The DNA Dependence of the ATPase Activity of Human DNA Topoisomerase IIα⁎

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We have purified human topoisomerase IIα from HeLa cells and studied its ATPase reaction. The ATPase activity is stimulated by DNA and shows apparent Michaelis-Menten kinetics. Although the ATPase activity of human topoisomerase IIα is lower than that of Saccharomyces cerevisiae, it is more active in decatenation, implying more efficient coupling of the ATPase to DNA strand passage under these conditions. Using plasmid pBR322 as the DNA cofactor, the reaction shows hyperstimulation by DNA at a base pair to enzyme dimer ratio of 100–200:1. When DNA fragments are used as the cofactor, the reaction requires > 100 base pairs to stimulate the activity and fragments of ~300 base pairs show hyperstimulation. This behavior can be rationalized in terms of the enzyme requiring fragments that can bind to both the DNA gate and the ATP-operated clamp in order for the ATPase reaction to be stimulated. Hyperstimulation is a consequence of the saturation of DNA with enzyme. The mechanistic implications of these results are discussed.

DNA topoisomerase IIα is a highly conserved enzyme that catalyzes topological changes in DNA (1, 2). These enzymes have been found in all cell types and are essential for cell viability. Their roles include maintenance of the level of intracellular DNA supercoiling, removing supercoils, which build up ahead of and behind transcription and replication complexes, and the decatenation of daughter chromosomes following replication. The topoisomerase reaction involves the breakage of DNA in one or both strands, the formation of protein-DNA covalent bonds, and the passage of another segment of DNA through the enzyme-stabilized break. In the case of type II enzymes, this DNA strand passage reaction generally requires the hydrolysis of ATP.

As a consequence of their essential roles in cells, DNA topoisomerases have become important drug targets. For example, the prokaryotic type II enzyme DNA gyrase is the target of a range of antibacterial agents such as the quinolone and coumarin drugs (3). The eukaryotic type I enzyme, DNA topoisomerase I, is the target of the antitumour agent camptothecin, and eukaryotic topoisomerase II is the target of a variety of antitumor drugs, which include amsacrine, epipodophyllotoxins, and mebarone (4, 5). Many of these compounds (e.g. quinolones, camptothecin, amsacrine, etc.) act by stabilizing a cleavable complex between the topoisomerase and DNA, in which the enzyme is covalently linked to the DNA. Arresting of DNA replication forks by this complex is thought to initiate events that lead to cell death (5). Other topoisomerase-targeted compounds act by different mechanisms, e.g. coumarin drugs (such as novobiocin) act as competitive inhibitors of the DNA gyrase ATPase reaction (6) and ICRF-159 is thought to stabilize the eukaryotic enzyme in a closed complex incapable of catalytic activity (7).

On the basis of the alignment of their amino acid sequences, DNA topoisomerases can be grouped into three subfamilies: type IA, type IB, and type II (8). All type II enzymes are evolutionarily and structurally related, each possessing two distinct catalytic centers: a DNA cleavage and rejoining site, and a site for ATP hydrolysis (9–13). The enzymes differ in their molecular masses and subunit composition, e.g. DNA gyrase from Escherichia coli consists of two subunits GyrA and GyrB of 97 and 90 kDa, which associate as an A2B2 complex (14). GyrA contains the DNA cleavage activity, while GyrB catalyzes ATP hydrolysis. Eukaryotic type II enzymes are homodimers where each monomer can be regarded as a fusion of a GyrB and GyrA subunit. Homology between eukaryotic and prokaryotic enzymes is closest in the N-terminal region and the region containing the active site for DNA cleavage (corresponding to the ATPase domain of GyrB and the N-terminal domain of GyrA, respectively), but the C termini tend to be divergent. The molecular sizes of the eukaryotic enzymes show some diversity; the enzyme from Saccharomyces cerevisiae (yeast topoisomerase II) has a monomer molecular mass of 164 kDa (15), whereas the two isoforms of the human enzyme α and β are 170 and 180 kDa (16).

