Communication between the ATPase and Cleavage/Religation Domains of Human Topoisomerase IIα*

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The DNA strand passage activity of eukaryotic topoisomerase II relies on a cascade of conformational changes triggered by ATP binding to the N-terminal domain of the enzyme. To investigate the interdomain communication between the ATPase and cleavage/religation domains of human topoisomerase IIα, we characterized a mutant enzyme that contains a deletion at the interface between the two domains, covering amino acids 350–407. The ATPase domain retained full activity with a rate of ATP hydrolysis that was severalfold higher than normal, but the ATPase activity was unaffected by DNA. The cleavage and religation activities of the enzyme were comparable with those of the wild-type enzyme both in the absence and presence of cancer chemotherapeutic agents. However, neither ATP nor a non-hydrolyzable ATP analog stimulated cleavage complex formation. Although both conserved domains retained full activity, the mutant enzyme was unable to coordinate these activities into strand passage. Our findings suggest that the normal conformational transitions occurring in the enzyme upon ATP binding are hampered or lacking in the mutant enzyme. Consistent with this hypothesis, the enzyme displayed an abnormal clamp closing activity. In summary, the region covering amino acids 350–407 in human topoisomerase IIα seems to be essential for correct interdomain communication and probably is involved in signaling ATP binding to the rest of the enzyme.

Human DNA topoisomerase II is a multifunctional and highly complex enzyme that is able to change the topological conformation of DNA in response to different physiological alterations (1–3). Topological changes mediated by the dimeric topoisomerase II enzyme require a strict control of the passage of duplex DNA through the whole subunit interface (4) and through another duplex coordinately cleaved by the enzyme, where a correct interdomain as well as intersubunit communication is fundamental.

Topoisomerase II consists of three distinct domains. The N-terminal and central domains are highly conserved among enzymes from different eukaryotic organisms and also share homology to the gyrase B and A subunits, respectively (5–9). The C-terminal domain is dispensable for in vitro catalytic activity and shows no sequence conservation (9–11). Central to the activity of the enzyme is its ability to bind and hydrolyze ATP as well as to cleave and religate DNA. The active site for ATP hydrolysis is encompassed in the N-terminal domain (12–14), while that for DNA cleavage/religation is located in the central domain (15).

Structural and biochemical data have suggested a rational model for the catalytic mechanism of eukaryotic topoisomerase II (16–19). According to this model, the two subunits of the enzyme form a heart-shaped ring structure, where the N-terminal domains protrude as a set of jaws functioning as an ATP operated clamp. In the absence of ATP, the enzyme assumes an open conformation with a gate in the N-terminal part of the enzyme. The open state can permit a DNA segment (the so-called G-segment) to enter the enzyme through the N-terminal face and bind to the cleavage/religation domain. Binding of the DNA segment will induce the first conformational change in the enzyme, which enables the active site tyrosines to move toward each other into a position whereby cleavage can occur. Upon binding of ATP and hydrolysis of the first ATP molecule, other conformational changes occur, facilitating dimerization of the N-terminal domains (20). In this process, the other duplex segment (the so-called T-segment) that has to be transported through the broken DNA is captured by the enzyme. Besides DNA trapping, the conformational changes also ensure the creation of a gate in the G-segment by separation of the two active site tyrosines covalently linked to the broken DNA ends.

In a recent study by Maxwell and co-workers (22), a human topoisomerase IIα fragment covering the N-terminal domain from amino acid 1 to 439 was shown to have an intrinsic ATPase activity, which could be further stimulated by the presence of DNA. In another study, a core domain of Drosophila topoisomerase II covering amino acids 406–1207 was demonstrated to have wild-type levels of cleavage/religation activity (23). The results from the two studies nicely illustrate that the individual domains in topoisomerase II still preserve their intrinsic activities even when separated from the rest of the enzyme, demonstrating that they fold up as independent cata-

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Uncoupling Domain Activities in Human DNA Topoisomerase IIα

lytic domains. However, strand passage activity requires a tightly coordinated communication between the individual domains. This is indicated by a study of the ATP consumption by topoisomerase II performed by Lindsley and Wang (24), where it was shown that a tight coupling exists between ATP usage and the DNA strand transport event under unsaturated ATP concentrations. Furthermore, several studies have revealed a stimulatory effect of ATP on topoisomerase II-mediated cleavage, also indicating the communication between enzyme subdomains (25, 26).

