A type IB topoisomerase with DNA repair activities

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Previously we have characterized type IB DNA topoisomerase V (topo V) in the hyperthermophile *Methanopyrus kandleri*. The enzyme has a powerful topoisomerase activity and is abundant in *M. kandleri*. Here we report two characterizations of topo V. First, we found that its N-terminal domain has sequence homology with both eukaryotic type IB topoisomerases and the integrase family of tyrosine recombinases. The C-terminal part of the sequence includes 12 repeats, each repeat consisting of two similar but distinct helix-hairpin-helix motifs; the same arrangement is seen in recombination protein RuvA and mammalian DNA polymerase β. Second, on the basis of sequence homology between topo V and polymerase β, we predict and demonstrate that topo V possesses apurinic/apyrimidinic (AP) site-processing activities that are important in base excision DNA repair: (*i*) it incises the phosphodiester backbone at the AP site, and (*ii*) 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. Topo V is thus the prototype for a new subfamily of type IB topoisomerases and is the first example of a topoisomerase with associated DNA repair activities.

All DNA topoisomerases are classified into types I and II, according to the nature of a transient break in DNA, single or double stranded, respectively (1). Type I enzymes are further classified into two families on the basis of their biochemical properties: type IA enzymes form a transient covalent bond to the 5′ phosphoryl of the broken strand, whereas type IB enzymes form a 3′-end covalent bond. Members of the same family share significant sequence similarities and are structurally related (2–5). The type IA family of topoisomerases, which includes topoisomerases I and III (6–8) and thermophilic reverse gyrases (9–12), is widespread in nature and not confined to prokaryotes solely. Until recently, type IB enzymes have been positively identified only in eukaryotes. We have previously purified and characterized type I DNA topoisomerase V (topo V) from the hyperthermophile *Methanopyrus kandleri* (13–15). The enzyme has the following properties in common with eukaryotic topoisomerase I, which distinguish it from all other known prokaryotic topoisomerases: (*i*) its activity is Mg2+-independent; (*ii*) it relaxes both positively and negatively supercoiled DNA; and (*iii*) it makes a covalent complex with the 3′ end of the broken DNA strand. The discovery of topo V suggests that type IB enzymes may also be ubiquitous and not confined to eukaryotes, as originally thought. We have now cloned and sequenced its gene by using peptidic sequence information. The finding of unexpected sequence homology with enzymes involved in DNA repair prompted us to examine whether topo V itself might have DNA repair activities.

Materials and Methods

N-Terminal Sequencing of Topo V and Development of DNA Probes. Sequencing of the N termini of topo V was performed on an Applied Biosystems 471A protein sequencer. Five hundred picomols of topo V (14) was digested with Endoproteinase Lys-C at 37°C for 1 h in buffer containing 25 mM Tris·HCl (pH 8.0) and 400 mM NaCl. The proteolytic fragments were separated by SDS/PAGE, blotted to Immobilon-P transfer membrane (Millipore), and subjected to 45 cycles of Edman degradation. The N-terminal sequence analysis of topo V and its N-terminal fragments was found to be ALVYDAEFVGSEREFEEERET-FLKGVKAYDGVLATRYLMERSSSA. The second N-terminal sequence was derived from a 35-kDa polypeptide and was determined to be KSGRQERSEEEWKEWLERKVGE-GRARRLIEYFGSA. The alanine in the first position indicates that the N terminus was processed.

The flanking seven amino acid stretches of the 45- and 35-aa sequences were used to prepare degenerate oligonucleotide primers. These primers were used in PCR to generate 135- and 105-bp nucleotide probes. The probes were then cloned and sequenced to confirm their usefulness.

Cloning and Sequencing of the *top5* Gene. Genomic DNA of *M. kandleri* and the λ library were prepared as described (16). Two uniformly labeled PCR probes were prepared by using the QuickPrime kit (Amersham Pharmacia) and (α-^{32}P) dCTP (NEN). With these probes, about 2,000 plaques were screened. Five λ clones hybridizing to both probes were isolated. The *top5* gene was cloned on a 6.5-kb *Eco*RI/*Pst*I fragment. This fragment was subcloned into pBluescript II SK(+), generating pAS65, and then used as a template for DNA sequencing. The problems in sequencing of guanine–cytosine-rich (60%) *Methanopyrus* DNA were resolved by the addition of ThermoFidelase (Fidelity Systems, Gaithersburg, MD) to an AmpliCycle kit (Perkin–Elmer). The topo V sequence within the cloned 6.5-kb *Eco*RI/*Pst*I genomic fragment was identified by comparing the translation product with the N-terminal 45- and internal 35-aa sequences derived from protein-sequencing experiments.

