Studies on DNA Topoisomerases I and II in Herpes Simplex Virus Type 2-infected Cells

By ASHOK R. BAPAT, FU-SHENG HAN, ZONGCHAO LIU, BING-SEN ZHOU AND YUNG-CHI CHENG*

Department of Pharmacology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514, U.S.A.

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SUMMARY

It has been suggested that herpes simplex virus (HSV) type 1 may induce a virus-specific DNA topoisomerase activity which copurifies with virus-induced DNA polymerase. We have examined DNA topoisomerase (TOPO) I and II activities in HSV-2-infected HeLa S3 cells. Both activities were partially purified using DEAE-cellulose, phosphocellulose and double-stranded DNA cellulose column chromatography. It was found that both activities could be separated from HSV-2-specific DNA polymerase. Throughout the purification TOPO I could be immunologically detected with a monoclonal antibody developed against human TOPO I. Regardless of the source, mock- or HSV-2-infected human cells, both types of topoisomerase were equally tolerant of 200 mM-KCl. There appeared to be no apparent heterogeneity of TOPO I in HeLa S3 cells through the course of the HSV-2 infection. We conclude that host cell topoisomerases are quite stable in HSV-2-infected HeLa S3 cells and that there is no evidence that HSV-2 is capable of inducing HSV-2-specific TOPO I and TOPO II activities.

The topology of eukaryotic DNA has implications in a number of events in DNA metabolism such as replication (Cozzarelli, 1980; Liu et al., 1980), transcription (Akrigg & Cook, 1980; Weisbrod, 1982), transposition (Sternglanz et al., 1981), recombination (Kikuchi & Nash, 1979), chromosome rearrangement (Vosberg & Vinograd, 1976), disentanglement of catenated DNA circles (Kreuzer & Cozzarelli, 1980; Liu et al., 1979; Mizuuchi et al., 1980) and viral integration (Ross, 1985). The necessary changes in DNA topology occurring during these events are carried out by a group of enzymes called DNA topoisomerases. These enzymes are capable of changing the topology of the DNA by coordinated breaking and rejoining of the phosphodiester backbone of DNA. Topoisomerases can be divided into two types: topoisomerase type I and type II (TOPO I and TOPO II). TOPO I changes the linking number in single steps by coordinated breaking and rejoining of one strand of the double helix whereas TOPO II changes the topological state of DNA by passing it through transient double-stranded breaks in the molecule, thus changing the linking number in steps of two. One major distinctive difference between these two enzymes is that TOPO II requires ATP to bring about necessary changes in DNA. The presence of both enzymes appears to be necessary for the viability of eukaryotic cells (Uemura & Yanagida, 1984).

The presence of TOPO I has been documented in a number of different systems including wheatgerm (Dynan et al., 1981), Escherichia coli (Wang, 1971), human KB cells (Champoux & Dulbecco, 1972), HeLa S3 cells (Liu & Miller, 1981), chicken erythrocytes (Pullybank & Ellison, 1982), calf thymus (Pullybank & Morgan, 1975), rat liver (Champoux & McConaugh, 1976), purified vaccinia virus (Bauer et al., 1977) and Rous sarcoma virus (Weiss & Faras, 1981). Recent reports describe isolation of this enzyme from vaccinia virus-infected mouse L cells.
Short communication

Fig. 1. Elution profiles of TOPO I, TOPO II and virus-specific DNA polymerase from a phosphocellulose column. Assays for virus-specific DNA polymerase contained the following: 50 mM-Tris-HCl pH 8.0, 5 mM-MgCl₂, 1 mM-DTT, 50 μg of heat-inactivated bovine serum albumin/ml, 150 mM-KCl, 50 μg of activated calf thymus DNA per ml, 100 μM-dATP, -dCTP, and -dGTP, 10 μM-[³H]TTP (10 μCi/ml). The reaction was started by adding the enzyme. After 30 min of incubation at 37°C, the reaction was placed on ice and acid-insoluble radioactivity was determined as described previously (Derse et al., 1982). The assay mixtures for TOPO I and TOPO II contained the following: 50 mM-HEPES pH 7.5, 20 mM-MgCl₂, 50 μg of heated BSA per ml, 5 mM-DTT. In addition, TOPO II mixtures also contained 150 mM-KCl and 1 mM-ATP. Fifteen μg of pBR322 DNA per ml for TOPO I and 0.3 μg of P4 DNA per ml for TOPO II were used in the assay mixtures. After 1 h of incubation at 37°C, the reaction was stopped and the products were analysed on 1% agarose gel.

