Topoisomerase I activity associated with human immunodeficiency virus (HIV) particles and equine infectious anemia virus core

E.Priel^{1,2}, S.D.Showalter³, M.Roberts⁴,
S.Oroszlan⁴, S.Segal¹, M.Aboud¹ and
D.G.Blair²

¹Microbiology and Immunology Unit, Faculty of Health Sciences, Ben-Gurion University, Beer-Sheva, Israel, ²National Cancer Institute, FCRDC, PO Box B, Frederick, MD 21702-1201, ³Program Resources, Inc., PO Box B, Frederick, MD 21702-1201 and ⁴Bionetics Research Inc., Basic Research Program, NCI-Frederick Cancer Research and Development Center, PO Box B, Frederick, MD 21702-1201, USA

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In the present study, we found a topoisomerase I (topo I) activity in two strains of human immunodeficiency virus type 1 (HIV-1) and equine infectious anemia virus (EIAV) particles. The topo I activity was located in the EIAV cores and differed from the cellular topo I in its ionic requirements and response to ATP, indicating that these were two distinct forms of this enzyme. Topo I activity was removed from the viral lysates and viral cores by anti-topo I antiserum. The only protein recognized by this antiserum was an 11.5 kd protein in HIV lysate and 11 kd in EIAV lysate. We showed that the 11 kd protein recognized by the anti-topo I antiserum is the EIAV p11 nucleocapsid protein. Furthermore, purified topo I protein blocked the binding of the antibodies to the p11 protein and vice versa, purified p11 protein blocked the binding of these antibodies to the cellular topo I. These results suggest that the EIAV p11 nucleocapsid protein and the cellular topo I share similar epitopes.
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Introduction

DNA topoisomerases are ubiquitous enzymes that modify the topological state of DNA via the breakage and rejoining of DNA strands (Wang, 1985, 1987; Vosberg, 1987). DNA topoisomerases are of two major types: type I enzymes catalyze the concerted breakage and rejoining of a single strand of the double helix and thus can change the linking number in steps of one. Type II DNA topoisomerase allows the concerted breakage of both DNA strands and changes the linking number in steps of two (Wang, 1985; Vosberg, 1987). Topo I was isolated and purified from several prokaryotic and eukaryotic cells and has been implicated in DNA replication and transcription (Fleischman *et al.*, 1984; Gilmour *et al.*, 1986; Zhang *et al.*, 1988). The enzyme acts catalytically at genes characterized by high rates of transcription and the heaviest enrichment for topo I is seen cytologically within the nucleus (Fleischman *et al.*, 1984). Recent studies showed that vaccinia virus encapsidates a type I topoisomerase that is encoded by the viral genome (Shuman and Moss, 1987).

Retroviruses establish persistent infection by integrating proviral DNA into the host cell genome (Varmus and Swanstrom, 1985; Varmus, 1988). It is likely that both provirus integration and expression require topological changes (Fujiwara and Mizuuchik, 1988; Varmus, 1988). It had been reported that topo I activity is associated with Rous sarcoma virus (Weis and Faras, 1981). Since DNA topoisomerases have been shown to be responsible for the induction of topological changes in DNA and to participate in many vital cellular reactions involving DNA, it was of interest to investigate whether a topoisomerase activity could be demonstrated in other retroviral particles and to characterize the protein that possesses this activity.

In this study, we show that a topo I activity with properties different from the cellular enzyme is present in purified human immunodeficiency virus (HIV) and equine infectious anemia virus (EIAV). We also show that topo I activity is present in purified EIAV cores and that EIAV p11 nucleocapsid protein and the cellular topo I share similar epitopes.

Results

Topo I activity in virions of HIV-1 and EIAV

In searching for topoisomerase activity in retroviruses, we used purified virions of HIV-III_B, HIV RF and EIAV which were lysed by 0.03% NP-40 and examined for their effect on a supercoiled plasmid DNA. Figure 1 illustrates results obtained with lysates of both HIV strains, showing a conversion of this DNA into an array of products (see Figure 1A, lanes 2, 4 and 6 for HIV-III_B and lanes 8, 10 and 12 for HIV RF).