The mechanism of eukaryotic topoisomerase II is now understood in some detail as a consequence of a number of structural and mechanistic studies (17–19). The enzyme binds a segment of DNA (~25 bp),1 which becomes the gate segment (or “G-segment”). The G-segment is cleaved in both strands with a 4-base stagger between the break sites. This leads to the formation of covalent bonds between the 5’-phosphates at the break site and the active-site tyrosines. Another segment of DNA (the “T-segment”) is captured by an ATP-operated clamp (comprising the N-terminal domains of the two subunits), which presents the T-segment to the double-stranded break in the G-segment and facilitates the strand passage reaction. Resolving of the break in the G-segment leads to a change in linking number of the DNA by 2, in the case of intramolecular reactions (e.g. DNA relaxation), or catenation or decatenation in the case of intermolecular strand passage.

Although the mechanism of topoisomerase II is now understood in some detail, the role of ATP hydrolysis remains to be clarified. ATP hydrolysis is normally required to drive reactions that are energetically unfavorable. Indeed, in the case of the prokaryotic type II topoisomerase, DNA gyrase, the requirement for ATP hydrolysis is clear. Gyrase can introduce negative supercoils into DNA, an energetically unfavorable

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1 The abbreviations used are: bp, base pair(s); G-segment, gate segment; T-segment, transported segment; kDNA, kinetoplast DNA; m-AMSA, amsacrine; G&T, enzyme-DNA complex involving both the G- and T-segments.

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reaction, which is coupled to ATP hydrolysis. In this case, there appears to be an approximate correspondence between the free energy available from the hydrolysis of ATP and the energy required to introduce supercoils (20–23). In the absence of ATP, gyrase can catalyze the relaxation of negative supercoils (an energetically favorable reaction) albeit less efficiently than the introduction of supercoils (24, 25). Eukaryotic topoisomerase II cannot introduce supercoils into DNA but relaxes DNA in an ATP-dependent reaction. Given that this is an energetically favorable reaction, it is unclear why ATP is required.

Early studies on topoisomerase II prepared from HeLa cells, *Drosophila melanogaster*, and calf thymus showed that the enzyme possessed a DNA-dependent ATPase activity (26–28). The degree of stimulation by DNA was 3–17-fold, depending on the source of the enzyme. More recent studies on the *Drosophila* enzyme have shown that phosphorylation by casein kinase II increases the rate of DNA-dependent ATP hydrolysis ~3-fold (29). This increase in ATPase activity mirrors the increase in recombination and catenation/dccatenuation activity by the enzyme, and it was concluded that modulation of the ATPase rate by phosphorylation determined the overall catalytic activity of the enzyme. Further experiments showed that the activation by phosphorylation could also be effected by protein kinase C (30). However, recent work on topoisomerase IIα from mouse showed no increase in enzyme activity following phosphorylation (31). This observation may simply reflect a difference in the properties of enzymes from mammalian and non-mammalian sources. A more detailed study of the ATPase reaction of yeast topoisomerase II has been carried out by Lindsley and Wang (32). The enzyme has an intrinsic ATPase activity, which is stimulated 19-fold by DNA. The DNA-independent reaction follows Michaelis-Menten kinetics with an estimated *k*ₐₚₑₙ of 1 s⁻¹. The DNA-dependent reaction shows cooperative binding of ATP to the two monomers in the enzyme dimer. Determination of the coupling between ATP usage and DNA strand passage showed that ~2 ATPs are hydrolyzed per strand passage event at low ATP concentrations, whereas at saturating ATP concentrations ~7–8 ATPs are hydrolyzed per event.

To further our understanding of ATP hydrolysis by DNA topoisomerase II, we have studied the ATPase reaction of the HeLa enzyme. Given the known effects of post-translational modifications on the activity of topoisomerase II, we have elected in the first instance to isolate the enzyme from a human cell line rather than a yeast clone to establish the properties of the native enzyme.

