A Human Topoisomerase IIα Heterodimer with Only One ATP Binding Site Can Go through Successive Catalytic Cycles*

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Eukaryotic DNA topoisomerase II is a dimeric nuclear enzyme essential for DNA metabolism and chromosome dynamics. It changes the topology of DNA by coupling binding and hydrolysis of two ATP molecules to the transport of one DNA duplex through a temporary break introduced in another. During this process the structurally and functionally complex enzyme passes through a cascade of conformational changes, which requires intra- and intersubunit communication. To study the importance of ATP binding and hydrolysis in relation to DNA strand transfer, we have purified and characterized a human topoisomerase IIα heterodimer with only one ATP binding site. The heterodimer was able to relax supercoiled DNA, although less efficiently than the wild type enzyme. It furthermore possessed a functional N-terminal clamp and was sensitive to ICRF-187. This demonstrates that human topoisomerase IIα can pass through all the conformations required for DNA strand passage and enzyme resetting with binding and hydrolysis of only one ATP. However, the heterodimer lacked the normal stimulatory effect of DNA on ATP binding and hydrolysis as well as the stimulatory effect of ATP on DNA cleavage. The results can be explained in a model, where efficient catalysis requires an extensive communication between the second ATP and the DNA segment to be cleaved.

DNA topoisomerase II is a multifunctional and highly complex enzyme, which uses the energy of ATP to resolve topological problems generated during DNA metabolic processes, including DNA replication, transcription, and recombination (1, 2). Beyond these functions, topoisomerase II is an abundant component of the mitotic chromosome scaffold (3), and it alleviates constraints in the DNA during chromosome segregation and condensation (1).

To preserve the topological integrity of the genome, the dimeric topoisomerase II enzyme strictly controls the passage of one duplex DNA (the T-segment) through another duplex (the G-segment) coordinately cleaved by the enzyme. ATP is required during the process to drive the enzyme through a series of conformations, which are essential to topoisomerase II catalysis.

During the last years, extensive research has provided valuable information about the usage of ATP during the reaction pathway of topoisomerase II, but still several steps in this highly complex process remain to be unraveled. A study of the ATP consumption in yeast topoisomerase II has indicated that a tight coupling exists between ATP utilization and DNA transport under unsaturated ATP concentrations (5). However, a human topoisomerase II enzyme lacking amino acids 350–407 at the interface between the ATPase and the cleavage/ligation domain was unable to perform strand passage, although ATPase and cleavage activities were intact (6). These results indicate that correct interdomain communication as well as signaling of ATP binding and hydrolysis to the rest of the enzyme are essential for the coupling of ATP consumption and DNA transport.

The binding of ATP is cooperative in the presence of DNA (5) and is thought to be stimulated by the binding of the G-segment (7). A study of a yeast topoisomerase II heterodimer having only one ATP binding site has demonstrated that conformational changes are induced in the entire enzyme upon ATP binding, because the enzyme was still able to trap circular DNA by closing the N-terminal clamp. This indicates that allosteric communication exists between the two enzyme subunits (8). Together with results obtained from studies employing nonhydrolyzable ATP analogues (9, 10), this initially led to the assumption that ATP binding triggers the conformational changes needed for one DNA transport event, whereas ATP hydrolysis and product release are exclusively coupled to enzyme resetting (4).

However, recent discoveries employing rapid quench techniques and pre-steady-state analysis of ATP hydrolysis in the yeast enzyme have revealed that hydrolysis of the two ATP molecules occurs sequentially with one of the two ATP molecules being rapidly hydrolyzed before the rate determining step in the reaction cycle (11). This sequential hydrolysis is explained in terms of a model, where hydrolysis of one ATP precedes and accelerates transport of the T-segment, although the reaction can occur in the absence of hydrolysis (12). Hydrolysis of the first ATP and release of Pi and ADP are supposed to induce conformational changes in the N-terminal ATPase region of the enzyme, which are transmitted to the central DNA cleavage/ligation domain, where large rotations are proposed to form a gate in the cleaved G-segment to allow transport of the T-segment. This conformational cascade is predicted to occur in both subunits, such that the enzyme maintains structural symmetry, despite the different nucleotide bound states of the two subunits (4, 12). Investigations of...
a yeast topoisomerase II heterodimer, which hydrolyzes only one of the two bound ATP molecules, have shown that this enzyme is able to perform DNA strand passage at the same rate as the wild type enzyme, although resetting of the enzyme is delayed, probably due to the bound unhydrolyzed ATP, which keeps the clamp closed. Based on these results, it has been hypothesized that the second hydrolysis event and the subsequent substrate release are linked to resetting of the enzyme (12). However, it is still unknown to what extend binding of the second ATP per se influences the individual steps in the catalytic cycle.

