The Interaction of a Topoisomerase-like Enzyme from Herpes Simplex Virus Type 1-infected Cells with Non-viral Circular DNA

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SUMMARY
An enzyme activity from herpes simplex virus type 1 (HSV-1)-infected baby hamster kidney cells has been identified which generates large networks of pBR322 DNA from a monomeric DNA substrate. Extracts derived from cells infected at the non-permissive temperature, with the early regulatory mutants of HSV-1, tsK and tsB2, did not contain activity, suggesting that the enzyme is virus-induced and may be virus-specific. The enzyme is similar to the DNA topoisomerases in that network formation was dependent upon the presence of Mg²⁺ and a DNA condensing agent, and ATP was not required. Following digestion with EcoRI, the networks could be resolved to a single, linear, monomeric species of pBR322 DNA.

INTRODUCTION
The winding of DNA around the histone proteins provides the first order of coiling in the chromatin of eukaryotes and some DNA viruses (Kornberg, 1977; Varshavsky et al., 1976). While some prokaryotic DNA is associated with histone-like proteins, supercoils in DNA are also maintained through the action of a class of enzymes known as DNA topoisomerases (Pettijohn & Pfenninger, 1980; for review, see Cozzarelli, 1980). Some of these, e.g. Escherichia coli DNA gyrase, are sensitive to the drug novobiocin (Higgins et al., 1978) while others, e.g. T4 topoisomerase type II, are relatively insensitive (Liu et al., 1979, 1980; Stetler et al., 1979).

Novobiocin will inhibit herpes simplex virus type 1 (HSV-1) DNA replication (Francke & Margolin, 1981) but the concentration required is higher than that needed to inhibit DNA gyrase. In this sense, HSV-1 DNA replication resembles the T4 system and this fact, together with the absence of a chromatin structure or associated histones in HSV DNA (Francke, 1977b; Leinbach & Summers, 1980; Sinden et al., 1982), prompted us to investigate the possibility that a topoisomerase-like enzyme may be involved in the replication of HSV-1 DNA.

In this report, we describe an enzymic activity found only in HSV-1-infected cells, which is capable of catenating closed circular DNA molecules. The ability to catenate two or more duplex DNA circles is a ubiquitous reaction common to all known topoisomerases (Cozzarelli, 1980) and our results therefore suggest that such an enzyme may play a role in the replication of HSV.

METHODS
Cells and viruses. BHK-21 clone 13 cells (MacPherson & Stoker, 1962) were grown in 10% calf serum (Colorado Serum Co.), 10% tryptose phosphate broth (Difco) and Dulbecco's modified Eagle's medium and transferred 1:10 every 3 to 4 days as described previously (Francke, 1977a).

Viruses used were: HSV-1 Glasgow strain 17 [HSV-1(17)] ts⁺ sym⁺ and temperature-sensitive DNA⁻ mutants of the same strain, tsB, tsD and tsK; HSV-1 strain KOS [HSV-1(KOS)] ts⁺ and the derived temperature-sensitive DNA⁻ mutants tsA1, tsA15, tsA16, tsA24, tsA42, tsB2, tsC4 and tsD9. Their isolation and characterization have
been described elsewhere (Brown et al., 1973; Dixon & Schaffer, 1980; Marsden et al., 1976; Preston, 1979; Schaffer et al., 1973, 1978; Weller et al., 1983).

Virus stocks were propagated in BHK cells and titrated by plaque assay as described by Francke (1977a, b).

**P**lasmid **D**NA. The plasmid pBR322 (Bolivar et al., 1977) was isolated from *E. coli* HB101 cells (Boy er & Roulland-Dussoix, 1969) according to the method of Humphreys et al. (1975) with minor modifications. The cells were gently lysed by the sequential addition of lysozyme, EDTA and Triton X-100. Following high-speed centrifugation, covalently closed circular pBR322 DNA was purified by two rounds of CsCl-ethidium bromide density gradient centrifugation. The ethidium bromide was removed by extraction with isooctyl alcohol and the DNA was dialysed into 10 mM-Tris-HCl pH 7-5, 1 mM-EDTA, and stored in frozen aliquots at −20 °C.