**EXPERIMENTAL PROCEDURES**

### Isolation of HeLa Topoisomerase IIα—Topoisomerase IIα was isolated from HeLa nuclear extracts (4C, Mons, Belgium) prepared from 2 x 10⁹ cells in 20 mM HEPES, pH 7.9, 100 mM KCl, 0.2 mM EDTA, 20% glycerol, 1 mM phenylmethylsulfonyl fluoride. The method of isolation is based on that described previously (16, 33). All procedures were performed at or below 4 °C where possible. Hydroxyapatite resin (50 g, Bio-Gel HTP) was pre-equilibrated with buffer HP + 150 mM potassium phosphate (buffer HP: 50 mM potassium phosphate, pH 7.0, 10 mM β-mercaptoethanol, 10% (v/v) glycerol, 1 mM phenylmethylsulfonyl fluoride). Nuclear extracts (60 ml) were gently mixed with the resin slurry for 15 min. The resin was collected by centrifugation (Beckman JT-21, JA-10 rotor, 3000 rpm, 5 min) and gently resuspended and given four 100-ml washes with buffer HP + 150 mM potassium phosphate to remove unbound proteins. The resin was then sequentially resuspended in, and centrifuged from, three 100-ml washes of buffer HP + 450 mM potassium phosphate and three 100-ml washes with buffer HP + 750 mM potassium phosphate. Each washing stage were tested for topoisomerase II activity as described below, and active supernatants were pooled for chromatographic separation (fraction I). Buffer HP + 850 mM potassium phosphate was added to fraction I until it reached a conductivity equivalent to, or greater than, buffer HP + 650 mM potassium phosphate.

### Fraction I was then loaded on a High Load Phenyl-Sepharose column (Pharmacia) equilibrated with buffer S plus 100 mM NaCl. This was eluted with a 25-ml gradient from 100 to 600 mM NaCl in buffer S. Active fractions from the Mono-S column were pooled and dialyzed against 50 mM Tris-HCl, pH 7.5, 100 mM KCl, 50% (v/v) glycerol, 0.5 mM EDTA, before storage as 100-μl aliquots at ~70 °C. Protein concentration was estimated by the method of Bradford (34), and topoisomerase II activity was estimated by the method described below. Wild-type yeast topoisomerase II, a C-terminal deletion mutant of yeast topoisomerase II (amino acids 1–1236), and an active-site mutant (Y783F) were kind gifts from E. Roberts and S. Gasser (Swiss Institute for Experimental Cancer Research, Lausanne, Switzerland).

### Assay for Topoisomerase II Activity—Samples were assayed for decatenation activity using kDNA (Topogen) as a substrate. Reaction mixtures contained 1–2 μl of sample in 20–30 μl of topoisomerase assay buffer (50 mM Tris-HCl, pH 8.0, 125 mM KCl, 10 mM MgCl₂, 1.25 mM ATP, 5 mM dithiothreitol, 0.5 mM EDTA, 150–200 ng of kDNA). Reactions were incubated for 30 min at 37 °C and stopped by addition of 10 μl of 100 mM Tris-HCl, pH 8.0, 40% (v/v) sucrose, 0.1 mM EDTA, 0.5 mg/ml bromophenol blue. Mixtures were then analyzed by agarose gel electrophoresis. One unit of topoisomerase II activity was defined as the amount of enzyme required to decatenate 200 ng of kDNA in 30 min at 37 °C. Gels were scanned and quantitated using a video camera linked to a UVP gel analysis program, version 2.0 (Ultraviolet Products Ltd., Cambridge, United Kingdom).

