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The QTK Loop Is Essential for the Communication between the N-Terminal ATPase Domain and the Central Cleavage−Ligation Region in Human Topoisomerase IIα[†]

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ABSTRACT: We have characterized a human topoisomerase IIα enzyme with a deletion of the conserved QTK loop, which extends from the transducer domain to the ATP-binding pocket in the GHKL domain. The loop has been suggested to play a role for interdomain communication in type II topoisomerases. The mutant enzyme performs only very low levels of strand passage, although it is able to cleave and ligate DNA as well as close the N-terminal clamp. Cleavage is nearly unaffected by ATP and ATP analogues relative to the wild-type enzyme. Although the enzyme is able to close the clamp, the clamp has altered characteristics, allowing trapping of DNA also in the absence of an ATP analogue. The enzyme furthermore retains intrinsic levels of ATPase activity, but the activity is not stimulated by DNA. Our observations demonstrate that the QTK loop is an important player for the interdomain communication in human topoisomerase IIα. First, the loop seems to play a role in keeping the N-terminal clamp in an open conformation when no nucleotide is present. Once the nucleotide binds, it facilitates clamp closure, although it is not essential for this event. The QTK loop, in contrast, is essential for the DNA-stimulated ATPase activity of human topoisomerase IIα.

DNA topoisomerase II is a multifunctional and highly complex enzyme that is able to change the topological conformation of DNA during DNA metabolic processes, including DNA replication, transcription, recombination, and chromosome segregation (*1*−*5*). Topoisomerase II enzymes mediate topological changes by introducing a transient break in one DNA duplex, the G segment, allowing coordinated transport of another intact DNA duplex, the T segment, through the gated DNA. During the process, ATP is required to drive the enzyme through a series of conformational changes depending on both interdomain and intersubunit communication (*6*−*9*).

The homodimeric eukaryotic topoisomerase II enzyme consists of three distinct regions. The active site for ATP hydrolysis is contained in the N-terminal region, and the DNA cleavage and ligation activities are held by the central core region (*10*). These two regions are highly conserved in topoisomerase II enzymes from different eukaryotic organisms, and they also share homology with the bacterial DNA topoisomerase II counterpart, DNA gyrase. The C-terminal domain varies between the type II topoisomerases and is dispensable for catalytic activity in vitro (*11*−*13*).

Structural data have demonstrated that the N-terminal region consists of two domains in each protomer (*14*, *15*). The most N-terminal domain shares homology with the GHKL-type ATPases and holds the ATP-binding site that dimerizes and forms a clamp upon nucleotide binding (*8*, *14*−*17*). The second domain is called the transducer domain, and this domain bridges the N-terminal ATPase domain with the core region and forms the walls of the clamp. ATP binding and clamp closure allow T-segment trapping. During clamp closure. the transducer domains approach one another, thereby creating a very tight cavity. Structural studies of yeast DNA topoisomerase II and human topoisomerase IIα have revealed that this cavity is too small to hold the T-segment, and it has been suggested that the transducer domain pushes the T-segment through the gate in the G-segment upon N-terminal clamp closure (*14*, *18*). Cleavage and ligation of the G-segment are managed by the core region of topoisomerase II. This central region is a heart-shaped dimer, as revealed by crystallization studies, and it consists of two domains, A′ and B′, exhibiting homology to the subunits of DNA gyrase, gyrase A and gyrase B, respectively (*19*). The A′ domain contains the active site tyrosine, which becomes covalently attached to the 5′-end of the DNA during cleavage (*10*). It also encompasses a large cavity holding the transported DNA after passage through the G-segment as well as the primary dimerization region in the enzyme (*19*, *20*). The B′ domain constitutes the interface between the transducer domain and the A′ domain and has a proposed role in the separation of the cleaved DNA ends, moving the A′

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domain by undergoing large conformational rearrangements in response to signals from the ATPase domain (*7*, *19*, *21*).

To ensure an effective catalytic cycle of topoisomerase II, a number of mechanistic events must be strictly coordinated. This is accomplished by extensive communication between the ATP-binding site and the DNA cleavage−ligation site. Thus, ATP binding stimulates G-segment cleavage, and binding of DNA stimulates ATP hydrolysis (*22*−*25*). Communication between the ATP-binding domain and the central domain of the enzyme, responsible for DNA cleavage and ligation, has been suggested to go through the transducer domain (*6*, *21*, *26*−*28*). Strong arguments for this have been obtained from structural studies (*18*, *26*, *29*) as well as two studies in which mutations in the C-terminal part of the transducer domain of human topoisomerase IIα were shown to result in a disturbed interdomain communication (*6*, *21*). The transducer domain contains a highly conserved QTK loop (amino acids 376−378 in human topoisomerase IIα) that extends into the ATP-binding pocket, where the lysine contacts the γ-phosphate of the bound nucleotide, most likely acting as a transition state stabilizer in the ATP hydrolysis reaction (*14*−*16*, *18*, *26*, *30*). The lysine has been proposed to be an important player in the interdomain communication between the ATP-binding site and the rest of the enzyme (*27*, *28*).

