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Proteolysis patterns of epitopically labeled yeast DNA topoisomerase II suggest an allosteric transition in the enzyme induced by ATP binding

(type II DNA topoisomerases/DNA gyrase/immunoblotting/protein footprinting)

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ABSTRACT **A cloned yeast *TOP2* gene was modified to produce yeast DNA topoisomerase II (EC 5.99.1.3) epitopically labeled at its amino or carboxyl terminus. Limited digestion with SV8 endoprotease shows three distinct protease-sensitive sites in each polypeptide of the dimeric enzyme. These sites were mapped by immunostaining of the end-labeled proteolytic fragments resolved by SDS/polyacrylamide gel electrophoresis; two of the mapped locations were confirmed by sequencing the amino ends of two unlabeled peptic fragments. Proteolytic cleavage by SV8 endoprotease at a pair of sites corresponding to the carboxyl sides of Glu-411 and Glu-680 is modulated by the binding of the nonhydrolyzable ATP analogs adenosine 5′-[β,γ-imido]triphosphate (5′-adenylyl imidodiphosphate) and adenosine 5′-[γ-thio]triphosphate: in their absence cleavage occurs predominantly at Glu-411; in the presence of either analog, cleavage occurs predominantly at Glu-680. These results are interpreted in terms of allosteric interdomainal movements in the type II DNA topoisomerase following the binding of ATP.**

Type II DNA topoisomerases (EC 5.99.1.3) are ubiquitous enzymes that form gates in DNA for the passage of one double-stranded DNA through another (1–4). *In vivo*, these enzymes participate in a number of processes involving DNA, including the segregation of pairs of newly replicated DNA molecules and the condensation and decondensation of chromosomes (5–7).

All type II DNA topoisomerases are evolutionarily and structurally related, and each possesses two distinct catalytic centers: a DNA breakage and rejoining site and a DNA-stimulated ATPase site (1–4). The enzyme breaks a double-stranded DNA by forming two DNA–protein phosphotyrosine linkages between the 5′ phosphoryl ends of the severed DNA strands and a pair of tyrosyl residues, one in each half of the multimeric enzyme. Following the passage of a DNA segment through the transiently broken DNA, rejoining of the strands occurs by a second transesterification reaction between the pair of 3′-hydroxyl groups on the broken DNA strands and the DNA–protein phosphotyrosine links. Biochemical experiments have identified Tyr-122 of the *Escherichia coli* DNA gyrase A subunit and Tyr-783 of the single-subunit *Saccharomyces cerevisiae* DNA topoisomerase II as the respective active-site tyrosine residues (8, 9). The DNA-stimulated ATPase site is located near the amino terminus of the B subunit of bacterial gyrase (10–14). From sequence comparisons, it can be readily inferred that the ATPase site is located near the amino end of eukaryotic DNA topoisomerase II or that of the gene 39-encoded subunit in the T-even phage DNA topoisomerases.

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Catalysis of DNA strand passage by type II DNA topoisomerases is strongly dependent on ATP hydrolysis (1–4). How ATP binding and hydrolysis are coupled to the various enzyme-mediated manipulations of the DNA strands is not clear. Important clues have been obtained, however, through the use of the nonhydrolyzable ATP analogs. Linking number measurements of enzyme-bound DNA rings, in the presence and absence of these analogs, suggest that nucleotide binding is sufficient for one double-stranded DNA passage event and that hydrolysis is required for returning the enzyme to its initial state (3, 4). Transient electric dichroism and exonuclease III protection assays have revealed changes in the DNA structure when a gyrase–DNA complex binds adenosine 5′-[β,γ-imido]triphosphate (Ado*PP*[NH]*P*) (15). Results from small-angle neutron scattering experiments indicate a slight conformational change in gyrase upon binding Ado*P*-*P*[NH]*P* (16). Recently, it has been reported that the ATP binding domain of *E. coli* DNA gyrase B subunit forms a dimer in the presence of Ado*PP*[NH]*P*, but not in its absence (14).