To investigate the coupling of ATP binding and hydrolysis to DNA strand passage and enzyme resetting, a human topoisomerase II heterodimer, constructed to bind only one ATP, has been purified and characterized. The results obtained with the heterodimer demonstrate that topoisomerase II can perform strand passage and resetting with only one ATP bound and hydrolyzed. However, the second ATP molecule facilitates interdomain communication, especially between the N-terminal ATP binding region and the central cleavage/ligation domain and is thus important for efficient catalysis.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Plasmids**—The Saccharomyces cerevisiae strain JEB1 (ura3–52 pir1–82 pep4 serialization) was used in this study (5’-GGATCCAGAACAATATCATATGGACCC-3’) containing the mutation that changes Gly-164 to isoleucine (boldface letters) and primer B (5’-GGATCCAGCCAGCATACTATAG-3’), which corresponds to the antisense strand and contains a NotI restriction site (underlined) were used. The 265-bp PCR fragment synthesized using these primers was gel-purified and used as antisense primer in a second round of PCR together with primer C (5’-CTAGGGCGACCCTCCATGGAAGTC-3’), which is located 5’ of the human topoisomerase II cDNA. The final PCR fragment was used to substitute the corresponding fragment of the hTOP2α cDNA in pHT212, which contains the human topoisomerase II cDNA. Construction of pHT212 was described in a previous study (13).

**Introduction of the G164I Point Mutation**—Two rounds of PCR were carried out to introduce the G164I point mutation in the human topoisomerase IIα cDNA. In the first round, primer A (5’-GTCGCTGCA-AATTATCATATGGACCC-3’) containing the mutation that changes Gly-164 to isoleucine (boldface letters) and primer B (5’-GGATCCAGAACAATATCATATGGACCC-3’), which corresponds to the antisense strand and contains a NotI restriction site (underlined) were used. The 265-bp PCR fragment synthesized using these primers was gel-purified and used as antisense primer in a second round of PCR together with primer C (5’-CTAGGGCGACCCTCCATGGAAGTC-3’), which is located 5’ of the human topoisomerase IIα cDNA. The final PCR fragment was used to substitute the corresponding fragment of the hTOP2α cDNA in pHT212, which contains the human topoisomerase IIα cDNA. Construction of pHT212 was described in a previous study (13). The fragment was used to replace the corresponding hTOP2α cDNA fragment in YEpWOB6. The hTOP2α cDNA was furthermore modified with a hexahistidine tag at the 3’-end as described by Bjerbaek et al. (6). The resulting plasmid was termed YEpWOBG164IHT. To fuse a hemagglutinin (HA) tag to the C-terminal end of human topoisomerase IIα for antibody detection, PCR was carried out using pH300 as a template. The antisense primer was designed using a stretch of 51 overhanging nucleotides containing the HA tag and an NheI restriction site, whereas the sense primer (5’-GGAGAGGTGGTAGACTACAC-3’) for cloning purposes contained a SpeI restriction site. The synthesized fragment was gel-purified and inserted at the 3’-end of the hTOP2α cDNA in YEpWOB6 employing the restriction sites NheI and SpeI. A DNA fragment encoding the glutathione S-transferase (GST) tag used for purification was subsequently ligated to the 3’-end of the HA-tagged hTOP2α cDNA. The GST sequence was amplified from pET-HTG (14) using a sense primer (5’-GGATCCAGGCTGGTCTGAGCCTATGTCG-3’) containing a NotI restriction site and a C-terminal hexahistidine and an antisense primer (5’-CCCTTTGCGGCCCTATATTGGGAGATGGTGGCACCACCCC-3’) containing a NdeI restriction site. The PCR fragment was inserted using the NheI and NdeI restriction sites. This resulted in plasmid YEpWOBHAGST.

To construct the plasmid used for the production of topoisomerase IIα heterodimers, YEpWOBG164I/WT (Fig. 1A), the hTOP2α cDNA fused to the HA and the GST tags together with the GAL1-promoter was excised from YEpWOBHAGST using the restriction enzymes SalI and NotI and inserted in YEpWOBG164IHT digested with NotI and XhoI. The plasmids YEpWOBWt/vt and YEpWOBG164I/G164I used for expression of the wild type and the homodimeric mutant enzyme, respectively, were constructed in a similar way. The constructs were sequenced to verify the presence of the mutations G164I.

**Yeast Transformation**—Yeast cells were transformed using electroporation and transferred to SC-media plates lacking uracil to select against the YEpWOB6-based constructs.