Structural studies of the archaeal topoisomerase VI bound to AMP-PNP or ADP suggest that nucleotide binding in type II topoisomerases leads to an ATP "restrained" conformation, where the clamp is closed and the transducer domain tightly packed against the GHKL domain, kept in place by the interaction between the loop lysine and the γ-phosphate of the bound nucleotide. The T-segment trapped upon clamp closure is thought to exert a pressure on the walls surrounding the cavity found in the transducer domain, which can be released by hydrolysis of one of the ATP molecules due to a transition of the enzyme subunit holding this ATP into a "relaxed" conformation. In this conformation, the loop and the rest of the transducer domain of the subunit are rotated with respect to the GHKL domain, resulting in an asymmetric structure with an increased width of the cavity (*18*, *26*, *29*). The relaxed conformation is supposed to favor P_i release and promote T-segment transport through a gate formed in the G-segment. Although several observations agree with the proposed model, biochemical and structural evidence to support this model is still lacking.

In this paper, we have purified and characterized a mutant human topoisomerase IIα enzyme lacking the QTK loop to investigate the role of this loop in interdomain and intersubunit communication. We find that the loop is important for several steps in the catalytic cycle of topoisomerase IIα and for the ability of the enzyme to efficiently pass through the series of conformational changes essential for optimal strand passage activity. Our data are in line with a transition of human topoisomerase IIα between a restrained and a relaxed conformation during catalysis, where the QTK loop is essential for this transition.

MATERIALS AND METHODS

Yeast Strains and Plasmids. *Saccharomyces cerevisiae* strain JEL1Δ*top1* (α *leu2 trp1 ura3-52 prb1-1122 pep4* Δ*his3*::*PGAL1-GAL4 top1*::*LEU2*) (kindly provided by J. C. Wang) was used for overexpression of recombinant human topoisomerase IIα. A modified version of the YEpWOB6 vector containing the human topoisomerase IIα cDNA under control of the yeast GAL1 promoter was used for overexpression of the hexahistidine-tagged human wild-type and mutant topoisomerase IIα enzymes. The topoisomerase IIα cDNA of YEpWOB6 was first modified with a hexahistidine tail at the C-terminus. For this purpose, a C-terminal fragment of topoisomerase IIα fused to a hexahistidine tail was generated via polymerase chain reaction (PCR) using pHT212 as the template (*12*). The 3′-primer was designed with a stretch of 54 overhanging nucleotides containing the hexahistidine tail and three restriction sites for cloning. The annealing segment of the primer was the reverse complementary sequence of the human c-Myc epitope (5′-TCC CCC CGG GGC GGC CGC CTC GAG CTA ATG ATG GTG GTG ATG GTG GCT CCC ACG GTT CAA GTC TTC TTC AGA GAT CAA C-3′). The 5′-primer sequence was identical to nucleotides 2973−2991 of the topoisomerase IIα cDNA sequence (5′-GAG AGA GTT GGA CTA CAC-3′). The generated PCR fragment was used to replace the corresponding fragment of human topoisomerase IIα in YEpWOB6 employing *Bln*I and *Xma*I as 5′- and 3′-cloning sites, respectively.

The PCR protocol QuikChange site-directed mutagenesis K kit (Stratagene) was subsequently used to delete the bases encoding Gln-376, Thr-377, and Lys-378 in the modified version of YEpWOB6. The primers used in the PCR were 5′-AAC CCA ACC TTT GAC TCT GAA AAC ATG ACT TTA CAA CCC AAG AG-3′ and 5′-GGT TGT AAA GTC TTT TCA GAG TCA AAG GT GGG TTT TCA AT-3′. The construct was sequenced to verify the deletion of the nine bases corresponding to Gln-376, Thr-377, and Lys-378.

Yeast Transformation. Yeast cells were transformed by using a modified version of the LiAc method of Ito et al. (*31*).

Human Topoisomerase IIα Overexpression and Purification. The recombinant human topoisomerase IIα enzymes were overexpressed in yeast strain JEL1Δ*top1* and purified through Ni^{2+}-nitriloacetic acid-agarose, heparin-Sepharose, and phosphocellulose column chromatography as described by Oestergaard et al. (*21*).

Topoisomerase II-Mediated DNA Relaxation. DNA relaxation was performed by incubating various concentrations of topoisomerase II with 6.5 nM negatively supercoiled pUC19 plasmid DNA in a total volume of 20 μL of buffer [50 mM Tris-HCl (pH 8), 140 mM KCl, 1 mM EDTA, and 8 mM $MgCl_2$ supplemented with 1 mM ATP]. The reaction mixtures were incubated at 37 °C and the reactions stopped after 10 min or at the indicated time points by the addition of SDS and EDTA to final concentrations of 0.1% and 10 mM, respectively. The samples were subjected to electrophoresis in 1% agarose gels in TBE buffer. DNA was stained with 1 μg/mL ethidium bromide and visualized with UV light.

Topoisomerase II-Mediated DNA Cleavage of Oligonucleotides. The following synthetic oligonucleotides were used in the cleavage assay: top strand (5′-TGCATGAGGATGACGA-TGAGCGCATTGTTAGATTGATG-3′) and bottom strand (5′-AATCATCAATCTAACAATGCGCTCATCGTCATCC-TCATGCA-3′). Hybridization and labeling of the synthetic oligonucleotides were performed by mixing 10 pmol of the top strand with 10 pmol of the complementary 41-mer (bottom strand) in 40 mM Tris-HCI (pH 7.5), 20 mM $MgCl_2$, and 50 mM NaCl in a 55 μL reaction volume. The mixture was heated to 68 °C for 2 min and allowed to cool slowly to room temperature. After hybridization, the top strand was labeled at the 3′-end by incubation of 5 pmol of hybrid DNA with 1 unit of Sequenase (USB) in 24 mM Tris-HCl (pH 7.5), 12 mM $MgCl_2$,

30 mM NaCl, 6 mM dithiothreitol, and 0.2 μM [α-^{32}P]dATP in a 30 μL reaction volume for 5 min at room temperature.

