The Domain Organization and Properties of Individual Domains of DNA Topoisomerase V, a Type 1B Topoisomerase with DNA Repair Activities*

Galina I. Belova‡‡, Rajendra Prasad‡§, Igor V. Nazimov‡, Samuel H. Wilson‡, and Alexei I. Slesarev¶¶

From the ¶¶M. M. Shemyakin and Yu A. Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 117871 Moscow, Russia, the ¶¶Laboratory of Structural Biology, NIEHS, National Institutes of Health, Research Triangle Park, North Carolina 27709, and the ¶¶Fidelity Systems, Inc., Gaithersburg, Maryland 20879

Topoisomerase V (Topo V) is a type IB (eukaryotic-like) DNA topoisomerase. It was discovered in the hyperthermophilic prokaryote *Methanopyrus kandleri* and is the only topoisomerase with associated apurinic/apyrimidinic (AP) site-processing activities. The structure of Topo V in the free and DNA-bound states was probed by limited proteolysis at 37 °C and 80 °C. The Topo V protein is comprised of (i) a 44-kDa NH2-terminal core subdomain, which contains the active site tyrosine residue for topoisomerase activity, (ii) an immediately adjacent 16-kDa subdomain that contains degenerate helix-hairpin-helix (HhH) motifs, (iii) a protease-sensitive 18-kDa HhH “hinge” region, and (iv) a 34-kDa COOH-terminal HhH domain. Three truncated Topo V polypeptides comprising the NH2-terminal 44-kDa and 16-kDa domains (Topo61), the 44- and 16-, and 18-kDa domains (Topo78), and the COOH-terminal 34-kDa domain (Topo34) were cloned, purified, and characterized. Both Topo61 and Topo78 are active topoisomerases, but in contrast to Topo V these enzymes are inhibited by high salt concentrations. Topo34 has strong DNA-binding ability but shows no topoisomerase activity. Finally, we demonstrate that Topo78 and Topo34 possess AP lyase activities that are important in base excision DNA repair. Thus, Topo V has at least two active sites capable of processing AP DNA. The significance of multiple HhH motifs for the Topo V processivity is discussed.

*Methanopyrus kandleri* DNA topoisomerase V (Topo V)1 (1–5) relaxes both negatively and positively supercoiled DNA in the temperature range from 60 to 122 °C by catalyzing the transient breakage of a phosphodiester bond in a single DNA strand (reviewed in Refs. 6–8). Topo V is active in an unsurpassable range of monovalent salt concentrations, from 0 to 0.65 M KCl or NaCl and from 0 to 3.1 M of potassium glutamate (K-Glu), and no metal cation or energy cofactor is required for Topo V activity. Cleavage of a phosphodiester bond in DNA involves a transesterification reaction in which the nucleophilic O-4 oxygen of the active site tyrosine (amino acid 226 in Topo V)2 (5) attacks the phosphodiester linkage (6). This results in the formation of a phosphotyrosine bond between the enzyme and the 3' end of the broken strand. This covalent intermediate can be trapped by denaturing the enzyme during catalysis with either SDS or alkali (1, 9). *In vitro* formation of covalent Topo V-DNA complexes involving regular duplex DNA is, however, very inefficient. The one cleavage site mapped so far resembles the consensus site for DNA cleavage by eukaryotic topoisomerase I in the absence of camptothecin (1).

Unlike other known DNA topoisomerases, Topo V has associated apurinic/apyrimidinic (AP) site-processing activities that are important in base excision DNA repair (5). The protein incises the phosphodiester backbone at the AP site, and then at the AP endonuclease-cleaved AP site, it removes the 5’-2’-deoxyribose-5-phosphate moiety so that a single-nucleotide gap with a 3’-hydroxyl and 5’-phosphate can be filled by a DNA polymerase. The importance of the integration of topoisomerase and DNA repair activities in Topo V remains to be understood but it was speculated that such association may prevent Topo V from the formation of covalent protein-DNA adducts in vivo (5).