**Human Topoisomerase IIα Induction, Overexpression, and Purification**—Expression of the recombinant hTOP2α enzymes in the yeast strain was induced by the addition of galactose to glucose-free medium (15). Yeast cells were extracted with 1 volume of 50 mM Tris-HCl, pH 7.8, 1 M NaCl and 1 volume of acid-washed glass beads (425–600 μm, Sigma). Further preparation of yeast extracts was done according to the procedure of Jensen et al. (13). The initial purification step using a 6-ml Ni2+-nitrilotriacetic acid-agarose column was performed as described previously by Biessack et al. (16). For further purification of the heterodimeric enzymes the fractions pooled from the Ni2+-column was loaded onto a 1-ml GST column (Amersham Biosciences), and elution was performed in a buffer containing 100 mM Tris-HCl, pH 7.5, 500 mM NaCl, 10% glycerol, 1 mM dithiothreitol, 1 mM EDTA, and 25 mM glutathione. The fractions containing topoisomerase II heterodimers were pooled, and after addition of glycerol to a final concentration of 40%, the enzymes were stored in liquid nitrogen. After each step of purification the eluted fractions were tested for topoisomerase II heterodimers on a silver-stained 6% polyacrylamide gel. Ruby Protein Gel staining (Molecular Probes) of 3–5% Tris acetate gradient gels (Novex Pre-Cast Gels) was performed to quantify the purified topoisomerase II heterodimers. The same procedure was used for purification of the wild type and homodimeric mutant enzymes, which were used as controls in the enzyme activity assays. All three enzymes were further purified according to the procedure described in Bjergbaek et al. (6) involving only Ni2+-nitrilotriacetic acid chromatography.

**Hydrolysis of ATP by Topoisomerase IIα**—The ATPase assay was performed using the method of Osheroff et al. (17). Yeast strain was induced and 150 μl topoisomerase II and 30 μl negatively supercoiled pUC19 and were carried out in 20 μl of 50 mM Tris-HCl, pH 7.9, 0.1 mM EDTA, 5 mM MgCl2, 2.5% glycerol, and 140 mM KCl containing a final concentration of 1 mM cold ATP and 0.08 μM of [γ-32P]ATP (3000 Ci/mmol, Amersham Biosciences). Mixtures were incubated at 37 °C, and 2.5-μl aliquots were taken at various time points and spotted onto thin-layer cellulose plates impregnated with polyethyleneimine (TLG plastic sheets, PEI Cellulose F, Merck). Chromatography was performed using freshly made 0.4 × NH4HCO3. Levels of free P, quantified by phosphorimaging (Molecular Imagex, Bio-Rad). The concentration of ATP hydrolyzed was determined by dividing the P, counts by the total number of counts in each lane and multiplying that fraction by the initial concentration of ATP.

**Topoisomerase IIα-mediated DNA Relaxation**—DNA relaxation was performed by incubating 2.2 mM topoisomerase IIα and 5.5 mM negatively supercoiled pUC19 in 50 mM Tris-HCl, pH 7.9, 0.1 mM EDTA, 5 mM MgCl2, 2.5% glycerol, and 140 mM KCl. Samples were incubated for 5 min at 37 °C and loaded onto a nitrocellulose filter using a slot blot apparatus (PR 648 Slot Blot Manifold, Amersham Biosciences). Each sample was washed 10 times in a buffer containing 50 mM Tris-HCl, pH 7.9, 0.1 mM EDTA, 5 mM MgCl2, 2.5% glycerol, and 140 mM KCl. Samples were stained with 0.5 μl of 0.8 mg/ml ethidium bromide and visualized by UV light using the Gel Doc 2000 from Bio-Rad.

**Topoisomerase IIα-mediated DNA Cleavage**—DNA cleavage was performed by incubating 6.6 mM topoisomerase IIα with 5.5 negatively supercoiled pUC19 in 50 mM Tris-HCl, pH 7.9, 0.1 mM EDTA, 5 mM MgCl2, 1 mM ATP, 2.5% glycerol, and 125 mM KCl for 10 min at 37 °C. Cleavage products were trapped by addition of SDS to 1%, and samples were treated with 2 μl of 0.8 mg/ml proteinase K before being subjected to electrophoresis in 1% agarose gels. Southern blotting was performed

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The abbreviations used are: HA, hemagglutinin; GST, glutathione S-transferase; AMPPNP, 5’-adenyl-β,γ-mididophosphate.
in the silver stain presented in Fig. 1B.

The Mutation G164I disrupts ATP Binding and Hydrolysis Activities of Human Topoisomerase IIa—To verify that ATP binding was completely blocked by the G164I point mutation, a homodimeric enzyme containing the mutation in both subunits was tested for its ability to bind and hydrolyze ATP. ATP binding was investigated using a nitrocellulose filter binding assay. The enzyme was incubated with plasmid DNA and a labeled ATP analogue, ATP[^32]P, to avoid hydrolysis and product release. After incubation at 37 °C, the reaction mixture was loaded onto a nitrocellulose filter followed by several washes in high salt to minimize nonspecific binding of the ATP analogue to the enzyme. The level of ATP[^32]P binding observed with the homodimeric G164I enzyme was similar to the level obtained with heat-inactivated wild type or mutant enzyme, strongly indicating that the observed activity was caused by residual unspecific binding (Fig. 2A).

The capability of the G164I homodimer to hydrolyze ATP was subsequently examined using thin-layer chromatography. The ATPase activity of the enzyme was equivalent to the background levels observed in the absence of enzyme (Fig. 2B). Taken together, these results demonstrate that conversion of Gly-164 to isoleucine in human topoisomerase IIα eliminates the enzymatic ATP binding and hydrolysis activities. Based on these findings, only the wild type subunit of the G164I/wt heterodimer is expected to bind and hydrolyze ATP.