For topoisomerase II-mediated DNA cleavage of the oligonucleotide duplex, 20 nM topoisomerase II was incubated with 1 nM labeled substrate in 20 μL of 10 mM Tris-HCl (pH 7.0), 5 mM $MgCl_2$, 140 mM KCl, and 0.1 mM EDTA for 10 min at 37 °C, and the reactions were stopped by the addition of SDS to a final concentration of 1%. The samples were subjected to sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) on 8% polyacrylamide gels, allowing for subsequent quantification of the covalent topoisomerase II−DNA cleavage complexes by PhosphorImager scanning (Molecular Imager, Bio-Rad). When topoisomerase II-mediated cleavage was conducted in the presence of ATP or AMPPNP, the concentration of the nucleotide was 1 mM.

Topoisomerase II-Mediated DNA Cleavage of Plasmid DNA. DNA cleavage was performed by incubating 50 nM topoisomerase II with 5.5 nM negatively supercoiled pUC19 plasmid DNA in 50 mM Tris-HCl (pH 7.9), 0.1 mM EDTA, 5 mM $MgCl_2$, 1 mM ATP, 2.5% glycerol, and 125 mM KCl for 10 min at 37 °C in the presence or absence of drug as indicated. Cleavage products were trapped by addition of SDS to a final concentration of 1%, and samples were treated with 2 μL of 0.8 mg/mL proteinase K before being subjected to electrophoresis in 1% agarose gels. A Molecular Imager was used for gel scanning. DNA was stained with 1 μg/mL ethidium bromide and visualized with UV light.

Topoisomerase II-Mediated DNA Ligation. Topoisomerase IIα DNA cleavage−ligation equilibria were established by use of a plasmid DNA substrate as described above. After incubation for 10 min, DNA ligation was initiated by shifting reaction mixtures from 37 to 0 °C, and reactions were stopped after 0, 5, 10, 20, and 40 s by the addition of 2 μL of 10% SDS followed by 1 μL of 375 mM EDTA (pH 8.0). Samples were treated with 2 μL of 0.8 mg/mL proteinase K before being subjected to electrophoresis in 1% agarose gels, containing 1 μg/mL ethidium bromide.

Topoisomerase II-Mediated Hydrolysis of ATP. Reaction mixtures contained 100 nM topoisomerase II and, when indicated, 5.5 nM negatively supercoiled pUC19 plasmid DNA. Reactions were conducted in 20 μL of 50 mM Tris-HCl (pH 8), 140 mM KCl, 1 mM EDTA, and 8 mM $MgCl_2$ containing a final concentration of 1 mM cold ATP and 2 μCi (0.033 μM) of [γ-^{32}P] 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(ethylenimine) (Baker-flex precoated flexible TLC sheets). Chromatography was performed using freshly made 0.4 M NH_4HCO_3. Levels of free PO_4 were quantified using a PhosphorImager (Molecular Imager, Bio-Rad).

Clamp Closing Assay. For clamp closing experiments, 20 nM topoisomerase II was preincubated with 6.5 nM negatively supercoiled pUC19 plasmid DNA for 5 min in a total volume of 20 μL of buffer [50 mM Tris-HCl (pH 8), 140 mM KCl, 1 mM EDTA, and 8 mM $MgCl_2$]. After preincubation, AMP-PNP was added to a final concentration of 1 mM and the reaction mixtures were incubated for a further 5 min. The reactions were next stopped by the addition of 80 μL of 800 mM NaCl. To trap enzyme−DNA catenanes, phenol extraction was performed by adding 1 volume of phenol. The samples were vortexed and centrifuged at 13000 rpm in an Eppendorf centrifuge for 10 min. The water phase was removed, ethanol-precipitated, and dissolved in 10 μL of TE buffer [10 mM Tris-HCl (pH 7.4) and 1 mM EDTA] for gel analysis. The combined phenol phase and phenol/water interphase were washed once in 4 M NaCl and once in 0.8 M NaCl. Upon removal of the water phase after the last wash, the remaining material was ethanol-precipitated and dissolved in 10 μL of TE buffer containing 1 mg/mL proteinase K. The samples were next subjected to electrophoresis in TBE buffer in a 1% agarose gel containing 1 μg/mL ethidium bromide.

RESULTS

Purification of Human Topoisomerase IIα Containing a Deletion of the QTK Loop. Previous studies have suggested that the QTK loop, constituted by the three amino acids, Gln-376, Thr-377, and Lys-378, in human topoisomerase IIα, plays an important role for enzyme catalysis, and the loop has been proposed to mediate communication between different regions of the enzyme (*21*, *26*, *27*). To study the implications of the QTK loop for interdomain communication in human topoisomerase IIα, we have characterized a mutant enzyme (ΔQTK) in which Gln-376, Thr-377, and Lys-378 have been deleted (Figure 1A). The QTK loop is highly conserved and extends from the transducer domain into the GHKL domain, contacting the nucleotide binding pocket (Figure 1B). For studies of the in vitro capabilities of ΔQTK, the enzyme fused to a hexahistidine tail at the C-terminal end was overexpressed in a *top1* null strain and purified to homogeneity as seen from the Coomassie-stained gel in Figure 1C.