**Topoisomerase preparation.** BHK cell topoisomerase II was prepared by the method of Miller et al. (1981) with minor modifications. All procedures were carried out at 4 °C unless otherwise indicated. Briefly, 4 × 10⁶ cells were lysed by Dounce homogenization and the nuclei were collected and washed by centrifugation. Pelleted nuclei were resuspended in 5 mM-potassium phosphate pH 7-5, 2 mM-MgCl₂, 1 mM-phenylmethylsulphonyl fluoride (PMSF; prepared as a 100 mM solution in isopropyl alcohol), 1 mM-2-mercaptoethanol, 0-5 mM-dithiothreitol (DTT) and 10 mM-EDTA and lysed by the slow addition of an equal volume of 100 mM-Tris–HCl pH 7-5, 2 mM-NaCl and 1 mM-PMSF. An identical volume of 18% (w/v) polyethylene glycol (PEG) in 1 M-NaCl was slowly added with stirring to give final concentrations of 6% PEG and 0-33 M-NaCl. The solution was centrifuged at 12000 g for 30 min and the supernatant was dialysed against two changes of 30 mM-potassium phosphate pH 7-0, 10 mM-2-mercaptoethanol, 1 mM-EDTA and 50% glycerol and stored at −20 °C.

**Topoisomerase assay.** The standard catenation reaction was a modification of that used by Hsieh & Brutlag (1980) as follows: a 40 μl reaction mixture contained final concentrations of 50 mM-Tris–HCl pH 7-75, 20 mM-KCl, 10 mM-MgCl₂, 0-5 mM-EDTA, 0-6 μg bovine serum albumin, 1 mM-ATP, 0-5 μg pBR322 form I DNA, 0-1 μg histone HI (optimized by prior titration) and 5 μl enzyme. The reaction was incubated at 37 °C for 10 to 60 min and stopped by the addition of SDS (final concn. 0-1%) and EDTA (final concn. 10 mM). Proteinase K (10 μg) was added and the samples were incubated for a further 30 min at 37 °C after which they were extracted twice with phenol and loaded onto a 0-7% horizontal agarose gel. Electrophoresis was carried out in 40 mM-Tris-acetate pH 8-0, 5 mM-sodium acetate, 10 mM-EDTA for 3 to 18 h at 1 to 6 V/cm. Gels were stained in 10 mM-Tris-HCl pH 7-5, containing 1 μg/ml ethidium bromide, washed and photographed under u.v. illumination.

**HSV-1 enzyme extraction.** Confluent monolayers of BHK cells were infected with the appropriate virus at an m.o.i. of 10 and incubated at 32 or 37 °C for 18 to 20 h post-infection or 39 °C for 15 to 18 h post-infection. The enzyme was prepared at 4 °C as follows: cells were washed in Tris-buffered saline and 5 ml hypotonic buffer (20 mM-HEPES pH 7-8, 0-1 mM-EDTA, 0-1 mM-DTT) was added per plate for 3 to 5 min. The buffer was removed and the cells were scraped from the dish. Approximately 2 × 10⁶ cells were contained in 150 μl of hypotonic lysate. KC1 (final concn. 80 mM) and NP40 (final concn. 0-02%) were added and the lysates were incubated at 4 °C for 20 min, then centrifuged for 5 min in a Beckman microfuge B. The supernatant was added to an equal volume of DEAE-Sephasel (Pharmacia) which had been washed exhaustively in buffer containing 20 mM-HEPES pH 7-0, 1 mM-EDTA, 1 mM-DTT and 80 mM-KCl. The enzyme was allowed to bind to DEAE for 1 h at 4 °C after which all unbound material was removed by washing in the same buffer by microfuge centrifugation. One-half vol. of 20 mM-HEPES pH 7-0, 1 mM-EDTA, 1 mM-DTT and 400 mM-KCl was added to the DEAE and incubated for 30 min at 4 °C. Following microfuge centrifugation the supernatant was removed and saved and another 0-5 vol. of HEPES buffer with 400 mM-KCl was added for an additional 30 min. Both supernatants were combined and dialysed against 10 mM-Tris–HCl pH 7-5, 1 mM-DTT, 150 mM-NaCl and 50% glycerol. The enzyme was stored at −20 °C.