To explore further how ATP usage is coupled to the type II DNA topoisomerase-mediated manipulations of DNA, we have studied the effects of ATP and its analogs on the sites of proteolytic cleavage in the yeast enzyme. In this communication, we show that the presence of Ado*PP*[NH]*P* or adenosine 5′-[γ-thio]triphosphate (ATP[γS]) alters the pattern of cleavage of yeast DNA topoisomerase II by protease SV8. By using yeast DNA topoisomerase II tagged at its amino or carboxyl end with an immunologically distinct oligopeptide, we have mapped the positions of exposed proteolytic sites in the absence and presence of ATP analogues. The results are interpreted in terms of allosteric interdomainal movements in the enzyme upon ATP binding.

MATERIALS AND METHODS

Materials. ATP, Ado*PP*[NH]*P*, adenosine 5′-[β,γ-methylene]triphosphate (Ado*PP*[CH_2]*P*), ATP[γS], and SV8 endoprotease were purchased from Boehringer Mannheim. The plasmid pMCT6, which contains the coding sequence of a decapeptide epitope of human c-myc protein (17) and was used in the construction of the expression vectors described below, was provided by Fernando Moya (Harvard University). A monoclonal antibody MYC 1-9E10.2 specific to this epitope was prepared from a hybridoma line (ATCC no. CRL 1729) by the Harvard University monoclonal facility.

Overexpression and Purification of Wild-Type, Mutant, and Epitopically Tagged *S. cerevisiae* DNA Topoisomerase II. Wild-type yeast DNA topoisomerase II was overexpressed in

Abbreviations: ATP[γS], adenosine 5′-[γ-thio]triphosphate; Ado*PP*[CH_2]*P*, adenosine 5′-[β,γ-methylene]triphosphate (5′-adenylyl methylenediphosphate); Ado*PP*[NH]*P*, adenosine 5′-[β,γ-imido]triphosphate (5′-adenylyl imidodiphosphate).

yeast from the inducible promoter PGAL1 in a multicopy expression plasmid, YEpTOP2-PGAL1 (9). Construction of the mutant Gly-144 → Ile by oligonucleotide directed mutagenesis will be described elsewhere. Derivatives of YEpTOP2-PGAL1 expressing the yeast enzyme tagged at its amino terminus (myc-*Sc*topoII) or carboxyl terminus (*Sc*topoII-myc) were constructed by standard recombinant DNA methodology. In the clone myc-*Sc*topoII, the beginning codons encode Met-*Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu*-Asn-Ser-Cys-Ser-Pro-Gly-Asp-Pro-Val-Thr; these are followed by the 1429 codons of wild-type yeast DNA topoisomerase II. The italicized decapeptide is the human c-myc epitope specifically recognized by the monoclonal antibody MYC 1-9E10.2 (17); the 10 codons following the italicized sequence were introduced during the cloning steps. In the clone *Sc*topoII-myc, the last 95 codons in the wild-type yeast enzyme are replaced by Met-*Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu*-Asn. These expression vectors were transformed into the yeast strain JEL1 (*α leu2 trp1 ura3-52 prb1-1122 pep4-3 Δhis3::PGAL10-GAL4*), the construction of which will be described elsewhere. In this strain, expression of GAL4 protein is induced by galactose, which improves the expression of genes linked to promoters that are activated by GAL4 protein. Purification of the various overexpressed proteins from JEL1 cells was done as described (9).

Proteolysis. Purified DNA topoisomerase II was preincubated for 10 min at 30°C with ATP, ATP analog, DNA, or their combinations, in a protease SV8 digestion buffer containing 50 mM Tris acetate (pH 7.5), 165 mM potassium acetate, 5 mM magnesium acetate, 5 mM 2-mercaptoethanol, and 10% (vol/vol) glycerol. SV8 endoprotease was then added, and digestion was carried out at 30°C for the length of time indicated in the figure legends. Proteolysis was stopped by adding an equal volume of SDS/PAGE sample-loading buffer (125 mM Tris·HCl, pH 6.8/20% glycerol/2% SDS/2% 2-mercaptoethanol/0.001% bromophenol blue) and boiling for 5 min. Samples were stored at −20°C and thawed before loading.