Deletion of the QTK Loop in Human Topoisomerase IIα Strongly Reduces the Strand Passage Activity. To examine the DNA strand passage activity of ΔQTK, a relaxation assay was performed in which the catalytic activity of the mutant enzyme was compared to that of the wild-type enzyme during a 10 min incubation. As seen from Figure 2 (top panel), the relaxation activity of ΔQTK is less than 1% of that of the wild-type enzyme. To examine if the decreased relaxation activity of ΔQTK corresponds to stoichiometric levels, where each enzyme goes through only one catalytic cycle, we have extended the incubation time of the relaxation reaction. However, nearly full relaxation was obtained after 160 min with the enzyme:DNA ratio used in the top panel, lane 8 (Figure 2, bottom panel). This demonstrates that ΔQTK can perform enzyme resetting and go through successive relaxation cycles, albeit very slowly. Thus, ΔQTK is slow in one or more of the steps involved in strand passage and catalytic turnover, or the enzyme is unable to efficiently coordinate the basic activities, N-terminal clamp closure, DNA cleavage and ligation, ATP hydrolysis, strand transport, and enzyme resetting into a fully functional catalytic mechanism.

The Cleavage−Ligation Region Is Active, but the Level of Baseline Cleavage Is Greatly Increased. 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 upon addition of a strong denaturing agent depends on both DNA cleavage and ligation. If ATP or an ATP analogue is present, strand passage is allowed and the equilibrium will be shifted toward cleavage, resulting in a 2−5-fold increase in the amount of cleavage complexes (*6*, *17*, *32*).

Because ΔQTK has a severely reduced relaxation activity, we investigated if the catalytic site for cleavage and ligation still constitutes a functional domain in the enzyme. Cleavage

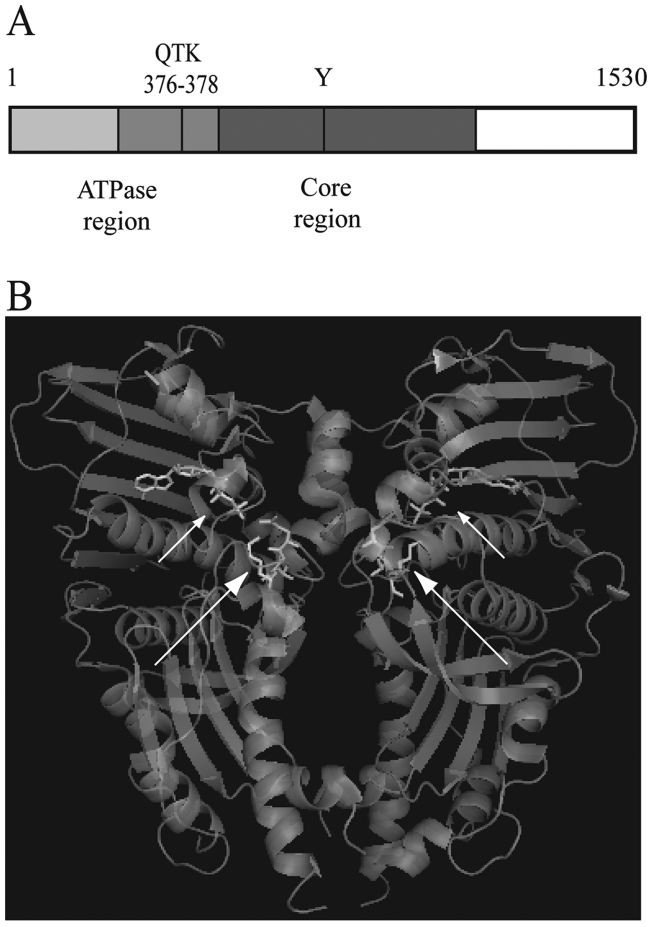

FIGURE 1: Purification of human topoisomerase IIα containing a deletion of the QTK loop. (A) Schematic representation of human topoisomerase IIα. Three amino acids, Gln-376, Thr-377, and Lys-378, constituting the QTK loop, were deleted. Numbers indicate amino acid positions, and Y indicates the position of the active site tyrosine. The GHKL domain is colored light gray, the transducer domain gray, the core region dark gray, and the C-terminal region white. (B) Structure of the N-terminal fragment of human topoisomerase IIα bound to AMP-PNP [modified with PYMOL from Protein Data Bank entry 1ZXM (*18*)], showing the position of the QTK loop. The monomers are colored in gray shades. The large and small arrows indicate the positions of the QTK loop and the bound AMP-PNP, respectively. (C) Purified ΔQTK and wild-type topoisomerase IIα. The homogeneity of the enzyme preparations was determined in a 10% SDS−polyacrylamide gel stained with Coomassie Blue. Protein size markers are indicated to the right of the gel. wt denotes wild-type human topoisomerase IIα.