Sequence analysis revealed that Topo V (984 residues, 112 kDa) can be divided into two parts: the NH2-terminal, ~300 amino acids topoisomerase domain, which contains the active site tyrosine in position 226, and the helix-hairpin-helix (HhH) motif (10–12)-containing domain (22 HhH motifs total) with the lysine- and arginine-rich COOH-terminal end (5).

Weak amino acid sequence similarity exists between Topo V and the eukaryotic type IB topoisomerases and the integrase family of tyrosine recombinases (5, 13, 14). The similarities are limited to the NH2-terminal 300 amino acids of Topo V and are most convincing in the region surrounding active site tyrosines of Topo V and recombinases. The recombinases are mechanistically related to type IB topoisomerases as, similar to topoisomerases, they make a covalent intermediate in which the 3’-phosphoryl group of DNA is esterified to the hydroxyl group of the conserved tyrosine residue (15, 16). A good example is *Escherichia coli* λ integrase, which can relax supercoiled DNA (17) and its truncated mutant, which lacks the COOH-terminal seven amino acids, has increased topoisomerase activity (18).

To further examine the domain structure of Topo V, we subjected the recombinant enzyme to limited proteolysis at different temperatures with endoproteinase Glu-C, trypsin,

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|| To whom correspondence should be addressed: Fidelity Systems, Inc., 7961 Cessna Ave., Gaithersburg, MD 20879. Tel.: 301-527-8775; Fax: 301-527-8250; E-mail: alex@fidelitysystems.com.

1 The abbreviations used are: Topo V, full-length topoisomerase V; Topo678, 75-3a fragment of Topo V; Topo61, 61-kDa fragment of Topo V; K-Glu, potassium glutamate; AP, apurinic/apyrimidinic; HhH, helix hairpin helix; dRP, 2-deoxyribose-5-phosphate; ss, single-stranded DNA.

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inhibitor (20 kDa), and mass markers (Amersham Biosciences) phosphorylase b (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin and thermolysin. The digestion patterns show that the topo-

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and thermolysin. The digestion patterns show that the topoisomerase core domain is a globular, tightly folded segment of the Topo V protein, while the COOH-terminal HhH-containing part is sensitive to proteolysis and is organized into several subdomains. We also find that noncovalent binding of duplex DNA results in protection of the COOH-terminal domains from proteolysis. We demonstrate that the Topo V protein lacking different parts of HhH domain remain fully active in relaxation of supercoiled DNA but become extremely sensitive to high salt concentrations. Finally we find that at least two different active sites capable of processing of AP site DNA are located within the HhH domains. Taken together, our results provide the first structural model for Topo V and provide important insights into the nature of its interaction with DNA. The results also provide further evidence that Topo V and eukaryotic topoisomerase I enzymes belong to different type IB families (5).

**TABLE I**

| Protease         | Polypeptide kDa | NH₂-terminal sequence | Digestion temperature °C |
|------------------|-----------------|------------------------|--------------------------|
| Endoproteinase   | 61              | 1 ALVYDAEFV            | 37                       |
| Glu-C            | 51              | 534 LKRKYGSASA        | 37                       |
| Trypsin          | 78              | 1 ALVYDAEFV            | 37                       |
|                  | 34              | 686 SGRERSEE          | 37                       |
|                  | 92              | 1 ALVYDAEF             | 37                       |
|                  | 16              | 538 YGSASAVR          | 37                       |
| Thermolysin      | 60              | 1 ALVYDAEFV           | 80                       |
|                  | 52              | 524 IVGDLEKAD         | 80                       |
|                  | 44              | 1 ALVYDAEFV           | 80                       |
|                  | 60              | 1 ALVYDAEF            | 37                       |
|                  | 52              | 524 IVGDLEKAD         | 37                       |
|                  | 45              | 582 AELYER            | 37                       |
|                  | 41              | 624 LLNIEG            | 37                       |
|                  | 34              | 686 SGRER             | 37                       |

**EXPERIMENTAL PROCEDURES**

**Cloning and Expression of Topo V Domains**—All plasmids were constructed by common subcloning techniques and propagated in DH5α (Invitrogen) strain of E. coli. The expression plasmid pET21TV encoding the full-size Topo V was previously described (5).

