Topoisomerase activity associated with SV40 large tumor antigen

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ABSTRACT

Purified preparations of simian virus 40 (SV40) large tumor antigen (LT) from three different sources, including LT expressed from a recombinant baculovirus, were found to relax negatively supercoiled cyclic DNA molecules, whether or not they contained SV40 sequences. Relaxation was stimulated by MgCl$_2$ but not by ATP, and inhibited by camptothecin, suggesting the involvement of an enzymatic activity similar to that of topoisomerase I (topo I). However, the pH requirements for relaxation by respectively LT and topo I are different. Also, antibodies reacting with LT inhibited relaxation by preparations of LT but not topo I, whereas antibodies inhibiting relaxation by topo I had no effect on relaxation by LT. Reconstruction experiments suggested that both procedures used to purify LT, immunoaffinity chromatography and DEAE-Sepharose chromatography, separate topo I from LT. Finally, relaxing activity was found in over 40 preparations of LT, and in the few instances where activity could not be found, it probably had been lost during storage, rather than absent from the start. Whereas these results seem to exclude that the activity being detected is that of a contaminant of LT, they would be consistent with this activity being that of a stable topo-LT complex, or else intrinsic to LT itself.

INTRODUCTION

The large tumor antigen (LT) of SV40 is a multifunctional protein involved in the initiation of viral DNA replication (1), the regulation of both early and late viral transcription (2–5), and the control of the expression of several cellular genes (6). These functions can be readily correlated with at least some of the well-known biochemical properties of LT, including nonspecific DNA binding (7), SV40 origin-binding (8,9), ATP-ase (10), DNA helicase (11) and RNA helicase (12) activities, and the ability to bind to DNA polymerase $\alpha$ (13), or to the p53 (14,15) and pRB (16) growth-regulating proteins. Interestingly, SV40 LT has also been shown to facilitate DNA renaturation as well as cyclization of double-stranded DNA (17). Perhaps the least understood role of SV40 LT, or for that matter polyomavirus LT, is that played in phenomena such as sister chromatid exchange (18–20), viral DNA integration into the host chromosome (21,22) and its reverse, viral DNA excision (23–26). It has generally been assumed that such recombination events would be dependent upon DNA replication (21,22,24–26), and that the need for LT in recombination would thus reflect its role in the initiation and/or propagation of replication (27). However, results from one of our laboratories have suggested for LT a role in recombination distinct from that played in replication (28), such that LT would directly determine the nature of the recombination product (29). Prior to the development of a suitable in vitro recombination system, we have re-examined the effect of LT on DNA structure, taking into consideration that recombinases consistently display topoisomerase activity (30). We report here that such is also the case for preparations of LT. The activity associated with these preparations is either inherent or tightly bound, to LT.

MATERIALS AND METHODS

DNA template

Plasmid pAT153 (31) was prepared from bacteria according to Guerry et al. (32) and purified by banding in CsCl-ethidium bromide gradients (33). Covalently closed, supercoiled DNA was electroeluted from a preparative 1% agarose gel and used in relaxation reactions.

Topoisomerases

Calf thymus topoisomerase I was purchased from GIBCO/BRL. Drosophila melanogaster topoisomerase I (34) was obtained from Stratagene. Human DNA topoisomerase II was kindly provided by Dr. P. Chartrand.

Inhibitors of DNA topoisomerases

Camptothecin, and its more potent form camptothecin-lactone, are both specific inhibitors for eukaryotic topoisomerase I (35), whereas oxolonic acid and m-AMSA are inhibitors of DNA
topoisomerase II (36). Camptothecin and oxolonic acid were purchased from Sigma, whereas m-AMSA and camptothecin lactone were generously provided by Nancita R. Lomax, National Cancer Institute (NIH, Bethesda, Md).

