Association of Type I DNA Topoisomerase with Herpes Simplex Virus

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SUMMARY

A topoisomerase activity is associated with herpes simplex virus type 1. The enzyme was recovered from purified virions which were disrupted with 6 M-guanidine-HCl followed by renaturation of extracted proteins. Based upon the following observations, the virion activity is classified as a type I topoisomerase: (i) the linking number of a unique DNA topoisomer is altered in steps of one; (ii) ATP and MgCl2 are not required for activity; (iii) the enzyme can be trapped in a covalent complex with DNA; (iv) the covalent linkage to DNA is through a 3' phosphoryl bond. A number of lines of evidence strongly indicate that the topoisomerase is external to the nucleocapsid. For example, the activity was released by treatment of intact virions with NP40, and subsequent washing steps extracted most residual activity. When guanidine extracts were prepared from nucleocapsids, topoisomerase activity was not detectable. Finally, DNA within the virion did not appear to contain covalently attached proteins with properties similar to topoisomerases. Thus, the enzyme appears to be a component of the envelope or tegument structure of the virion.

INTRODUCTION

DNA topoisomerases are enzymes that transiently break the phosphodiester backbone of DNA and then reseal the free DNA ends (for reviews, see Champoux, 1978; Gellert, 1981; Liu, 1983). The cycle of breakage and rejoicing of DNA allows for strand passage and alterations in DNA topology. The biological functions of these enzymes are probably diverse and have been implicated in central genetic events such as transcription (Akrigg & Cook, 1980; Sternglanz et al., 1981; Weisbrod, 1982), recombination (Kikuchi & Nash, 1979), transposition (Sternglanz et al., 1981), DNA replication (Liu et al., 1980) and segregation of daughter DNA molecules after S phase (DiNardo et al., 1984). In both prokaryotes and eukaryotes these enzymes appear ubiquitous and have been isolated from a variety of sources (Gellert, 1981; Liu, 1983). Topoisomerases have also been identified in vaccinia virus cores (Bauer et al., 1977) and simian virus 40 (SV40) minichromosomes (Tsubota et al., 1978).

To date, all topoisomerases can be divided into two categories. The type I enzymes catalyse the breaking and rejoicing of only one strand of DNA at a time, while type II enzymes appear to introduce transient double-strand breaks (Gellert, 1981; Liu, 1983). The two classes will relax supercoiled DNA although the reaction mechanisms are clearly different (Brown & Cozzarelli, 1981), and each can be distinguished by reaction cofactor requirements. Notably, type II enzymes require ATP and MgCl2, whereas type I topoisomerases do not. In addition, type II topoisomerases will decatenate interlocking DNA rings (Marini et al., 1980). Topoisomerases are characterized by their ability to form stable (probably covalent) bonds with DNA during the reaction sequence (Prell & Vosberg, 1980; Sander & Hsieh, 1983; Trask et al., 1984). Indeed, this feature led to the development of an assay which detects the covalent DNA/topoisomerase intermediate (Trask et al., 1984).

We have investigated the possibility that topoisomerases play a role in the replication of herpes simplex virus (HSV). Recently, a topoisomerase was reported to co-purify with the HSV-induced DNA polymerase and evidence was provided that this topoisomerase may be unique to
infected cells (Biswal et al., 1983). Leary & Francke (1984) also reported that HSV induced a topoisomerase activity as assayed by catenation of DNA. In this work, we present evidence that a type I topoisomerase activity is associated with purified virions and appears to be localized outside the nucleocapsid.

METHODS

Cells and viruses. Primary rabbit kidney (RK) cells were prepared from 7- to 10-day-old rabbits by standard methods. These cells were passed a maximum of three times prior to use. Vero cells were cultivated in Dulbecco’s MEM (Flow Laboratories) supplemented with 10% foetal bovine serum (Gibco), 100 µg streptomycin/ml, 100 units penicillin/ml and 0.075% NaHCO₃ for cultures in closed vessels or 0.225% NaHCO₃ for cultures in open vessels. RK cells were similarly grown in MEM containing 5% newborn calf serum and 2.5% foetal bovine serum.