The G164I/wt Heterodimer Is Able to Perform DNA Relaxation—Yeast topoisomerase II has been shown to hydrolyze only one ATP during transport of the T-segment, whereas the second ATP is thought to be essential for resetting of the enzyme (12). To investigate the importance of the second ATP for human topoisomerase IIα catalysis, we investigated the relaxation activity of G164I/wt. Interestingly, the enzyme was found to relax supercoiled DNA, although less efficiently than the wild type enzyme (Fig. 3). As anticipated, the G164I homodimer was not able to perform relaxation. Because the enzyme:DNA molar ratio was only 1:2.5, the level of relaxation obtained by the heterodimer clearly demonstrates that each enzyme molecule performs more than one DNA strand passage event. The data illustrate that human topoisomerase IIα can go through all the conformational changes required for DNA strand passage and resetting of the enzyme, with binding and hydrolysis of ATP taking place in only one subunit of the enzyme.

The G164I/wt Heterodimer Possesses a Normal Clamp Closing Activity—Binding of the first ATP molecule to topoisomerase II is presumed to induce closure of the N-terminal clamp, and this is followed by binding of a second ATP (8). To test if the lack of a second ATP molecule influences clamp closing, we analyzed the heterodimer in a clamp closing assay. The assay is based on a stable closure of the N-terminal clamp upon binding of a nonhydrolyzable ATP analogue. If the wild type enzyme is incubated with equal amounts of linear and circular DNA before the addition of the ATP analogue, AMPPNP, only circular DNA will become trapped by the enzyme, and the interlinked enzyme-DNA complexes can be collected from a water-phenol interface (Fig. 4, lanes 1 and 2) (6). When G164I/wt was incubated in the presence of AMPPNP, the amount of supercoiled DNA trapped in the interphase was equivalent to the amount trapped by the wild type enzyme (Fig. 4, compare lanes 1 and 2 with lanes 7 and 8) showing that the heterodimer has retained the ability to close the N-terminal clamp. When the enzymes were incubated with ATP rather than the ATP analogue, no DNA trapping took place, demonstrating that G164I/wt like the wild type enzyme reopens the clamp upon ATP hydrolysis (Fig. 4, lanes 6 and 12). Furthermore, neither...
enzyme allowed trapping of DNA upon addition of SDS, which disrupts the interlink between enzyme and DNA (Fig. 4, lanes 4 and 10) or in the absence of AMPPNP, where clamp closing does not occur (data not shown). Together, the results show that the heterodimeric N-terminal clamp operates with a mechanism similar to the clamp in the wild type enzyme. Thus, binding and hydrolysis of one ATP molecule is sufficient to allow both clamp closing and reopening in agreement with the observed relaxation activity.

The G164I/wt Heterodimer Is Sensitive to ICRF-187—The bisdioxopiperazines, ICRF-193, ICRF-187, and ICRF-159, are catalytic inhibitors of topoisomerase II, which have been shown to lock the enzyme on DNA by trapping it in the closed clamp form (19, 20). Several point mutations leading to resistance have been suggested that bisdioxopiperazines bind to the enzyme-ADP complex after DNA transport has occurred and have no effect on hydrolysis of either the first or the second ATP. Instead the drugs inhibit dissociation of the second ADP, thereby preventing the enzyme from catalytic turnover (25). To test whether the heterodimer can adopt the conformation required for bisdioxopiperazine interaction and further investigate how these drugs perturb the mechanism of topoisomerase II, we investigated the effect of ICRF-187 on the ability of G164I/wt to perform DNA relaxation. Fig. 5 illustrates that 100 μM ICRF-187 decreases the relaxation activity of the heterodimer, whereas activity is completely abolished at a concentration of 500 μM. These results predict that, when binding and hydrolysis of only one ATP is possible, the enzyme is still able to enter a definite conformational state, where recognition and binding of ICRF-187 can occur, resulting in a trapping of the enzyme in the closed clamp form.

DNA Cleavage by the G164I/wt Heterodimer Is Unaffected by ATP—Because G164I/wt has a reduced relaxation activity, we investigated if the catalytic site for cleavage and ligation located in the central domain of topoisomerase II still constitutes a full functional domain. For this purpose cleavage experiments were performed with G164I/wt employing the circular DNA substrate used for relaxation. When topoisomerase II and DNA are incubated, a cleavage/ligation equilibrium is normally established, where the amount of enzyme-DNA cleavage complex intermediates that can be trapped, when a denaturating agent is added, depends on both the DNA cleavage and ligation rates. If ATP or an ATP analogue is present, strand passage is allowed, and the equilibrium will be shifted toward cleavage, resulting in a 3- to 5-fold increase in cleavage complex formation (6, 10, 26). When cleavage was carried out in the absence of nucleotide, cleavage complex formation by G164I/wt and the wild type enzyme was similar (Fig. 6). However, in contrast to the 4-fold stimulation of cleavage obtained with the wild type enzyme in the presence ATP or the ATP analogue, AMPPNP, cleavage complex formation by G164I/wt was not influenced by nucleotide (Fig. 6), demonstrating a role of the second ATP molecule in this stimulation. In conclusion, these results suggest that the central domain of G164I/wt operates normally with respect to DNA binding, cleavage, and ligation. However, the inability of ATP or the ATP analogue to stimulate cleavage indicates an impaired ability of the heterodimer to transmit.