In order to investigate the communication between the ATPase and cleavage/religation domains of human topoisomerase IIα, we characterized a mutant enzyme having a deletion at the interface between the two domains. The enzyme contained both ATPase and cleavage/religation activities, but no strand passage occurred. Furthermore, the DNA cleavage activity was independent of ATP, and rates of ATP hydrolysis were unaffected by the presence of DNA. Finally, the mutant enzyme lacked a normal clamp closing activity. In summary, the deleted region seems to be essential for correct interdomain communication and probably is involved in signaling ATP binding to the rest of the enzyme.

EXPERIMENTAL PROCEDURES

Yeast Strains and Plasmids—The Saccharomyces cerevisiae strains BJ201 (Mata ura3-52 trp1 pep4:HY3 prb1 can1 top2:TRP1) and JEL1ΔTop1 (kindly provided by J. C. Wang) were used for complementation and overexpression of topoisomerase II constructs, respectively. Plasmid pBY105 contains the yeast TPI promoter inserted into the polylinker region of the LEU2/ARS-CEN plasmid pRS315, which was used as the backbone for pHT212 and pHT350–407, carrying the wild-type human TOP2α cDNA and the human TOP2α cDNA with a deletion spanning amino acids 350–407, respectively. Both pHT212 and pHT350–407 contain a bicomposite tag at the C-terminal end consisting of the c-Myc epitope and a hexahistidine tail (9). Modified versions of YEpWOB6 were used for overexpression of the hexahistidine-tagged human topoisomerase IIα and h350–407 enzymes.

Construction of Plasmids—The construction of pHT212 and pHT350–407 was described by Jensen et al. (9). pHT212 was used as the positive control. pHT350–407 was transformed into BJ201, and cells were transferred to media plates containing 5 mg/ml -fluoro-orotic acid (1 mg/ml) to select against the URA3 plasmid carrying the corresponding fragment from pHT350–407 enzyme to complement the deficiency of the h350–407 enzyme in the YEpWOB6 system, the overexpression of the h350–407 enzymes.

To generate polymerase chain reaction fragment was used to replace the aeg-specific fragment from pHT350–407 enzyme to complement the deficiency of the h350–407 enzyme in the YEpWOB6 system, the overexpression of the h350–407 enzymes.
topoisomerase II and, when indicated, 1 μg of negatively supercoiled pHBR32. Reactions were carried out in 20 μl of assay buffer containing a final concentration of 1 mM cold ATP and 1 mM [γ-32P]ATP (3000 Ci/mmol; Amersham Pharmacia Biotech). Mixtures were incubated at 37 °C, and 2.5-μl aliquots were removed at various times and spotted onto thin layer cellulose plates impregnated with poly(ethyleneimine) (Baker-flex precoated flexible TLC sheets). Chromatography was performed using freshly made 0.4 M NH4HCO3. Levels of free PO4 were quantified using a PhosphorImager.

Clamp Closing Assay—For clamp closing experiments, 0.06 pmol of topoisomerase II was preincubated with 6 ng of supercoiled pHBR32 and 6 ng of linearized pHBR32 at 37 °C for 5 min in a total volume of 20 μl of assay buffer. After preincubation, AMP-PNP or ATP was added to a final concentration of 1 mM, and the reactions were incubated for an additional 5 min at 37 °C. The reactions were next stopped by the addition of either NaCl or SDS to final concentrations of 800 mM and 1%, respectively. The sample volume was increased to 70 μl by adding 50 μl of an 800 mM NaCl solution. To trap enzyme-DNA catenanes, phenol extraction was performed by adding 1 volume of phenol. The samples were vortexed and centrifuged at 13,000 rpm in an Eppendorf centrifuge for 15 min. The water phase was removed, and 35 μl of it was ethanol-precipitated and dissolved in 10 μl of TE buffer for gel volume analysis. The combined phenol and phenol interphase was washed three times with 500 μl of 800 mM NaCl after increasing the sample volume to approximately 100 μl with phenol. Upon removal of the water phase after the last wash, the remaining material was ethanol-precipitated and dissolved in 10 μl of Proteinase K buffer containing 1 mg/ml Proteinase K and 0.5% SDS. The samples were next subjected to electrophoresis in 1% agarose gels in TBE. Southern blotting was performed using Zeta-Probe GT membranes (Bio-Rad), and random primed plasmids were used for hybridization (Roche Molecular Biochemicals). A PhosphorImager (Molecular Dynamics) was used for gel scanning.