Recombinant Proteins. The *top5* gene and the gene fragment covering amino acids 1–532 were amplified by PCR from pAS65 and cloned into pET21b or pET14 vectors (Novagen), generating pET21TV and pET14T61 expression plasmids, which encode full-size topo V and its 61-kDa N-terminal fragments, respectively. Proteins were overexpressed in *Escherichia coli* BL21(DE3) pLysS cells (Novagen) and purified essentially as described (14).

Mutagenesis. The tyrosine residues were replaced with phenylalanine in the *top5* gene by using a site-directed mutagenesis kit (QuickChange, Stratagene) on pET21bTV and pET14T61. After mutagenesis, the presence of desired nucleotide changes was verified by DNA sequencing. Mutant proteins were isolated as described above.

Topoisomerase, Apurinic/Apyrimidinic (AP) Lyase, and 2-Deoxyribose 5-Phosphate (dRP) Lyase Assays. The topoisomerase activity was determined as described (13), except that the truncated topo V

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Abbreviations: HhH, helix-hairpin-helix; HTH, helix-turn-helix; dRP, 2-deoxyribose 5-phosphate; AP, apurinic/apyrimidinic; topo V, topoisomerase V; β-pol, polymerase β.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF311944).

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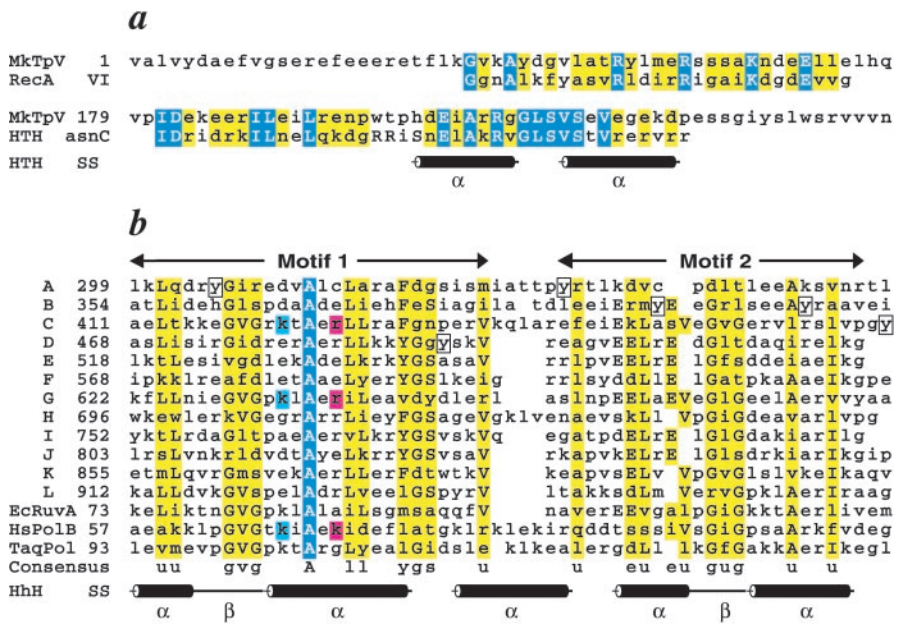

Fig. 1. (*a*) Motifs conserved between topo V, RecA, and leucine-responsive regulator signature sequences. Topo V amino acid region 236–298 made no hits in databases and is not shown. A short region between positions 677–695 connecting repeats G and H and the 19-aa residues at the end of the sequence is not shown for simplicity. Invariant residues are shown on blue backgrounds with white lettering. Conservative positions are highlighted on the yellow background. (*b*) Structure of topo V HhH motifs. Backgrounds of Lys-68 and Lys-72 of β-pol and corresponding positions in C and G repeats of topo V are colored cyan and magenta, respectively. Secondary structures in *a* and *b* were predicted by using JPRED (//jura.ebi.ac.uk:8888/). Cylinders represent α-helices, and lines between them (*b*) represent β-hairpins. Tyrosines that have been substituted for phenylalanines by mutagenesis are boxed (see Fig. 2*a*). MkTpV, *M. kandleri* topo V; HTH asnC, the three-element fingerprint that provides a signature for the HTH motif of the asnC bacterial regulatory proteins; HTH SS, secondary structure of the HTH motif; A–L, topo V's HhH repeats; EcRuvA, *E. coli* RuvA protein, HsPolB, human polymerase β; TaqPol, *T. aquaticus* polymerase I; HhH SS, secondary structure of HhH motifs. ALSCRIPT (30) was used to illustrate the alignments.