The assay conditions for this enzyme were essentially the same as those described for the topoisomerase above except that ATP was omitted. Individual differences will be pointed out as they arise in Results.

**Electron microscopy.** DNA was spread onto Parlodin-coated grids by the Kleinschmidt formamide procedure as described by Davis et al. (1971). Following uranyl acetate staining and tungsten shadowing (Williams, 1977), the DNA was visualized in a Philips EM201 microscope.

**Enzymes and chemicals.** Lysozyme and proteinase K were purchased from Sigma and *Eco*RI was obtained from Bethesda Research Laboratories. Histone HI was purchased from Boehringer Mannheim.

**RESULTS**

**Network formation in extracts from HSV-1 wild-type and temperature-sensitive mutant-infected cells**

In order to identify a topoisomerase-like enzyme in HSV-1-infected cells, cell-free extracts were screened for their ability to catenate plasmid DNA. We chose this reaction because all known topoisomerases are capable of catenating duplex DNA circles, thereby generating multimers of the original monomeric DNA (Cozzarelli, 1980). The source of enzyme was a
Fig. 1. Catenating activity in HSV-1(17) ts+ and tsK and mock-infected BHK cells. Extracts prepared from mock-infected or cells infected with HSV-1 ts+ or tsK at 32 or 39 °C were assayed for catenating activity at either 32 °C or 39 °C, in the absence of ATP and analysed by gel electrophoresis as described in Methods. Lanes 1 and 7, HSV-1, ts+, 32 °C infection; lanes 2 and 8, HSV-1, ts+, 39 °C infection; lanes 3 and 9, HSV-tsK, 32 °C infection; lanes 4 and 10, HSV-1, tsK, 39 °C infection; lanes 5 and 11, mock 32 °C infection; lanes 6 and 12, mock 39 °C infection. Incubation of the extracts was at 32 °C for 15 min (lanes 1 to 6) or 39 °C for 10 min (lanes 7 to 12). The reactions were terminated and the products analysed as described in Methods. The symbols used are: I, supercoiled; II, nicked circular; Ir, relaxed closed circular; N, networks.

Fig. 2. Effect of temperature shift on the catenating activity induced by HSV-1 tsK. Extracts were prepared from cells infected with HSV-1(17) ts+ or tsK at 32 °C, before or after shift up to 39 °C. The standard catenating assay was performed at 32 °C for 15 min in the absence of ATP as described in Methods. Lane 1, HSV-1, ts+, 18 h post-infection, 32 °C; lane 2, HSV-1, tsK, 18 h, 39 °C; lane 3, HSV-1, tsK, 18 h, 32 °C; lane 4, HSV-1, tsK, 22 h, 32 °C; lane 5, HSV-1, tsK, 18 h at 32 °C plus 2 h shift up to 39 °C; lane 6, HSV-1, tsK, 18 h at 32 °C plus 4 h shift up to 39 °C. The reactions were stopped and analysed as described in Methods. The symbols used are defined in the legend to Fig. 1.

DEAE-purified, DNA-free, HSV-1-infected cell extract and the substrate was purified pBR322 form I (supercoiled) DNA. This plasmid was chosen as the structure and DNA sequence are well characterized (Sutcliffe, 1978), it can be obtained in large quantities as a supercoiled molecule and it is a convenient, defined, non-specific substrate for a potential topoisomerase.