Stocks of HSV-1, strain KOS (Smith, 1964) were maintained by low multiplicity passage as previously described (Parris et al., 1978). Virions were prepared from the extracellular medium and cytosol fraction of RK cells and were further fractionated on linear 20 to 60% (w/w) sucrose gradients exactly as described previously (Parris et al., 1980). The virus band was collected and pelleted (35000 g for 15 min). In some cases, virions were extracted with 1% (v/v) NP40 (on ice for 15 min) followed by centrifugation (35000 g for 60 min) to pellet nucleocapsids.

Guanidine-HCl extraction of virions and nucleocapsids. Extraction of proteins from intact virions or nucleocapsids can be effectively accomplished by gentle denaturation. Ionic detergents irreversibly denature many proteins and were avoided. We modified the polypeptide renaturation protocol described by Hager & Burgess (1980). Briefly, pelleted virions or nucleocapsids were resuspended directly in 6 M-guanidine-HCl, 200 µg bovine serum albumin (BSA)/ml. The extraction was allowed to proceed for 30 min on ice, followed by dialysis for 18 to 24 h against 10 mM-Tris HCl pH 7.6, 100 mM-NaCl, 1 mM-EDTA (TNE). When large amounts of virions were treated in this way, a precipitate was sometimes observed after dialysis; however, in terms of topoisomerase I activity, removing the precipitate did not affect enzyme recovery. Topoisomerase activity was quite stable to freezing and thawing cycles.

Preparation of DNA substrates. Thymidine ([¹H]TdR)-labelled and non-labelled SV40 supercoiled DNA (form I) were prepared as described previously (Trask et al., 1984). HSV DNA was prepared from purified virions as described by Parris et al. (1980) and nick-translation by standard methods. Phage lambda DNA was obtained from commercial sources. This DNA was 5' end labelled as described previously (Trask & Muller, 1983) after digestion with HaeIII. To label 3' ends, DNA was digested with HpaII followed by end repair with T4 DNA polymerase. End-labelled DNAs were phenol-extracted and twice ethanol-precipitated. Phage lambda DNA was also labelled using DNA polymerase I to repair exonuclease III-digested DNA (James & Leffak, 1984). Supercoiled pBR322 was prepared by standard methods (Trask et al., 1984). A single topoisomer of unique linking number was prepared by incubating 200 µg supercoiled DNA with purified avian topoisomerase I followed by separation of various topoisomers by agarose gel electrophoresis. To resolve the ladder of topoisomerase bands, 25 cm gels were run at 2 V/cm at room temperature for 24 h in Tris-acetate buffer (Trask et al., 1984). An adjacent lane was removed, stained with ethidium bromide and used as reference to cut out a single topoisomer from the unstained portion of the gel. The DNA was electroeluted, precipitated directly with ethanol and washed extensively with 70% ethanol. This DNA served as a substrate for topoisomerases. The topoisomerase was efficiently relaxed by avian topoisomerase I and the HSV-associated enzyme.

Detection of topoisomerase activity. Topoisomerase I was assayed by relaxation of supercoiled SV40 DNA. The 30 µl reactions contained 0.25 µg SV40 DNA in 10 mM-Tris- HCl pH 7.5, 1 mM-EDTA, 20 µg BSA/ml (buffer A) and either 100 or 200 mM-NaCl. Optimal activity was observed in 100 mM-NaCl with purified avian topoisomerase I (Trask & Muller, 1983) and in 200 mM-NaCl with the HSV-associated topoisomerase I. Reactions were incubated for 30 to 60 min at 30 °C, terminated with 0.5% (v/v) SDS and digested with proteinase K (100 µg/ml) for 15 min at 56 °C followed by addition of bromphenol blue/glycerol (0-005% and 4%, respectively). The products were analysed on 1% agarose gels containing 0.5 µg ethidium bromide/ml as described previously (Trask et al., 1984) to separate nicked and circular DNA forms. To resolve DNA topoisomers, ethidium bromide was not included in gels or electrophoresis buffer. Assays designed to measure covalent DNA/topoisomerase intermediates (SDS-K⁺ assays) were carried out exactly as described by Trask et al. (1984) according to the 'analytical method'.