These products were subjected to a second electrophoretic dimension in the presence of a high concentration of ethidium bromide (EtBr) (Minfred *et al.*, 1986). As illustrated in Figure 1B, all of these products moved in the second electrophoretic direction at the same rate. It is thus evident that they were closed circular DNA molecules with a varying degree of relaxation, rather than linear molecules of different sizes or nicked circles since EtBr can introduce positive supercoiling only in closed DNA circles. Such a relaxation activity is typical of topo I and as shown in lanes 3, 5, 7, 9 and 13, it was indeed blocked by the well-known topo I specific inhibitor, camptothecin (CPT) (Hsiang *et al.*, 1985; Andoh *et al.*, 1987; Gupta *et al.*, 1988; Nitiss and Wang, 1988).

The relaxation activity was dose dependent. Densitometric analysis of the results depicted in Figure 1A, lanes 2, 4, 6, 8, 10 and 12 revealed that 42% of the input substrate DNA was relaxed by 2.6 µg of viral lysate proteins, 56% by 5.2 µg and 80% by 20 µg. Figure 1C illustrates a similar relaxation activity in the lysate of EIAV (lane 2) which was also sensitive to the inhibitory effect of CPT (lane 3).

Localization of topo I in the viral core

In attempts to determine more specifically the location site of topo I within the viral particle, we looked for its activity

Fig. 1. Topoisomerase I activity in purified HIV and EIAV particles. (A) First dimension gel electrophoresis; (B) second dimension gel electrophoresis. Topoisomerase I activity was assayed in HIV-1 lysates, of the IIIb (A,B, lanes 2–7) and RF (A,B, lanes 8–13) strains and in EIAV lysates (C, lanes 2 and 3) using increased amounts of viral proteins: 2.6 μ g protein (A,B, lanes 2, 3, 8 and 9), 5.2 μ g (A,B, lanes 4, 5, 10 and 11), 20.8 μ g (A,B, lanes 6, 7, 12, 13 and C, lanes 2 and 3). The reaction was performed in the absence (A,B, lanes 2, 4, 6, 8, 10 and 12) or presence (A,B, lanes 3, 5, 7, 9, 11, 13 and C, lane 3) of 400 μ M CPT. Lane 1 contains control supercoiled pUC19 plasmid. The bands labeled I, II, I_o, I_R are supercoiled, open, completely relaxed, partially relaxed circular DNA, respectively.

in the viral core. For this purpose, we analyzed only core preparations which were proven, by electron microscopy, to be clear of intact virions and confirmed by SDS–PAGE to contain only core proteins such as p11 and p26 (Roberts and Oroszlan, 1989). Figure 2 illustrates a representative experiment with EIAV cores, providing clear evidence for topo I activity in these cores (lane 2).

Furthermore, densitometric comparison between this core activity and the respective whole virion activity shown in Figure 1C, lane 2, revealed that the specific activity of this enzyme (defined as μ g of substrate DNA converted into relaxed DNA products in 30 min per μ g lysate proteins) was 0.02 for the whole virus and 0.08 for the EIAV cores, i.e. 4-fold enrichment of this enzyme in the viral cores. It is therefore evident that topo I concentrates primarily, if not exclusively, within the viral core.

Enzymatic differences between the viral and the cellular topo I

Since it has been shown that cellular enzymes can be packaged in virus particles during viral assembly and budding from the host cell (Temin and Baltimore, 1972), we asked whether the observed viral topo I activity reflected cellular topo I molecules randomly trapped in the viral cores or was it exerted by a distinct virus specific form of this enzyme. To resolve this question, we compared the enzymatic properties of the viral topo I with nuclear topo I derived from the uninfected cells of the same lines used for propagating

Fig. 2. Topo I activity in EIAV cores. EIAV cores were purified as described (Roberts and Oroszlan, 1989) and lysed by 0.03% NP-40. Core lysate 8 μ g was added to a complete topo I reaction mixture (lane 2) and in the presence of 1:50 dilution of anti-topo I antiserum (lane 3). Lane 1 contains untreated pUC19 plasmid DNA. The topo I assay and the labeled bands are as described in Figure 1.

