occurred at concentrations of Mn^{2+} greater than 1 mM (9). Therefore at Mn^{2+} concentrations greater than 1 mM, only endonuclease activity occurs, whereas both endonuclease and exonuclease activity occur at all concentrations of Mg^{2+}. HSV-1 and HSV-2 endonuclease showed the same response to divalent cations.

Effect of polyamines. Putrescine, spermidine, and spermine were tested for their effect on the endonuclease activity (Fig. 4). Putrescine had virtually no effect on endonuclease activity, whereas spermidine and spermine were found to be inhibitory. Spermidine gave a similar inhibitory effect on endonuclease activity over the same concentration range to that observed for the exonuclease, although spermidine was somewhat less inhibitory on endonuclease activity than on exonuclease activity (9). In contrast to the exonuclease (9), 50% inhibition of the endonuclease activity occurred at about a 10-fold-lower concentration of spermine than that required for 50% inhibition of exonuclease (Fig. 4). However, it should be considered that the difference in the effect of spermine on HSV endonuclease and exonuclease may, in part, reflect a different effect of spermine on supercoiled DNA (substrate for endonuclease) compared to linear DNA (substrate for exonuclease).

Substrate specificity. Since the fluorescence assay for endonuclease activity only measures the decrease in the quantity of covalently closed circular DNA, agarose gel electrophoresis was used to examine the products of HSV endonuclease action to obtain information regarding the substrate specificity and mechanism of action of the enzyme.

The action of HSV endonuclease on two different form I DNA substrates was compared (Fig. 5). The enzymatic reaction was done in the presence of 5 mM Mn^{2+} so that only endonuclease activity would be observed. With both substrates, form I DNA was first converted to nicked open circular (form II) DNA, indicating that a cleavage in one DNA strand was first made. Form II DNA was then converted to linear, duplex (form III) DNA which was subsequently degraded to small oligonucleotide fragments (Fig. 5). Electrophoresis of the products after denaturation produced a two-band pattern from PM2 DNA and a one-band pattern from pMB9 DNA (Fig. 5). The pattern obtained after denaturation represented complementary single-stranded DNAs (14) of unit length which are separated in the case of PM2 DNA but are

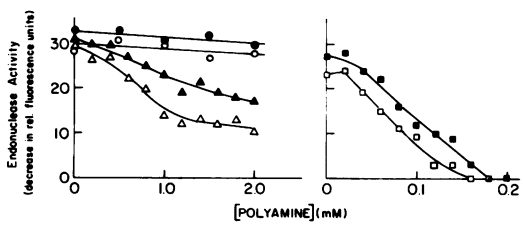

FIG. 4. *Effects of polyamines on HSV endonuclease. The activity of purified HSV-1 (closed symbols) and HSV-2 (open symbols) endonuclease was determined in the standard assay mixture in the presence of the indicated concentrations of putrescine* (●, ○), *spermidine* (▲, △), *and spermine* (■, □).