Gel Electrophoresis and Electroblotting. Protein samples were analyzed by SDS/PAGE in 8%, 12%, or 5–10% linear-gradient gels with 4% stacking gels in all cases (18). All of the proteolytic patterns shown in the figures were obtained with the gradient gels; the molecular weights of polypeptides estimated from their electrophoretic mobilities in the gradient gels were confirmed from corresponding values estimated from experiments with constant percentage running gels. Gels were stained in Coomassie blue, destained, and photographed, or bands were blotted onto nitrocellulose membranes for immunochemical staining by using an electrophoretic transferring unit (Bio-Rad). Following electroblotting, the membranes were probed sequentially with anti-human c-myc monoclonal antibody and goat anti-mouse antibodies linked to horseradish peroxidase (Bio-Rad) as described (19). Sequencing of the amino ends of purified polypeptides was performed by William S. Lane of the Harvard Microchemical Facility, using an Applied Biosystems model 477A protein sequenator connected on-line to a model 120A high-pressure liquid chromatograph.

RESULTS

Cleavage of Yeast DNA Topoisomerase II by Protease SV8 Is Altered by the Presence of Ado*PP*[NH]*P* or ATP[γS], but Not by ATP. A time course of digestion of *S. cerevisiae* DNA topoisomerase II with endoprotease SV8 is shown in Fig. 1. This protease cleaves on the carboxyl side of glutamate or aspartate residues, and thus there are numerous potential cleavage sites in the dimeric yeast enzyme with 1429 amino acids (164 kDa) in each polypeptide. However, cleavage of the native protein occurred at a small number of sites to give

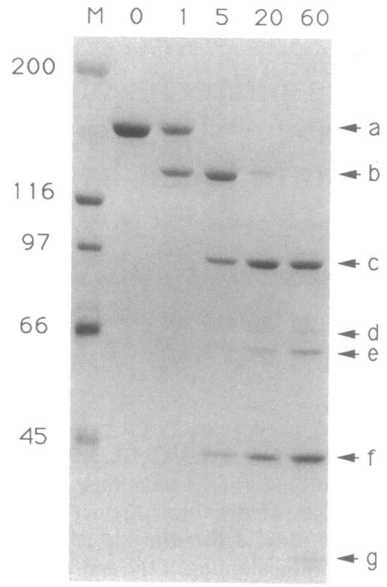

FIG. 1. Proteolytic cleavage of yeast DNA topoisomerase II by SV8 endoprotease. The yeast enzyme (320 μg/ml) was incubated with the protease (10 μg/ml) at 30°C, and proteolytic fragments were resolved by electrophoresis in a 5–10% linear gradient polyacrylamide gel containing SDS. The gel was stained with Coomassie blue and photographed. The digestion time for each sample in minutes is indicated at the top of each lane. The lane labeled M contained markers with their molecular masses shown in kDa to the left of the lane.

six readily discernible fragments with apparent molecular masses of 137, 90, 77, 60, 47, 30, and 27 kDa from their electrophoretic mobilities in SDS/polyacrylamide gels. Seven of these, including the full-length polypeptide, can be seen in Fig. 1, and their positions are marked by arrows a–g; the 27-kDa fragment is not visible in Fig. 1 and will be identified in later sections.

The sizes of the fragments and the intensities of the bands and their dependence on proteolytic digestion time suggest that the six fragments were derived from cleavages at three sites A, B, and C, approximately 400, 660, and 1200 amino acids from the amino terminus of the intact polypeptide, respectively. At the early times of digestion, cleavage was mostly at site C, yielding the 137- and 27-kDa fragments. At longer digestion times, the 137-kDa polypeptide was cut mainly at site A to yield the 90- and 47-kDa fragments; some cutting of the 90-kDa fragment at site B was also observable, yielding the 30- and 60-kDa fragments. The 77-kDa polypeptide appeared to be generated by infrequent cleavage of the 137-kDa polypeptide at site B, which would also produce some of the same 60-kDa fragment mentioned above. Confirmation of these assignments will be presented in a later section.

Fig. 2 depicts the results of protease SV8 digestion in the presence of ATP or its analogs. A comparison of the samples run in lane 2 (no ATP) and lane 3 (1 mM ATP) shows that ATP had little effect on the SV8 endoprotease sensitivity of yeast DNA topoisomerase II.