Fig. 3. Comparison of topo I activity in virus particles with that from uninfected cells. The effect of Mg^{2+} , KCl, Ca^{2+} and ATP on topo I activity from HIV particles: (A) 20 μ g of proteins from HIV-IIIb (lanes 2–6), HIV-RF (lanes 7–11); (B) EIAV (lanes 2–6) was added to a complete reaction mixture (A, lanes 2, 7 and B, lane 2), or to the mixture without $MgCl_2$ (A, lanes 4, 8 and B, lane 4), without KCl (A, lanes 3, 9 and B, lane 3), without Mg^{2+} and in the presence of 10 mM $CaCl_2$ (A, lanes 5, 10 and B, lane 5) or in the presence of 1 mM ATP (A, lanes 6, 11 and B, lane 6). Lane 1 shows control pUC19 plasmid DNA incubated without proteins. The labeled bands are as described in Figure 1. (C) Topoisomerase I activity in nuclear extract from H9 cells. The nuclear extract was obtained as described elsewhere (Auer *et al.*, 1982). 20 μ g of total protein were added to a complete reaction mixture (lane 2) or to the mixture without $MgCl_2$ (lane 3), without KCl (lane 4), without $MgCl_2$ and in the presence of 10 mM $CaCl_2$ (lane 5), or in the presence of 1 mM ATP (lane 6). The topo I assay and the labeled bands are as described in Figure 1.

the respective viruses. Figure 3A shows that the enzyme activity in both HIV strains required either Mg^{2+} (lanes 4 and 8) or Ca^{2+} (lanes 5 and 10) and was optimized by 20 mM KCl (lanes 3 and 9). However, it was remarkably inhibited by 1 mM ATP (lanes 6 and 11). Similar proper-

Fig. 4. Immunodepletion of topo I activity from lysates of HIV-IIIB (lanes 2–4), HIV-RF (lanes 5–7) and EIAV (lanes 8–10). Lysates were immunoprecipitated with human anti-topo I serum (lanes 3, 6 and 9) or normal human serum (lanes 4, 7 and 10). The retroviral lysates were treated for 1 h at 4°C with a 50-fold dilution of anti-topo I or normal human serum. Lanes 2, 5 and 8 represent control lysates not exposed to antisera. Protein A sepharose was added to each sample and the immunocomplexes were precipitated. Topo I activity was determined in the supernatant. Lane 1 contains pUC19 plasmid DNA.

ties were seen with the EIAV enzyme (Figure 3B) which also required Mg^{2+} (lane 3) or Ca^{2+} (lane 5), optimized by 20 mM KCl (lane 4) and inhibited by 1 mM ATP (lane 6). By contrast, Figure 3C shows that the nuclear topo I of uninfected H9 cells (which were used for propagating both of the tested HIV strains), did not require Mg^{2+} , Ca^{2+} nor KCl (compare lanes 2, 3, 4 and 5), and was not significantly affected by 1 mM ATP (lane 6). Similar results were obtained with the nuclear extract of CF2Th cells which we used for propagating EIAV (data not shown). This dependency on Mg^{2+} , Ca^{2+} , as well as the marginal effect of ATP, could not be attributed to an overdose of the extracts that we might use in these particular experiments, since they were observed in repeated experiments with up to 100-fold dilutions of these extracts. These differences suggest that the viral and the host cell topo I activities reflected two distinct forms of this enzyme.

Inhibition of the viral topo I activity and recognition of the viral p11 protein by an anticellular topo I antiserum

In a further attempt to elucidate the possible relatedness of the viral topo I to the host cell enzyme, we looked in the next experiment for any antigenic homology between them. We used for this purpose an autoimmune antiserum derived from scleroderma patients. This antiserum has been shown by Shero *et al.* (1986) to react with human cellular topo I. As illustrated in Figure 4, immunoprecipitation with this antiserum eliminated topo I activity from all of the viral lysates (lanes 3, 6 and 9). Similar data were obtained when this antiserum was reacted with lysates of the viral cores (results with EIAV cores are depicted in Figure 2, lane 3). Normal human serum was ineffective in this respect when tested with virion lysates (Figure 4, lanes 4, 7 and 10). As could be expected, the anti-topo I antiserum also eliminated the topo I activity from the nuclear extracts of H9 and CF2Th cells (data not shown).