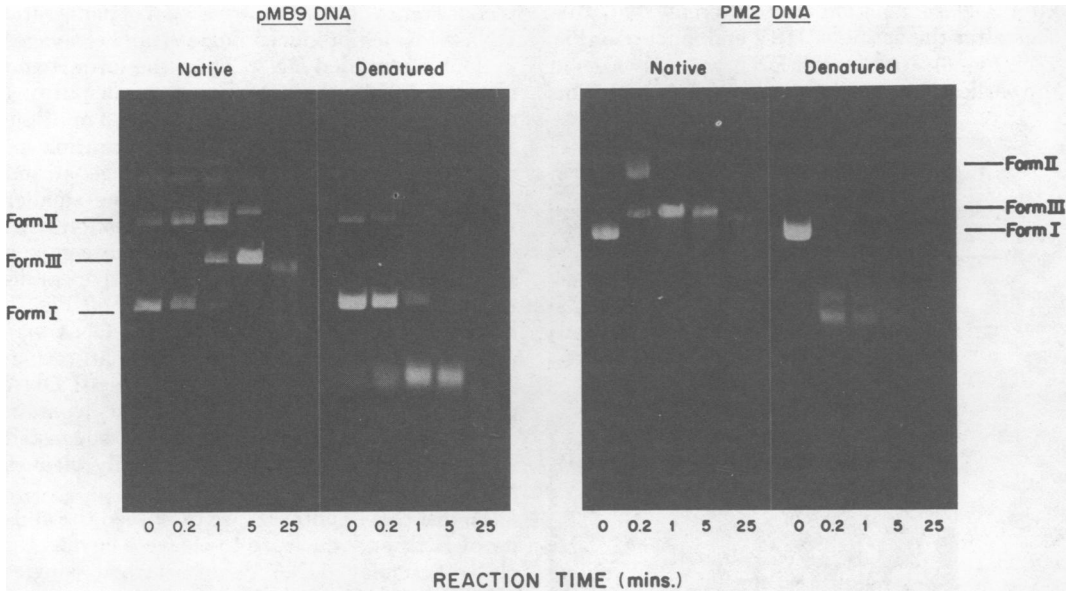

FIG. 5. *Agarose gel electrophoresis of the products derived from the digestion of pMB9 DNA and PM2 DNA with purified HSV-2 DNase. pMB9 DNA and PM2 DNA were reacted with 1.0 unit of HSV-2 DNase in the standard assay mixture but in the presence of 5 mM Mn^{2+} each of the times indicated, and the native and denatured products were electrophoresed on agarose gel.*

not in the case of pMB9 DNA. The HSV endonucleases do not produce sequence-specific single-strand cleavages, since if this were the case, a unique set of single-strand DNA fragments (after denaturation) would be expected from the two different substrates (28, 29). The action of HSV endonuclease on both pMB9 and PM2 DNA is essentially the same (Fig. 5), although the activity of the endonuclease seems significantly greater on the PM2 DNA than on the PMB9 DNA.

The products obtained when the enzymatic reaction is performed in Mg^{2+} compared to Mn^{2+} are shown in Fig. 6. Overall degradation to small oligonucleotides is more rapid in the presence of Mg^{2+}, and for this reason the data shown in Fig. 6 represent the activity of the HSV DNase in Mg^{2+} at half the enzyme concentration as that for Mn^{2+}. Even under these conditions it would appear that an incision of form I DNA occurs more rapidly in the presence of Mn^{2+} than in Mg^{2+}. This result differs from that obtained early in the reaction where equal rates of loss of form I DNA in the presence of either Mg^{2+} or Mn^{2+} were observed (Fig. 3). However, it is difficult to directly compare the overall degradation patterns seen by agarose gel electrophoresis with the quantitative fluorescence assay where only the loss of the first 20% of the substrate was linear with time. The products of the reaction are themselves substrates for the HSV endonucleases, and it should be remembered that, in the presence of Mg^{2+}, interpretation of the electrophoretic pattern is further complicated by the presence of exonuclease activity. Comparison of the pattern of fragments obtained after 25 min of reaction in Mg^{2+} or Mn^{2+} (Fig. 6) shows that linear (form III) molecules are degraded more rapidly in the presence of Mg^{2+}. This is consistent with the added presence of HSV exonuclease in Mg^{2+}. Thus, the apparent slowness of conversion of small amounts of form I DNA to form II DNA, in Mg^{2+}, in the presence of larger amounts of form II and form III DNA (Fig. 6, Mg^{2+}, native, 1 and 5 min of reaction) may also be explained by the fact that form II and form III DNA are substrates for the exonuclease whereas form I DNA is not, and that in a reaction mixture where form II and form III DNA predominate, these substrates are degraded preferentially to form I DNA by a rate-limiting amount of enzyme.