![Fig. 2.](image1) The mutation G164I in human topoisomerase IIα disrupts ATP binding and hydrolysis activities. A, the ATP binding activity of human topoisomerase IIα was measured using a nitrocellulose filter binding assay, where various amounts of wild type enzyme (wt), G164I homodimer (HI G164I), heat-inactivated G164I homodimer (HI G164I), or heat-inactivated wild type enzyme (HI wt) were incubated with 0.3 nM [35S]ATP in the presence of 5.5 nM plasmid DNA. Levels of ATP binding are relative to the amount of binding observed in the presence of 120 nM wild type enzyme (set to 1). B, the ATPase activity of human topoisomerase IIα was measured by thin-layer chromatography. 150 nM enzyme was incubated with 1 mM cold ATP and 0.08 μM [γ-32P]ATP in the presence of 30 nM plasmid DNA. Aliquots were withdrawn at the indicated time points and loaded onto thin-layer cellulose plates. Quantification was done using a PhosphorImager. Error bars represent the standard deviations from three independent experiments.

![Fig. 3.](image2) The G164I/wt heterodimer is able to perform DNA relaxation. Samples containing 5.5 nM supercoiled plasmid DNA and 2.2 nM either wild type enzyme, G164I/wt heterodimer, or G164I homodimer were incubated at 37 °C and stopped with SDS at the time points indicated above each lane. N, RC, and SC indicate the position of nicked, relaxed circular, and supercoiled circular DNA, respectively.
supercoiled circular DNA, respectively. Reactions were stopped by addition of NaCl or SDS to final concentrations of 800 mM or 1%, respectively.

**FIG. 4.** The G164I/wt heterodimer is able to perform N-terminal clamp closure. Upper panel, Southern blot showing a clamp closing experiment, where 1 nM enzyme was incubated with 2.5 nM supercoiled and 2.5 nM linearized plasmid DNA. ATP or AMPPNP was added to a final concentration of 1 mM after preincubation of enzyme and DNA. Reactions were stopped by addition of NaCl or SDS to final concentrations of 800 mM or 1%, respectively. w and i indicate phenol-water and phenol interphases, respectively. RC, L, and SC indicate relaxed circular, linear, and supercoiled circular DNA, respectively. Lower panel, histogram of the clamp closing experiment described in A. Levels of supercoiled plasmid DNA trapped in the interphase were quantified using a PhosphorImager and are represented in arbitrary units relative to the amount of DNA trapped by the wild type enzyme in the presence of AMPPNP and NaCl. The error bars represent the standard deviations from three independent experiments. Southern blotting was performed using random primed plasmids as probes.

**FIG. 5.** The G164I/wt heterodimer is sensitive to ICRF-187. A relaxation assay was performed, where samples containing 5.5 nM supercoiled plasmid DNA and 2.2 nM of either wild type enzyme or G164I/wt heterodimer were incubated at 37 °C in the absence or presence of ICRF-187 as indicated above each lane and subsequently stopped with SDS. N, RC, L, and SC indicate nicked, relaxed circular, linear, and supercoiled circular DNA, respectively.

information between the N-terminal ATPase and the central cleavage/ligation domains, which might contribute to the lower relaxation activity observed with G164I/wt.

ATP Binding and Hydrolysis Activities of the G164I/wt Heterodimer Are Independent of DNA—It has previously been presumed that the second ATP in topoisomerase II plays a role during enzyme resetting (4, 12). However, the cleavage results presented in Fig. 6 strongly indicate that binding of a second ATP molecule is also essential for conformational changes initiated earlier in the catalytic cycle. To further study this hypothesis, we examined the ability of DNA to stimulate the ATP binding and hydrolysis activities of G164I/wt. Because the ATP binding and hydrolysis assays require higher amounts of enzyme than that obtained from the two-affinity tag purification method, another purification was performed using only one affinity tag, in this case a hexahistidine tag on the G164I mutant subunit (see “Experimental Procedures”). The purified enzyme fractions, which contained both G164I/wt heterodimers and G164I homodimers, were suitable for analysis given that the ATP binding- and hydrolysis activities of G164I/wt. Because the ATP binding and hydrolysis assays require higher amounts of enzyme than that obtained from the two-affinity tag purification method, another purification was performed using only one affinity tag, in this case a hexahistidine tag on the G164I mutant subunit (see “Experimental Procedures”). The purified enzyme fractions, which contained both G164I/wt heterodimers and G164I homodimers, were suitable for analysis given that the ATP binding- and hydrolysis activities of G164I/wt.