**SV40 LT**

**LT from monkey cells.** Initial experiments were carried out with LT purchased from Molecular Biology Resources Inc. (Milwaukee WI, 53210: catalog no. 5800-02). This LT had been immunopurified from BSC-40 monkey kidney cells infected with the SV40 mutant cs 1085 (2) by a modification of a published procedure (37), using PAB419 monoclonal anti-LT antibody (38). Since this antibody recognizes the amino-terminus of LT (38), the resulting preparation consisted of not only LT but also small-T (ST) and was ~90% pure, as judged by SDS-PAGE followed by silver staining. We also purified SV40 LT by essentially the same procedure (37) but from growing COS-1 cells (39), making use of KT3, a monoclonal antibody specific for LT (see below). The resulting preparation consisted of over 90% LT, with no detectable ST.

**LT from insect cells.** SV40 LT was also produced in *Spodoptera frugiperda* cells (Sf9 strain) that either were kindly provided by Dr E. Carstens or purchased from Invitrogen Corp.. Sf9 cells growing in suspension culture were infected with a recombinant *Autographa californica* virus expressing SV40 LT but not ST, vE55SvST (40), a generous gift from Dr C. Prives. LT was immunopurified as stated above (37; see also Purification of LT), using PAB419 monoclonal antibody (38). Resulting preparations were more than 90% pure, as shown in Fig. 1. Mutant LTs were purified by a slightly different procedure (41), from Sf9 cells infected with various *A. californica* recombinant viruses encoding either a deleted or a substituted LT protein (42, 43).

**Antibodies**

PAbss were either purchased (PAB419 and PAB416) from Oncogene Science, Inc. (Manhasset, N.Y.), or generated as ascitic fluid, from mice injected with either KT3 hybridoma cells (44), a generous gift from Dr G. Walter, or with PAB419 hybridoma cells (38), kindly provided by Dr C. Prives. The serum of a scleroderma patient with a high titer of auto-antibodies against DNA topoisomerase I (45) was generously provided by Dr E. Durban.

**Cells and cell extracts**

**Monkey cells.** COS-1 cells were grown at 37°C in DME minimal essential medium supplemented with 10% fetal calf serum. After the monolayer cultures had been washed twice with cold PBS, the cells were scraped and suspended in buffer-A (20 mM Tris-Cl pH 8.0; 200 mM NaCl; 1 mM EDTA; 1 mM DTT; 10% glycerol; 0.1% NP40 and 200 μg/ml of phenylmethylsulfonylfluoride), and allowed to swell on ice for 15 min. After the cells had been homogenized with a pestle, the debris were removed by centrifugation at 10,000 × g for 20 min at 4°C.

**Insect cells.** Sf9 cells were grown in suspension at 27°C in Grace's medium (46) supplemented with 10% fetal calf serum. They were infected at a density of 2 x 10^6 cells per ml with recombinant baculovirus (about 1 PFU per cell), diluted to 3 x 10^5 cells per ml, further incubated at 27°C, and harvested three days after infection. After centrifugation at 4000 rpm for 20 min, the pellet from one liter of cell suspension was suspended in 20 ml of Cowie lysis buffer (50 mM Tris-HCl pH8.0; 150 mM NaCl; 1% NP40; 1 mM DTT; 0.1% aprotinin; 0.01% leupeptin; 0.35 mM phenylmethylsulfonylfluoride), and kept on ice for 30 min. After removal of cell debris (2000 rpm, for 15 min, at 4°C), nuclei were pelleted by centrifugation for 30 min at 20000 rpm and 4°C, and the supernatant (S100) was collected.