Another feature of the HSV endonuclease reaction was obtained by comparison to the action of DNase I on PM2 DNA. The action of DNase I on PM2 DNA gave a similar pattern to that produced by HSV endonuclease under nondenaturing conditions (Fig. 7). Form I DNA was converted to form II and then form II was converted to form III which was subsequently degraded to small oligonucleotide fragments. However, the pattern obtained upon denaturation

after DNase I action was different than that seen after the action of HSV endonuclease (Fig. 7, cf. Fig. 6). Unit length DNA was only seen at the earliest time of digestion with DNase I. This is consistent with the known action of pancreatic DNase I which produces single-strand cleavages in double-stranded DNA. Thus, the form II and form III DNAs observed under nondenaturing conditions after DNase I action contain multiple single-strand cleavages, and denaturation of these products yielded a range of single-strand fragments whose average size became smaller with increasing time of DNase I digestion (Fig. 7). In contrast, when HSV endonuclease gave a similar pattern to DNase I under native conditions (Fig. 6, Mn^{2+}, native, 0.2 min, cf. Fig. 7, native, 1-min reaction), unit length DNA was predominantly observed after denaturation. This indicated that form II and form III DNA produced by HSV endonuclease in Mn^{2+} contain relatively few internal nicks which suggested that HSV endonuclease preferentially cleaves single-stranded DNA.

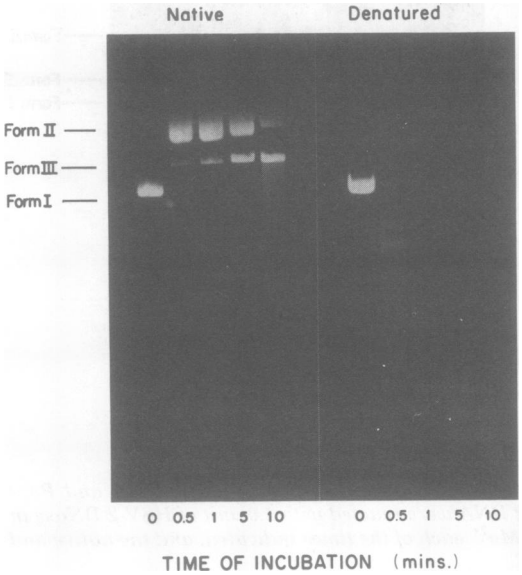

FIG. 6. *Agarose gel electrophoresis of the products derived from the digestion of PM2 DNA with HSV-2 DNase in the presence of Mg^{2+} or Mn^{2+}. PM2 DNA was treated in the standard assay mixture with 0.5 unit of HSV-2 DNase but in the presence of 5 mM Mg^{2+} or 1.0 unit of HSV-2 DNase in the presence of 5 mM Mn^{2+} for the indicated times. At each of the times indicated, native and denatured products were electrophoresed on agarose gel.*

To test this hypothesis, we examined the ability of HSV endonuclease to cleave a covalently closed circular DNA which lacked single-stranded regions. Such a substrate was prepared (see Materials and Methods) by treating form I PM2 DNA with nicking-closing enzyme to yield nonsuperhelical covalently closed circular DNA (form I'). This form of DNA was a poor substrate for HSV endonuclease and was cleaved about 60 times more slowly than form I DNA (Fig. 8) in the presence of either Mg^{2+} or Mn^{2+}. Control experiments showed that the slow rate of cleavage by HSV endonuclease of form I' DNA was not due to inhibition by other proteins present in the enzyme extract containing the nicking-closing enzyme. In contrast, DNase I, which can