TOPO I is also reported to copurify with virus-specific DNA polymerase from HSV-1-infected cells (Biswal et al., 1983). However, it is uncertain whether this activity was virus-specific. TOPO II has been purified from a variety of sources including Xenopus oocytes (Benedetti et al., 1983), E. coli (Staudenbauer & Orr, 1981), T4-infected cells (Liu et al., 1979), HeLa cells (Miller et al., 1981) and calf thymus (Halligan et al., 1985). The presence of TOPO II has not been studied in animal virus systems. In this report we provide the results of our investigation directed towards identification and characterization of TOPO I and TOPO II from HSV type 2 (HSV-2)-infected HeLa S3 cells.

A 11 culture of HeLa S3 cells (1.2 × 10⁶) in stationary growth phase was infected with HSV-2 (strain 333) virus stock (free of producer cell debris) at a m.o.i. of 5 p.f.u./cell. After a 1 h adsorption period, the virus inoculum was removed. The cells were resuspended in RPMI 1640 containing 2% foetal calf serum and were harvested 13 h post-infection. The cell pellet was washed with phosphate-buffered saline (PBS), centrifuged and extracted with 400 mM-phosphate pH 7.5 containing 2 mM-EDTA, 2 mM-dithiothreitol (DTT), 1 mM-phenylmethylsulphonyl fluoride (PMSF, a protease inhibitor) and 25% glycerol. The crude cell-free extract was subjected to a multistep purification procedure for virus-specific DNA polymerase. All the procedures were performed at 4°C and all the buffers contained 2 mM-DTT, 2 mM-EDTA, 1 mM-PMSF and 25% glycerol. TOPO I, TOPO II and DNA polymerase were assayed according to previously published methods (Liu et al., 1979; Pullybank & Ellison, 1982; Derse et al., 1982). Briefly, the crude extract was applied to a DEAE–cellulose column (15 ml) to remove the nucleic acids. The high salt wash (450 mM-phosphate at pH 7.5) containing proteins was
dialysed against 50 mM-phosphate pH 7.5, (buffer A) and then applied to a second DEAE-cellulose column (60 ml) which was pre-equilibrated with buffer A. The DNA polymerase was eluted with a linear phosphate gradient (50 mM to 1.25 M) and the fractions were assayed for TOPO I, TOPO II and DNA polymerase activities. We observed that all three activities coeluted, and so the active fractions were pooled, dialysed overnight against buffer A and applied to a phosphocellulose column (60 ml) which was pre-equilibrated with buffer A. This column was washed with 75 ml of buffer A and then eluted with a linear phosphate gradient (50 mM to 1.25 M). The fractions were assayed for TOPO I, TOPO II and DNA polymerase. The profile of these activities is shown in Fig. 1.

TOPO I and TOPO II activities were not only separated from virus-specific DNA polymerase but also from each other. Fractions with TOPO I activity (66 to 72) were pooled, dialysed overnight against buffer A and then applied to a single-stranded DNA cellulose column (7 ml) that had been equilibrated with buffer A. The TOPO I activity was eluted with a linear KCl gradient (0 to 1.25 M). The profile of TOPO I activity is shown in Fig. 2. The fractions from this purification step were dot-blotted onto a nitrocellulose paper and were immunostained. Fig. 2 shows that the fractions with TOPO I activity were also recognized by an antibody to human TOPO I (Han et al., 1985). In order to examine whether the molecular weights of TOPO I and TOPO II purified from HSV-2-infected cells were the same as host cellular enzymes, we subjected the fractions from the phosphocellulose column that contained either TOPO I or TOPO II activity to Western blot analysis. The proteins separated on a SDS-polyacrylamide gel by electrophoresis were transferred to a nitrocellulose paper and then stained using antibodies against human TOPO I (Han et al., 1985) and TOPO II (kindly provided by Leroy Liu, Johns

Fig. 2. Profile of TOPO I from a double-stranded DNA cellulose column. The fractions were assayed as described in Fig. 1(b) and were also dot blotted and immunostained with human TOPO I antibody (a).
Hopkins University, Baltimore, Md., U.S.A.). These antibodies were capable of recognizing proteins of identical molecular weights from both mock- and virus-infected sources (data not shown). To characterize these enzymes (purified through a phosphocellulose column) further, we investigated the effect of KCl on their activities (Champoux et al., 1979; Ross, 1985). Fig. 3 shows the results of this experiment which indicated that the enzymes were equally tolerant of salt and thus appeared to be similar in nature.