**Purification of LT**

**COS-1 cell extract.** The extract from 25 petri dishes (10 cm in diameter) was mixed with an immunomatrix consisting of KT3 covalently attached (47) to protein A-Sepharose beads (Pharmacia LKB, 10 mg of IgG/ml of Sepharose). After overnight incubation, a packed column was made from the mixture and washed sequentially with 2 ml of buffer-B (50 mM Tris-HCl, pH 8.0; 500 mM NaCl; 1 mM EDTA; 1 mM DTT; 10% glycerol; 1% NP40) and 2 ml of buffer-C (the same as buffer-B, but buffered at pH 9 and containing no NP40), and finally with the elution buffer (20 mM Tris—HCl, pH 11; 500 mM NaCl; 1 mM EDTA; 1 mM DTT; 10% glycerol). One ml-fractions were collected directly into neutralizing buffer (500 mM Tris—HCl, pH 7.0; 1 mM EDTA; 1 mM DTT; 10% glycerol), and then dialyzed against buffer-D (10 mM Tris—HCl, pH 8.0; 100 mM NaCl; 1 mM EDTA; 1 mM DTT; 50% glycerol). A typical yield was 20—25 μg of nearly homogeneous LT, as judged by SDS-PAGE followed by silver staining.

**Sf9 cells.** The S100 from one liter of cell suspension was mixed with 3 ml of a matrix consisting of PAB419 coupled to protein A-Sepharose (10 mg IgG/ml of 30% Sepharose). The column made after overnight incubation was washed as stated above, except that 10 ml of buffer-B and 5 ml of buffer-C were now used. The pH11 elution fractions (1 ml each) were collected in the same neutralizing buffer as before, and they were analyzed by SDS-PAGE followed by silver staining. Fractions containing the bulk of LT were pooled, dialyzed against buffer-E (same than buffer-D, but with 100 mM KCl instead of NaCl), aliquoted and stored at —80°C. Typically, one liter of cell suspension yielded 100 to 500 μg of purified LT.

Mutant LTs were purified according to Simanis and Lane (41) and eluted from the same immunomatrix with 50% ethylene glycol; 0.02 M Tris—HCl pH8.5; 0.5 M NaCl; 0.001 M EDTA; 10% glycerol.

**Ion exchange chromatography**

Immunopurified LT was further purified on DEAE-Sepharose. In preliminary experiments, we noted that topol I, whether from calf thymus or *D. melanogaster*, was eluted from such matrix in 120 mM NaCl solution, whereas LT was eluted at about 200 mM NaCl. Therefore, we used the following procedure to repurify LT. Ten μg of immunopurified LT diluted in 1 ml of buffer I (25 mM Tris—HCl, pH8.0; 120 mM NaCl; 10 mM EDTA; 1% NP40 and 200 μg/ml of phenylmethylsulfonylfluoride) were applied to a 0.4 ml column of DEAE-Sepharose 50% (Pharmacia) equilibrated in the same buffer. After 3 washes of 1 ml each with buffer I, elution was carried out with 3 x 1 ml of buffer 2 (same as buffer 1, but with 500 mM NaCl), LT being recovered in the first of these three fractions. When 40 units (Gibco/BRL) of calf thymus topol I were used instead of LT in such a fractionation, relaxing activity was quantitatively recovered in the flowthrough of the column.

**Relaxation reactions**

Relaxation of negatively supercoiled pAT153 DNA was performed in our standard buffer (50 mM Tris—HCl, pH8.0;
balance. B/A ratios were calculated and corrected using the B/A to topoisomers (B), were cut out and weighed on a precision quantitated by scanning autoradiograms with a densitometer of supercoiled DNA (form I) into relaxed topoisomers was represented at least 100 such units. Our D. melanogaster) (Corning 750 system). Peaks corresponding to form I (A), and acceptable for comparison between different samples. Conversion under optimal conditions, especially with commercial enzymes. Of such preparations of LT contained in general one of our units of topo activity per 50-150 ng of purified LT. Of such preparations of LT, 25 to 50 ng of pAT153 DNA had to be used in most relaxation reactions, making it necessary to resort to a radioactive probe for the detection of topoisomers. Ideally, meaningful comparisons between the requirements for relaxation by LT and topo I demanded that similar amounts of enzymatically active protein be used in all instances. Meeting this requirement was clearly difficult here, due to the very high specific activity of topo I, and to the low activity associated with LT preparations. Hence, for all experiments, we attempted to define optimal conditions that would allow only partial relaxation of the DNA template, thus making sure that we would never work in enzyme excess. Also, we defined our own topoisomerase unit. This unit was defined as the amount of topo I or LT that would convert 50% of fully supercoiled pAT153 DNA into partly or totally relaxed DNA, using 25 ng of DNA in the reaction and the standard conditions described at the beginning of this section. On the basis of the manufacturer technical information, one commercial unit of topo I (whether from calf thymus or from D. melanogaster) represented at least 100 such units. Our preparations of LT contained in general one of our units of topo activity per 50 – 150 ng of purified LT. Of such preparations of LT, and of commercial preparations of topo I, we then used either one unit or two units, versus 25 ng or 50 ng of DNA, respectively. Because of the highly catalytic activity of topoisomerases, a 50% conversion could not always be obtained under optimal conditions, especially with commercial enzymes. Thus, a 10% to 90% range of conversion was considered acceptable for comparison between different samples. Conversion of supercoiled DNA (form I) into relaxed topoisomers was quantitated by scanning autoradiograms with a densitometer (Corning 750 system). Peaks corresponding to form I (A), and to topoisomers (B), were cut out and weighed on a precision balance. B/A ratios were calculated and corrected using the B/A ratio obtained for untreated pAT153 DNA run in the same gel.