To evaluate these observations better the viral lysates were subjected to Western blot analysis using this anti-topo I antiserum. As can be seen from Figure 5A, this antiserum specifically recognized an 11 kd protein in the EIAV lysate (lane 1) and an 11.5 kd protein in the lysates of both HIV strains (lanes 3 and 4). To verify the specificity of the immunoreaction with the 11–11.5 kd proteins, we tested this antiserum with another viral protein such as the HIV reverse transcriptase (Figure 5, lane 2), but no interaction could be demonstrated. For comparison, we analyzed also the nuclear and cytoplasmic extracts of H9 (Figure 5A, lanes

Fig. 5. Western blot analysis of HIV, EIAV proteins and nuclear cell extracts. (A) Anti-topo I antiserum, (B) normal human serum. EIAV proteins (A, lane 1 and B, lane 3), HIV reverse transcriptase (a gift from S. Hughes, BRI, Frederick, MD); (A, lane 2) HIV-RF proteins (A, lane 3 and B, lane 4), HIV-IIIB proteins (A, lane 4), CF2Th cell nuclear extract (A, lane 5 and B, lane 1), CF2Th cell cytoplasmic extract (A, lane 6), H9 cell nuclear extract (A, lane 7, and B, lane 2), H9 cytoplasmic extract (A, lane 8), were analyzed by Western blot using the protein A gold kit (Bio-Rad). Nuclear extracts were prepared from H9 or CF2Th cells as described elsewhere (Auer *et al.*, 1982). SDS-PAGE in 10% gels, and electroblotting to nitrocellulose as described in Materials and methods. The immunodetection was done with 1:2500 dilution of human autoimmune anti-topo I serum or human normal serum using the immunoblot protein A gold assay (Bio-Rad). Marker proteins indicate $M_r \times 10^{-3}$ (BRL).

5 and 6) and CF2Th (Figure 5A, lanes 7 and 8) cells. As can be seen, this antiserum recognized in the nuclear extracts the typical 110 kd cellular topo I protein, but not any protein at the size of 11–11.5 kd. Normal human serum did not interact with either the cellular 100 kd or the viral 11–11.5 kd proteins, but it did bind to other cellular proteins ranging between 14 kd and 30 kd (Figure 5B).

An 11 kd nucleocapsid named p11 has been reported in EIAV cores (Henderson *et al.*, 1987). It was therefore of interest to determine whether the 11 kd protein recognized by this antiserum is identical to p11. For this purpose, lysates of EIAV cores were first immunoprecipitated with the anti-topo I antiserum. Then the immunoprecipitate and the remaining supernatant, as well as the complete lysate, were subjected to Western blot analysis using anti-EIAV p11 specific antiserum. As can be seen from Figure 6A, the

Fig. 6. Western blot analysis of EIAV cores (A) and EIAV infected CF2Th cells (B). (A) 8 μ g of core proteins were treated for 1 h at 4°C with a 50-fold dilution of anti-topo I antiserum. The immunocomplexes were precipitated. Samples from the supernatant (lane 1), immunocomplexes (lane 2) and core proteins (lane 3) were analyzed by Western blot using anti-p11 antiserum. (B) Western blot analysis of CF2Th cells (lanes 1 and 2) and EIAV infected CF2Th cells (lanes 3 and 4). One hundred microgram proteins from cytoplasmic cell extracts (lanes 1 and 3) or nuclear cell extracts (lanes 2 and 4) were analyzed using topo I antiserum [1:2500 dilution (I), p11 antiserum 1:1000 dilution (II), or p26 antiserum 1:1000 dilution (III)]. Marker protein indicated as $M_r \times 10^{-3}$ (BRL) is shown in lane 5.

anti-EIAV p11 antiserum recognized the 11 kD protein in the immunoprecipitate (lane 2), whereas the supernatant was almost completely depleted of this protein (compare the supernatant in lane 1 with the complete core lysate in lane 3).