In Fig. 1 the results of such a screening process are shown. Wild-type HSV-1 strain 17-infected cells were incubated at 32 °C (lane 1) and 39 °C (lane 2). The cells were harvested, extracts were prepared and were reacted with pBR322 DNA at 32 °C under the conditions described. In both cases, large networks of DNA were formed which were unable to enter the gel and little or no form I DNA, circular nicked form II DNA, or circular relaxed form Ir DNA remained in the reaction mixtures. Small amounts of monomer form II and dimer forms I and II DNA were always present in the unreacted pBR322 DNA I substrate (see Fig. 5, lane 9 for control DNA). During the reaction a ladder of relaxed closed circular DNA (form Ir) was generated. It is not clear if this was associated with the catenating activity, or whether it resulted from an unwinding activity (topoisomerase type I) also present in these preparations (Champoux & Dulbecco, 1972). When the same extracts were assayed at 39 °C the results were identical (lanes 7 and 8). Mock-infected cells, treated in the same way, contained no such activity at any temperature tested (lanes 5, 6, 11, 12), but did have the ability to relax DNA (see Ir in these lanes). The HSV-1(17) temperature-sensitive mutant, tsK, was deficient in catenating activity at 32 °C and 39 °C, when harvested following infection in vivo at 39 °C (lanes 4 and 10). However, extracts from tsK-infected cells contained catenating activity at either temperature in vitro,
Table 1. Activity induced by DNA− temperature-sensitive mutants of HSV-1*

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* Extracts were prepared from cells infected with each virus at an m.o.i. of 10, at 18 h post-infection for 32 °C infection and 15 h post-infection for 39 °C infection, as described in Methods. Catenating activity was assayed without ATP as described. (+) Indicates the presence of networks at the top of the gel and (−) indicates their absence.

When made from cells infected in vivo at the lower permissive temperature of 32 °C. Preincubation at 39 °C of an extract from cells infected with tsK at 32 °C did not abolish the activity (data not shown), suggesting that the protein(s) involved was not temperature-sensitive in vitro, but that the synthesis of catenating activity was temperature-sensitive in vivo. To examine this in more detail, we infected cells with tsK and, after 18 h at 32 °C, shifted them to 39 °C for 2 or 4 h before harvesting. The results are shown in Fig. 2. Lanes 1 and 2 are reactions with extracts prepared from HSV-1 ts+ infected cells at 32 °C and HSV-1 tsK-infected cells at 39 °C, representing positive and negative controls respectively. In lanes 3 and 4 it can be seen again that tsK-infected cell extracts contained catenating activity at 32 °C and this activity did not decay by 22 h post-infection at 32 °C. When these cells were shifted, after 18 h at 32 °C, to 39 °C for 2 or 4 additional h (lanes 5 and 6 respectively), catenating activity remained and did not decay. It is therefore clear that the tsK gene product is required for the turn-on of synthesis of protein(s) involved in network formation, but that the activity itself, once synthesized in tsK-infected cells at the permissive temperature, is not temperature-sensitive under temperature shift conditions in vivo.

We then screened a series of DNA− temperature-sensitive mutant-infected cells for catenating activity and the results are shown in Table 1. Only extracts from cells infected at 39 °C with the early regulatory mutants, tsK and tsB2, were deficient in catenating activity. The tsD mutation is known to map in the same gene as tsK, but its control over viral DNA replication is less stringent (Marsden et al., 1976), which would explain the small amount of DNA at the top of the gel that was detected using extracts derived from tsD-infected cells incubated at 39 °C. In Table 1, the reaction for tsD at 39 °C was therefore designated as + / −. Extracts from tsB2-infected cells behaved in the same way as those from cells infected with tsK, which confirmed the requirement for an immediate-early function for the expression of catenating activity.