The polymerase chain reaction was used to amplify segments of the topo5 gene from position 1 to 2055 (pET1478), from 1 to 1596 (pET14T78), and from 2056 to 2952 (pET14T34). The 5’ ends of the amplification products contained the NcoI restriction site followed by an initiating methionine codon for residue Met1. The 3’ ends contained stop codons immediately adjacent to the codon for residue Lys534 in case of pET14T78, residue Asp532 in case of pET14T61, and natural Topo V COOH terminus in case of pET14T34, followed by NdeI restriction site in all constructs. The polymerase chain reaction products were digested with NcoI-NdeI and cloned into the plasmid pET14b (Novagen). The resulting plasmids, called pET14T78, pET14T61, and pET14T34 carried the coding sequences for a (i) 78-kDa COOH-truncated form of Topo V called Topo78 that starts at the natural Topo V NH₂ terminus and ends at Lys534, (ii) a 61-kDa COOH-truncated form of Topo V called Topo61 that starts at the natural Topo V NH₂ terminus and ends at Asp532, and (iii) a 34-kDa NH₂-truncated form of Topo V called Topo34 that starts at a methionine immediately 5’ of Gly534 and ends at the natural Topo V COOH terminus. All subcloned sequences that had been subjected to polymerase chain reaction were sequenced to confirm proper construction of the initiation signal and to ensure that no other mutations were introduced. Proteins were overexpressed in E. coli BL21(DE3) pLysS cells (Novagen) and purified essentially as described earlier (2).

**Topoisomerase and DNA Binding Assays**—The standard topoisomerase activity assay was performed by incubating Topo78 or Topo61 with 0.2 μg of supercoiled pBluescript II SK DNA (Stratagene) in 30 mM Tris-HCl, pH 7.0 (at 75 °C), 5 mM EDTA, and 0.1 mM potassium glutato
FIG. 2. Domain organization of Topo V. A, full-length Topo V can be divided in two major structural parts, the NH2-terminal and COOH-terminal containing HhH motifs. NH2-terminal domain and a small part of C-terminal domain form the 44-kDa core subdomain (residues Met1-Leu530), highly resistant to proteolysis. COOH-terminal domain consists of three subdomains: I (residues Leu381-Ser523), II (residues Ile524-Lys685), and III (residues Ser686-Gly984), defined by accessibility to proteolytic cleavage that have been defined by amino-terminal sequencing. Arrows indicate sites of proteolytic cleavage that have been defined by amino-terminal sequencing. Broken arrow indicates an approximate position of the of the thermolysin cleavage. B, schematic representation of expressed Topo V protein; full-length topoisomerase V (Topo V), COOH-terminally truncated Topo V at Lys685 (Topo78) and Asp532 (Topo61), respectively; NH2-terminally truncated Topo V (Topo34), which initiates translation with an engineered methionine immediately upstream of Gly286. C, purified proteins (4 μg each) were fractionated by 12% SDS-PAGE and visualized with Coomassie Blue staining. The positions of molecular mass protein markers are indicated on the left hand side. Lane 1, recombinant full-length Topo V (TV); lane 2, recombinant Topo78 (T-78); lane 3, recombinant Topo61 (T-61); lane 4, recombinant Topo34 (T-34).

Apurinic/Apyrimidinic (AP) Lyase and 2-Deoxycytidine 5-Phosphate (dRP) Lyase Assays—AP lyase and dRP lyase assays were performed essentially as described (19–21). The standard assay of an AP lyase activity consisted in an incubation of 0.05 μg of Topo78 or 0.04 μg of Topo34 with 20 mM 3P-labeled (3P) duplex DNA substrate containing an AP site at position 21 (topo V strand) and 5′-ACAGCCATTCAAGTTAACAAATATTAGCCTGATAGCT-3′, in 50 mM Hepes, pH 7.5, 1 mM EDTA, and 0.03 μM NaCl. The assay was performed at 60 °C for 5 min. The reaction products were stabilized by addition of NaBH4 (340 mM) and then separated by electrophoresis in a 20% polyacrylamide gel (7 M urea, 90 mM Tris/64 mM boric acid/2.5 mM EDTA, pH 8.5). Gels were dried and visualized by autoradiography. The dRP lyase activity was assayed under similar reaction conditions as for AP lyase, except that the uracil-DNA glycosylase-reacted DNA substrate was further treated with AP endonuclease in the presence of 5 mM MgCl2, to generate an incised AP-DNA. Trapping of covalent complexes of Topo78 and Topo61 with DNA by NaBH4 was performed as described previously (1, 21).