In the presence of 1 mM Ado*PP*[NH]*P* (Fig. 2, lane 4) or ATP[γS] (Fig. 2, lane 5), however, the proteolysis pattern was significantly altered. Both analogues are strong inhibitors of ATP hydrolysis by eukaryotic DNA topoisomerase II (20), and the rates of hydrolysis of these analogues themselves by yeast DNA topoisomerase II were at least 2 orders of magnitude lower than that of ATP (data not shown). Cleavage of the yeast enzyme at site C to give the 137-kDa and 27-kDa fragments was not significantly affected by the presence of these nonhydrolyzable analogs. However, cleav-

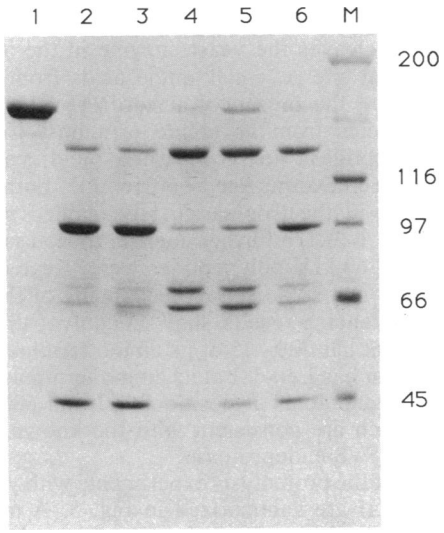

FIG. 2. Effects of ATP and ATP analogs on the cleavage of yeast DNA topoisomerase II by SV8 endoprotease. Purified yeast DNA topoisomerase II (320 μg/ml) was incubated at 30°C for 10 min in the SV8 digestion buffer with or without adenine nucleotide. SV8 endoprotease was then added to all samples except the no-protease control to a final concentration of 10 μg/ml. Incubation was continued for 20 min, and the samples were then prepared for electrophoresis as described. Lanes: 1, no-protease control; 2, sample digested in the absence of added adenine nucleotide; 3–6, samples proteolyzed in the presence of 1 mM ATP (lane 3), Ado*PP*[NH]*P* (lane 4), ATP[γS] (lane 5), or Ado*PP*[CH_2]*P* (lane 6). The rightmost lane labeled M contained protein markers with molecular masses indicated in kDa on the right margin.

age of the 137-kDa fragment occurred predominantly at site B in the presence of Ado*PP*[NH]*P* or ATP[γS] rather than at site A, leading to an increase in the intensities of the 77- and 60-kDa pair and a corresponding decrease in the intensities of the 90- and 47-kDa pair (in Fig. 2, compare lanes 4 and 5 with lanes 2 and 3). Ado*PP*[CH_2]*P* did not significantly affect the proteolysis pattern (Fig. 2, lane 6). Significantly, in the presence or absence of ATP or its nonhydrolyzable analogs, there was little accumulation of the 30-kDa fragment after 20 min of digestion. This indicates that cleavage can occur at either site A or B depending on nucleotide binding, but rarely at both.

Switching Between the Two SV8 Protease-Sensitive Sites in Yeast DNA Topoisomerase II by Ado*PP*[NH]*P* Requires a Functional ATPase Site in the Enzyme. To test whether the switching between the two SV8 endopeptidase sites in yeast DNA topoisomerase II by Ado*PP*[NH]*P* or ATP[γS] is due to specific binding of the nonhydrolyzable analog to a functional ATPase site of the enzyme, a point mutant of the enzyme was obtained by site-directed mutagenesis, in which Gly-144 was replaced by an isoleucine residue. As will be reported elsewhere, in this mutant enzyme both ATPase and DNA topoisomerase activity are down by at least 2 orders of magnitude from those of the wild-type enzyme.