Reaction requirements for network formation

In order to characterize further the catenating activity in HSV-1-infected cells, we performed the following experiments. An extract derived from HSV-1(17) wild-type-infected cells was boiled for 2 min before assay and all activity was destroyed (Fig. 3, lane 1). When aliquots were incubated over time at 37 °C, a peak of catenating activity occurred at 6 to 12 min (Fig. 3, lanes 5 to 8). At the earlier times (2 and 4 min), small quantities of slower migrating bands were
HSV protein–pBR322 DNA interaction

Fig. 3. Time course of the catenating reaction. Extracts were prepared at 18 h post-infection from cells infected at 37 °C with HSV-1(17) ts+. The standard catenating assay was performed at 37 °C, in the absence of ATP as described in Methods, with the following changes. Lane 1, 5 μl of extract heated to 100 °C for 2 min prior to 10 min incubation at 37 °C with pBR322 DNA and reaction buffer. The remaining lanes (2 to 12) represent standard reactions incubated for various times at 37 °C: lane 2, 0 min; lane 3, 2 min; lane 4, 4 min; lane 5, 6 min; lane 6, 8 min; lane 7, 10 min; lane 8, 12 min; lane 9, 14 min; lane 10, 16 min; lane 11, 18 min; lane 12, 20 min. The reactions were terminated and the product analysed as described in Methods. The symbols used are defined in the legend to Fig. 1.

Fig. 4. Requirements for the catenating reaction. HSV-1(17) ts+-infected cell extracts (37 °C) were prepared at 18 h post-infection and assayed for catenating activity at 37 °C under the conditions described in Methods with the following additions and deletions. Lane 1, no histone H1; lane 2, H1/DNA (w/w) ratio of 0.13; lane 3, H1/DNA (w/w) ratio of 0.2; lane 4, H1/DNA (w/w) ratio of 0.5; lane 5, H1/DNA ratio of 1.0; lane 6, H1/DNA (w/w) ratio of 3.0; lane 7, no MgCl2; lane 8, no DTT. The reactions were stopped and analysed as described. The symbols used are defined in the legend to Fig. 1.

generated, which may represent intermediate multimers of pBR322 synthesized before the formation of the larger networks. The enzyme preparations were relatively crude extracts and so were not entirely free of the HSV-1 alkaline exonuclease (Strobel-Fidler & Francke, 1980). Some evidence for nucleolytic activity can be seen at the later incubation times in Fig. 3.

The importance of the DNA condensing agent (histone H1) is shown in Fig. 4. In the absence of histone, no networks were formed (lane 1) and there was some degradation of DNA, presumably again through the action of nuclease. A peak of catenating activity occurred at an H1/DNA (w/w) ratio of 0.2 to 0.5 (lanes 3 and 4) but at higher H1 concentrations, there was no reaction (lanes 5 and 6). However, at these higher H1 concentrations, the DNA was not degraded, presumably due to the protective action of the histone on the DNA. The requirement for divalent cations was absolute (lane 7), while that for DTT was not as stringent (lane 8).

The topoisomerase type II enzymes are known to require ATP in order to catenate DNA (Cozzarelli, 1980) and the catenated product is stable during phenol extraction. The BHK topoisomerase type II enzyme was isolated according to the method of Miller et al. (1981) as described in Methods. Fig. 5 shows a comparison of this enzyme and the HSV-1 enzyme incubated in the presence or absence of ATP. In order to be sure of detecting even small amounts of catenated DNA on the gels, twice the amounts of enzyme and DNA were incubated in the reaction. In lanes 1 and 2, it can be seen that with the BHK cell enzyme no catenated DNA was generated in the absence of ATP, whereas in lane 6 a large amount of catenated DNA was generated in the presence of ATP and the product was stable during phenol extraction (lane 5).
Fig. 5. Comparison of the BHK topoisomerase type II with the HSV-1 catenating enzyme. The BHK cell topoisomerase type II enzyme was prepared as described in Methods. The HSV-1 extract was from cells infected with HSV-1(17)ts+ for 18 h at 37 °C. The reaction conditions were standard except that 1 to 2 μg DNA and 10 μl extract were used. Also, 1 mM-ATP was included where indicated. Incubation was at 37 °C for 15 min and the reactions were stopped with or without phenol extraction as indicated. Lanes 1 and 5, BHK topo II, phenol extraction; lanes 2 and 6, BHK topo II, no phenol; lanes 3 and 7, HSV-1 enzyme, phenol extraction; lanes 4 and 8, HSV-1 enzyme, no phenol; lanes 1 to 4, reactions did not contain ATP; lanes 5 to 8, 1 mM-ATP was included in the reaction; lane 9, unreacted pBR322 DNA. The symbols used are defined in the legend to Fig. 1.