RESULTS

Purification and Characterization of a Human Topoisomerase IIa Enzyme Lacking the Interface between the ATPase and the Cleavage/Religation Domains—ATP binding and hydrolysis by topoisomerase IIa is known to mediate sequential conformational changes in the enzyme reaching from the N-terminal clamp to the C-terminal dimerization region (16–20). In order to investigate the communication between the ATPase and the cleavage/religation domains of human topoisomerase IIa, we characterized a mutant enzyme (hΔ350–407) that contains a deletion of amino acids 350–407 at the very C-terminal end of the N-terminal ATPase domain (Fig. 1A). The deletion mutant has been presented earlier as one in a series of human topoisomerase IIa mutants all having deletions in highly conserved subdomains (9). In a complementation assay using an s. cerevisiae top2 deletion strain, hΔ350–407 failed to sustain mitotic growth in contrast to a wild-type human topoisomerase IIa enzyme, suggesting that the mutant enzyme has lost its in vivo activity (Fig. 1B). For studies of the in vitro capabilities of hΔ350–407, the mutant enzyme fused to a hexahistidine tail at the C-terminal end was overexpressed in a yeast top1 null strain and purified to homogeneity as seen from the Coomassie-stained gel in Fig. 1C.

To investigate the DNA strand passage activity of hΔ350–407, a DNA relaxation assay was performed, where the catalytic activity of the mutant enzyme was compared with that of the wild-type enzyme (Fig. 2). While the wild-type enzyme relaxed all the supercoiled DNA within 10 min, the deletion mutant showed no sign of relaxation up to 15 min. Thus, consistent with the lack of in vivo complementation, hΔ350–407 appears to have lost its in vitro relaxation activity, or it is diminished to under detectable levels. A similar lack of DNA strand passage was observed in a DNA decatenation assay (data not shown). These results demonstrate that deletion of the C-terminal 57 amino acids of the ATPase region in human topoisomerase IIa is detrimental to the enzyme. Either it abrogates the DNA strand passage activity of the enzyme, or it disrupts correct folding of the protein.

Characterization of the N-terminal ATPase Domain of hΔ350–407—In order to determine whether the individual domains of hΔ350–407 still retain their residual activities although the enzyme is unable to convert these activities to full catalysis, we analyzed the capability of the N-terminal domain of the deletion mutant to hydrolyze ATP. According to the model presented by Lindsley, ATP hydrolysis is responsible for
Characterization of the Central Cleavage/Religation Domain

Earlier studies on topoisomerase II-mediated ATP hydrolysis have shown that this activity is stimulated in the presence of DNA, both when the ATPase region is embodied in a full-length enzyme or in an N-terminal topoisomerase II fragment (22, 24, 31–34). In the present study, the ATPase activity of the wild-type human topoisomerase IIα enzyme was stimulated 2–3-fold in the presence of supercoiled plasmid DNA (Fig. 3, inset). However, no stimulation was observed with hΔ350–407, indicating that the ATPase activity of the mutant enzyme is unaffected by DNA. The significant rise in the ATPase activity and its DNA independence combined with the lack of strand passage activity indicate an uncoupling of the N-terminal domain from the rest of the enzyme. The uncoupling might be caused either by an inability of the mutant enzyme to transmit essential conformational changes as a result of ATP binding and hydrolysis or an inability to interact properly with DNA.

Characterization of the Central Cleavage/Religation Domain of hΔ350–407—In order to test if the central domain of the hΔ350–407 enzyme still retained its intrinsic DNA binding and cleavage activities, cleavage experiments were performed using either oligonucleotides or supercoiled circular DNA as substrate.