RESULTS

Association of topoisomerase with the virion

Proteins which stably or covalently bind DNA can be detected by a procedure we refer to as the SDS-K⁺ method (Trask et al., 1984). Stable DNA-binding proteins are operationally defined by this assay as proteins which are not dissociated from the DNA by treatment with the ionic detergent SDS. SDS binds proteins but not nucleic acids; therefore, upon addition of KCl a precipitate is formed which traps free protein, as well as those DNA molecules stably attached
HSV topoisomerase activity was assayed by measuring conversion of supercoiled to relaxed DNA. The DNA forms were separated by electrophoresis in a 1% agarose gel containing 0.5 µg ethidium bromide/ml. Lane 1, supercoiled DNA marker (form I); lane 2, relaxed DNA marker (form II); lanes 3 to 6, increasing amounts of virion extract (2, 5, 10 and 17 µl, respectively). Lane 7 shows a mixing experiment of 10 µl virion extract and 1 unit purified avian topoisomerase I. The positions of open circular (O.C.) and HSV-1 (HSV) DNA are indicated. (b) SDS–K⁺ reactions. Reactions (final vol. 0.1 ml in buffer A) containing 40000 d.p.m. of nick-translated HSV-1-³²P-labelled DNA and the indicated volume of extract in buffer A were incubated at 30 °C for 60 min. SDS–K⁺-precipitable DNA was then determined. Background precipitation was determined by proteinase K digestion (after SDS addition) prior to adding KCl. O, SDS–K⁺-precipitable ³²P-labelled DNA; Δ, SDS–K⁺-precipitable ³H-labelled DNA; ●, SDS–K⁺-precipitable ³²P-labelled DNA after proteinase K treatment; ▲, SDS–K⁺-precipitable ³H-labelled DNA after proteinase K treatment.

Intact virions, labelled with [³H]TdR for 48 h prior to harvesting, were purified and treated with 6 M-guanidine–HCl to disperse and release nucleoproteins. The extracts were then dialysed against a Tris/NaCl buffer to remove the guanidine and allow the polypeptides to renature. Reconstruction experiments with infected cell extracts (containing topoisomerase I) or purified topoisomerase I clearly demonstrated that the enzyme activity could be recovered using this approach. Fig. 1(a) shows that topoisomerase activity was recovered based upon the conversion of supercoiled DNA (form I) to relaxed DNA (form II). The amount of activity was relatively low in that complete conversion to relaxed DNA was not observed; however, this does not appear to be due to a diffusible inhibitor since the virion extract did not inhibit purified cellular topoisomerase I (Fig. 1a, lane 7). Stable DNA-binding proteins are present in these extracts as shown by the SDS–K⁺ titration in Fig. 1(b). The protein(s) stably bound both exogenously added HSV DNA and endogenous virion DNA. This suggests that a topoisomerase is released from the virion by guanidine extraction and that the enzyme is free to diffuse and react in trans with non-virion associated DNA as well as virion DNA from the disrupted particles. Additional data (below) support the claim that most of the enzyme is not stably bound to virion DNA prior to extraction with guanidine–HCl.
Characterization of the virion-associated topoisomerase

As shown in Fig. 2 (lanes 3, 4), the virion-associated enzyme required 0·2 m-NaCl for maximum activity. Dithiothreitol was strongly inhibitory (lane 5) and 10 mM-MgCl₂ (in the presence of 0·1 m-NaCl, lane 6) did not influence the reaction. In the absence of NaCl, addition of MgCl₂ resulted in extensive nicking of DNA (producing open circular forms, lane 8) indicating the presence of a nuclease which was inhibited by NaCl. The addition of MnCl₂ caused nicking of the substrate DNA in the presence and absence of 0·1 m-NaCl (lanes 7, 9). The fact that divalent cations were not required for activity (compare lanes 3 and 6, Fig. 2) suggests that a type I topoisomerase is present.

Topoisomerases act by altering DNA topology. These enzymes cause alterations in a parameter called linking number (for review, see Crick, 1976), which is the number of times one strand of DNA is linked to the other. The linking number can only be changed by breaking one or both strands of the helix. Type I topoisomerases alter the linking number of circular DNA in steps of one while type II enzymes do so in steps of two (for review, see Gellert, 1981; Liu, 1983); thus, the change in linking number of an isolated topoisomer is a diagnostic test for the presence of type I enzymes. A topoisomer with a single linking number was isolated by preparative gel
HSV topoisomerase

Fig. 4. Release of topoisomerase from virions by NP40 treatment. Virions were purified as described. Approximately 2 × 10⁸ p.f.u./ml were recovered from the sucrose gradient. Various amounts were assayed for topoisomerase I in buffer A containing 0.2 M-NaCl with or without 1% (v/v) NP40. The reaction products were analysed as described in the legend to Fig. 2. Lane 1, supercoiled DNA marker; lane 2, supercoiled DNA incubated with purified topoisomerase I; lane 3, as in lane 2 but with 1% NP40 in the reaction; lanes 4 to 8, assays with intact virions without NP40 (1, 2, 4, 8 and 16 μl, respectively); lanes 9 to 13, as in lanes 4 to 8 but with 1% NP40 in the reaction.