Of interest to note in this context is that when extracts of CF2Th cells chronically infected with EIAV were similarly analyzed, the anti-topo I antiserum recognized the pr55^{gag} and pr49^{gag} proteins (Figure 6BI, lane 4). These gag proteins were also recognized by the anti-EIAV p11 (Figure 6BII, lane 4) and anti-EIAV p26 (Figure 6BIII, lane 4), antisera. pr55^{gag} is the precursor for p15, p26 and p9, whereas pr49^{gag} is an intermediate cleavage product of pr55^{gag} consisting of p15, p26 and p11 (Henderson *et al.*, 1987). The anti-p26 antiserum recognized also the pr43^{gag} (Figure 6BIII, lane 4) which was not detected by the anti-p11 (Figure 6B, lane 4) or by the anti-topo I (Figure 6B, lane 4) antisera. pr43^{gag} is another intermediate product of pr55^{gag} which consists of p26 and p15 EIAV proteins only (Henderson *et al.*, 1987). This set of experiments further substantiates our notion that the anti-topo I antiserum specifically interacts with the viral p11 nucleocapsid protein, by showing that gag precursor proteins are recognized by this antiserum only if they still contain p11.

Recognition of topo I and p11 proteins by the same antibodies

It was unclear, however, from these experiments whether or not the viral p11 and the cellular topo I proteins were recognized by the same antibodies or whether this antiserum may have contained separate species of antibodies for each of these proteins. To clarify this question, we isolated from this antiserum the antibodies which were specifically bound to the EIAV p11 protein on a preparation blot as detailed

in Materials and methods. These specific homogeneous antibodies were found in a subsequent experiment to interact specifically with both the viral p11 (Figure 7A, lane 3) and the cellular topo I proteins (Figure 7B, lane 3). Furthermore, highly purified EIAV p11 protein was found to compete out the binding of these antibodies, not only to the viral p11 band (Figure 7C, lane 2), but also to the cellular topo I band (Figure 7D, lane 2) and vice versa, purified calf thymus topo I competed out the binding of these antibodies to the cellular topo I (Figure 7E, lane 2), as well as to the viral p11 band (Figure 7C, lane 4). Taken together, these data prove that topo I and p11 were both recognized by the same antibodies and share similar epitopes.

Discussion

In this study we demonstrated the presence of topo I in two strains of HIV-1 and in EIAV, whereas in experiments to be reported elsewhere, we found this enzyme also in MoMLV. These findings, together with a previous report of topo I in RSV (Weis and Faras, 1981), may suggest that topo I is probably a general feature of retroviruses. Such an enzyme would comply with the apparent needs of these viruses for specific topological DNA modification to accomplish their integration and subsequent expression.

The viral topo I discovered in our experiments was found to localize in the viral core, thus ruling out a trivial argument that it might merely reflect a non-specific contamination of the viral preparation by the cellular topo I of their host cells. Furthermore, the viral and the host cell enzymes were strikingly different from each other in their ionic requirements and response to ATP. The viral topo I was found to depend on Mg^{2+} or Ca^{2+} and to require KCl for

Fig. 7. Affinity purification of the specific p11 antibodies from the human autoimmune topo I antiserum. The specific p11 antibodies from the topo I antiserum were purified as described in Materials and methods and were used to probe the EIAV p11 nucleocapsid protein (A, lane 3) and the H9 nuclear extracts (B, lane 3). A and B, lane 1, are the EIAV lysate and the H9 nuclear extracts probed with the human autoimmune topo I antiserum, respectively, and A and B, lane 2, are the EIAV lysates and the H9 nuclear extracts probed with the p11 antiserum. The bands shown in A, lanes 2 and 3, were enhanced by silver (Bio-Rad kit). (C)–(E) Competition tests using purified calf thymus topo I protein and purified p11 protein. The affinity purified p11 antibodies were used to probe the EIAV p11 protein (C, lanes 1 and 3), or the H9 nuclear extract (D, lane 1 and E, lane 1) in the presence of 14 μ g of topo I protein (C, lane 4 and E, lane 2) or 10 μ g of p11 protein (C, lane 2 and D, lane 2).