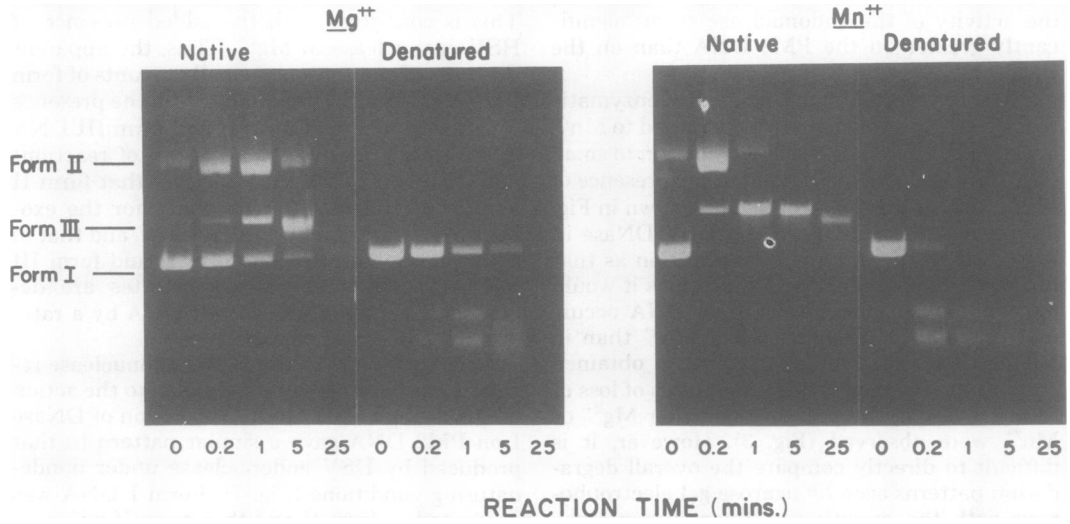

FIG. 7. *Agarose gel electrophoresis of the products derived from digestion of PM2 DNA with DNase I. PM2 DNA was reacted with 0.5 ng of DNase I for each of the times indicated in the assay mixture described in the text. At each of the times indicated, native and denatured products were electrophoresed on agarose gel.*

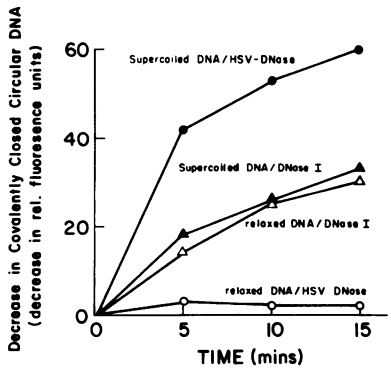

FIG. 8. *Action of HSV DNase and DNase I on form I and form I' DNA. Circular supercoiled PM2 DNA (closed symbols) and circular nonsuperhelical PM2 DNA (open symbols) were treated with 0.2 unit of HSV-2 DNase (●, ○) or 0.07 ng of DNase I (▲, △) in the standard assay mixtures for the times indicated. Cleavage of circular DNA was determined using the fluorescence assay.*

make endonucleolytic single-strand cleavages within a double-stranded DNA molecule, showed the same rate of activity on form I and form I' DNA (Fig. 8).

It has been shown that supercoiled, form I DNA contains regions of non-base-paired, single-stranded DNA (1, 5, 17), with the greatest amount of single-strandedness occurring at higher superhelical densities (36). As superhelical DNA is relaxed, single-stranded regions progressively disappear (36). Thus, conversion of form I DNA to form II DNA by HSV endonuclease probably occurs predominantly by cleavage in a single-stranded region. Conversely, slow cleavage of form I' DNA by HSV endonuclease would appear to result from the lack of single-stranded regions. Faster cleavage of form I PM2 DNA compared to form I pMB9 DNA (Fig. 5) may also be related to superhelical density since native PM2 DNA which has the highest naturally occurring superhelical density known (36) would have a greater degree of single strandedness compared to form I pMB9 DNA. Endonucleolytic degradation of form III DNA by HSV endonuclease must therefore occur primarily from the ends of the molecules where the DNA transiently acquires a single-stranded nature. A significant number of internal cleavages, nevertheless, do occur, since the fluorescence intensity of form III DNA and the denatured products decreases with time of digestion (Fig. 5 and 6). If degradation occurred exclusively from the ends, the fluorescence intensity of the bands should remain almost constant while the molecular weight of the fragment progressively decreases.

The HSV-1 and HSV-2 DNases were virtually identical in all properties examined, but the HSV-2 DNase had 2.48 times as much endonuclease activity per unit of exonuclease as the HSV-1 DNase.

DISCUSSION

The data presented in this report indicate that the protein specified by both HSV-1 and HSV-2 which catalyzes exonucleolytic cleavage also possesses an associated endonuclease activity. It would seem unlikely that the endonuclease activity which copurifies and also cosediments with the exonuclease is of cellular origin. In this regard it is particularly relevant that both activities showed the same slight decrease in apparent molecular weight in the presence of 0.5 M KCl and that the distribution of the activities were coincident (Fig. 1).