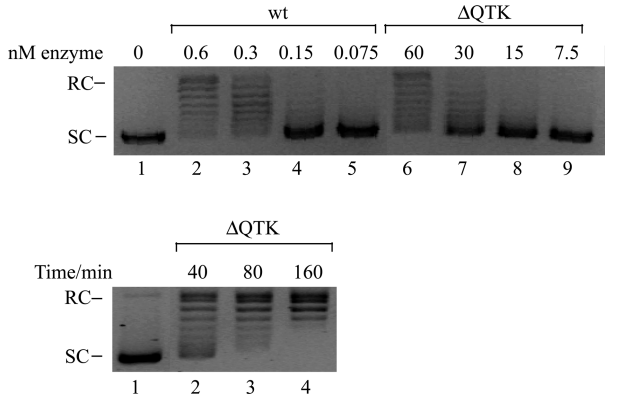

FIGURE 2: ΔQTK retains less than 1% strand passage activity relative to the wild-type enzyme. In the top panel, ΔQTK and the wild-type enzyme were investigated in a DNA relaxation assay using the indicated enzyme concentrations and 10 min incubation time. In the bottom panel, a relaxation assay was performed with 15 nM ΔQTK using the indicated incubation times. The products were analyzed on 1% agarose gels. The enzymes used in the reactions are indicated above the lanes. Lane 1 of the bottom panel contained the DNA control. The positions of supercoiled (SC) and relaxed (RC) circular plasmid DNA are indicated. wt denotes wild-type human topoisomerase IIα.

experiments were therefore performed with the negatively supercoiled DNA substrate used for relaxation (Figure 3A). Relative to the wild-type enzyme, ΔQTK has a much higher baseline level of cleavage in the absence of nucleotide, and the cleavage−ligation equilibrium is only vaguely affected by the presence of nucleotide (Figure 3A). In the presence of ATP, the wild-type enzyme relaxes the DNA, whereas ΔQTK mainly cleaves the DNA, in support of its very slow catalytic cycle.

To further investigate the cleavage reaction, we have used a 40-mer DNA duplex substrate, which more readily allows measurements of cleavage levels. As shown in the histogram presented in Figure 3B, the level of baseline cleavage is 2.5-fold higher in ΔQTK than in the wild-type enzyme. However, whereas the amount of cleavage complexes trapped with the wild-type enzyme is increased ~3.5-fold when AMP-PNP is present, the analogue does not further increase the level of cleavage by ΔQTK.

To examine if the increased level of cleavage observed with ΔQTK in the absence of nucleotide can be caused by an inability of the enzyme to ligate the cleaved DNA, a ligation assay was set up. A cleavage−ligation equilibrium was first established between topoisomerase II and supercoiled plasmid DNA. Ligation was next followed after a shift in the temperature to 0 °C, which inhibits further cleavage (*33*). As shown in Figure 3C, ΔQTK is able to ligate DNA to an extent similar to that of the wild-type enzyme, and the initial ligation rates of the two enzymes are comparable (data not shown). Thus, the increased cleavage level obtained with ΔQTK cannot be explained by an inability of the enzyme to perform ligation.

Topoisomerase II poisons like mAMSA and VM26 stabilize topoisomerase II−DNA cleavage complexes by inhibiting topoisomerase II-mediated DNA ligation (*2*, *34*, *35*). To investigate if the central cleavage−ligation domain of ΔQTK responds to such drugs, cleavage reactions were performed in the presence of 20 μM mAMSA. As shown in Figure 3D, mAMSA stimulates cleavage with ΔQTK in support of the ability of the enzyme to perform ligation. Relative to the stimulation observed with the wild-type enzyme, the stimulation with ΔQTK is weak, probably reflecting the high basal cleavage level of ΔQTK, which may be close to the upper limit of the enzyme.