proteins were assayed in low salt (0–100 mM NaCl). AP lyase and dRP lyase assays were performed essentially as described (17–19). Briefly, the reaction mixture for the AP lyase activity of topo V consisted of 20 nM ^{32}P-labeled (3′) duplex DNA substrate containing an AP site at position 21 (top strand, 5′-AGCTACCATGCCTGCACGAAUTAAGCAATTCGTA-ATCATGGTCATAGCT-3′), 50 mM Hepes (pH 7.5), 1 mM EDTA, and 0.2 M NaCl. The assay was performed at 60°C for 5 min. The reaction products were stabilized by addition of $NaBH_4$ (340 mM) and then separated by electrophoresis in a 20% polyacrylamide gel (7 M urea, 90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3). Gels were dried and visualized by autoradiography. To quantify the product, gels were scanned on a Molecular Dynamics PhosphorImager model 450, and the data were analyzed by using IMAGEQUANT software. 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 $MgCl_2$ to generate an incised AP-DNA.

Trapping of topo V–DNA covalent complex by alkali or $NaBH_4$ was performed as described previously (13, 19).

Gel Retardation Assay. Reaction mixture containing 200 nM topo V, 20 nM ^{32}P-labeled substrates (double-stranded oligonucleotides containing uracil, AP, or dRP sites at position 21), 50 mM Hepes, pH 7.5, 0.2 M NaCl was incubated for 5 min at 37°C. An equal volume of 2× Tris·borate EDTA buffer with 20% glycerol was added to the binding reaction, and the samples were analyzed by 6% PAGE. The extent of binding was visualized by autoradiography.

Results and Discussion

Cloning and Sequence Analysis of TOP5. Topo V has been cloned on a 6.5-kb *Eco*RI/*Pst*I genomic fragment, which was identified by screening of a *M. kandleri* λ library with two PCR probes derived from N-terminal and internal protein sequences of topo V. Sequence analysis of topo V revealed an ORF encoding a protein of 988 amino acids, with a predicted molecular mass of 112 kDa. This is in a very good agreement with the molecular mass of 110 kDa deduced from SDS/PAGE (2). The coding sequence agrees with the protein-sequencing data, although there are 5 codons for RVKVV directly 5′ of the codon for the first alanine. The translation starting point of the *top5* gene has not yet been identified. On the basis of our sequencing data, we assume that the initiator codon is the first GTG (valine) preceding GCG (alanine). The lack of a good promoter sequence upstream of the *top5* gene, the presence of a probable endonuclease V gene that terminates within 12 nt of the topo V initiator GTG, and the presence of the several stop codons after *top5* suggest that *top5* may be the terminal gene of a multicistronic message.

Analysis of the topo V sequence with the dot-matrix program DOTTER (20) revealed that the sequence starting at position ≈300 contains a number of repeats. Protein databases were then screened separately with the 300 N-terminal and 684 C-terminal amino acids by using PRINTSSCAN (http://www.bioinf.man.ac.uk/dbbrowser/PRINTS), FASTA (http://www.ebi.ac.uk/fasta3), and BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) programs to detect similarities. We could not retrieve any topoisomerase sequence in either case. However, PRINTSSCAN searches with the N-terminal 300 aa gave significant scores with the RecA motif VI sequence and with the HTHasnC fingerprint of the leucine-responsive bacterial regulatory proteins from the PRINTS database (Fig. 1*a*). The RecA motif VI includes the RecA signature nonapeptide encoded by PROSITE pattern RECA (PS00321). This region is part of the monomer–monomer interface in a RecA filament. HTHasnC is a three-element fingerprint that provides a signature for the helix-turn-helix motif (HTH) of the asnC proteins. Different parts of the 684 C-terminal amino