Results depicted in Fig. 3 show that the presence or absence of Ado*PP*[NH]*P* has no effect on the SV8-proteolysis pattern of the mutant protein (in Fig. 3, compare lanes 7 and 8 for the mutant protein with lanes 2 and 3 for the wild-type protein). Because the wild-type and mutant enzymes differ by a single amino acid in their ATPase sites, this result strongly suggests that the Ado*PP*[NH]*P*-induced switching of a protease SV8 cleavage site from A to B in the wild-type enzyme is due to a conformational change triggered by the specific binding of the nonhydrolyzable analog to a functional ATPase site. Data in Fig. 3 also show that the 137-kDa polypeptide is significantly more resistant to proteolytic cleavage in the presence of DNA, for either the Gly-144 → Ile mutant or the wild-type enzyme.

Mapping the Proteolytic Cleavage Sites by Epitope-Tagging of the Amino or Carboxyl Terminus of Yeast DNA Topoisomerase II. To facilitate the mapping of the endopeptidase cleavage sites in the 1429-amino acid yeast polypeptide, plasmids were constructed to express yeast DNA topoisomerase II with an immunologically distinct oligopeptide added to its amino or carboxyl terminus. A decapeptide present in the human c-myc protein was selected for immunotagging (17) because of the availability of a highly specific monoclonal antibody for this epitope. In the enzyme myc-*Sc*topoII, the myc tag plus a short peptide spacer is added to the amino terminus of yeast DNA topoisomerase II; in the enzyme *Sc*topoII-myc, the carboxyl-terminal 95 amino acids of the yeast enzyme, which is located in a region known to be nonessential for activity (S. Worland, P. R. Caron and J.C.W., unpublished data), are replaced with the human c-myc decapeptide epitope. Genetic complementation and biochemical experiments show that both immunotagged enzymes are active *in vivo* and *in vitro* (data not shown).

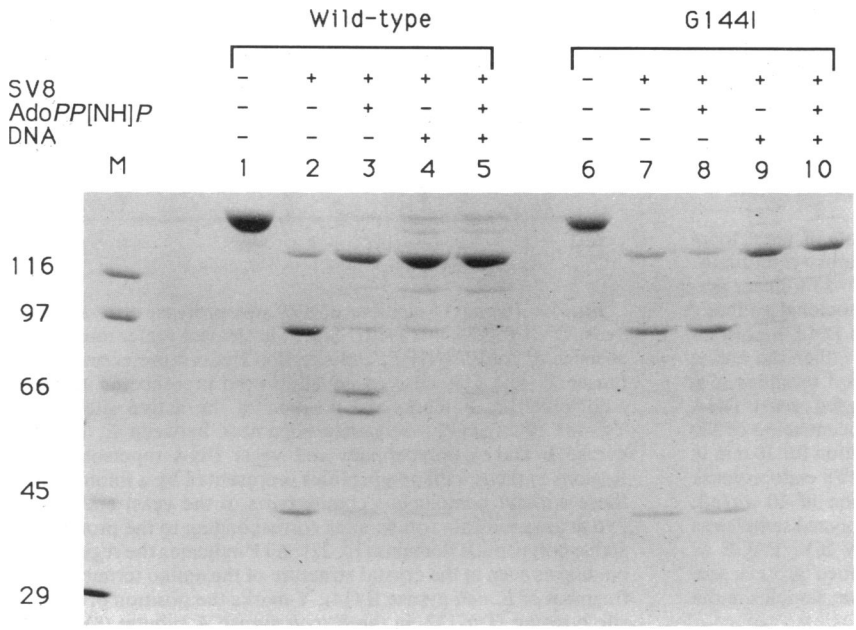

FIG. 3. Analysis of SV8 peptidic fragments of wild-type and Gly-144 → Ile mutant yeast DNA topoisomerase II by SDS/PAGE. Wild-type or Gly-144 → Ile mutant DNA topoisomerase II (200 μg/ml) was incubated for 10 min in SV8 digestion buffer with or without 2 mM Ado*PP*[NH]*P* or 200 μM (in nucleotides) of a plasmid DNA, as specified above each lane (the minus and plus sign respectively indicate absence and presence of the reactant). SV8 endoprotease was added to all samples except the no-protease controls to a final concentration of 8 μg/ml, and incubation was continued for 20 min. The molecular masses in kDa of protein markers run in the lane labeled M are shown to the left of the markers.