### Assay for ATPase Activity—ATP hydrolysis by topoisomerase IIα was linked to the oxidation of NADH (35) and the reaction monitored spectrophotometrically at 340 nm (Perkin Elmer Lambda 4, Unicam SP1800 or a Bio-Tek ELX340 microplate reader). The change in absorbance of the mixture was related to the hydrolysis of ATP by using *A*₅₅₀ = 6220 cm⁻¹. Assays (typically 200 μl) were performed in topoisomerase assay buffer containing 400 mM phosphate pyruvate, 50 mM NADH, and 5 μl of pyruvate kinase/lactate dehydrogenase enzymes (all from Sigma) at 37 °C. Reaction vessels were incubated in the assay mix overlaid with nuclease-free mineral oil (Sigma) to avoid losses due to evaporation. The rate of ATP hydrolysis was calculated from at least 5 min of continuous data collection after an initial equilibration period. In assays containing drugs, novobiocin (Sigma) was added as an 10 mM stock in water. Other topoisomerase II inhibitors (m-AMSA, from Sigma; etoposide and teniposide, from Bristol-Meyers; ICRF-159, a gift from Dr. J. R. Jenkins, University of Leicester; and merbarone, a gift from NCI, National Institutes of Health, Bethesda, MD) were added to ATPase reaction mixes as 2–10 mM stock solutions in Me2SO; the final MeSO₂ concentration was always below 1.5%.

### Production of DNA Fragments—DNA fragments (108–437 bp) were produced using polymerase chain reaction centered upon a 40-bp fragment from pBR322 previously described as a preferential binding site for the supromoter (36). Where inappropriate priming from pBR322 was a problem, a purified 606-bp fragment (from positions 1419–413 of pBR322) was used as a template. Upon completion of polymerase chain reaction, reaction mixtures were loaded onto a 5% agarose gel (Bio-Gel HTP) pre-equilibrated with 10 mM sodium phosphate, pH 7.2. DNA fragments were eluted with a 20-ml gradient of buffer from 10 to 600 mM sodium phosphate, pH 7.2. Fra-
Isolation of HeLa Topoisomerase IIα—HeLa topoisomerase IIα was isolated to high purity (>95%) as estimated from a silver-stained SDS-polyacrylamide gel (Fig. 1, panel A). A Western blot of a similar gel (Fig. 1, panel B) using an anti-topoisomerase IIα antibody (kind gift of Dr. J. R. Jenkins, University of Leicester) shows a single band at 170 kDa, consistent with human topoisomerase IIα (27). From a nuclear extract of 2 × 10^10 HeLa cells, 100 μg of topoisomerase IIα was isolated.

Intrinsic and DNA-stimulated ATPase of HeLa Topoisomerase IIα—Fig. 2 shows the increase of ATP hydrolysis by topoisomerase IIα upon the addition of supercoiled closed-circular plasmid pBR322 DNA. Straight line fits to each set of data give values of 0.055 ATP molecules hydrolyzed/s/dimer for the intrinsic ATPase rate and a 10-fold increase to 0.56 s^-1/dimer for the DNA-stimulated rate at 37 °C. The DNA base pair to enzyme dimer (bp:dimer) ratios were kept above 1000:1 for all readings in this assay to ensure a saturating DNA concentration at all enzyme concentrations (32). No significant difference was seen in the DNA-stimulated ATPase rate when using relaxed closed circular or linear pBR322 (added supercoiled pBR322 would be relaxed within the first few minutes of the ATPase reaction). Both the intrinsic and DNA-stimulated rates observed are low when compared with yeast and Drosophila DNA topoisomerase II. Yeast topoisomerase II has reported intrinsic and DNA-stimulated rates of 0.37 s^-1 and 7 s^-1 at 30 °C (32), and under assay conditions identical to those for the HeLa enzyme, a DNA-stimulated rate of 3 s^-1 was observed (data not shown). Drosophila has an ATPase rate of 5–13 s^-1 at 30 °C, depending on the phosphorylation state of the enzyme (29, 30). However, the rates observed are more comparable to the intrinsic and DNA-stimulated rates measured for DNA gyrase, −0.05 s^-1 and 1 s^-1 at 25 °C (37), and for calf thymus topoisomerase II, 0.3 and 1.1 s^-1 (28). HeLa topoisomerase IIα was, however, very active in a decatenation assay. Under the conditions described under “Experimental Procedures,” it gave a consistently faster rate of decatenation than the yeast enzyme (Fig. 3). Rates of decatenation calculated from Fig. 3 show human topoisomerase IIα to be at least 5 times more active. Considering that the 5-fold increase in activity is achieved while hydrolyzing ATP at a 15-fold slower rate, it would suggest that, under these assay conditions, human topoisomerase IIα is more efficient in terms of coupling ATP hydrolysis to strand passage than yeast topoisomerase II.