RESULTS AND DISCUSSION

Domain Organization of Topo V as Revealed by Limited Proteolysis.—To examine the domain structure of Topo V, we subjected the recombinant full-length Topo V protein to limited proteolytic digestion with a variety of proteases. Endoproteinase Glu-C, trypsin, and thermolysin were found to produce the most informative digestion patterns (Fig. 1). Endoproteinase Glu-C cleaves preferentially at a single site yielding two polypeptides of ~61 and ~51 kDa (Fig. 1A). The sum of the apparent molecular masses of these fragments is equivalent to that of the calculated molecular mass of the full-length protein. NH2-terminal sequencing of these cleavage products by automated Edman chemistry revealed that the 61-kDa polypeptide originates from the amino terminus and the 51-kDa fragment arises via cleavage between amino acids Glu333 and Leu524 (Table I).

Trypsin produces a different digestion pattern. It cleaves Topo V initially between Lys865 and Ser866 to yield a large amino-terminal polypeptide of ~78 kDa and a smaller carboxyl fragment of about 34 kDa (Fig. 1B, lane 3). At increased levels of trypsin the 78-kDa fragment is further converted to the amino-terminal ~62 kDa and carboxyl-terminal ~16 kDa polypeptides via cleavage between Lys537 and Tyr538 (Fig. 1B, lanes 4, 5) as determined by N-terminal protein microsequencing.

Limited proteolysis of Topo V with thermolysin was performed at 37 and 80 °C. The rationale of choosing proteolysis at 80 °C was that this temperature is close to the optimal temperature of Topo V activities (1, 2), as compared with 37 °C (Fig. 1C). Results shown in Fig. 1C indicate that at low concentrations of thermolysin the initial cleavage pattern remains relatively unaffected at different incubation temperatures (Fig. 1C, lanes 2, 3, and 6). In this case thermolysin cleaves Topo V between Ser253 and Ile254 to yield an NH2-terminal polypeptide of ~60 kDa and a COOH-terminal fragment of ~52 kDa. At 80 °C the amino-terminal 60-kDa fragment gets further digested to the NH2-terminal ~44-kDa with a probable carboxyl terminus at Ile186, given the size of produced fragment and the specificity of thermolysin, while the COOH-terminal 52-kDa is cleaved into the number of low molecular weight peptides (Fig. 1C, lanes 4, 5). At 37 °C the 60-kDa polypeptide remains largely resistant to thermolysin digestion, and the NH2-terminal 44-kDa was not detected at all. The 52-kDa carboxyl fragment is degraded stepwise to the ~45-kDa, 41-kDa, and 34-kDa fragments from Ala582 to Leu624, respectively, and, eventually, to the ~34-kDa polypeptide, which is resistant to further digestion and starts at residue Ser666 (Fig. 1C, lanes 7–9). Thus, the thermolysin sensitivity of the 60-kDa NH2-terminal domain at high temperature suggests a significant conformational change, which in turn may activate Topo V
activity. It is worthy to note that Topo V shows no appreciable topoisomerase activity below 60 °C (1).

The revealed domain boundaries parallel those predicted from the sequence analysis of Topo V and from sequence comparisons with other type IB topoisomerases and the integrase family of tyrosine recombinases (5). Namely, the core topoisomerase domain, which is composed of ~300 NH₂-terminal amino acids and contained the active site tyrosine (Tyr226), appears to be a tightly packed globular subdomain and is highly resistant to proteolysis under all conditions tested. In contrast, the rest of the Topo V sequence is apparently organized into more flexible subdomain structures, which can undergo rearrangements at high temperatures.