It has been observed that some of the host enzymes which are present in enriched amounts during the S phase apparently lose their activity after virus infection (Caradonna & Cheng, 1980). Topoisomerase activities, especially TOPO I, appear to be cell cycle-dependent. Although the conclusions are conflicting, the main body of evidence suggests that high levels of TOPO I activity exist during the S phase, while very little activity is present in either G1 or G2 (Champoux et al., 1979; Poddar & Bauer, 1986; Rosenberg et al., 1976). The presence of different molecular weight species as identified by the monoclonal antibody raised against TOPO I have been observed in human leukaemic cells (Han et al., 1985). In order to examine the fate of host TOPO I with respect to its size during the course of viral infection, the suspension culture was infected with HSV-2 (strain 333). At various times post-infection, cell suspension samples were removed, centrifuged, washed with PBS and the cell pellet was extracted into 50 mM-Tris–HCl (pH 7.5). The crude extract was electrophoresed on a 10% SDS–PAGE gel and immunostained as described earlier. Fig. 4 shows the progress of the virus infection as observed using the commercial antibody against HSV-2 extract (obtained from Dako Corporation, Santa Barbara, Ca., U.S.A.) and monoclonal antibody against human TOPO I. This monoclonal antibody was capable of recognizing a protein of approximately 100K, the apparent molecular weight of human TOPO I. The levels of this enzyme (as observed by this method) appeared to have remained constant through the course of virus infection. The presence of virus-induced deoxyribonuclease activity, which destroyed the DNA substrates, hindered the detection of either TOPO I or TOPO II activities in the crude extracts.
In this report, we have presented results which indicate that host TOPO I and TOPO II activities are present in HSV-2-infected cells and appear to function. In no case did we observe TOPO I or TOPO II activities which were not recognized by antibodies capable of recognizing human cellular enzymes. Furthermore, enzymes derived from virus-infected and mock-infected cells had similar responses to salt. This suggests that HSV-2 is unlikely to induce virus-specific TOPO I or TOPO II in HSV-2-infected cells, although we can not rule out the possibilities of, first, coelution of viral and human topoisomerases through purification and/or, second, minor modifications of host enzymes by HSV-2. It can be concluded that there was no apparent degradation of TOPO I in HSV-2-infected HeLa S3 cells after HSV-2 infection although why this TOPO I behaved differently from other host S phase enriched enzymes with respect to stability in HSV-infected cells is unclear. Attempts to examine either TOPO I or TOPO II activities during the course of virus infection were hindered by the induction of virus-specific deoxyribonuclease in virus-infected cells. It should be noted that unlike a recent report which indicated that in HSV-1-infected cells TOPO I copurified with virus-specific DNA polymerase (Biswal et al., 1983; a result which we could not reproduce using procedures similar to those indicated in this communication) our studies clearly indicated that both TOPO I and TOPO II activities were separable from virus-specific DNA polymerase in HSV-2-infected cells. When a similar procedure was used to purify HSV-1-specific DNA polymerase to near homogeneity, no associated TOPO I activity could be detected (unpublished results).

The biological function of TOPO I in eukaryotes is not yet well understood. In yeast, it appears to be non-essential. It is conceivable that these topoisomerase activities may be critical for viral DNA or RNA synthesis and for the maturation process. The presence of host TOPO I and TOPO II activities in HSV-2-infected cells may fulfil the needs of this virus, thus removing the need for HSV-2 to induce its own DNA topoisomerases. If so, this suggests opportunistic behaviour by the virus in using cellular enzymes for its propagation. A possibility still exists, however, that HSV-2 may be capable of inducing unstable topoisomerases which may not be detected through the purification steps. The functional role of these enzymes during viral replication needs to be further investigated.

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REFERENCES


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