50 mM KCl; 10 mM MgCl₂; 1 mM DTT; 30 μg/ml of BSA), unless otherwise indicated, for 30 min at 37°C in a total volume of 20 μl. Except for the experiment shown in Fig. 2 (see legend), reactions were stopped by the addition of 1 μl of 10 mg/ml proteinase K and 2 μl of 10% SDS. After a further 30 min incubation at 50°C followed by phenol-chloroform extraction, samples were electrophoresed through a 1% agarose gel, blotted onto a Hybond-N⁺ nylon membrane (Amersham Corp.), and hybridized with a ³²P-labelled pAT153 probe, as described elsewhere (29). Because of the relatively low topo activity of LT preparations, 25 to 50 ng of pAT153 DNA had to be used in most relaxation reactions, making it necessary to resort to a radioactive probe for the detection of topoisomers. Ideally, meaningful comparisons between the requirements for relaxation by LT and topo I demanded that similar amounts of enzymatically active protein be used in all instances. Meeting this requirement was clearly difficult here, due to the very high specific activity of topo I, and to the low activity associated with LT preparations. Hence, for all experiments, we attempted to define optimal conditions that would allow only partial relaxation of the DNA template, thus making sure that we would never work in enzyme excess. Also, we defined our own topoisomerase unit. This unit was defined as the amount of topo I or LT that would convert 50% of fully supercoiled pAT153 DNA into partly or totally relaxed DNA, using 25 ng of DNA in the reaction and the standard conditions described at the beginning of this section. On the basis of the manufacturer technical information, one commercial unit of topo I (whether from calf thymus or from D. melanogaster) represented at least 100 such units. Our preparations of LT contained in general one of our units of topo activity per 50 – 150 ng of purified LT. Of such preparations of LT, and of commercial preparations of topo I, we then used either one unit or two units, versus 25 ng or 50 ng of DNA, respectively. Because of the highly catalytic activity of topoisomerases, a 50% conversion could not always be obtained under optimal conditions, especially with commercial enzymes. Thus, a 10% to 90% range of conversion was considered acceptable for comparison between different samples. Conversion of supercoiled DNA (form I) into relaxed topoisomers was quantitated by scanning autoradiograms with a densitometer (Corning 750 system). Peaks corresponding to form I (A), and to topoisomers (B), were cut out and weighed on a precision balance. B/A ratios were calculated and corrected using the B/A ratio obtained for untreated pAT153 DNA run in the same gel.