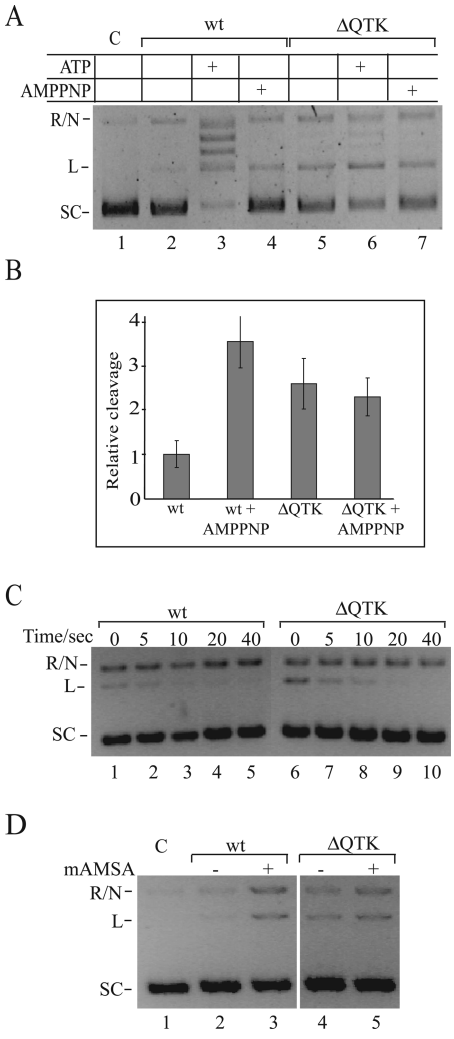

FIGURE 3: The cleavage−ligation domain in ΔQTK is active, but cleavage is only vaguely affected by ATP or AMP-PNP. (A) A cleavage assay was performed in which ΔQTK or wild-type human topoisomerase IIα was incubated with supercoiled plasmid DNA in the absence or presence of ATP or AMP-PNP as indicated. Cleavage products were investigated in a 1% agarose gel after SDS and proteinase K treatment: C, DNA control; SC, supercoiled circular plasmid DNA; L, linear plasmid DNA; R/N, relaxed or nicked circular plasmid DNA. (B) A cleavage experiment was performed using an end-labeled 40 bp DNA duplex as a substrate, and cleavage complexes were directly analyzed in an 8% SDS−polyacrylamide gel after SDS treatment. The obtained cleavage levels were quantified using a PhosphorImager and are presented in arbitrary units relative to the cleavage obtained with the wild-type enzyme in the absence of nucleotide. The results are the means ± standard deviation of three independent experiments. wt denotes wild-type human topoisomerase IIα. (C) To investigate the DNA ligation ability of ΔQTK relative to that of the wild-type enzyme, a cleavage assay was first set up with ΔQTK and the wild-type enzyme using supercoiled plasmid DNA as the substrate. After incubation for 10 min, DNA ligation was initiated by shifting the samples from 37 to 0 °C. Samples were withdrawn at the indicated time points, reactions stopped with SDS, and mixtures treated with proteinase K before they were analyzed in a 1% agarose gel. DNA ligation is revealed as a disappearance of the linear cleavage products: SC, supercoiled circular plasmid DNA; L, linear plasmid DNA; R/N, relaxed or nicked circular plasmid DNA; wt, wild-type human topoisomerase IIα. (D) A cleavage experiment was performed as described for panel A but in the presence of 20 μM mAMSA: C, DNA control; SC, supercoiled circular plasmid DNA; L, linear plasmid DNA; R/N, relaxed or nicked circular plasmid DNA.

Altogether the results demonstrate that the central domain harboring the catalytic site for cleavage and ligation is active in ΔQTK. However, the reduced effect of ATP or AMP-PNP on enzyme-mediated cleavage suggests that ΔQTK is severely impaired in its ability to transmit information from the N-terminal ATPase domain to the central cleavage−ligation domain. Alternatively, the conformation normally induced upon nucleotide binding already exists in ΔQTK.

ΔQTK Has Intrinsic but Not DNA-Stimulated ATPase Activity. To investigate if ΔQTK shows abnormalities in its ability to bind and hydrolyze ATP, the ATPase activity of the mutant enzyme was investigated in the absence or presence of DNA and compared to that of the wild-type enzyme. As seen in Figure 4, the intrinsic ATPase activity obtained in the absence of DNA is comparable for ΔQTK and the wild-type enzyme. Thus, ΔQTK retains the ability to both bind and hydrolyze ATP.

In the wild-type enzyme, the ATPase activity is stimulated ~20-fold in the presence of negatively supercoiled plasmid DNA, but no stimulation is observed with DNA in ΔQTK (Figure 4). The exact events leading to DNA-mediated stimulation of the ATPase activity of topoisomerase II are not known. In a previous study, DNA was shown to have a reduced effect on the ATPase activity of the cleavage-deficient topoisomerase IIα enzyme, Y805S, which was suggested to be due to the inability of the enzyme to perform strand passage (*28*). In this study, DNA stimulates the ATPase activity of Y805S ~2-fold, although no stimulation is seen in ΔQTK, which still holds some strand transfer activity. On the basis of these observations, an intimate correlation between strand transfer and DNA-mediated ATPase stimulation does not seem to exist in topoisomerase IIα.

In conclusion, the QTK loop is not necessary for basic levels of ATP hydrolysis but clearly plays an essential role in the mechanism that allows DNA to stimulate ATP hydrolysis.

ΔQTK Has Retained Its Ability To Close the N-Terminal Clamp. 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 (*36*, *37*). To test whether ΔQTK has retained this important function of the N-terminus, a clamp closing assay was performed (Figure 5A) [modified from that of Bjergbaek et al. (*6*)]. ΔQTK or the wild-type enzyme was preincubated with supercoiled plasmid DNA before the addition of ATP or AMP-PNP. When AMP-PNP is added to the wild-type enzyme, it leads to the formation of salt stable topoisomerase II−DNA interlinked complexes, which can be collected from a phenol/water interphase. When ΔQTK is incubated in the presence of AMP-PNP, the amount of supercoiled DNA trapped in the interphase is equivalent to the amount trapped by the wild-type enzyme (Figure 5B), showing that the mutant enzyme has retained the ability to close the N-terminal clamp. In contrast to the wild-type enzyme, ΔQTK also traps DNA in the absence of nucleotide and in the presence of ATP. Thus, although ΔQTK is able to perform clamp closure, it has lost the strict regulation of the clamp.

DISCUSSION

To understand the importance of the QTK loop for the coupling among ATP binding and hydrolysis, DNA gate opening, T-segment transport, and enzyme resetting in human topoisomerase IIα, we have characterized a mutant enzyme (ΔQTK) that has a deletion of the QTK loop. On the basis of structural studies performed on archaeal topoisomerase VI, it has been suggested that the loop is involved in a transition from an ATP restrained to a relaxed conformation upon ATP hydrolysis (*26*, *29*, *38*).

Our characterization has revealed that ΔQTK, although capable of N-terminal clamp closure, ATP hydrolysis, and DNA cleavage and ligation, has a very low strand passage activity. The regulation of the clamp is not as strict as in the wild-type enzyme, and the cleavage−ligation equilibrium is shifted toward cleavage. Furthermore, the mutant enzyme lacks normal ATP-stimulated DNA cleavage and DNA-stimulated ATP hydrolysis.