When the purified, immunotagged enzymes were digested with SV8 endoprotease, the proteolysis patterns were identical to those shown in Figs. 1–3, except (*i*) that the shortening of the carboxyl-terminal region of yeast DNA topoisomerase II in *Sc*topoII-myc reduced its apparent molecular mass to about 155 kDa and (*ii*) that cleavage at site C of the shortened enzyme gave a carboxyl-terminal fragment of ≈18 kDa, instead of 27 kDa in the untagged or amino-end-tagged proteins.

Fig. 4 shows an immunoblot of the proteolyzed samples after resolution of the polypeptides by SDS/gel electophoresis. For *Sc*topoII-myc, the only polypeptides recognized by the monoclonal myc-antibody are the intact protein and the 18-kDa fragment, confirming the assignment of site C described above; the presence or absence of Ado*PP*[NH]*P* showed no effect on cleavage at this site (in Fig. 4, compare lanes 5 and 6), in agreement with the results shown in Figs. 2 and 3.

For the protein myc-*Sc*topoII, the intact protein, the 137-kDa polypeptide, the 47-kDa fragment derived from the 137-kDa polypeptide in the absence of Ado*PP*[NH]*P*, and the 77-kDa polypeptide derived from the 137-kDa protein in the presence of Ado*PP*[NH]*P* are all recognized by the anti-human c-myc antibody. These results show clearly that SV8 endopeptidase cleaves the yeast enzyme in the absence of Ado*PP*[NH]*P* at site A, ≈400 amino acids from its amino terminus, and in the presence of Ado*PP*[NH]*P* at site B, ≈660 amino acids from its amino terminus. Cutting the 137-kDa polypeptide at site A or B would yield, respectively, a 90-kDa or a 60-kDa untagged polypeptide, both of which were observed in the Coomassie blue-stained proteolysis products (Figs. 1–3). To further confirm these assignments, the 90-kDa and 60-kDa polypeptides were isolated after gel electrophoresis and subjected to sequencing of their amino ends. The sequencing results show unequivocally that the 90-kDa fragment has Arg-412 at its amino terminus, and the 60-kDa fragment has Leu-681 at its amino terminus (data not shown); the amino acids at positions 411 and 680 are both glutamate, which are consistent with the known sequence preferences of SV8 endoprotease.

The results of the proteolysis experiments with yeast DNA topoisomerase II are summarized in Fig. 5. A map of the cleavage sites is displayed. For comparison, the homologously aligned *E. coli* DNA gyrase B and A polypeptides and two known protease-sensitive sites in them are also shown in the figure.