When the HSV-1 enzyme was assayed in the same way, large networks of DNA were formed, irrespective of the absence or presence of ATP (lanes 3, 4 and 7, 8) and in all cases the product remained stable during phenol extraction.

Products of the reaction

To analyse further the reaction products of the HSV-1-associated enzyme, we concentrated the DNA product by ethanol precipitation and examined one aliquot by electron microscopy and digested the other with the restriction enzyme EcoRI. Fig. 6(a, b) shows two different sizes of DNA networks formed following reaction of the infected cell enzyme with monomer pBR322 DNA. The majority of the DNA was intact with a few double-stranded breaks. The networks appear similar to published electron micrographs of catenated circular DNA (Hsieh & Brutlag, 1980; Kreuzer & Cozzarelli, 1980). When we incubated the BHK topoisomerase type II enzyme with pBR322 DNA, in the presence of ATP, we could identify similar structures by electron microscopy. However, monomer circles of pBR322 were the only identifiable structure following incubation of pBR322 DNA with the BHK topoisomerase type I (data not shown). pBR322 DNA contains a single cleavage site for EcoRI and the enzyme therefore generates linear, unit length molecules from the circular substrate (Sutcliffe, 1978). If these networks are catenated (interlocked) DNA, rather than having been generated by some sort of recombination event, digestion with EcoRI should resolve them into one single band on the gel, corresponding to linear, monomeric pBR322 DNA (form III). In Fig. 7 it can be seen that, following EcoRI
Fig. 6. Electron micrograph of catenated pBR322 DNA. Supercoiled pBR322 DNA was reacted with a standard HSV-1-infected cell extract under the conditions described in Methods, in the absence of ATP. The reaction was stopped with SDS, proteinase K and phenol extraction. The DNA product was precipitated with ethanol, redissolved in 10 mM-Tris-HCl pH 7.5, 1 mM-EDTA, spread onto Parlodin-coated grids, stained and visualized in a Philips EM201 electron microscope as described in Methods. (a) and (b) are two different sizes of networks formed during the reaction. Bar markers represent 1 μm.
Fig. 7. *EcoRI* digestion of the catenated product. Catenated pBR322 DNA was generated by an HSV-
1-infected cell extract in the absence of ATP, as described in Methods. The DNA was precipitated in
ethanol, redissolved in H2O and digested with 5 h units of *EcoRI* for 15 min at 37 °C in 100 mM-Tris-
HCl pH 7-2, 5 mM-MgCl2, 2 mM-2-mercaptoethanol and 50 mM-NaCl. The reaction was stopped by
phenol extraction and the products were analysed on a 0-7% agarose gel as described. Lane 1, unreacted
pBR322; lane 2, catenated DNA; lane 3, pBR322 DNA digested with *EcoRI*; lane 4, catenated DNA
digested with *EcoRI*. The symbols used are: I, supercoiled; II, nicked circular; III, linear; N, networks.

digestion, both the original substrate and the reacted DNA (lanes 1 and 2) migrated as linear
pBR322 DNA (lanes 3 and 4). The inaccessibility of some of the *EcoRI* sites in the large
networks would explain the small amount of undigested DNA that remained at the top of the gel
in lane 4.