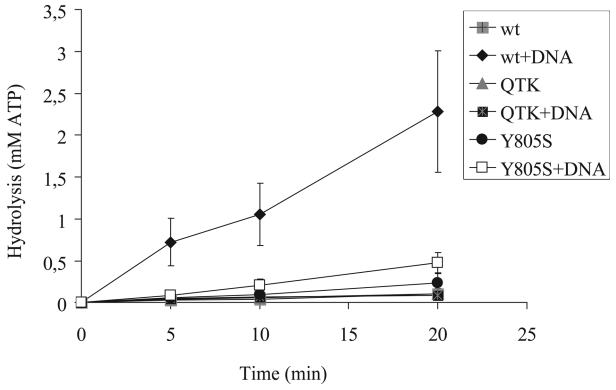

FIGURE 4: ΔQTK binds and hydrolyzes ATP, but DNA does not stimulate ATPase activity. The ATPase activity of either wild-type human topoisomerase IIα, ΔQTK, or Y805S was measured by incubating the enzyme with [γ-^{32}P]ATP at 37 °C in the presence or absence of DNA. The samples were removed at the indicated time points and spotted onto thin layer cellulose plates. The levels of free phosphate were measured using a Molecular Imager (Bio-Rad). The results are the means ± standard deviation of three independent experiments. wt denotes wild-type human topoisomerase IIα.

The activities still remaining in the N-terminal ATPase and central core regions demonstrate that the two catalytic domains are still able to fold into active entities in the mutant enzyme. However, deletion of the QTK loop seems to inhibit the communication pathway between the two catalytic domains, resulting in an enzyme with very low levels of strand passage activity. Our data suggest that removal of the QTK loop in human topoisomerase IIα favors a conformation in which the clamp is closed and both subunits of the enzyme are in a relaxed conformation with the DNA gate open.

Clamp Closure. Although ΔQTK still retains the ability to close the N-terminal clamp, deletion of the QTK loop disturbs clamp function. Thus, capture of circular DNA occurs not only upon binding of an ATP analogue but also in the absence of nucleotide and in the presence of ATP (Figure 5). This suggests that ΔQTK favors the closed clamp conformation and that one of the roles of the QTK loop in the wild-type enzyme is to prevent dimerization of the N-terminal domain, when ATP is absent. This idea is supported by observations made with crystal structures of the structurally similar topoisomerase VI enzyme, where the loop is located toward the center axis of the enzyme in the absence of nucleotide. This orientation of the loop could inhibit clamp closure simply by steric hindrance (*14*, *26*, *29*). In line with a role of the QTK loop as a regulator of clamp closure, a study by Hu et al. (*27*) has demonstrated that a change of the lysine residue of the QTK loop to glutamic acid in *Drosophila* topoisomerase II results in an enzyme with reduced clamp closure activity. In this mutant, the interaction between the lysine and the γ-phosphate of ATP cannot take place, and as a possible consequence of this, the loop does not easily change its position upon ATP binding. However, since binding of the nucleotide still

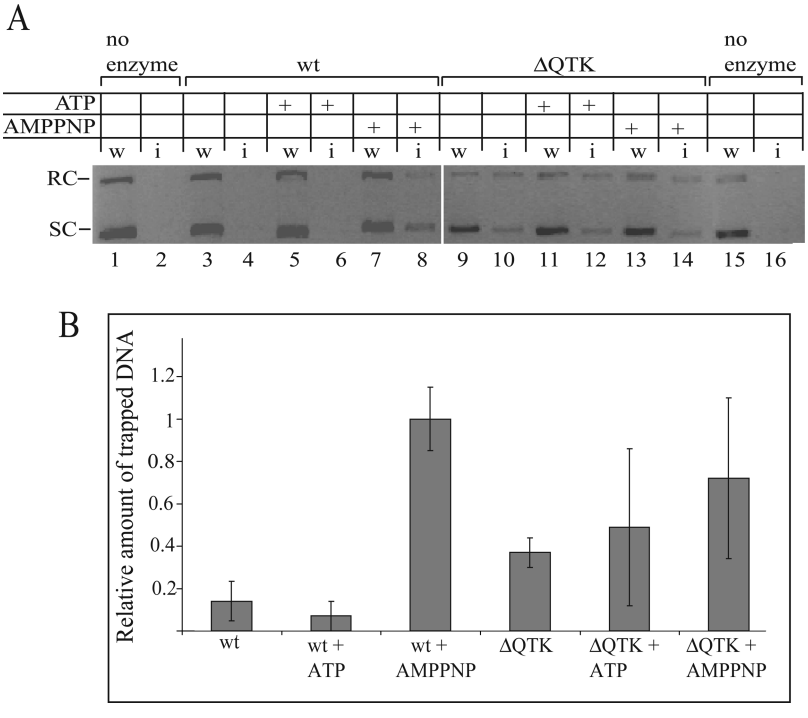

FIGURE 5: ΔQTK retains clamp closure activity but has lost the ability to regulate the clamp properly. (A) A clamp closing experiment was performed with either ΔQTK or the wild-type enzyme using circular plasmid DNA as the substrate. ATP or AMP-PNP was added as indicated after preincubation of the enzyme and DNA. Trapped complexes were recovered from the phenol/water interphase after phenol extraction and analyzed in a 1% agarose gel after proteinase K treatment together with the water phase samples. RC and SC denote relaxed circular and supercoiled circular plasmid DNA, respectively. wt denotes wild-type human topoisomerase IIα; i and w denote the phenol/water interphase and water phase, respectively. (B) Histogram of clamp closing experiments performed as described for panel A. The amount of supercoiled plasmid DNA trapped by the wild-type enzyme in the presence of AMP-PNP relative to the total amount of supercoiled plasmid DNA was set to 1. The results are the means ± standard deviation of three independent experiments.