The suicide substrate, which is schematically illustrated in Fig. 4A (upper panel) consists of a 16-base-long 5’-recessed top strand with only three nucleotides 5’ to the cleavage position and a 28-base-long bottom strand. Use of the suicide substrate has been demonstrated to cause an uncoupling of the cleavage and ligation half-reactions due to the release of the trinucleotide 5’ to the cleavage position on the top strand (29). The substrate was labeled at the 3’-end of the recessed top strand and incubated with either hΔ350–407 or the wild-type enzyme. Samples were withdrawn at different times, and after termination of the cleavage reaction by SDS, the protein-linked cleavage complexes were isolated from a phenol/water interface. Samples were analyzed in a 12% denaturing polyacrylamide gel after proteinase K treatment (Fig. 4A, middle panel). As seen from a schematic presentation of the obtained cleavage levels (Fig. 4A, lower panel), the mutant enzyme cleaved the suicide substrate to a level similar to the wild-type enzyme, indicating that the cleavage domain retained normal DNA binding and cleavage activities.

The topoisomerase II cleavage complex generated upon cleavage of a suicide substrate is kinetically competent. As demonstrated previously, such a complex is able to perform ligation if a suitable ligation substrate is added to the cleavage mixture as schematically illustrated in Fig. 4B (upper panel) (29). To investigate if the central domain of hΔ350–407 also withholds ligation activity, topoisomerase II-DNA cleavage complexes were prepared for the ligation assay as described under "Experimental Procedures." After an increase in the salt concentration to inhibit further cleavage, ligation was initiated by the addition of a 45-mer DNA oligonucleotide able to hybridize to the bottom strand of the cleaved substrate. At different times, aliquots were taken and treated with SDS and proteinase K before analysis in a 12% polyacrylamide gel. The results are depicted graphically in Fig. 4B (lower panel), where the levels of ligation at different times are given relative to the amount of initial cleaved material to take into account differences in the cleavage level at the start of ligation. The relative rates of ligation as visualized from the slope of the curves are comparable for the two enzymes, further substantiating that the central domain is folded into an entity retaining normal properties.

The optimal conditions for topoisomerase II-mediated cleavage of small oligonucleotides vary to some extent from those giving maximum cleavage of longer duplexes including circular DNA (29). Also, whereas oligonucleotides only require contacts to a very restricted area of the cleavage domain, circular DNA might contact the enzyme in place of both the T- and G-segments (33). Cleavage of circular DNA or long duplex DNA substrates might therefore require a higher extent of correct interdomain communication. This is also indicated from the stimulatory effect of ATP on topoisomerase II-mediated cleavage of such substrates (25, 26) as compared with the negligible effect of ATP on cleavage of small oligonucleotides (35). Therefore, in order to investigate if hΔ350–407, although operating normally on oligonucleotides, has an altered behavior toward longer substrates, we performed cleavage experiments using supercoiled plasmid DNA as substrate. As shown in Fig. 5A and schematically presented in Fig. 5B, hΔ350–407 is able to cleave supercoiled DNA to almost the same level as the wild-type enzyme (compare lanes 4 and 5 with lanes 2 and 3). Therefore, the mutant enzyme also appeared to display normal DNA binding and cleavage activity with supercoiled plasmid substrates. This conclusion is further supported by the similar response of the hΔ350–407 and the wild-type enzymes to the two anti-tumor agents VM26 and amsacrine with respect to cleavage complex formation (Fig. 5C).

To further test if cleavage mediated by hΔ350–407 is still influenced by ATP binding to the ATPase domain, topoisomerase II-mediated cleavage of the supercoiled substrate was performed in the presence of ATP or the ATP analog, AMP-PNP.
In contrast to the 2–3-fold stimulation of cleavage obtained with the wild-type enzyme, cleavage complex formation by hΔ350–407 was not enhanced by the presence of ATP or the ATP analog (Fig. 5A, compare lanes 6, 7, 10, and 11 with lanes 8, 9, 12, and 13). In conclusion, our data suggest that the central domain operates normally with respect to DNA binding, cleavage, and religation and therefore constitutes a full functional domain in the mutant enzyme. However, the inability of ATP to stimulate the cleavage reaction of the enzyme favors the hypothesis of an uncoupling of the ATPase and cleavage/religation domains.