**FIG. 6.** DNA cleavage mediated by the G164I/wt heterodimer is unaffected by ATP and AMPPNP. Upper panel, 6.6 nM of either wild type enzyme or G164I/wt heterodimer was incubated with 5.5 nM supercoiled plasmid DNA at 37 °C in the presence or absence of 1 mM ATP or AMPPNP as indicated. Samples were stopped after 10 min by the addition of SDS to 1%, and following proteinase K treatment they were subjected to electrophoresis in a 1% agarose gel containing ethidium bromide. N, RC, L, and SC indicate nicked, relaxed circular, linear, and supercoiled circular DNA, respectively. Lower panel, histogram of the cleavage experiment described in A. Levels of DNA cleavage obtained in the absence or presence of AMPPNP were quantified using a PhosphorImager and are represented in arbitrary units relative to the cleavage obtained with the wild type enzyme in the absence of nucleotide. The error bars represent the standard deviations from three independent experiments.
Fig. 7. The ATP binding and hydrolysis activities of the G164I/wt heterodimer are independent of DNA. A, the ATP binding activity was measured using a nitrocellulose filter binding assay, where 150 nM of either wild type enzyme or G164I/wt heterodimer was incubated with 0.3 nM [35S]ATP in the absence or presence of 30 nM plasmid DNA. Levels of ATP binding in the presence of DNA are relative to the amount of binding observed in the absence of DNA (set to 1). B, the ATPase activity was measured by thin-layer chromatography. 150 nM enzyme was incubated with 1 mM cold ATP and 0.08 nM [γ-32P]ATP in the absence or presence of 30 nM plasmid DNA. Aliquots were taken at various time points and loaded onto thin-layer cellulose plates. Quantification was done using a PhosphorImager. Error bars represent the standard deviations from three independent experiments.

DISCUSSION

The current mechanistic model of topoisomerase II catalysis involves a complex sequential mechanism of ATP hydrolysis, where binding and hydrolysis of ATP drive the enzyme through a series of conformations. Whereas hydrolysis of one ATP is thought to accelerate transport of the T-segment through a gate formed upon cleavage of the G-segment, it is presently unclear how the second ATP is correlated to the remaining steps of the catalytic cycle, although a connection to the resetting of the enzyme has been suggested (11, 12). In the present study we have investigated the coupling between ATP binding/hydrolysis and DNA strand passage/resetting by studying a human topoisomerase IIα heterodimer, G164I/wt, constructed to bind ATP only in one of the two subunits.

Investigation of the enzyme having the G164I mutation in both subunits showed that it was unable to bind and hydrolyze ATP, demonstrating that Gly-164 is essential for ATP interaction in human topoisomerase IIα. Based on this, we assume that the heterodimer only binds and hydrolyzes one ATP molecule, although we cannot exclude the possibility that a second ATP molecule binds to the wild type subunit in the heterodimer upon hydrolysis of the first ATP. In this case a second ATP could contribute to the driving force for one catalytic cycle.

The amino acid residue mutated in G164I/wt is located in the highly conserved Walker A motif, which is a glycine-rich region found in the ATP binding domain of all known type II topoisomerases (29). Assuming that the ATP binding domain folds into a structure similar to the one formed in gyrase B, Gly-164 would be located on a flexible loop of the Walker A motif. This motif is in close contact with another loop, which extends from an enzyme domain located further toward the cleavage/ligation domain. Both loops contact the γ-phosphate of the bound nucleotide as revealed from the crystal structure of gyrase B (18), and in this way information regarding the nucleotide bound state of the enzyme could be transmitted to the central domain.

In a recent report on yeast topoisomerase II, it has been suggested that release of the γ-phosphate from the first ATP hydrolysis event triggers conformational changes associated with DNA transport. Such a model predicts that conformational changes in the two subunits rely on a signal initiated in only one subunit (30). Our results demonstrate that human topoisomerase IIα still mediates DNA relaxation with only one ATP bound and hydrolyzed, in support of this model. In agreement with the relaxation data, our G164I/wt heterodimer was found to close the N-terminal clamp properly, demonstrating that binding of one ATP induces a conformational change in both subunits leading to dimerization of the N-terminal arms. These findings correlate with results obtained with the yeast homologue, which also possessed a normal clamp-closing activity, although ATP binding only occurred in one subunit (8).

The ability of G164I/wt to perform clamp closure was further manifested through its sensitivity to the bisdioxopiperazine ICRF-187. Recent data have indicated that ATP binding is required for efficient binding of bisdioxopiperazines to the enzyme (25). In fact, investigations performed by Lindsley and coworkers have suggested that bisdioxopiperazines bind to the enzyme-ADP complex after hydrolysis of both ATPs and after dissociation of the products of the first hydrolysis (25). The sensitivity of G164I/wt to ICRF-187 predicts that binding and hydrolysis of only one ATP induce a specific conformation in the N-terminal clamp, where binding of the drug is possible. This suggests that the N-terminal conformation adopted by the G164I/wt heterodimer after hydrolysis of the first ATP at a point becomes similar to the conformation attained by the wild type enzyme after hydrolysis of the second ATP.