RESULTS AND DISCUSSION

Purified LT carries an activity which has similarities with that of topoisomerase I

As described in detail in Materials and Methods, we have used in our experiments three different preparations of LT, all purified by immunoaffinity chromatography. Commercially available LT had been purified from BSC-40 cells infected with cs 1085, a virus mutant overproducing LT (2); because PAb419 had been used to prepare the immunoaffinity column, the resulting preparation contained not only LT but also ST (38). We also prepared LT in our own laboratory from growing COS-1 cells, using monoclonal KT3 (44). As this antibody is directed to the C-terminal end of LT, the protein purified with it consists of LT exclusively. Thirdly, we purified LT from Spodoptera frugiperda cells infected with a recombinant baculovirus encoding LT only (40). All three preparations of LT appeared to possess the same topoisomerase activity. Finally, LT immunopurified from infected insect cells was subjected to a second cycle of purification by DEAE-Sepharose chromatography, yielding a fourth type of LT preparation which also had relaxing activity.

Our initial observations indicated that LT removed negative supercoils from covalently-closed form I DNA, regardless of whether this DNA contained SV40 sequences or not (not shown). Indeed, relaxation was observed with not only SV40 DNA itself, but with plasmids like pAT153. Hence, we decided to carry out further experiments using pAT153 as template (Materials and Methods). Next, we found that LT-relaxing activity required Mg²⁺ as a cofactor, and was slightly inhibited by ATP (Fig. 2). Such inhibition presumably resulted from partial chelation of Mg²⁺ ions by ATP. As expected, control experiments demonstrated that such effects of MgCl₂ and ATP were similar to those of the same compounds on relaxation by calf thymus topo I (mammalian, or M-topo I), whereas relaxation by human topoisomerase II was found to require ATP (not shown). Various inhibitors known to be effective on relaxation by topoisomerases I and II were tested for their effect on relaxation by LT. Thus, oxolonic acid and m-AMSA (36) were found to be inactive or largely inactive (not shown). Camptothecin and camptothecin
present in the cell extract? Or alternatively, was the extract so
Secondly, how did the relaxing activity behave when topo I was
loaded with relaxing activity, that the immunoaffinity column did
Finally, the complex could have been in such low abundance that
would have co-purified with LT itself, and its structure would
have been such that antibodies directed against LT, but not those
from the patient's serum, is discernible (A and B).
proportion of the total relaxing activity
would not have been detected after SDS-PAGE electrophoresis
Represent a significant proportion of the total relaxing activity
would have interfered with relaxation.
Lactone (35) however strongly inhibited relaxation by LT or M-
topo I (not shown). DNA relaxation by LT thus resulted from an
activity (LT-topo) with strong similarities with that of topo I,
whether inherent to LT, bound to LT, or simply contaminating the
LT preparations.
Effect of antibodies (Ab) on relaxation
Monoclonal Ab raised against LT were tested for their effect on relaxation by LT-topo or M-topo I. Both PAb416 and PAb419
clearly inhibited relaxation by LT-topo, whereas PAb419 had no effect on relaxation by topo I (Fig. 3). The effect of PAb416 on relaxation by topo I, however, was variable (not shown). We also looked for a possible inhibition of relaxation by human serum containing polyclonal auto-antibodies against human topo I (see Materials and Methods). Such Ab strongly inhibited relaxation by M-topo I (Fig.3), and also insect topo I or I-topo I (not shown), while having no detectable effect on relaxation by LT-topo (Fig.3). These data cannot be easily reconciled with LT-topo activity being that of a cellular topo I contaminating the LT preparations.
LT-topo and topo I do not copurify
Conceivably, LT-topo activity could have been carried by a protein complex consisting of LT and topo I. Such a complex
would have co-purified with LT itself, and its structure would
have been such that antibodies directed against LT, but not those
directed against topo I, would have interfered with relaxation.
Finally, the complex could have been in such low abundance that
it would not have been detected after SDS-PAGE electrophoresis of
the immunopurified protein (Fig. 1). We decided to investigate
three separate issues concerning the reliability of our LT
purification procedure. First, did the relaxing activity of LT-topo
represent a significant proportion of the total relaxing activity
present in the cell extract? Or alternatively, was the extract so
loaded with relaxing activity, that the immunoaffinity column did
not clearly separate the LT protein from the bulk of this activity?
Secondly, how did the relaxing activity behave when topo I was