optimal activity, whereas the host cell topo I did not show any of these ionic requirements. In addition, ATP was highly inhibitory for the viral, but not for the cellular topo I. In this respect, the host cell enzyme resembled most other cellular topo I derived from a wide variety of eukaryotic sources (Wang, 1985, 1987; Vosberg, 1987; Thrash *et al.*, 1985). On the other hand, the viral enzyme resembled in its unique ionic requirement the topo I from bacteria (Dyran *et al.*, 1981; Fukata and Fukasawa, 1982) and *Xenopus laevis* oocytes (Attardi *et al.*, 1981). These fundamental differences suggest that the viral and the host cell enzymes are two distinct forms of topo I and argue against the possibility that the viral enzymatic activity reflected cellular topo I molecules randomly trapped within the virions during their assembly. However, the findings cannot exclude the possibility that such molecules are packaged within the viral cores via a specific pathway which introduces certain changes in their structure or enzymatic activity to comply with specific requirements of the virus. These changes were probably

reflected by the above described differences. Moreover, our finding that anti-cellular topo I antiserum could block the viral topo I activity might even support this possibility. However, Western blot analysis showed that this antiserum recognized in viral lysates only an 11–11.5 kD protein. Furthermore, affinity purified antibodies isolated from this antiserum provided clear evidence that this protein was identified by the same antibodies which recognize the cellular topo I, and not by distinct antibodies that might exist in this antiserum.

In addition, the topo I antiserum recognized the viral *gag* precursor proteins pr55^{gag} and pr49^{gag} intermediate product which contained the p11 component (Henderson *et al.*, 1987), but not the pr43^{gag} intermediate product from which the p11 fragment was already cleaved out (Henderson *et al.*, 1987). It is therefore evident that the 11 kD protein identified in the viral lysates was the mature viral p11 nucleocapsid protein. This was directly confirmed by showing that anti-EIAV p11 antiserum recognized the 11 kD protein precipitated by the anti-topo I antiserum. Moreover, purified calf thymus topo I protein blocked the recognition of p11 proteins by the affinity purified antibodies and, vice versa, the purified p11 blocked the recognition of topo I. These results suggest that the EIAV p11 and topo I proteins share common epitopes.

It should be emphasized that using computer alignment (Bestfit; Devereux *et al.*, 1984), we could not find any significant amino acid sequence homology between the p11 protein (Stephens *et al.*, 1986) and cellular topo I from any of the eukaryotic (D'arpa *et al.*, 1988) or bacterial (Tse-Dinh and Wang, 1986) sources studied so far, nor from the vaccinia topo I (Shuman and Moss, 1987). Yet, it is well known by now that the immunological specificity of antigens is primarily determined by their three-dimensional configuration. Although Western blot analysis denatured protein structure due to the presence of SDS, it is still possible that similar steric configuration of such structures may be created by certain different amino acid sequences which permit their identification by the same antibodies. Nevertheless, our data indicate that a topo I activity is present in the retroviral core and that the EIAV p11 nucleocapsid and the cellular topo I share similar epitopes.

The question now arises, is there any connection between these two findings? According to the results presented here, we cannot yet attribute the viral topo I activity to the p11 nucleocapsid protein. It is still possible that this activity was related to some non-viral protein that happened to be the same size as the viral p11 protein and to manifest antigenic cross-reactivity with the p11 and the cellular topo I. To resolve this problem more directly, we are now trying to purify to homogeneity an enzymatically active topo I from the viral cores and hope to be able to compare its size and amino acid sequences with corresponding reported parameters of the viral p11 protein. It also remains to be determined whether this enzyme plays a role in the retroviral infectious cycle. If topo I does play a role in the viral life cycle, it could provide a new basis for the chemotherapy of retroviruses, including AIDS.

Materials and methods

Materials

CPT was obtained from the Division of Cancer Treatment, Natural Products Branch, National Cancer Institute. Stock solutions of CPT 10 mM in 100%

dimethylsulfoxide were stored in aliquots at -20°C . Human autoimmune anti-topo I was kindly provided by Dr W.C.Earnshaw, Johns Hopkins University, and Dr N.F.Rothfield, University of Connecticut. Anti-EIAV serum and EIAV p26 antiserum were prepared as described elsewhere (Henderson *et al.*, 1987).