Characterization of the N-Terminal Clamp Closing Activity of hΔ350–407—The existence of two functional domains in hΔ350–407, combined with the lack of strand passage activity, strongly suggests that the two domains of the enzyme are unable to communicate. Several studies suggest that the mode of communication in topoisomerase II is through a cascade of conformational changes taking place in the enzyme upon ATP binding and hydrolysis, starting with the trapping of a T-segment by N-terminal clamp closure (16, 19, 20, 36, 37). The changes are transmitted to the rest of the enzyme, leading to a coordinated separation of the two active site tyrosines. The newly created gate in the G-segment allows the T-segment to pass through and leave the enzyme after opening of the primary C-terminal dimerization region. To investigate if the initial conformational changes including N-terminal clamp closure occur in hΔ350–407 upon ATP binding, we performed a clamp closing assay taking advantage of phenol extraction for collection of enzyme-DNA complexes that have become interlinked due to enzyme clamp closure. The hΔ350–407 or wild-type enzyme was incubated with a DNA mixture containing equal amounts of circular and linear DNA, where most of the enzyme-DNA complexes were recovered from a phenol/water interphase. Complexes were subsequently ethanol-precipitated and treated with proteinase K, before they were subjected to electrophoresis in a 12% polyacrylamide gel. Lane 1, DNA size marker increasing in steps of two bases; lane 2, labeled DNA substrate; lanes 3–8, time course of the cleavage reaction performed with the wild-type enzyme; lanes 9–14, time course of the cleavage reaction performed with the hΔ350–407 enzyme. S, the cleavage substrate remaining in the interphase after phenol extraction. CI, the cleavage product, for which migration was retarded with +1 base due to partial proteinase K digestion. Lower panel, schematic representation of the time course experiment shown in the middle panel. Cleavage levels were measured by PhosphorImager scanning and are presented in arbitrary units relative to the cleavage level obtained with the wild-type enzyme after 90 min. B, investigation of the suicide ligation reaction performed with the hΔ350–407 and wild-type enzymes. Upper panel, schematic illustration of the topoisomerase II-mediated ligation reaction on the 5′-recessed substrate performed by topoisomerase II covalently linked to the cleaved suicide substrate. The 45-mer added to the reaction is the incoming ligation substrate, which in the 3′ end is complementary to the single-stranded region of the bottom strand. The arrowheads indicate the position of topoisomerase II-mediated cleavage. The asterisk represents radioactive labeling. Lower panel, graphic illustration of a topoisomerase II-mediated time course ligation experiment using the wild-type or hΔ350–407 enzyme. A suicide cleavage reaction was performed as described in A. After 60 min, the cleavage reaction was stopped by the addition of salt, thereby preventing further cleavage during the ligation reaction. Ligation was initiated by the addition of a 45-mer ligation substrate in a 200-fold molar excess relative to the cleavage substrate. Samples were withdrawn at the indicated times, treated with SDS, and subjected to electrophoresis in a 12% denaturing polyacrylamide gel. Levels of ligation were measured by PhosphorImager scanning of the gel and are expressed in arbitrary units relative to initial cleaved material.
the supercoiled DNA and none of the linear DNA being found in a protein-linked form in the interphase, in accordance with the principles of DNA trapping due to enzyme clamp closure (Fig. 6, upper panel). In contrast, only a trace amount of the supercoiled DNA was found in the interphase for hΔ350–407, and enzyme-mediated trapping of DNA occurred independent of the ATP analog. The efficiency of the clamp closure event for the mutant enzyme relative to the wild-type enzyme is depicted graphically in the histogram presented in Fig. 6 (lower panel). The results strongly indicate that the mutant enzyme is disturbed in its clamp closing activity, being unable to stably close the clamp in the presence of high salt, although it still binds and hydrolyzes ATP. The increased ATPase activity of the mutant enzyme might be caused by a slight change in the affinity of the ATP analog at the level of the enzyme-DNA interactions during strand passage. The negligible amount of supercoiled DNA trapped by the mutant enzyme independent of the ATP analog might be caused by a slight change in the affinity of the enzyme for the G-segment in the central domain, as also suggested from the inability to totally reverse enzyme-DNA binding in the presence of 1 M salt (data not shown).
DISCUSSION