subjected to the purification procedure designed to isolate LT?
Finally, what happened if topo I was mixed with LT prior to
purification? Figure 4 largely answers these questions. It shows
first that when an extract of S.fragiperda cells infected with a
recombinant baculovirus expressing LT is fractionated by
immunoaffinity chromatography, relaxing activity is detected only
in those fractions in which LT can be detected by SDS-PAGE
(Fig. 4A). However, this result is somewhat ambiguous since
the flowthrough and pH8 wash contain enough nuclease to
preclude the detection of relaxing activity. Yet, in a separate
experiment, we verified that processing the pH11 fractions
containing LT through a second cycle of immunopurification did
not allow the detection of relaxing activity separable from the
LT protein (not shown). Next, we subjected a large amount of
M-topo I (50 commercial units) to the immunopurification used
to isolate LT, and recovered all of the relaxing activity in the
flowthrough and washes (Fig. 4B). Then, we mixed 10 µg of
purified LT with 50 commercial units of M-topo I and subjected
the mixture to immunopurification: hence, two peaks of activity
were noted, one in the flowthrough and washes, of a magnitude
expected from the introduction of 50 units of M-topo I in the
mixture, and another one in the pH11 elution buffer, of the magnitude expected for the LT-topo (Fig. 4B). Fifty commercial
units of topo I, and 10 µg of purified LT, represent approximately
5000 and 100, respectively, of our units (see Materials and
Methods). Finally, a LT preparation having lost all relaxing
activity upon storage at −20°C was mixed with 50 commercial
units of M-topo I and the mixture was fractionated. Relaxing
activity was then found in the flowthrough and washes, and not
in the fraction containing LT (Fig. 4D). We realized that this
experiment could be criticized from having been conducted with
two 'mammalian' proteins, one of which, SV40 LT, had however
been generated in insect cells. We thus repeated the experiment
shown in Fig. 4D with the same LT preparation, but with I-topo I,
and got the same results as before, indicating lack of binding of
the added I-topo I to LT. Altogether, these results suggest
that the relaxing activity which we detected in LT preparations
is neither due to inadequate purification of LT from a host topo I
existing as a free protein, nor to the fact that LT and topo I
form readily a high affinity complex.
Effect of pH on relaxation
While attempting to study the relative affinities of topo I and LT-
topo for respectively single-stranded and double-stranded DNA,
we noticed that the two relaxing activities reacted differently to
changes in salt concentration or pH (Fig. 5A). First, we noticed that
the relaxing activity of LT-topo, unlike that of M-topo I,
was markedly inhibited when NaCl was added to our standard
buffer to the final concentration of 50 mM (Fig. 5A, compare
lanes 1 and 2). Also some differences in relaxation were noted
as a function of pH, suggesting that LT-topo but not topo I
was largely inactive at pH6 (Fig. 5A, compare lanes 1 and 3).
We thus decided to further investigate the effects of pH on relaxation.
Figure 5B shows that M-topo I has activity over a broader pH
range than LT-topo, whether LT is purified from insect cells (I-
LT) or from COS-1 cells (M-LT). This effect is not due to
buffering of the solution by the protein itself, since every reaction
mixture contained 30 µg/ml of BSA (Materials and Methods).
Furthermore, I-topo I was found to behave in this respect as M-
topo I (not shown). These results are as yet of unclear
significance, but may indicate an intrinsic difference between the
enzymatic activities of topo I and LT-topo.
Figure 4. Reliability of the immunopurification procedure. The starting material is either an extract from baculovirus-infected cells (A), or M-topo I (B), or a mixture of M-topo I and immunopurified LT, with (C) or without (D) LT-topo activity. In all instances, the material is processed through an immunoaffinity column that includes PAB19 (see Materials and Methods). One ml-fractions are collected in all cases, except in panel A for the flowthrough (FT, 10 ml), and the pH8 and pH9 washes (5 ml each). From each fraction, 5 ml were used for the detection of relaxing activity, while 40 ml (except for B) were subjected to SDS-PAGE followed by silver staining. The percentage of conversion of supercoiled DNA into relaxed topoisomers (histogram) was determined as detailed in Materials and Methods, except for the FT and pH8 fractions in panel A, where severe nicking of DNA rendered topo activity undetectable. Vertical arrows below panels A, C and D stand for the relative amount of LT detected by SDS-PAGE in the respective fractions. Thus, in C and D, LT could be detected for concentrations which varied between 6 µg (largest arrows) and 0.3 µg (shortest arrows) per ml of eluate. Also, in C and D, each of the pH8 and pH9 solutions was used to perform three successive 1 ml washes.