Table I shows the effects of various DNA topoisomerase II inhibitors on the DNA-stimulated ATPase rate of human topoisomerase IIα. Assays contained 13 nm enzyme dimer and 2 μM (in base pairs) supercoiled pBR322. We find that all of the DNA topoisomerase poisons tested (m-AMSA, etoposide, and teniposide) and ICRF-159 inhibit ATPase activity by a maximum of approximately 80% of the control, but do not abolish DNA-stimulated ATPase. These data are consistent with previous studies (38). However, merbarone, which does not stabilize an enzyme-DNA cleavable complex, showed minimal inhibition of the ATPase reaction. The effect of novobiocin, a potent inhibitor of the ATPase activity of DNA gyrase (39, 40), on the DNA-stimulated ATPase reaction was investigated. No change in rate was seen at concentrations of novobiocin up to 10 μM, although some effect was seen at higher concentrations (0.1–
ATPase assays containing 10 nM enzyme dimer and 1 μM (in base pairs) supercoiled pBR322 were incubated with the topoisomerase inhibitors indicated. The DNA-stimulated ATPase rate for each reaction was plotted against the concentration of drug in the reaction mix and ID<sub>50</sub> values estimated as the concentration that gave 50% of maximal inhibition. It should be noted that in no case was the ATPase activity of the enzyme completely inhibited by drug; DNA-stimulated activity was reduced to approximately 20% of control values for all compounds except novobiocin and merbarone.

| Compound      | ID<sub>50</sub> μM |
|---------------|-------------------|
| Novobiocin    | 700               |
| m-AMSA        | 11                |
| Etoposide     | 11                |
| Teniposide    | 7                 |
| ICRF-159      | 5                 |
| Merbarone     | >100              |

TABLE I
Effects of topoisomerase II inhibitors on the catalytic activity of human topoisomerase IIa

Effect of DNA Concentration on ATPase Rate—Fig. 4 shows the effect of adding different concentrations of supercoiled pBR322 to ATPase assay mixtures containing topoisomerase IIa. DNA stimulates the ATPase reaction until a critical point is reached, whereupon the ATPase rate decreases with increasing DNA concentration, and then levels off at a rate similar to that seen in the DNA-stimulated data in Fig. 2. This pattern is apparent at different enzyme concentrations, and the spike is centered around a bp:dimer ratio of approximately 100–200 (Fig. 4, inset). The ATPase rates measured in this spike region are very sensitive to both the DNA concentration and to the monovalent cation concentration of the assay mixture.

Similar observations have been made with wild-type yeast topoisomerase II. These experiments were performed in both the assay buffer described under “Experimental Procedures” and that previously described for the yeast enzyme (32). In both cases, ATPase activity was increased and then decreased with DNA addition. The effect was most marked under assay conditions used in this study, but nonetheless apparent under assay conditions described previously (32). To address the question of a possible regulatory role of the C-terminal region of the enzyme affecting the DNA stimulation of the ATPase rate, experiments were performed using a truncated yeast enzyme containing the first 1236 amino acids. This enzyme gave a similar spike response upon DNA stimulation (data not shown).