The ability of G164I/wt to perform more than stoichiometric relaxation demonstrates that the enzyme is able to go through successive catalytic cycles. Binding and hydrolysis of only one ATP molecule is therefore sufficient for resetting of the enzyme, although we cannot exclude that the decreased relaxation activity observed with the heterodimer to some extent is caused by a less efficient resetting. However, the lack of DNA-stimulated ATP binding and hydrolysis and the lack of ATP-stimulated DNA cleavage observed in the heterodimer demonstrate that steps in the catalytic cycle prior to the resetting event are affected, when the second ATP is inhibited from binding. Thus, although the clamp closing data suggest that the intersubunit communication is retained in the N-terminal region, lack of a second ATP seems to influence the intrasubunit communication at least between the ATP binding region and the central cleavage/ligation domain.

Studies of a yeast topoisomerase II mutant lacking cleavage activity have indicated that DNA stimulation of the ATP binding rate is primarily mediated through G-segment binding, and
It has been suggested that binding of the T-segment might be required to obtain full stimulation of ATP hydrolysis (7). Additionally, investigation of wild type human topoisomerase IIα using DNA fragments of different lengths has revealed that stimulation of the ATPase rate may be dependent upon both G- and T-segment binding (28). According to these results, an impaired ability to bind either of the two DNA duplexes could cause the DNA-independent ATP binding and hydrolysis activities observed by G164I/wt. However, the cleavage results showed that the mutation at Gly-164 did not affect the intrinsic cleavage activity, indicating that the mutant binds the G-segment normally. The relaxation data further demonstrated that the enzyme is able to capture a T-segment. Thus, it seems unlikely that the inability of DNA to stimulate the ATP binding and hydrolysis activities of the heterodimer are caused by impaired DNA interactions. It is, however, more likely that the stimulatory effect of DNA on ATP binding is normally exerted through binding of the second ATP after clamp closure, which would correlate with the earlier observation that ATP binding in topoisomerase IIα is cooperative only in the presence of DNA (5). In that case we would not expect any DNA stimulation of ATP binding in G164I/wt.

Like ATP binding, ATP hydrolysis in G164I/wt is unaffected by DNA binding, and the enzyme does not show the normal stimulatory effect of ATP on DNA cleavage. When topoisomerase II-mediated cleavage is performed in the presence of ATP or an ATP analogue, the DNA cleavage/ligation equilbrium is normally shifted toward cleavage. This shift has been suggested to result from a delay in the ligation reaction due to formation of a transient gate in the cleaved G-segment that can accommodate T-segment transport (10, 31). However, the heterodimer lacked the stimulatory effect of ATP on cleavage complex formation, although the active site tyrosines were able to move apart to allow transport of the T-segment. Based on these results, binding of the second ATP molecule could be required for stabilization of a gate already formed upon binding of the first ATP, or it could influence the rate of cleavage per se. Alternatively, if signaling between the ATP binding domain and the cleavage/ligation domain requires a functional interaction between the two ATP binding sites, the stimulatory effect of the nucleotide on cleavage could be abolished in the heterodimer.

Cleavage might indirectly be related to a DNA-dependent stimulation of ATP hydrolysis. This has been suggested earlier from studies with yeast topoisomerase II, where an active site mutant unable to perform DNA cleavage was found to have a decreased rate of ATP hydrolysis in the presence of DNA. The data were explained by an inability of the mutant enzyme to adopt a specific cleavage conformation needed for the stimulatory effect of DNA on ATP hydrolysis or by an inability of the enzyme to interact properly with the T-segment, thus causing a decreased ATP hydrolysis (7). In the G164I/wt heterodimer, ATP hydrolysis is unaffected by DNA, although the heterodimer can trap a T-segment. It is therefore more likely that binding of the second ATP molecule, either directly or indirectly, is necessary for the ability of DNA to stimulate the rate of ATP hydrolysis at least of the first ATP molecule. Such a coupling between binding of the second ATP and hydrolysis of the first would seem natural, because it would place the different events in the correct order, ensuring that G-segment binding and cleavage have occurred before ATP hydrolysis allows T-segment transport.