Table 1. Virion-associated topoisomerase forms a stable bond at 3' sites

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>DNA substrate*</th>
<th>SDS-K⁺ precipitate (d.p.m.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>3' end label</td>
<td>563</td>
</tr>
<tr>
<td>None</td>
<td>5' end label</td>
<td>695</td>
</tr>
<tr>
<td>Topoisomerase I</td>
<td>3' end label</td>
<td>628</td>
</tr>
<tr>
<td>Topoisomerase I</td>
<td>5' end label</td>
<td>3227</td>
</tr>
<tr>
<td>Virion topoisomerase</td>
<td>3' end label</td>
<td>508</td>
</tr>
<tr>
<td>Virion topoisomerase</td>
<td>5' end label</td>
<td>4100</td>
</tr>
</tbody>
</table>

* DNA substrates were labelled at the 3' or 5' end as described. Reactions were carried out in buffer A and contained either purified avian topoisomerase I or virion-associated topoisomerase (see legend to Fig. 1) in a final vol. of 25 μl. The DNA substrates (2.4 × 10⁴ d.p.m./reaction) were heat-denatured just prior to use. Reactions were incubated for 30 min at 30 °C and SDS-K⁺-precipitable DNA measured.

The virion-associated topoisomerase I stability binds to 3' ends

Eukaryotic topoisomerase I will spontaneously break down single-stranded DNA (Champoux, 1976; Been & Champoux, 1981; Edwards et al., 1980; Trask & Muller, 1983). It has been proposed that the resealing step cannot efficiently occur with denatured DNA since nicking is followed by free diffusion of contiguous DNA ends (Liu, 1983). Moreover, the eukaryotic enzyme is bound to the 3' phosphoryl end at the site of the nick in single-stranded DNA (Prell & Vosberg, 1980; Halligan et al., 1982; Trask & Muller, 1983). The virion topoisomerase also displays this characteristic. We prepared 3' or 5' end-labelled DNA fragments and compared the binding of the virion topoisomerase to each of these substrates using the SDS-K⁺ method to detect stable nucleoprotein complexes. Single-stranded DNA was used in these experiments (Table 1). For comparison, background levels of SDS-K⁺-precipitable DNA were determined from reactions lacking enzyme (500 to 600 d.p.m.). A control reaction with purified avian
Table 2. Distribution of topoisomerase I activity after extraction with NP40

| Extraction step* | C.p.m. in I r DNA† | Activity remaining (%)
|------------------|--------------------|------------------------
| Form I DNA (background) | 340                | –                      |
| Positive control (purified topoisomerase I) | 2096               | –                      |
| Guanidine extract of intact virions | 2075               | 100                    |
| 1st NP40 extract | 1129               | 54                     |
| 2nd NP40 extract | 865                | 42                     |
| 3rd NP40 extract | 381                | 18                     |
| Guanidine extract after 3rd NP40 treatment | 400                | 19                     |

* Virions were purified from Vero cells as described in Methods. The starting preparation contained approx. 3 × 10⁸ p.f.u. Intact virions were divided into two aliquots. One was pelleted and extracted directly with guanidine-HCl and the level of topoisomerase I activity in this material was taken as 100%. A second aliquot was subjected to NP40 (1%) treatments in TNE buffer followed by centrifugation to pellet the nucleocapsids. Assays for topoisomerase I activity were performed on each pellet fraction after resuspension in NP40, TNE buffer. After the third wash with NP40, the nucleocapsids were pelleted and resuspended directly in 6 M-guanidine-HCl and dialysed to renature any remaining enzyme. In all extractions and washing steps, volumes were kept constant and 2 µl of each fraction assayed.