Determination of k<sub>cat</sub> and K<sub>m</sub> Values for the ATPase Reaction of HeLa Topoisomerase IIa—It became apparent that the measurement of kinetic parameters for the ATPase rate of HeLa topoisomerase IIa is complicated by the sensitivity of the system to DNA concentration. Fig. 5 demonstrates the effect of substrate (ATP) on the measured ATPase rate of HeLa topoisomerase IIa in the presence of two different DNA concentrations, giving bp:dimer ratios of 130 (open circles) and 2000 (filled circles). Enzyme at a bp:dimer ratio of 130 gives a k<sub>cat</sub> of 2.17 ± 0.13 s<sup>-1</sup> and a K<sub>m</sub> of 0.78 ± 0.12 mM. Similar calculations for enzyme at a bp:dimer ratio of 2000 give a k<sub>cat</sub> of 0.59 ± 0.04 s<sup>-1</sup> and a K<sub>m</sub> of 0.56 ± 0.17 mM. Again these parameters are different to those quoted for yeast topoisomerase II (k<sub>cat</sub> of 8 s<sup>-1</sup> and K<sub>m</sub> of 0.13 μM; ref. 32).

Effects of DNA Length on HeLa Topoisomerase IIa ATPase Rate—To investigate further the DNA stimulation of HeLa topoisomerase IIa, DNA fragments were produced of various lengths from 40 to 890 bp. Fig. 6 shows the effects of these DNA fragments on the ATPase rate of HeLa topoisomerase IIa when added in equimolar amounts. Addition of a 40-bp fragment induces no increase in rate, whereas fragments of >100 stimulate the rate. When the size of the added DNA fragment reaches ~300 bp, the ATPase rate increases markedly, to a maximum of around 1.2 s<sup>-1</sup>. This rise is then followed by a fall in rate and a leveling off, forming “spike” and plateau regions in the graph similar to those seen in Fig. 4, with ATPase rates eventually returning to that seen in the DNA-stimulated data for Fig. 2. In Fig. 7, we show the effect of higher DNA:enzyme ratios on the ATPase reactions with selected fragments. Fragments of less than 300 bp stimulated an increase in ATPase rate when added at 2-fold and 4-fold excess over enzyme dimer concentration. However, those fragments of over 300 bp showed a decrease in ATPase activity upon addition of additional DNA.
The rate of ATP hydrolysis of topoisomerase IIa (10 nM enzyme dimer) was measured as described under “Experimental Procedures” in the presence of DNA fragments of various lengths. In all experiments, the fragments were added in a ratio of 2 DNA molecules to each topoisomerase IIa dimer (i.e. 20 nM). The ATP concentration was 1.25 mM throughout. Data are plotted as ATP molecules hydrolyzed per dimer per second and represent the DNA-stimulated rate observed minus the DNA-independent rate for each experiment.

**DISCUSSION**

We have isolated to high purity human topoisomerase IIα from HeLa cells and investigated the ATP hydrolysis activity of this enzyme. We considered it important to obtain data on the human enzyme from HeLa cells as a prelude to work on enzyme overexpressed in yeast. Proteins overexpressed in such clones are ubiquitously known as human, but some carry a modified N terminus (41, 42) and may therefore differ in their biochemical characteristics from the native enzyme. This is particularly true for the ATP hydrolysis of the enzymes, as this is known to be carried out by the N-terminal domain (43). In addition, enzymes derived from yeast will not necessarily have undergone the same post-translational modifications as those from human cells. This is important with respect to phosphorylation of the enzyme, as this may play a major role in determining the ATPase activity (29, 30). The N-terminal region contains a phosphorylation site at serine 29 (44), and the availability of this residue for phosphorylation in enzyme cloned in yeast may be affected, as the N-terminal portion of this enzyme contains a yeast-derived peptide followed by the human enzyme from serine 29 onward (42).