The ability of eukaryotic topoisomerase II to change the topological conformation of DNA is based on a highly controlled communication between the individual subdomains in the dimeric enzyme, allowing transport of a DNA duplex through the whole intersubunit channel and through a second duplex hold and cleaved by the enzyme (19). In the present study, we investigated the interdomain communication between the N-terminal ATPase and the central cleavage/religation domains by studying a human topoisomerase IIα enzyme that contained a deletion at the interface between the two domains, spanning amino acids 350–407. In accordance with a lack of strand passage activity of the mutant enzyme, hΔ350–407 was unable to sustain mitotic growth of a yeast top2 null strain. The mutant enzyme displayed a high ATPase activity and cleaved either suicide substrates or supercoiled plasmid DNA to wild-type levels. These results indicate that the enzyme consists of two functional domains that have lost their ability to coordinate their activities into strand passage. Further supporting an uncoupling of the N-terminal and central domain activities is the observation that the N-terminal ATPase activity was unaffected by DNA and that the cleavage/religation activity of the central domain was not stimulated by ATP. An examination of the clamp closing activity of the N-terminal domain revealed an inability of the mutant enzyme to close the clamp properly, strongly favoring the hypothesis that the mutant enzyme has lost its interdomain communication due to a failure in the generation and/or transmission of the correct conformational changes upon ATP binding and hydrolysis.

The region deleted in hΔ350–407 constitutes a highly conserved domain of human topoisomerase IIα, implying that this region is very important for overall enzyme activity (9). This is further supported by results obtained from a linker insertion analysis, which showed that even a 2-amino acid linker inserted at position 350 or 407 was detrimental for enzyme activity, whereas similar insertions in regions flanking other highly conserved domains were tolerated by the enzyme (9). Based on homology to DNA gyrase, the deleted region constitutes the wall of a cavity existing in the dimeric form of the N-terminal region of Gyr B, as visualized after crystallization of this fragment of the gyrase enzyme (13). Assuming that the N-terminal region of Gyr B and human topoisomerase IIα fold into similar structures, the outer skeleton of the enzyme would probably be left undisturbed after the deletion. The overall frame structure of the N-terminal domain in the mutant enzyme would thus be kept intact, as also indicated from the ability of the enzyme to still efficiently hydrolyze ATP and cleave DNA. The size of the cavity in Gyr B is 20 Å, large enough to accommodate a DNA duplex, and it has been suggested that the cavity is a DNA binding pocket that binds the T-segment to be transported during the strand passage reaction (13). In light of this, hΔ350–407 might suffer from an inability to interact properly with the T-segment, which eventually would disturb the whole communication pathway in the enzyme.

As revealed by biochemical and structural analyses of both eukaryotic topoisomerase II and the prokaryotic DNA gyrase, ATP binding and subsequent hydrolysis of one of the bound ATP molecules trigger a series of conformational changes resulting in N-terminal clamp closure and T-segment transport through the intersubunit channel (17, 19, 20). For the gyrase enzyme, the crystal structure of the N-terminal fragment of Gyr B has revealed that ATP upon binding contacts amino acids located in the wall facing the cavity encompassing the T-segment (13). These contacts were suggested to provide a mechanism for signaling ATP binding to the rest of the enzyme. Since this contact region is lacking in our deletion mutant, ATP binding and hydrolysis might not be sensed properly by the enzyme and transmitted further to allow correct T-segment binding and/or movements as well as further conformational changes in the enzyme.

Several studies performed either with fragments of DNA gyrase or eukaryotic topoisomerase II or with the full-length enzymes have shown a stimulatory effect of DNA on topoisomerase II-catalyzed ATP hydrolysis (22, 24, 31–33, 38). Although it is still unclear whether this stimulation is caused by T- and/or G-segment binding, an N-terminal fragment of human topoisomerase II covering amino acids 1–439 (and therefore lacking the ability to interact with the G-segment) displayed a DNA-stimulated ATPase activity. This result favors the involvement of the T-segment in stimulating ATP hydrolysis (22) and supports the hypothesis of a hampered T-segment interaction in hΔ350–407, which displayed a DNA-independent ATP hydrolysis although its interaction to the G-segment was normal. In a study of a gyrase mutant having a point mutation at Arg286 located in the wall of the cavity in the N-terminal part of Gyr B, the enzyme was also suggested to have a disturbed T-segment interaction (38). The gyrase mutant showed several similarities to hΔ350–407, in that it had a DNA-independent ATPase activity and cleaved DNA, but did not perform ATP-dependent DNA strand passage. Wang and co-workers (34) have recently shown that the stimulatory effect of DNA on the ATPase activity of topoisomerase II fragments is increased if the enzyme fragment besides the N-terminal ATPase domain also holds the B’ region of the enzyme. Since this region normally is involved in G-segment binding (19), the result strongly indicates that the G-segment influences topoisomerase II-catalyzed ATP hydrolysis. To this end, Lindsley and co-workers (39) have recently suggested, based on results obtained with yeast topoisomerase II, that binding of the G-segment per se primarily stimulates ATP binding to the enzyme, whereas binding of the T-segment stimulates ATP hydrolysis, so that both DNA segments affect the activities of the ATPase domain. hΔ350–407 efficiently binds and cleaves the G-segment. A potential stimulatory effect of the G-segment on topoisomerase II-catalyzed ATP hydrolysis might in the mutant enzyme be prevented due to loss of communication between the N-terminal and central domains.