A careful comparison of the properties of the HSV-specified exo-endo-nuclease with the properties of known mammalian nucleolytic enzymes (31) indicates that the viral enzymes are unique. Recently described endonucleases from KB cells (32, 34) are clearly different from the HSV enzymes. To date no DNase of mammalian origin has been attributed to have both exo- and endonuclease activities, although this may simply be due to the fact that endo- or exonucleases have not been carefully examined for the counterpart activity. For example, snake venom phosphodiesterase, often referred to as an exonuclease and in use for almost 20 years, has recently been found to possess an intrinsic endonuclease activity specific for single-stranded DNA (25).

The endonuclease activity of the HSV-1 and HSV-2 DNase appeared to be very similar in properties and mode of action. No difference was found between the two enzymes with the exception that the HSV-2 DNase had more than two times as much endonuclease activity per unit of exonuclease as the HSV-1 DNase. However, some differences between the exonuclease and endonuclease activity of each polypeptide were observed. The endo- and exonuclease activities responded differently to Mn^{2+}. Endonuclease activity was observed at all concentrations of Mn^{2+}, and Mn^{2+} was equally effective as a cofactor as Mg^{2+} for endonuclease activity (Fig. 3), whereas little if any exonuclease activity occurred at concentrations of Mn^{2+} greater than 1 mM (9). The optimum concentration of Mg^{2+} for endonuclease activity was 2 mM (Fig. 3), whereas it was 5 mM for exonuclease activity (9). Also HSV endonuclease was inhibited by a 10-fold-lower concentration of spermine compared to the exonuclease (Fig. 4, reference 9).

The definitions of exonuclease and endonuclease have changed during the years due to the discovery of new nucleases with unique and specific substrate requirements. It is now agreed that an exonuclease requires a free DNA end for its action and will not cleave circular DNA, whereas only those enzymes which cleave circular DNA are classified as endonucleases (18, 19). The HSV DNases clearly have the ability to cleave circular DNA, and we have recognized the endonuclease activity on this basis. Although the data suggested that the endonucleases recognized single-strand regions in supercoiled DNA, this does not imply that the nick was necessarily confined to that region. It is equally possible that only binding requires a single-strand region and hydrolysis occurs at a distant site. With a supercoiled molecule this is indeed possible because the high degree of folding may bring together, in close physical proximity, regions of the molecule which are distinct from each other in primary sequence. However, it should be remembered that it was only possible to study the endonuclease activity alone in the presence of Mn^{2+}, and therefore it is not unlikely that the HSV endonucleases have greater specificity in the presence of Mg^{2+}. In this regard it should be pointed out that several enzymes, including restriction endonucleases and DNase I, lose specificity in the presence of Mn^{2+} compared to their action in the presence of Mg^{2+} (10, 21, 24).

The presence of the endonuclease in the HSV-infected cell raises some interesting questions with regard to its possible involvement in viral DNA replication. It was recently observed that, during herpesvirus DNA replication, some viral DNA exists in sizes greater than mature length and in a variety of forms (2, 12, 13), and it is possible that the HSV endonuclease is involved in the processing of these molecules. It has been shown, for example, that the single-strand-specific S1 nuclease converts supercoiled polyoma DNA to unit length rods (8), and it has been implied that an endonuclease in nuclei of 3T3 cells carries out a similar function (20). If HSV DNA replicates via a rolling circle mechanism (11), the viral DNA should be protected from the action of the HSV exonuclease, although replication forks and other single-stranded regions in duplex DNA may be particularly susceptible to HSV endonucleases.

ACKNOWLEDGMENTS

We thank David Kowalski for his advice on the use of the fluorescent endonuclease assay and for many stimulating discussions. We also thank David Kowalski and Terry Beerman for their critical review of this manuscript.

This work was supported by Research Grant No. CH-29B from the American Cancer Society.

Y.-C. C. is a Scholar of the Leukemia Society of America.

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