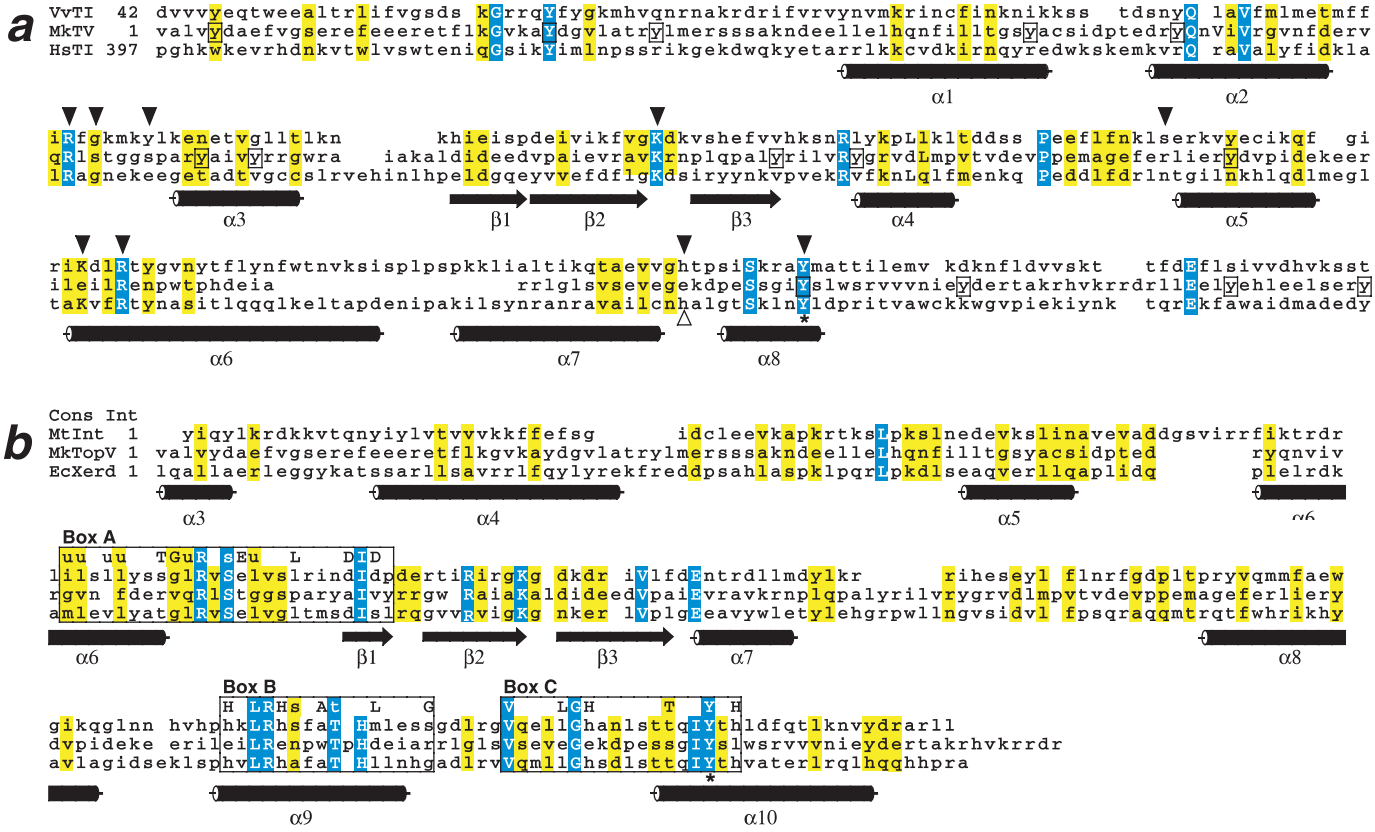

Fig. 2. (*a*) The 270-aa N-terminal part of topo V is aligned with the homologous segments of Vaccinia virus topoisomerase I (VvTI) and human topoisomerase I (HsTI). Sequence gaps are indicated by spaces. Conserved and homologous regions are marked as in Fig. 1. The active-site tyrosine is indicated by the asterisk. Residues important for DNA cleavage and religation chemistry are denoted by filled arrowheads according to ref. 4. The unfilled arrowhead denotes the insertion position of a nonconserved linker in the human enzyme. The secondary structure of vaccinia topoisomerase is shown underneath. (*b*) The 252-aa N-terminal part of topo V is aligned with *Methanobacterium thermoautotrophicum* integrase, *E. coli* XerD recombinase sequences, and Box A, B, C consensus sequences derived from the alignment of 75 prokaryotic recombinases (26). The secondary structure of *Eco* XerD recombinase is shown under its sequence.

acid region exhibited similarities with recombination protein RuvA, human DNA polymerase β (β-pol), *Taq* DNA polymerase (Fig. 1*b*), Endonuclease III, NAD-dependent DNA ligases, and other proteins (not shown). In all cases, the homology was confined to 20–25 amino acids, corresponding with the helix-hairpin-helix (HhH) DNA-binding motif (21). This motif, identified in 14 homologous families of DNA-binding proteins (22), provides nonsequence-specific interactions of proteins with DNA and contrasts with the sequence-specific interactions of other motifs such as HTH motifs (23). A DOTTER-assisted visual inspection of the 684 C-terminal amino acids revealed 12 repeats, each ≈50 aa long. All repeats consist of two similar but distinct HhH motifs, and the same arrangement is seen in recombination protein RuvA and mammalian β-pol. We found that truncated topo V that lacks of repeats E–L (Topo-61) (Fig. 1*b*) remains fully competent for topoisomerase activity, but its mode of action changes from a highly processive (14) mode to a distributive one (unpublished work).