With the above structural information in mind, we next sought to gain insight into the domain(s) of Topo V that either interact with DNA or undergo a conformational change upon binding to DNA. To this end, we compared the proteolytic digestion pattern of recombinant Topo V in the presence and absence of plasmid DNA. After performing preliminary experiments to ensure that DNA does not act as general inhibitor of thermolysin (see “Experimental Procedures”), we subjected full-length Topo V to digestion with increasing quantities of protease in the presence or absence of plasmid DNA. (Fig. 1, A and B, lanes 6–9, C and D, lanes 6–9). The DNA:protein mass ratio (2:1) was used to ensure that DNA-protein complexes remained soluble (data not shown). At 37 °C the cleavage pattern remains essentially the same with and without DNA for all proteases. A remarkably different picture was observed with thermolysin when the proteolytic pattern was compared with and without DNA at 80 °C (Fig. 1, C and D, compare lanes 1–5). Thus, the specificity of interaction of the 52-kDa domain of Topo V to DNA at 80 °C increases greatly. It also implies that a conformational change may occur in this domain at high temperature upon DNA binding.

Based on limited proteolysis results we concluded that Topo V is organized into four subdomains. The ~380 NH₂-terminal amino acids form the 44-kDa core topoisomerase subdomain (residues Met⁴-Ile⁴⁸⁶), which contains ~4 HhH motifs. This subdomain is highly resistant to proteolysis. The remaining ~580 COOH-terminal amino acids residues are organized into HhH motifs-containing domain, which can be divided into three subdomains: subdomain I (residues Leu⁸³¹-Ser⁹²³), subdomain II (residues Ile⁵²⁴-Lys⁶⁸⁵), and subdomain III (residues Ser⁶⁸⁶-Gly⁸⁸⁴), defined by accessibility to endoproteinase Glu-C, trypsin, or thermolysin (Fig. 2A). The 16-kDa subdomain I and the 34-kDa subdomain III are relatively resistant to digestion, while the 18-kDa region is sensitive to proteolysis and can serve as a hinge between two other subdomains.

Properties of Topo V Domains—First to investigate the biochemical activities residing in these various subdomains of Topo V, we constructed three expression plasmids that encode the following sequences (see “Experimental Procedures”). The recombinant 78-kDa and 61-kDa polypeptides consists of the 44-, 16-, and 18-kDa, and 44- and 16-kDa subdomain, respectively (Fig. 2), whereas, Topo34 subdomain contains the COOH-terminal residues of the Topo V without the first 685 NH₂-terminal amino acids (Fig. 2). The recombinant proteins were expressed in E. coli and purified to apparent homogeneity (Fig. 2B). We found that both Topo78 and Topo61 possess the topoisomerase activity, while Topo34 does not.

To evaluate the temperature dependence of the Topo78 and Topo61 on relaxation activity we incubated these polypeptides in the standard topoisomerase assay buffer at different temperatures with negatively supercoiled pBluescript DNA (Fig. 3, A and B). All these enzymes show almost no relaxation activity below 60 °C. The same low temperature barrier was originally observed for full-size Topo V (1). Both Topo78 and Topo61 produce fully relaxed duplex DNA at 70–75 °C, whereas at higher temperature (above 80 °C) both enzymes generate highly unwound forms of DNA (Fig. 3). This effect, named unlinking (i.e. the substantial reduction in DNA linking number), is caused by DNA melting, and had been described in detail earlier (2, 22).