seems to facilitate clamp closure in ΔQTK, the interaction between the QTK loop and the γ-phosphate is not a prerequisite for clamp closure but merely facilitates correct operation of the clamp. The fact that ΔQTK traps circular DNA in the presence of ATP probably reflects the very low ATPase activity in ΔQTK, which inhibits reopening of the clamp.

ATP Hydrolysis and Strand Passage. In ΔQTK, the strand passage activity is reduced to less than 1% of that of the wild-type enzyme (Figure 2), and DNA does not stimulate ATP hydrolysis (Figure 4). The very low strand passage activity is not caused by an inability of the enzyme to reset as it is able to perform multiple catalytic cycles (Figure 2). Rather, our data indicate that ΔQTK, due to the lack of the loop in both subunits, is unable to attain the optimal strand transfer conformation, where one subunit is relaxed and the other restrained. In ΔQTK, both subunits are expected to favor the relaxed conformation. Thus, the T-segment trapped upon clamp closure fails to generate the pressure that normally would trigger ATP hydrolysis, in line with the inability of ΔQTK to perform DNA-stimulated ATP hydrolysis. The consequence of bypassing this step is a severe reduction in the strand transfer activity. Furthermore, the reduced level of hydrolysis may exert a major effect on enzyme resetting, contributing to the slow relaxation activity of ΔQTK. It is also possible that ΔQTK, having both subunits in a relaxed conformation, can accommodate the T-segment in the N-terminal cavity without a fully opened gate in the G-segment, which could contribute to the reduced strand transfer activity.

N-Terminal Control of the DNA Gate. Structure determinations of type II topoisomerases have so far been unable to demonstrate how the conformational changes, taking place in the N-terminal region upon ATP binding, are connected to DNA cleavage and formation of a gate in the G-segment. Relative to the wild-type enzyme, ΔQTK has a very high cleavage level in the absence of nucleotide, but in contrast to the wild-type enzyme, the level of cleavage is only vaguely if at all increased by nucleotide binding (Figure 3) (*17*). This indicates that ΔQTK already in the absence of nucleotide favors a conformation with near-optimal cleavage as expected for an enzyme with the G-segment opened (*29*, *38*). Our data thus point to the existence of an open gate in the relaxed enzyme conformation and strongly suggest that there is an intimate correlation among QTK loop operation, clamp closure, and gate opening in human topoisomerase IIα. The importance of the N-terminal region for DNA gate formation has also been stressed by studies performed with a yeast topoisomerase II mutant having a deletion of the N-terminal region (*39*). Although the yeast enzyme was able to cleave the G-segment, the DNA gate was not opened to allow T-segment transport.

Role of the QTK Loop for Catalytic Activity. The obtained results strongly indicate that the interaction between ATP and the QTK loop of the N-terminal region coordinates the conformational changes in topoisomerase IIα needed for an efficient catalytic reaction. Our data fit into a model very similar to the model presented for archaeal topoisomerase VI (*26*, *29*). In this model, ATP binding to human topoisomerase IIα will lead to a conformational change in the enzyme, resulting in a movement of the QTK loop into a position where hydrogen binding between the γ-phosphate of the nucleotide and the lysine residue of the loop is allowed, and closure of the N-terminal clamp will take place. The enzyme is now in an ATP-restrained conformation. The loop may here play a role as a hinge in regulating clamp operation, simply by inhibiting clamp closure in the absence of nucleotide due to steric hindrance. Along with clamp closure, ATP binding will lead to a stimulation of cleavage. This occurs due to a transmission of the movements in the GHKL domain and of the QTK loop via the transducer domain further down to the cleavage−ligation region, where the result will be a cleavage event. Since the cavity formed upon clamp closure is too small to accommodate a DNA helix when a gate has not yet been formed in the cleaved G-segment, a trapped T-segment will exert a pressure on the walls of the transducer domain. This pressure is sensed by the QTK loop interacting with the γ-phosphate of the bound ATP. The pressure will be released upon hydrolysis of the first ATP molecule due to a movement of the loop contacting this ATP, which simultaneously allows P_i release. The conformational change obtained by this movement is again transmitted to the α-helical path of the transducer domain and subsequently to the cleavage−ligation region, where it will lead to formation of a gate in the G-segment allowing T-segment transport. In this way, both the G-segment and the T-segment are required to obtain full DNA-stimulated ATP hydrolysis, the G-segment to ensure that the cavity is kept in a restrained conformation upon clamp closure and T-segment trapping and the T-segment to exert the final pressure leading to ATP hydrolysis and movement of the QTK loop. The enzyme conformation generated upon T-segment expulsion and/or DNA ligation in the presence of a closed N-terminal clamp may affect the interaction between the QTK loop and ATP in the second subunit, resulting in hydrolysis of this ATP molecule. The second subunit will subsequently enter the relaxed state, and the clamp will reopen, leading to enzyme resetting.

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