Identification of an Active Site Tyrosine in Topo V and Similarity with Tyrosine Recombinases and Type IB Topoisomerases. In all topoisomerases, DNA cleavage is initiated by the attack on the phosphodiester bond by a tyrosine residue to form a covalent DNA-protein transient intermediate (1, 24). The conserved type IB active site motif Ser-Lys-x-x-Tyr (4) does not occur in the topo V sequence. Therefore, we have replaced by site-directed mutagenesis all 22 tyrosines by phenylalanines within the 532 N-terminal amino acids of topo V (corresponding Ys are boxed in Figs. 1*b* and 3*a*). All recombinant proteins were purified, and their relaxation activity was investigated. Most of the mutant topoisomerases relaxed supercoiled DNA with the same level of activity as did wild-type topo V. Tyr-5→Phe, Tyr-30→Phe, and Tyr-289→Phe mutant proteins had decreased topoisomerase activity (2- to 5-fold; data not shown), and only the Tyr-226→Phe mutant revealed no topoisomerase activity. Further evidence that Tyr-226 may be the active site thyrosine comes from the label transfer experiments (13, 25), where the transfer of a radioactive phosphate occurred from a uniformly labeled DNA to the protein through the formation of a covalent DNA-(3′-phosphotyrosyl)–enzyme complex, in the case of topo V, but did not occur with its Tyr-226→Phe mutant (Fig. 4*b*). This experiment proves that no protein–DNA covalent complex is formed in case of mutant topo V.

Using Tyr-226 as an anchoring point, we tried to locally align ≈300 N-terminal amino acid residues of topo V with C-terminal amino acid residues of eukaryotic topoisomerase I proteins and the family of integrase proteins of tyrosine recombinases (26). In Fig. 2*a*, the N-terminal sequence of topo V is aligned with Vaccinia virus and human topoisomerase I C-terminal fragments. The overall homology is very weak. However, it could be significant that the majority of conserved residues identified by mutagenesis as being important for DNA cleavage and religation chemistry in eukaryotic topoisomerases I (4) are conserved in topo V (Fig. 2*a*). Specifically, three of five side chain amino acid residues (other than the active site tyrosine) that participate in transesterification chemistry and are conserved in every member