† Topoisomerase I activity was measured by relaxation of SV40 ³H-labelled DNA and separation of supercoiled and relaxed forms by ethidium bromide/agarose gels. The relaxed DNA band (I r) was sliced from the gel, dissolved in 0.5 ml H₂O₂ (68 °C for 6 h) and radioactivity determined by liquid scintillation spectrometry. All data are corrected for background which is the amount of relaxed DNA present in supercoiled DNA substrate.

topoisomerase I demonstrated that only 5' labelled fragments were precipitated in agreement with previous data showing a 3' phosphoryl linkage of the avian enzyme (Trask & Muller, 1983). Essentially identical results were obtained with the virion topoisomerase. These results illustrate the specificity of the SDS-K⁺ assay in detecting topoisomerase I in the guanidine-HCl extract and show that the virion-associated enzyme behaves like a typical eukaryotic type I topoisomerase in this respect.

Release of the virion topoisomerase by NP40

We determined if the virion topoisomerase was associated with the envelope/tegument structure by assaying intact virions for relaxation activity in the presence and absence of the non-ionic detergent NP40. Control experiments with purified avian topoisomerase I demonstrated that NP40 at 1% (v/v) did not inhibit the enzymic activity (compare lanes 2 and 3 in Fig. 4). Additional experiments (not shown) with purified topoisomerase I also demonstrated that NP40 did not activate the enzyme. In this particular preparation, purified, intact virions displayed no detectable topoisomerase activity over a wide range of concentrations (lanes 4 to 8). In some cases, low levels of activity were detected in intact virions. We have noticed that freshly prepared, intact virions frequently have topoisomerase activity in the absence of the detergent; however, this activity was removed by pelleting the virions and resuspending in fresh buffer. For unknown reasons, the activity associated with intact virions was quite variable from one preparation to the next. The amount of topoisomerase activity was clearly and consistently increased if 1% NP40 was included in the reaction mixtures. This is illustrated in lanes 9 to 13 of Fig. 4. Upon addition of NP40, relaxation activity was generally increased by 10- to 30-fold. These data indicate that a substantial amount of enzyme is released by agents which disrupt the envelope and release tegument proteins (Roizman & Furlong, 1974; Spear & Roizman, 1972). As shown above (Fig. 3), the activity present in the NP40 extract is a type I topoisomerase and very likely the same activity we identified in the guanidine extract. Based upon a variety of biochemical tests (such as reaction requirements for SDS-K⁺ and relaxation assays), the guanidine-extracted activity and the NP40 activity are identical (data not shown).

To investigate further the association of the enzyme with the envelope/tegument fraction, intact virions were subjected to a series of NP40 extractions and washing steps (Table 2). In
order to quantify the activity, labelled SV40 DNA (form I) was used as substrate and the formation of I' DNA was determined by cutting out bands from the gel followed by liquid scintillation counting. The recovery of topoisomerase activity after each NP40 extraction was compared to the level of enzyme activity (as 100%) in guanidine extracts of unfractionated virions. The first NP40 extraction removed about half of the topoisomerase I activity, and after two more washes, approximately 80% of the activity was extracted. The final pellet of nucleocapsids was then extracted with 6 M-guanidine-HCl, dialysed and assayed. The small amount of activity (20%) which was not extracted by NP40 was quantitatively recovered after guanidine extraction. This experiment was repeated six times using different virion preparations which had been prepared from either Vero or RK cells. In all cases, NP40 effectively extracted most (>80%) of the topoisomerase activity. The 100% value (2075 c.p.m. in I' DNA) reported in Table 2 is based upon a reaction in which all of the substrate DNA was relaxed; therefore, we have underestimated the amount of activity in the guanidine extract of intact virions. As a result, the recovery of activity in the various washes is probably less than that reported in Table 2. Collectively, these results strongly suggest that the enzyme is not inside the nucleocapsid, but more likely is a constituent of either the envelope or tegument.

From the data in Table 2 we cannot rule out the possibility that topoisomerase is stably bound to the genome within the particle. In particular, we considered that guanidine-HCl might act like SDS and denature topoisomerase bound to DNA, thereby producing a covalent complex. To determine if stable binding proteins exist in association with viral DNA in nucleocapsids, SDS-K+ assays were carried out on a preparation of [3H]TdR-labelled virions. The purified virions were either acid-precipitated for total radioactivity or SDS-K+-precipitated (allowing rapid denaturation of protein) to determine the fraction of virion DNA stably bound to protein. As shown in Fig. 5, less than 10% of the total DNA was precipitated by SDS-K+ treatment. Since background levels of SDS-K+ precipitates (determined by proteinase K treatment) were not significantly different from untreated virions, it appears that little if any virion DNA exists as a stable DNA/protein ensemble which can be detected by the SDS-K+ method.