Viruses

HIV-IIIIB and HIV-RF were obtained from Larry Arthur, Biological Products Lab, PRI. The virus had been banded twice in sucrose gradients, pelleted and resuspended in TEN (10 mM Tris, 1 mM EDTA, 0.1 mM NaCl). EIAV was propagated in CF2Th cells, banded twice in Ficoll gradients, pelleted and resuspended in 10 mM Tris-HCl, pH 9.0.

Cells

H9 cells were grown in RPMI supplemented with 15% fetal bovine serum. CF2Th cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% bovine calf serum. All cultures were routinely checked for mycoplasma contamination.

Topo I assay

Virus particles were lysed by adding NP-40 to a 0.03% final concentration and incubating at 37°C for 20 min. The amount of protein was determined by using the Bio-Rad reagent and specific amounts of viral proteins were added (as indicated) to a topo I reaction mixture contained in a final volume of 25 μl : 20 mM Tris-HCl, pH 8.1, 10 mM MgCl_2 , 0.5 mM Na_2EDTA , 1 mM DTT, 20 mM KCl, 20 $\mu\text{g}/\text{ml}$ bovine serum albumin, 1 μg pUC19 plasmid DNA (Vieira and Messing, 1982). Where indicated, CPT was added to a final concentration of 400 μM . The mixture was incubated at 37°C for 30 min, and the reaction was terminated by adding 5 μl of stopping buffer (final concentration: 1% SDS, 15% glycerol, 0.5% bromophenol blue, 50 mM EDTA, pH 8). The reaction products were analyzed by electrophoresis in 1% agarose using TBE buffer (0.089 M Tris-HCl, 0.089 M boric acid, 0.062 M EDTA) at 1 V/cm. The gel was stained by EtBr (1 $\mu\text{g}/\text{ml}$) and photographed using a short wavelength UV lamp. Loss of supercoiled DNA substrate relative to total DNA present was calculated based upon densitometric scans of photographic negatives using a dual wavelength TLC scanner CS-930.

Two-dimensional gel electrophoresis

This was performed as described elsewhere (Minfrod *et al.*, 1986). Briefly, the agarose gel was rotated 90° and 1 $\mu\text{g}/\text{ml}$ EtBr was added to the TBE electrophoresis buffer. Electrophoresis for the second dimension was at 70 V for 2 h.

Western blot analysis

200 μg of viral proteins or 100 μg of nuclear or cytoplasmic extracts were electrophoresed in 10% SDS gel (Lewis and Laemmli, 1982) and electroblotted to nitrocellulose, as described (Towbin *et al.*, 1979). The immunodetection was done with 1:2500 dilution of human autoimmune anti-topo I serum or 1:1000 dilution of anti-p11 EIAV nucleocapsid protein serum using the immunoblot protein A gold assay (Bio-Rad).

Immunoprecipitation assay

Viral particles or EIAV cores were lysed by adding NP-40 to a 0.03% final concentration. The retroviral lysate was treated for 1 h at 4°C with a 50-fold dilution of anti-topo I serum or normal human serum. Protein A Sepharose (0.1 g/ml) was added to each sample for 1 h at 4°C and the immunocomplexes were precipitated. Topo I activity was determined in the supernatant.

Affinity purification of the p11 antibodies from the topo I antiserum

We have used an affinity method described by Smith and Fisher (1984) to elute the antibodies specifically bound to the EIAV p11 protein immobilized on nitrocellulose after blot transfer from SDS-polyacrylamide gel.

The nitrocellulose blots containing five different lanes of EIAV lysates were incubated with topo I antiserum overnight and washed with Tween-TBS (20 mM Tris, 500 mM NaCl, pH 7.5, 0.05% Tween). The region containing the p11 bands were then eluted with three 30 s washes with 5 mM glycine-HCl, pH 2.3, 500 mM NaCl, 0.5% Tween 20, 100 $\mu\text{g}/\text{ml}$ BSA; these eluates were immediately neutralized by Na_2PO_4 to a final concentration of 50 mM. These eluted antibodies were used in a second Western blot as indicated in the individual figure legends.

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