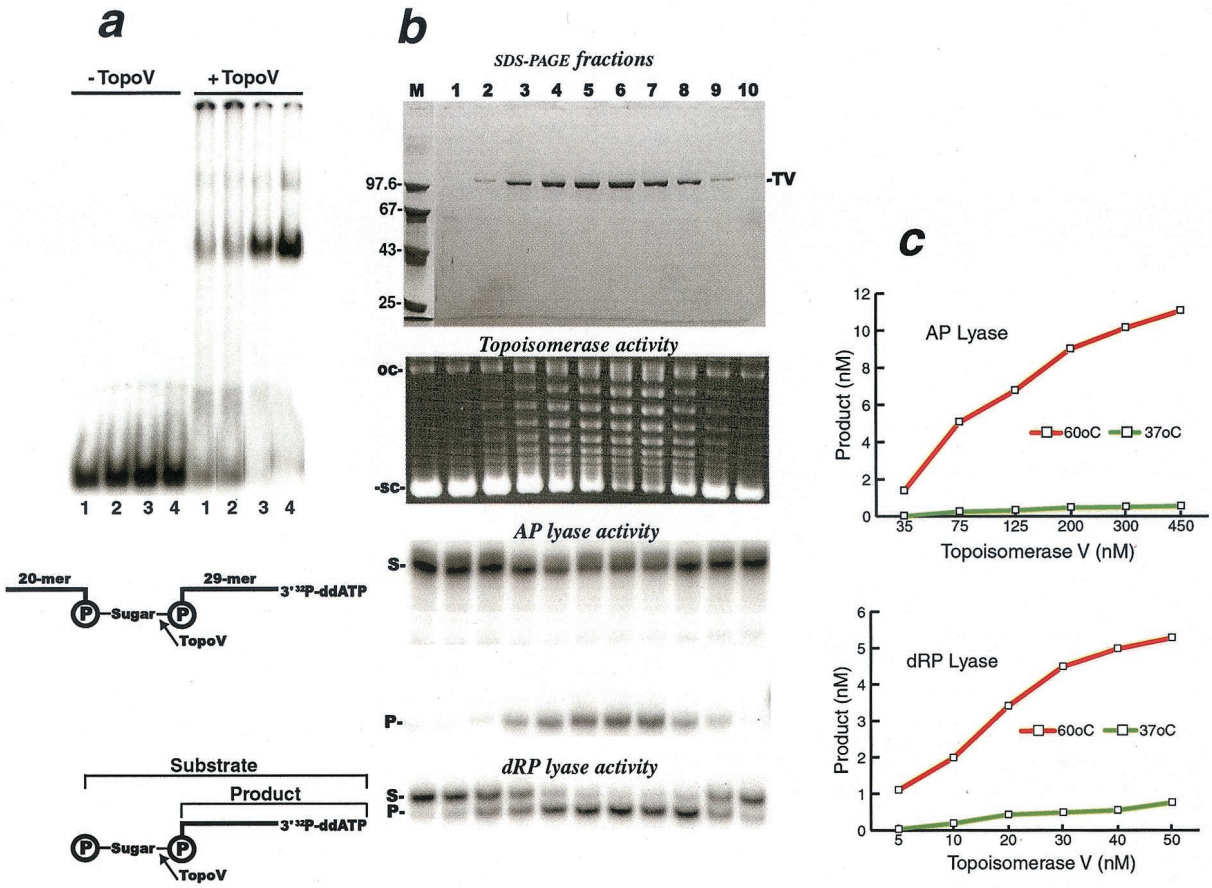

Fig. 3. AP and dRP lyase activities of topo V. (*a*) Gel retardation assay was performed with a ^{32}P-labeled double-stranded 49-mer oligonucleotide containing no mismatch (1), G-U mismatch (2), dRP site (3), and dRP site (4). (*b*) Topo V fractions after heparin chromatography were analyzed by SDS/PAGE, and the same fractions were assayed for topoisomerase activity, AP lyase, and dRPase activities. Drawings to the left of the AP and dRP lyase sections represent AP and dRP site DNAs, respectively, and illustrate DNA substrates and the expected products formed as a result of topo V-catalyzed incision of 5′-terminal deoxyribose-phosphate. oc, -sc indicate positions of open circular and negatively supercoiled forms of plasmid DNA; P, S indicate positions of substrate and product DNAs in AP lyase and dRPase assays. (c) AP and dRP lyase activities of topo V at 37°C and 60°C.

of the type IB family (Arg-130, Lys-167, and Arg-223 in the vaccinia sequence) are also present in topo V sequence. However, it is worthy to note that two other conserved amino acids (Lys-220 and His-265 in the vaccinia sequence) are not present in topo V.

Fig. 2*b* shows the alignment of topo V with two recombinases. Again, the overall homology is scant, particularly in the N-terminal part of sequence. The highest degree of homology between topo V 300 N-terminal amino acids and two integrase sequences occurs in conservative clusters, designated Boxes A, B, and C, according to ref. 26 (Fig. 2*b*). The presence of global similarities between recombinases and three clusters were revealed by sequence analysis of 75 prokaryotic recombinase proteins (26).

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 (27, 28). A good example of the intimate relationship between prokaryotic recombinases and type IB topoisomerases is *E. coli* λ integrase, which can relax supercoiled DNA (29) and its truncated mutant, which lacks the C-terminal seven amino acids, has increased topoisomerase activity (30). Therefore, it is most instructive that the revealed sequence similarities between topo V on one side and type IB topoisomerases and recombinases on the other are confined to amino acid residues that are important for transesterification chemistry (in the case of topoisomerases) or located within the clusters of global similarities (in the case of recombinases). Whether the nonconserved residues can be accommodated into the common fold of the tyrosine recombinase and type IB topoisomerase remains to be seen. Considering that *M. kandleri*, from which topo V was isolated, is positioned outstandingly deep within the 16S rRNA-based phylogenetic tree (31, 32), the N-terminal domain of topo V may represent a catalytic domain of a last common ancestral strand transferase.

DNA Repair Activities of Topo V. The HhH motifs in repeat C of topo V are arranged very similarly to the HhH motifs of human β-pol (Fig. 1*b*). Residues Lys-68 and Lys-72 in the 8-kDa N-terminal domain of β-pol were found to be essential for the removal of the dRP-containing termini and for the binding of the 8-kDa domain to single-stranded DNA, respectively (17). Recently, it has been also shown that Lys-72 is the sole Schiff base nucleophile in the 8-kDa domain of β-pol (33). We found that the corresponding positions in C and G repeats of topo V are either conserved or have homologous substitutions. This prompted us to see whether topo V and its domains possess AP lyase and dRP lyase activities.