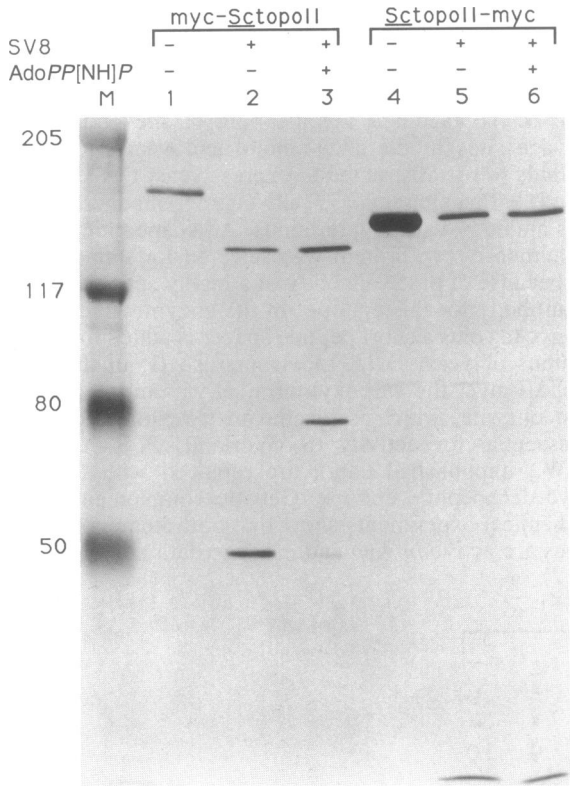

FIG. 4. Immunostaining of proteolytic fragments of yeast topoisomerase II epitopically tagged at their amino or carboxyl terminus. A nitrocellulose blot of an SDS/polyacrylamide 5–10% linear gradient gel was probed with anti-human c-myc monoclonal antibody MYC 1-9E10.2, as described. Samples of purified DNA topoisomerase II with a decapeptide human c-myc tag at either the amino terminus (myc-*Sc*topoII, 50 μg/ml) or the carboxyl terminus (*Sc*topoII-myc, 100 μg/ml) were mixed with untagged yeast DNA topoisomerase II to give a total topoisomerase concentration of 320 μg/ml in the SV8 digestion buffer. After preincubation for 10 min in the presence or absence of 1 mM Ado*PP*[NH]*P*, SV8 endoprotease was added to the samples to a final concentration of 10 μg/ml; proteolysis was terminated after 20 min. The proteolyzed samples as well as the undigested controls were analyzed by SDS/PAGE as described. The lane labeled M contained prestained protein size markers with the indicated molecular masses in kDa; samples in the other lanes are specified above the lanes.

DISCUSSION

As summarized in Fig. 5, SV8 endoprotease cleaves yeast DNA topoisomerase II at three prominent sites A, B, and C. Sites A and B correspond to the carboxyl side of Glu-411 and Glu-680, respectively, and site C is around amino acid 1200. Two protease-sensitive sites in *E. coli* DNA gyrase have been well characterized. One is responsible for the formation of the 47-kDa carboxyl-terminal fragment of *E. coli* gyrase B subunit, termed GyrB′, and is known to have Arg-394 at its amino end (12). The other proteolytic site is in the *E. coli* gyrase A subunit, dividing the polypeptide into a 64-kDa amino-terminal and a 33-kDa carboxyl terminal region (21). These two sites in *E. coli* DNA gyrase correspond to sites A and C, respectively, in the yeast enzyme; the position of site B in the yeast enzyme corresponds closely to the *E. coli* gyrase B/gyrase A junction, and lies probably within the beginning part of the gyrase A polypeptide (see Fig. 5).

The protease-sensitive sites in the 1429-amino acid yeast DNA topoisomerase II were mapped by the use of proteins immunotagged at their amino or carboxyl end. Although "footprinting" of nucleic acids has been widely used to study their structural features and interactions with other small or

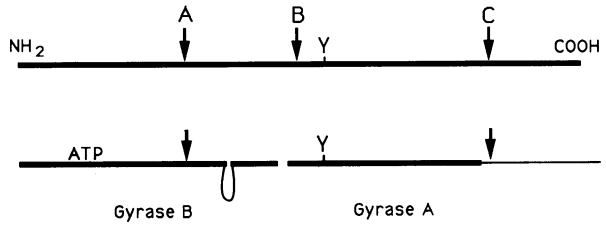

FIG. 5. (*Upper*) Locations of SV8 endoprotease cleavage sites in yeast DNA topoisomerase II. Site A is cleaved preferentially in the absence of Ado*PP*[NH]*P*, and site B is cleaved preferentially in its presence. Site C is cleaved equally in the presence or absence of Ado*PP*[NH]*P*. Y marks the position of the active site tyrosine, Tyr-783 (9). (*Lower*) Sequence alignment between *E. coli* DNA gyrase B and A polypeptides and yeast DNA topoisomerase II. Regions in the *E. coli* polypeptides represented by a thinner line are those without homologous counterparts in the yeast enzyme. The two arrows indicate tryptic sites corresponding to the production of stable polypeptide domains (14, 21). ATP indicates the region of ATP binding as seen in the crystal structure of the amino terminal 43-kDa fragment of *E. coli* gyrase B (14); Y marks the position of the active site tyrosine, Tyr-122, in the *E. coli* gyrase A subunit (8).

large molecules, there are only a few examples of the application of this approach to polypeptides. Radiolabeling at one unique end of a protein was used in several protein-footprinting experiments (22), and immuno-tagging of protein termini was used in others (23–25). These two approaches exemplify, respectively, how direct and indirect end-labeling, analogous to DNA-sequencing technology, can be applied to polypeptides. One application of these techniques would involve the use of endopeptidases with stringent recognition sequences, such as factor X_a and thrombin. An appropriate oligopeptide containing both the "restriction endoprotease" recognition sequence and the epitope tag could be placed at various positions within the polypeptide of interest. The native polypeptide could then be probed either with a less stringent protease (e.g., trypsin) or chemically modified with a residue-specific reagent. The polypeptides would then be denatured and digested to completion with the "restriction endoprotease" followed by electrophoretic resolution and immunoblotting.