DISCUSSION

In this report, we have identified and characterized a topoisomerase associated with HSV-1. When virions were disrupted with guanidine-HCl followed by renaturation of extracted proteins, a type I topoisomerase was demonstrated based upon the following observations: (i) it does not require MgCl₂ or ATP; (ii) it forms an SDS-resistant bond (probably covalent) with double- and single-stranded DNA; (iii) the protein is bound to single-stranded DNA through a 3' phosphoryl link as reported with other eukaryotic type I enzymes (Halligan et al., 1982; Prell & Vosberg, 1980; Trask & Muller, 1983); (iv) the enzyme alters DNA linking number of a unique topoisomer in steps of one.
It is possible that topoisomerase activity could be mimicked by a deoxyribonuclease and DNA ligase acting in combination; however, the guanidine–HCl extracts failed to convert open circular DNA to covalently closed circular forms. For example, in Fig. 3, the topoisomer was contaminated with a small amount of open circular DNA. After incubation with the guanidine extract, the intensity of this nicked species remained the same or increased slightly (compare lane 4 with 5 and 6) showing that a contaminating nicking activity might convert circular DNA to nicked forms (see also Fig. 2); however, there does not appear to be an activity which reverses this cycle. Moreover, the conditions under which we detect the topoisomerase I activity are not conducive to ligase activity.

A type I topoisomerase is also released from intact virions by NP40 treatment and can be effectively washed away from nucleocapsids. This observation suggests a location external to the nucleocapsid, perhaps as a tegument or envelope component. Although we did not show the data, the purified virions appeared relatively free of contaminating cellular debris based upon electron microscopy. In addition, virions purified on dextran T-10 gradients contain topoisomerase I activity. The data do not rigorously exclude the possibility that some enzyme is intimately bound to DNA within the particle; however, this seems unlikely since labelled DNA in virions was not precipitated with SDS-K+ (Fig. 5).

It is conceivable that the virion-associated topoisomerase is simply a cellular contaminant adventitiously trapped in the envelope. During maturation and egress, herpesvirus acquires its envelope from the nuclear membrane (Roizman & Furlong, 1974); thus, if cellular topoisomerase I is enriched at this site, adventitious capture is possible. On the other hand, we have evidence that the nuclear location of the cellular topoisomerase I is clearly unrelated to the membrane or the nuclear matrix (M. T. Muller, unpublished results). Specific antibodies against host and putative viral activities will be required to resolve this issue directly.

The identification of a topoisomerase activity in the virion raises interesting questions worthy of some speculation. The observation might imply a role for the enzyme in immediate-early transcription. It is intriguing, for example, that a trans-acting regulatory element in virions has been reported to stimulate transcription from immediate-early promoters (Post et al., 1981; Mackem & Roizman, 1982; Cordingley et al., 1983; Preston et al., 1984). This element apparently is a structural component of the virion and is localized outside the nucleocapsid (Batterson & Roizman, 1983); furthermore, a major tegument species has been tentatively identified as the trans-active function (Campbell et al., 1984). A DNA target sequence has been identified which is evidently required for trans activation by the structural element (Preston et al., 1984); therefore, a DNA-binding protein is likely to be involved. A topoisomerase is a reasonable candidate as a component in trans activation; however, the HSV topoisomerase I does not appear to be sequence-specific (at least in vitro). While sequence-specific binding of topoisomerase to HSV immediate-early regulatory sites in vivo cannot be excluded, it is quite possible that the enzyme acts to facilitate the initiation of the viral replication cycle by modulating DNA topology in such a way as to influence the binding of RNA polymerase to viral sequences. In any event, a fundamental requirement for topoisomerase activity is indicated because the HSV-1 genome is thought to circularize after infection (Jacob et al., 1979; Davison & Wilkie, 1983). Circularization apparently occurs immediately after infection (Poffenberger & Roizman, 1985) which implies that a need for topoisomerase activity very early in the infectious cycle could be met by introducing the enzyme as a virion component.

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