We performed experiments to show that topo V possesses the AP lyase and dRP lyase activities. First, we found in a gel-retardation assay that topo V has better affinity to AP- and

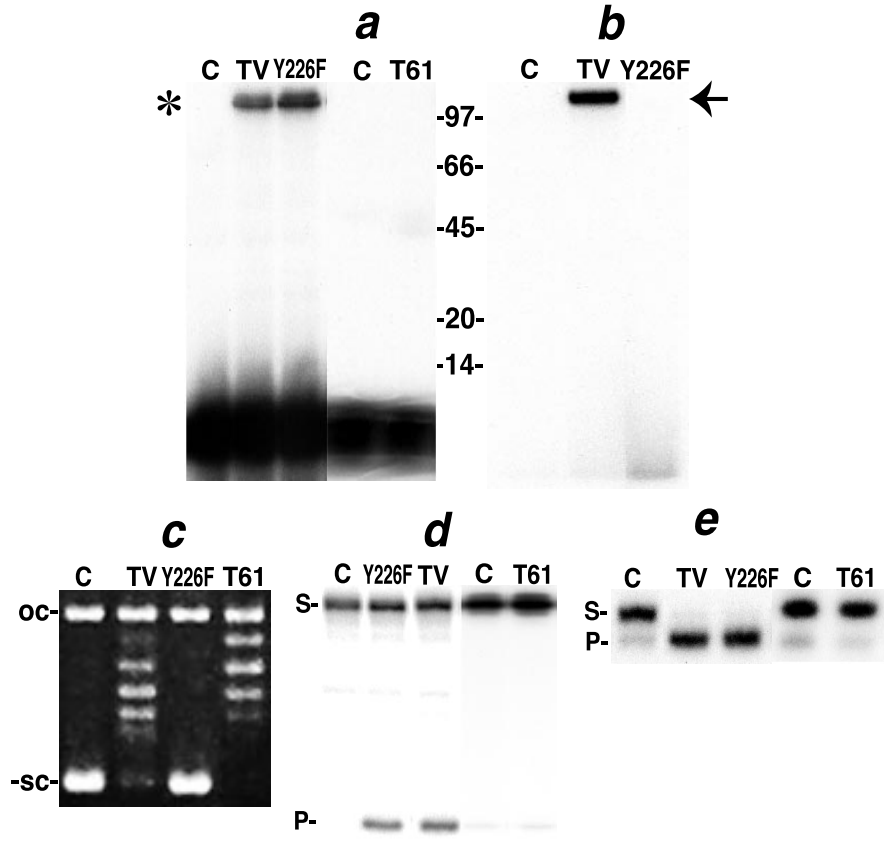

Fig. 4. Biochemical activities of topo V, its Tyr-226→Phe mutant, and Topo-61. (*a*) The topo V (TV), topo V Tyr-226→Phe mutant protein or Topo-61 (T61) protein covalent complex with dRP site containing DNA was trapped by the addition of $NaBH_4$; C, no protein was added. Samples were analyzed by SDS/PAGE. The asterisk indicates the position of the $NaBH_4$-mediated topoisomerase–DNA complex. The migration distances of the protein molecular size markers are marked between *a* and *b*. (*b*) The topo V or topo V Tyr-226→Phe mutant protein was assayed in formation of the covalent topoisomerase–DNA complex by treatment with alkali. The arrow points at the position of the topoisomerase–DNA complex. *c*, *d*, and *e* show the topoisomerase, AP lyase, and dRP lyase activities of topo V, the topo V Tyr-226→Phe mutant protein, and Topo-61.

dRP-site-containing DNA as compared with normal DNA (no mismatch) or G-U mismatch DNA (Fig. 3). In this experiment, topo V was preincubated with ^{32}P-labeled duplex oligonucleotide containing either no mismatch (lane 1), G-U mismatch (lane 2), and AP site (lane 3) or a dRP site at the 5′ end in the gap, i.e., DNA substrate in lane 3 was treated with both uracil–DNA glycosylase and AP endonuclease (lane 4) and followed by analysis of complexes by nondenaturing PAGE. This experiment revealed that topo V has the preference to the dRP-group containing DNA when compared with other substrates (Fig. 3*a*, lane 4).

Next, several experiments were performed to show that both AP and dRP lyase activities are associated with the topo V protein rather than a contaminating protein coming from the host *E. coli*. Recombinant topo V was purified to near homogeneity (>95% purity) and in our purification scheme, the first step involves boiling of the crude cell lysate and removal of the denatured proteins by centrifugation. Therefore, the presence of a protein with AP or dRP lyase activity originating from *E. coli* is unlikely. Furthermore, protein fractions after heparin chromatography were analyzed for topoisomerase, AP lyase, and dRP lyase, and the peak of these activities coincides with the protein peak (Fig. 3*b*). These results suggest that AP and dRP lyase activities are intrinsic to topo V.