Although the ATPase activity of hΔ350–407 is independent of DNA, the mutant enzyme had a very high rate of ATP hydrolysis compared with the wild-type enzyme. However, our study of the N-terminal clamp closing activity of hΔ350–407 revealed that, in contrast to the wild-type enzyme, the mutant enzyme was unable to keep the clamp tightly closed in high salt, since only very limited amounts of DNA were trapped by the enzyme in the presence of an ATP analog. The dimerization of the N-terminal arms, which occurs upon ATP binding, has been suggested to be a prerequisite for ATP hydrolysis, since dimerization might bring together key residues in both halves of the enzyme, forming a pocket essential for ATP hydrolysis (13). However, our data on hΔ350–407 demonstrate that ATP hydrolysis can occur efficiently in the absence of correct N-terminal dimerization.

The model recently presented by Lindsley on topoisomerase II action suggests that ATP hydrolysis occurs sequentially, where hydrolysis of the first ATP precedes transport of the T-segment and hydrolysis of the second ATP is responsible for enzyme turn over (40, 41). The very high level of ATP hydrolysis observed in hΔ350–407 can be interpreted in light of the lack of DNA strand passage activity in the mutant enzyme. Thus, due to the inability of the enzyme to perform DNA strand passage, it avoids the time lag generated as a consequence of the whole cascade of conformational changes it has to undergo.
from the time of ATP binding to ATP hydrolysis of the second ATP molecule. Our observations to some extent correlate with observations obtained previously by Lindsley and Wang (24) using yeast topoisomerase II, where they found that uncoupling of ATP hydrolysis and DNA strand passage due to high ATP concentrations correlated with an increased rate of ATP hydrolysis.

Besides the stimulatory effect of DNA on topoisomerase II-catalyzed ATP hydrolysis, the communication between the N-terminal and central domains is also manifested during topoisomerase II-mediated DNA cleavage, where ATP shifts the DNA cleavage/religation equilibrium toward cleavage (25, 42). In h350–407, however, the stimulatory effect of ATP on cleavage was lacking. Studies of topoisomerase II-mediated cleavage have revealed that two different cleavage/religation equilibriums exist (43). One is the pre-strand passage equilibrium, which occurs in the absence of ATP and therefore in the absence of strand passage. The other is the post-strand passage equilibrium existing in the presence of ATP or an ATP analog, which involves T-segment transport through a gate in the G-segment formed by a separation of the two active site tyrosines (43). The conformation of the enzyme is presumed to be different in the two situations. In the pre-strand passage equilibrium, the N-terminal arms most likely are in an open conformation and the tyrosines close together. However, in the post-strand passage equilibrium, the N-terminal arms might be closed and the tyrosines allowed to move back and forth between a closed and a widely separated conformation, thereby shifting the equilibrium toward cleavage. Our data on h350–407 are consistent with the mutant enzyme being unable to switch to the post-strand passage equilibrium in the presence of ATP. The mutant enzyme stays in the pre-strand passage equilibrium under all conditions.

In conclusion, the highly conserved region deleted in h350–407 is essential for correct two-way communication, which normally exists between the N-terminal and central cleavage/religation domains in eukaryotic topoisomerase II, resulting in a loss of DNA strand passage activity. Thus, although the two domains retain their normal intrinsic activities, the DNA cleavage activity of the enzyme becomes independent of ATP, and the ATPase activity does not respond to DNA due to the lack of interdomain communication. The mutant enzyme is unable to perform the correct conformational changes upon ATP binding and hydrolysis as evident from an inability to perform a correct clamp closure. The enzyme might also suffer from a disturbed T-segment trapping, which could by itself hamper clamp closure and inhibit DNA strand passage.

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