The three SV8-sensitive sites in the yeast enzyme suggest that the polypeptide consists of at least four distinct domains, with the protease-sensitive sites as their dividers. The domain at the carboxyl terminus is nonessential for the catalytic reactions of type II DNA topoisomerases (P. R. Caron, S. Worland, and J.C.W., unpublished; T.-S. Hsieh, personal communication). For the domain NH_2–A, from the amino end of the intact protein to site A, the three-dimensional structure of the *E. coli* enzyme is known (14); the ATPase site is located in the amino half of this domain (10–14). The domain AB between sites A and B contains three oligopeptides that are present in all known type II DNA topoisomerases: Glu-Gly-Asp-Ser-Ala-Pro-Leu-(Arg or Lys)-Gly-Lys-(Ile, Leu, or Met)-Leu-Asn, and Ile-Met-(Thr or Ala)-Asp-(Gln or Ala)-Asp. The domain BC between sites B and C also contains several highly conserved oligopeptides in its amino-portion, including the dipeptide Arg-Tyr, in which the active site tyrosine resides.

From sequence comparisons of type II DNA topoisomerases, in eukaryotic DNA topoisomerase II, the amino-end domain NH_2–A and the domain AB are most likely adjacent to each other; the same is true for the domains AB and BC. In *E. coli* DNA gyrase, the domains corresponding to NH_2–A and AB form the globular gyrase B subunit. Because the NH_2–A domain in *E. coli* gyrase appears to be easily lost from the rest of the enzyme upon limited proteolysis (10–12), the association between NH_2–A and AB is probably rather weak, at least in the absence of bound ATP. The domains corresponding to AB and BC/C–COOH in *E. coli* DNA gyrase can combine to give the ATP-independent activity DNA topoisomerase II′ (10–12), and since the carboxyl-terminal domain of gyrase A is known to be nonessential for activity (21), AB and BC must interact with each other in the holoenzyme, at least in the absence of ATP. Furthermore, in *E. coli* DNA gyrase, all known *gyrB* mutations leading to nalidixic acid resistance are located within two of the highly conserved motifs Gly-Gly-Asp-Ser-Ala and Pro-Leu-(Arg or Lys)-Gly-Lys-(Ile, Leu, or Met)-Leu-Asn in the AB domain (24, 25). All *gyrA* mutations leading to nalidixic acid resistance, on the other hand, were found in a cluster centering around Ser-83 in the beginning part of domain BC (26). Thus, it seems likely that the two highly conserved *E. coli* gyrase B motifs, the *E. coli* gyrase A Ser-83 region, and the active-site Tyr-122 of gyrase A are all located near each other to form the DNA breakage and rejoining pocket.

The data presented in *Results* indicate that the binding of the nonhydrolyzable ATP analog Ado*PP*[NH]*P* or ATP[γS] to yeast DNA topoisomerase II is associated with movements of the domains in the enzyme; as a consequence, site A becomes less sensitive to cleavage by SV8 endoprotease, while at the same time site B becomes more so. The simplest interpretation of this switching between protease-sensitive sites is that ATP-binding induces a conformation change in the domain containing the ATPase site, which in turn triggers allosteric changes cascading across adjacent domains. The presence of ATP itself does not cause prominent changes in the proteolysis pattern of the enzyme, presumably because bound ATP is hydrolyzed faster than the release of the hydrolysis products ADP and P_i, and thus only a small percentage of the enzyme is in the ATP-bound form at any given time.

The cascading of allosteric changes across adjacent domains provides a way of communication between the ATPase site and the DNA binding site(s) in type II DNA topoisomerases. An understanding of the mechanism of reactions catalyzed by these enzymes is thus critically dependent on the elucidation of these allosteric changes. The results presented in this communication demonstrate that probing epitopically tagged enzymes by proteolytic reagents can provide much needed information on these changes.

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