It is difficult to follow the unlinking reaction on the supercoiled substrate because the products migrate close to, or with, the substrate on the gel. To overcome this problem a simpler assay was designed, in which a relaxed DNA substrate is used to allow the detection of Topo78 and Topo61 unlinking activities easily. The results of this assay are shown in Fig. 3. Topo78 unlinks DNA at 95 °C in the range 2–25 mM NaCl (Fig. 3C), whereas 50 mM NaCl appears to be inhibitory for the Topo78 unlinking activity (Fig. 3C). This is in sharp contrast to full-length Topo V, which can unlink DNA in 200 mM NaCl (2). Results shown in Fig. 3D compare the unlinking activities of Topo78 and Topo61 at 95 °C in the presence of 5 mM NaCl and 5 mM Na₂EDTA (I), 5 mM MgCl₂ (II) or 5 mM CaCl₂ (III). The unlinking activity of Topo78 is almost the same in 25 mM NaCl or in 5 mM NaCl with 5 mM MgCl₂ or CaCl₂ suggesting that the Topo 78 activity was similar to the full-length Topo V activity (2). Thus, the unlinking activity depends on the actual ionic strength of the buffer rather than on specific ions. Topo61 on the other hand, while showing a similar ionic dependence, has
zymes relax supercoiled DNA in a range of 10 to 100 mM NaCl, chose to investigate their salt dependence in detail. Both enzymes relax supercoiled DNA in a range of 10 to 100 mM NaCl (Fig. 3D, lane 13). This could be due to the intensive nicking of duplex DNA, which then melts and appears as single-stranded species at the bottom of the gel. Further experiments are required to elucidate this effect.

Since both Topo78 and Topo61 require much lower salt concentrations for unlinking activity than full-length Topo V, we chose to investigate their salt dependence in detail. Both enzymes relax supercoiled DNA in a range of 10 to 100 mM NaCl/KCl concentrations with an optimum around 30 mM (Fig. 4, A, B, and F and Table II). The highest activity was at ~35 mM for both salts. The replacement of the Cl\(^-\) anion by glu\(^-\) widens the range of Na\(^+\) and K\(^+\) concentrations for enzyme activities up to 250 mM for Topo78 (Fig. 4, C and D) and 150 mM for Topo61 (Fig. 4E). In K-Glu and Na-Glu Topo78 acts somewhat processively, as demonstrated by the appearance of fully relaxed topoisomeres before the disappearance of supercoiled substrate (Fig. 4, C and D).

We had previously established that MgCl\(_2\) had no effect on the relaxation activity of Topo V at the optimal monovalent salt concentrations (see Table II). This was not the case for Topo78 and Topo61. Initially, we found that the highest effect of Mg\(^2+\) and Ca\(^2+\) on their relaxing activities was at 5 mM over a range of NaCl or KCl (data not shown). In the next experiment using different concentrations of Topo78 or Topo61 we compared the relaxation activity of enzymes in 30 mM NaCl (NaCl optimum) and in the presence of 5 mM MgCl\(_2\) or 5 mM CaCl\(_2\) (Fig. 4, G and H). These experiments reveal that divalent metal cations stimulate Topo78 and Topo61 by ~2- and 3-fold, respectively (Fig. 4, G and H). The difference in the effect of divalent ions on Topo V on the one side and on Topo78 and Topo61 on the other can be explained by the fact that Topo V requires higher monovalent salt concentrations for the optimal activity as compared with these polypeptides (Table II). Essentially similar results as described above were obtained in experiments with positively supercoiled plasmid DNA (data not shown).

From comparisons of Topo78 and Topo61 with full-length Topo V two major conclusions can be drawn. First, the specific topoisomerase activity of Topo78 and Topo61 is 10-, and 1000-fold less than that of Topo V, respectively. Second, the optimal and maximal salt concentrations for Topo78 and Topo61 are about 10 times lower than those of Topo V. Taking into account that Topo78 and Topo61 were obtained by progressive trimming the COOH terminus, which contains a number of HhH motifs, we speculated that the HhH motifs play a crucial role in Topo V interactions with DNA at high salt concentrations. These motifs may form a protein structure that anchors the enzyme on DNA in high salt. Furthermore, these motifs render high processivity to Topo V in the very broad range of salt concentrations. Topo78 has a very weak processivity as compared with full-length Topo V, whereas Topo61 was completely lacking processivity.