Together, our data can be explained in a model in which the catalytic cycle begins with binding of a G-segment and one ATP molecule to the enzyme. Binding of the first ATP will lead to clamp closure and consequently trapping of a T-segment. After clamp closure the G-segment will facilitate binding of another ATP molecule. This ATP could now stimulate G-segment cleavage or stabilize the gate in the G-segment. Either through this event or via the T-segment the second ATP will stimulate hydrolysis of the first ATP molecule, which will force the T-segment through the subunit interface and the gate in the G-segment. Because DNA ligation is favored over cleavage in the normal cleavage/ligation equilibrium, the gate in the G-segment might be passively ligated following strand passage. At this step, the T-segment needs to be expelled through the primary dimerization region, and the enzyme subsequently resets with opening of the N-terminal clamp. Either one or both of these events might be facilitated by hydrolysis of the second ATP. Although the precise details of some of these steps are not yet known, it is clear from the present study that binding of a second ATP is not essential for DNA transfer and enzyme resetting but exerts an important function in the communication between the N-terminal ATPase domain and the cleavage/ligation domain to promote efficient catalysis.

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REFERENCES

1. Wang, J. C. (1996) Annu. Rev. Biochem. 65, 635–692
2. Burden, D. A., and Osheroff, N. (1998) Biochim. Biophys. Acta 1400, 139–154
3. Strick, R., and Laemmli, U. K. (1995) Cell 83, 1137–1148
4. Berger, J. M., Gamblin, S. J., Harrison, S. C., and Wang, J. C. (1996) Nature 379, 225–232
5. Lindsley, J. S., and Wang, J. C. (1993) J. Biol. Chem. 268, 8096–8104
6. Bjergbaek, L., Kingma, P., Nielsen, I., Wang, Y., Westergaard, O., Osheroff, N., and Andersen, A. H. (2000) J. Biol. Chem. 275, 13041–13048
7. Morris, S. K., Harkins, T. T., Tennyson, R. B., and Lindsley, J. E. (1999) J. Biol. Chem. 274, 3446–3452
8. Lindsley, J. S., and Wang, J. C. (1993) Nature 361, 749–750
9. Rocca, J., and Wang, J. C. (1992) Cell 71, 833–840
10. Osheroff, N. (1986) J. Biol. Chem. 261, 9944–9950
11. Harkins, T. T., Lewis, T. J., and Lindsley, J. E. (1998) Biochemistry 37, 7299–7312
12. Baird, C. L., Harkins, T. T., Morris, S. K., and Lindsley, J. E. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 13695–13699
13. Jensen, S., Andersen, A. H., Kjeldsen, E., Biersack, H., Olsen, E. H., Andersen, T. B., Westergaard, O., and Jakobsen, B. K. (1998) Mol. Cell. Biol. 18, 3866–3877
14. Jensen, T. H., Jensen, A., and Kjems, J. (1995) Gene (Amst.) 162, 235–237
15. Worland, S. T., and Wang, J. C. (1989) J. Biol. Chem. 264, 4412–4418
16. Biersack, H., Jensen, S., Gromova, I., Nielsen, I. S., Westergaard, O., and Andersen, A. H. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 8288–8293
17. Osheroff, N., Shelton, E. R., and Brutlag, D. L. (1985) J. Biol. Chem. 258, 9536–9543
18. Wigley, B. D., Davies, G. J., Dodson, E. J., Maxwell, A., and Dodson, G. (1991) Nature 351, 624–629
19. Chang, S., Hu, T., and Hsieh, T. S. (1998) J. Biol. Chem. 273, 19822–19828
20. Rocca, J., Ishida, R., Berger, J. M., Andoh, T., and Wang, J. C. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 1781–1785
21. Patel, S., Jatrawi, E., Creighton, A. M., Austin, C. A., and Fisher, L. M. (2000) Mol. Pharmacol. 58, 560–568
22. Sehested, M., Wessel, I., Jensen, L. H., Holm, B., Oliveri, R. S., Kenrick, S., Creighton, A. M., Nitiss, J. L., and Jensen, P. B. (1998) Cancer Res. 58, 1460–1468
23. Wessel, I., Jensen, L. H., Jensen, P. B., Falck, J., Rose, A., Roerth, M., Nitiss, J. L., and Sehested, M. (1999) Cancer Res. 59, 3442–3450
24. Wessel, I., Jensen, L. H., Benodin-Cornaire, A., Sorensen, T. K., Nitiss, J. L., Jensen, P. B., and Sehested, M. (2002) FEBS Lett. 520, 161–166
25. Morris, S. K., Baird, C. L., and Lindsley, J. E. (2000) J. Biol. Chem. 275, 2633–2638
26. Robinson, M. J., and Osheroff, N. (1991) Biochemistry 30, 1897–1813
27. Gardiner, L. P., Roper, D. I., Hammond, T. B., and Maxwell, A. (1998) Biochemistry 37, 16997–17004
28. Hammond, T. B., and Maxwell, A. (1997) J. Biol. Chem. 272, 32696–32703
29. O’Dea, M. H., Tamura, J. K., and Gellert, M. (1986) J. Biol. Chem. 271, 9723–9729
30. Baird, C. L., Gordon, M. S., Andrenyak, D. M., Marecek, J. F., and Lindsley, J. E. (2001) J. Biol. Chem. 276, 27893–27898
31. Robinson, M. J., and Osheroff, N. (1999) Biochemistry 28, 2511–2515

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