In agreement with the thermophilic nature of topo V, its AP and dRP lyase activities are much higher at 60°C than at 37°C (Fig. 3*c*). We failed to measure AP and dRP lyase activities at higher than 60°C because the AP site containing DNA was unstable at higher temperatures.

Similar to β-pol (18, 19, 34), the topo V dRP lyase activity occurs via β-elimination as opposed to hydrolysis. We demonstrated this by trapping the protein with preincised AP DNA by using $NaBH_4$ as the Schiff base reducing reagent. Topo V–DNA complex was detected and identified by autoradiography (Fig. 4*a*). This result implies that the chemical mechanism of the topo V 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 Tyr-226→Phe mutant of topo V is able neither to relax supercoiled DNA nor to make a covalent phosphotyrosine bond with DNA (Fig. 4 *b* and *c*). However, it makes the $NaBH_4$-mediated covalent complex with dRP site-containing DNA and has the same level of AP and dRP lyase activities as intact topo V (Fig. 4 *a*, *c*–*e*). By contrast, truncated topo V, which lacks 452 C-terminal amino acid residues (Topo-61), retains topoisomerase activity (Fig. 4*c*) but shows no appreciable AP and dRP lyase activities (Fig. 4 *d* and *e*). Topo-61 also failed to make the $NaBH_4$-mediated covalent complex with dRP site-containing DNA (Fig. 4*a*). These experiments suggest that 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.

Deamination of cytosine to uracil is the most common promutagenic change in DNA, and it is greatly increased at the elevated growth temperatures of hyperthermophiles. Recent

findings indicate that in hyperthermophiles, enzymes involved in a conventional excision-repair pathway required for the essential replacement of uracil to cytosine may be different from those of *E. coli* or eukaryotes. In particular, uracil–DNA glycosylases (UDG) of hyperthermophilic *Thermotoga maritima* and *Archaeoglobus fulgidus* differ significantly from the *E. coli*/eukaryotic/viral UDG enzymes at the amino acid sequence level (35, 36). DNA polymerases from hyperthermophilic archaea (including *Vent* and *Pfu*) specifically recognize the presence of uracil in a template strand and stall DNA synthesis before mutagenic incorporation of adenine (37). In this context, DNA topo V with its AP and dRP lyase activities contributes further to the unusual features of hyperthermophilic enzymes involved in base-excision repair.

Why important DNA repair activities are fused to a topoisomerase domain in the case of topo V remains to be understood. It is possible that a C-terminal HhH motif has been multiplied within topo V to allow its binding to DNA at high intracellular ionic conditions of *M. kandleri* [>3 M [K^+] (38)], and the lyase activity of the enzyme is merely an incidental consequence of tight binding to AP sites. Perhaps the HhH domain targets topo V to these sites not for lyase repair but to trigger topoisomerase action, for example, to resolve intermediates made during repair of these sites by homologous recombination. It is tempting also to consider that the HhH domain with its lyase activities could work in concert with topoisomerase activity. *M. kandleri* thrives at "black smoker" conditions, at temperatures up to 110°C (38), and therefore has a high probability of occurrence of DNA lesions. DNA lesions greatly enhance type IB topoisomerase I-mediated DNA cleavage and formation of covalent protein–DNA adducts (39). In eukaryotes, topoisomerase I lesions can be repaired by a Tyr-DNA phosphodiesterase, which is absent in prokaryotes (40). Thus the integration of DNA repair activity highly specific for AP sites with the topo V activity in a single protein might prevent topo V from becoming a poison for *M. kandleri* cells. A regular type IB topoisomerase would produce protein–DNA adducts with high frequency in or around AP sites. In the case of topo V, its HhH domain, containing AP lyase or dRPase active sites, may bind specifically to an AP site containing DNA and initiate base-excision repair, thereby preventing topo V from making chromosomal aberrations. In this regard, it may be important that topo V is an abundant protein in *M. kandleri* (more than 3,000 copies/cell) with a very high turnover rate (about 4 cycles/s/enzyme monomer at 108°C) (14).

Topo V, with its multiple activities, may also represent an example of an efficient usage of genetic material in *M. kandleri*, considering its relatively small genome size (1.8 Mb). In this regard, it may be important that topo V contain a signature motif of leucine-responsive regulatory proteins and therefore might also act as a transcriptional regulator, in addition to being a topoisomerase and an AP lyase.

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