LT mutants

We have undertaken to examine whether some deleted or substituted LT genes (42,43), all expressed from baculovirus vectors, would encode an LT without, or with a modified, LT-topo activity. Interesting preliminary results have been obtained with mutants defective in nonspecific DNA binding activity. For instance, mutant proteins 226VA and 185ST, which both have about 20 to 30% of wild-type levels of DNA binding activity (D.T. Simmons, unpublished results) appear as effective as wild-type LT in relaxing DNA (Fig. 6), while showing the pH-dependence (not shown) already observed for wild-type LT (Fig. 5B). However, mutant protein 203HN, which has no detectable binding activity (D.T. Simmons, unpublished results) appeared to have little or no relaxing activity (Fig. 6). These results are consistent with relaxation by LT-topo being due to either LT itself, or a complex that would include LT and a cellular topoisomerase distinct from both topo I and topo II.

Figure 5. Effect of salt and pH on relaxation. A: Two units of LT-topo (left) or M-topo I (right) were used to relax 50 ng of pAT153 DNA in standard buffer (lanes 1), the same buffer plus 50 mM NaCl (lanes 2), or (lanes 3) pH6 buffer (standard buffer with 50 mM NaCl instead of 50 mM KCl, and 5 mM TRIS-HCl-5 mM sodium phosphate, pH6, see ref. 50). pAT: untreated DNA. B: Two units of LT-topo, from either insect cells (I-LT) or monkey cells (M-LT), were used to relax 50 ng of pAT153 DNA in the same buffer as in panel A (lanes 3) but brought to different pH values. pAT: untreated DNA.
CONCLUSION

The results summarized above indicate that preparations of SV40 LT purified by immunoaffinity chromatography, whether produced in a research or a commercial laboratory, consistently contain an activity, designated here as LT-topo, which relaxes negatively supercoiled cyclic DNA. Judging from its ATP-independence and sensitivity to inhibitors, this activity appears similar to that of topo I. Re-purification of immunopurified LT by DEAE-Sepharose chromatography, which does separate topo I from LT, does not however dissociate LT-topo from LT. Antibodies directed against LT inhibit LT-topo, while having no effect on relaxation by topo I. The opposite is true with antibodies against topo I. Finally, the two activities, topo I and LT-topo, are exerted over different pH ranges. In our opinion, these findings clearly exclude the possibility that the preparations of LT which we have tested, regardless of their origin, are contaminated by a type I topoisomerase. We are aware of the fact that topo I is a highly catalytic enzyme, often almost undetectable as a protein, yet we cannot see how the hypothesis of a contamination can be reconciled with our results, in particular those pertaining to the effect of antibodies. Our data however do not clearly make the distinction between two remaining interpretations: (1) LT-topo is inherent to LT, or to a form of LT; (2) LT-topo results from a stable association between LT and a cellular protein distinct from topo I and topo II, such that the complex displays a unique relaxing activity. Even the latter interpretation can be reconciled with PAbS against LT inhibiting relaxation by LT-topo, or with a mutant LT having lost LT-topo activity. However, it is difficult to resist the idea that this complex would then be rather specific. In such case, the hypothetical cellular topoisomerase would have to be highly conserved throughout the evolution to interact similarly with LT, whether being of mammalian or insect origin.

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