Human topoisomerase IIα was found to have an intrinsic ATPase rate of approximately 0.05 ATP molecules hydrolyzed/s/dimer at 37 °C (Fig. 2), the DNA-stimulated ATPase rate is at least 10-fold higher than this. The degree of stimulation correlates well with the 3–17-fold stimulation of other type II topoisomerases upon addition of DNA (26–28, 32). However, the actual rate of ATP hydrolysis is low compared with other eukaryotic enzymes. Rates observed for enzymes derived from non-mammalian sources are some 6–16 times faster than those for the human enzyme (30, 32), but those observed with calf thymus topoisomerase II are similar (28). The slow rate of ATP hydrolysis does not translate to low topoisomerase activity when comparing human and yeast enzymes. The human enzyme has a 5-fold higher rate of decatenation of kDNA under the reaction conditions herein and is thus apparently more efficient than the yeast enzyme in coupling the ATPase reaction to decatenation (Fig. 3). Under other reaction conditions, he yeast enzyme may show more efficient coupling.

We find that the DNA-independent and DNA-dependent ATPase reactions of topoisomerase IIa conform to the Michaelis-Menten paradigm. For the DNA-dependent reaction, the values of $k_{\text{cat}}$ are dependent upon the DNA:enzyme ratio. In work with the yeast enzyme, in the absence of DNA the enzyme was Michaelian, but in the presence of DNA it showed marked cooperativity in terms of ATP binding (32). Given that the topoisomerase II dimer has two ATP binding sites, some level of cooperativity is likely. The fact that we see no evidence of this in our experiments may reflect the value of the individual rate constants in the reaction pathway. Indeed, this situation obtains with DNA gyrase where ATP binding is known to be cooperative but binding and ATPase experiments do not necessarily show evidence of cooperativity (22, 45, 46).

The coumarin drug novobiocin competitively inhibits the DNA gyrase ATPase reaction (6) and has some reported activity against *Drosophila* topoisomerase II (38). We find that novobiocin has very weak activity against the human enzyme (Table I). The effects of antitumor compounds on the ATPase rate of topoisomerase II (Table I) show inhibition by intercalating and non-intercalating poisons (m-AMSA, etoposide, and teniposide) and by the ATP-operated clamp binding compound ICRF-159 (7, 47). Merbarone shows little inhibition of ATP hydrolysis at concentrations tested. None of the compounds completely abolished the rate of ATP hydrolysis, even at high drug concentrations; a similar observation was seen with some antitumor agents tested against the DNA-stimulated ATPase of *Drosophila* topoisomerase II (38). This suggests that because these compounds interact with the enzyme-DNA cleavable complex (m-AMSA, etoposide, and teniposide), or with the ATP dependent clamp (ICRF-159), they interfere with the topoisomerase reaction cycle and alter the rate of ATP hydrolysis in an indirect manner. The exception to this is merbarone, which has no effect upon the ATPase reaction at concentrations known to inhibit enzyme activity (33, 48).

The most striking aspect of the observed DNA-stimulated rate of ATPase is the unusual dependence on DNA concentration (Fig. 4). Human topoisomerase IIa incubated with increasing concentrations of pBR322 shows maximal stimulation at a
hydrolysis is dependent upon cleavage of the G-segment. Taken together, these data indicate that the G-segment must be bound and cleaved and the T-segment must be present in the ATP-operated clamp to stimulate the rate of ATP hydrolysis (this situation is referred to as the “G&T” complex). At low concentrations of the 40-bp fragment, the predominant DNA-enzyme complex is believed to be that shown in Fig. 8 (panel a, i), where the DNA is shown bound as a G-segment (but could alternatively be bound as a lone T-segment), and this gives no measurable increase in ATPase rate. The production of the G&T complex, and the consequent stimulation of ATPase rate, can be forced by the addition of a large excess of DNA as two fragments are bound per enzyme dimer (one at the DNA cleavage site and the other at the ATP-operated clamp).