**Binding of Topo34 to DNA**—The COOH terminus domain (Topo34) spans amino acids 687–984 and consists exclusively of HhH motifs (5). There is no topoisomerase activity associated with Topo34. This is not surprising, as the only active site tyrosine of Topo V is located in its NH$_2$-terminal part and is absent in Topo34. To investigate DNA-binding properties of Topo34 we incubated different amounts of Topo34 at 80 °C in 1 μM K-Glu with different forms of DNA: negatively supercoiled plasmid DNA, linear duplex DNA, and ssDNA (Fig. 5). Protein-DNA complexes were then analyzed in electrophoretic mobility shift assays (23). At the protein:DNA mass ratio 1:3 to 2:1 (Fig. 5, A and B) Topo34 interacts with both supercoiled and linear DNA. It is interesting to note that the protein more readily binds an open circular DNA form, which is topologically equivalent to linear DNA, than supercoiled DNA (Fig. 5A, lanes 3–6).
in A were separated by electrophoresis in a 20% polyacrylamide gel containing 8 M urea. Photographs of the autoradiograms are shown. Complexes of proteins and dRP site containing-DNA were trapped by NaBH₄. Samples were analyzed by SDS-polyacrylamide gel electrophoresis.

Another interesting fact is that Topo34 enhances the electrophoretic mobility of DNA, rather than retards it, which is indicative of DNA compaction by the protein. In other words, its way of interaction with duplex DNA superficially resembles that of archaeal histones (23, 24) rather than transcription factors. The latter retards DNA in gel-shift assays.

Fig. 5C shows the results of the Topo34 interaction with single stranded DNA. There is no change in the mobility of ssDNA at protein:DNA ratios from 1:10 to 1:1 (data not shown). A slight retardation of DNA occurs at the protein:DNA ratio of 1.6:1 and higher (Fig. 5C, lanes 2–4). In all protein:DNA ratios used complexes are retarded in a gel, i.e. apparently no DNA compensation occurs. Compared with duplex DNA, about 5× more Topo34 is required to achieve a noticeable retardation in the mobility of ssDNA upon binding with Topo34. The results of electrophoretic mobility shift assays of Topo34 parallel our earlier finding that Topo V is not inhibited by ssDNA in the presence of 1 M K-Glu or in 0.3 M NaCl and 2.2 M betaine (3) and further prove that the Topo V HhH motifs render unique DNA binding properties of Topo V.

**DNA Repair Activities of Topo V Domains**—We have demonstrated earlier that Topo V possesses two AP site-processing activities that are important in base excision DNA repair (25). Topo V can incise the phosphodiester backbone of the AP site, and also from the AP endonuclease-cleaved AP site it can remove the 5′-deoxyribose 5′-phosphate moiety so that the one-nucleotide gap with a 3′-hydroxyl and 5′-phosphate is generated, which can be filled by a DNA polymerase (5). The Tyr<sup>226</sup>→Phe mutant of Topo V, which is not able to relax supercoiled DNA or to make a covalent phosphotyrosine bond with DNA, has, however, the same level of AP and dRP lyase activities as intact Topo V. Moreover, we have shown that Topo61 failed to repair activities or to make the NaBH₄-mediated covalent complex with dRP site-containing DNA (5).

We performed several experiments to determine whether or not Topo78 and Topo34 possess DNA repair activities. The
results demonstrates that both proteins possess AP lyase (Fig. 6A) and dRP lyase (Fig. 6B) activities. Similar to DNA polymerase β (20, 21, 26), the lyase activity of Topo78 and Topo34 dRP is catalyzed via β-elimination as opposed to hydrolysis. We determined this activity by trapping the proteins with precised AP DNA by using NaBH₄ as the Schiff base-reducing reagent. Topo78-DNA and Topo34-DNA complexes were detected and identified by autoradiography (Fig. 6C). This result implies that the chemical mechanism of the Topo78 and Topo34 dRP lyase reaction proceeds through an imine-DNA intermediate and that the active-site residue responsible for dRP release must contain a primary amine. The Tyr²²⁶ → Phe mutant of Topo78, which has no topoisomerase activity, traps the NaBH₄-mediated covalent complex with dRP site-containing DNA and has the same level of AP and dRP lyase activities as intact Topo V (Fig. 6, A–C).

Thus, these experiments further suggest that the topoisomerase and DNA repair activities of Topo V reside in different parts of the protein and the DNA repair activity is dispensable for the topoisomerase catalytic activity. Finally, these experiments established that Topo V has at least two different active centers capable of processing AP site DNA.

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