DISCUSSION
In this report we present evidence for the existence of a new HSV-1 infection-associated
enzyme which resembles the DNA topoisomerases in that it interacted with circular non-viral
DNA to form large catenated networks which did not migrate into 0-7% agarose gels.

The data obtained using temperature-sensitive mutants of HSV-1, together with the absence
of catenating activity in mock-infected cells, strongly suggest that the enzyme induction is virus-
specific or the enzyme is in fact virus-coded. The VP175 immediate-early HSV-1 protein,
defined by the *tsK* and *tsB2* mutants (Preston, 1979; Dixon & Schaffer, 1980), is likely to control
the synthesis of the catenating enzyme in a regulatory fashion (Honess & Roizman, 1974), rather
than be directly responsible for the observed activity. We could not demonstrate temperature
sensitivity *in vitro* or *in vivo* with extracts obtained from *tsK*-infected cells, but could show
temperature sensitivity in the synthesis of the enzyme in both *tsK* and *tsB2*-infected cells.
Clearly, DNA synthesis is not required for the expression of enzymic activity as the other
DNA- mutants tested, which define the major DNA-binding protein locus (Weller et al., 1983)
and the DNA polymerase locus (Chartrand et al., 1980; Purifoy et al., 1977; Purifoy & Powell,
1981), were positive for catenating activity at the non-permissive temperature. A recent report
by Biswal et al. (1983) presents evidence for an HSV-1-induced unwinding enzyme activity that
co-purifies with the major peak of the HSV-1 DNA polymerase on DEAE-cellulose. When
assayed in the same way, the minor peak of polymerase activity had the ability to generate
concatemers of SV40 DNA. In attempting purification of our observed catenating activity, we
have found that it is chromatographically distinct from the major peak of the viral DNA
polymerase and the alkaline nuclease. It is very likely that the activity can be found in the minor peak. We have also observed the activity in HSV-2-infected cells (our unpublished data). It is therefore possible that one of the DNA-binding proteins common to both serotypes (Bookout & Levy, 1980) is responsible for catenating activity.

The DNA topoisomerase enzymes are divided into two classes. Both types of enzymes have the ability to relax supercoiled DNA with (type II), or without (type I), added ATP. Type 1 enzymes are capable of reversibly catenating DNA rings in the absence of ATP, in the presence of a defined amount of DNA condensing agent (histone or spermidine), and they require that at least one strand of each duplex contains a nick (Tse & Wang, 1980; Brown & Cozzarelli, 1981). Type II enzymes can also reversibly catenate duplex DNA rings, have a strict requirement for ATP and condensing agent, and do not require that the DNA be nicked (Liu et al., 1980; Hsieh & Brutlag, 1980). The mock-infected BHK cells, or cells infected with tsK or tsB2 at 39°C, retained the ability to relax supercoiled DNA in the absence of ATP, suggesting that this property is separate from the infected cell enzyme's ability to catenate pBR322.

The evidence presented here suggests that the enzyme from HSV-1-infected cells is a topoisomerase-like enzyme for the following reasons: (i) large networks of DNA were formed, (ii) the reaction required a defined concentration of DNA condensing agent and, (iii) the networks could be resolved to one single, linear pBR322 DNA isomer following EcoRI digestion.

While some topoisomerases contain more than one subunit (Cozzarelli, 1980), a discussion of subunit structure with respect to the observed activity in this report is premature. The preparations used were crude and until purification is complete it would be impossible to determine the subunit structure and precisely define the viral or cellular origin of the protein(s) involved. In addition, the presence of the known contaminants in our preparations (unwinding enzyme and nuclease) preclude a more accurate quantification of the catenating activity and a more rigorous analysis of the products. For the present it is sufficient to state that the presence of the enzyme activity is directly related to HSV-1 infection and that this is controlled by the tsK and tsB2 loci of the viral genome.

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