Addition of 108–263-bp fragments at low concentrations results in an increase in the ATPase rate, and, in general, the degree of ATPase rate stimulation is proportional to the length of the DNA fragment added (Fig. 6). This is thought to be due to longer DNA fragments being more flexible and, therefore, together, these data indicate that the G-segment must be bound and cleaved and the T-segment must be present in the ATP-operated clamp to stimulate the rate of ATP hydrolysis (this situation is referred to as the “G&T” complex). At low concentrations of the 40-bp fragment, the predominant DNA-enzyme complex is believed to be that shown in Fig. 8 (panel a, i), where the DNA is shown bound as a G-segment (but could alternatively be bound as a lone T-segment), and this gives no measurable increase in ATPase rate. The production of the G&T complex, and the consequent stimulation of ATPase rate, can be forced by the addition of a large excess of DNA as two fragments are bound per enzyme dimer (one at the DNA cleavage site and the other at the ATP-operated clamp).

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more likely to bind both the gate and clamp simultaneously and forming a G&T complex using one DNA fragment (Fig. 8, panel a, iii). It is worth noting that, in DNA gyrase, there are enzyme-DNA interactions that are thought to promote the binding of DNA to the unoccupied binding site (52), greatly increasing the probability of forming a G&T complex. This may explain the all or nothing characteristics of the dependence of the ATPase activity on DNA length seen with this enzyme (37). Eukaryotic topoisomerase II probably does not wrap DNA in the same manner as gyrase (53); hence, DNA flexibility may be a major factor in determining whether or not a G&T complex is formed.

An increase in the rate of ATP hydrolysis is seen upon addition of excess DNA fragments of these lengths (108–263 bp, Fig. 7). This may simply reflect increased probability of DNA binding at high DNA concentrations or the possibility of two fragments being bound per enzyme dimer.

DNA fragments of 300–350 bp show hyperstimulation of the ATPase activity as manifested by the spike in Fig. 6. This phenomenon is similar to the hyperstimulation by pBR322 and is approximately double the bp:dimer ratio seen at the spike upon addition of pBR322. At DNA lengths greater than approximately 450 bp per dimer (achieved by adding longer fragments or a higher concentration of pBR322), this crowding effect diminishes as the enzymes become more separated along the DNA molecule and the ATPase rates observed decreased to plateau levels. Higher concentrations of DNA fragments of 312–330 bp also decrease the rate of ATPase (Fig. 7), as this provides more DNA for single dimer-DNA complexes and therefore reduces the degree of saturation of the enzyme on the DNA.

For this model to be valid, the interaction of two adjacent topoisomerase II dimers must bring about an increase in the rate of ATP hydrolysis of the enzymes, and we have two possible explanations for this. One is that one or more of the conformations shown in Fig. 8 (panel b, i–iv) results in stabilization of the G&T complex. In effect, the T-segment becomes trapped in the ATP-operated clamp, either due to steric effects from the adjacent enzyme-DNA complex inhibiting strand passage (e.g. Fig. 8, panel b, i) or via competition between two enzymes to transport the same piece of DNA (e.g. Fig. 8, panel b, iv). T-segment passage by yeast DNA topoisomerase II is known to be inhibited at high enzyme DNA ratios (170 bp/dimer) (54), and this would result in an increase in the ATPase rate as the G&T complex is effectively stabilized. A second explanation is that, while in close proximity, the enzymes undergo protein-protein interactions and form tetratomers. This protein-protein interaction leads to enhanced ATPase activity. Such tetratomers have been seen in yeast topoisomerase II, and their production is promoted by the addition of short pieces of DNA (55). The human enzyme shows some evidence for formation of such tetratomers, as determined by its sedimentation characteristics (data not shown). Why these complexes, proposed to be structural components of the chromosome, should exhibit raised rates of ATP hydrolysis is unknown. Indeed, in the yeast model the removal of the C terminus reduced the formation of tetratomers (55) and hence our observation that the yeast C-terminal deletion mutant showed spike characteristics does not favor this particular explanation of the data.

In summary, we have found that the ATPase reaction of HeLa topoisomerase IIα is stimulated by DNA in a length-dependent fashion and that hyperstimulation occurs at certain enzyme-DNA ratios. The physiological relevance of these observations is unclear, and we do not know the effects of phosphorylation on the DNA dependence of the ATPase reaction. Such issues are currently under investigation.

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