THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 272, No. 18, Issue of May 2, pp. 12132–12137, 1997
Printed in U.S.A.

Study of Yeast DNA Topoisomerase II and Its Truncation Derivatives by Transmission Electron Microscopy*

(Received for publication, November 19, 1996, and in revised form, January 30, 1997)

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The 1429-amino acid residue long yeast DNA topoisomerase II and three of its deletion derivatives, a C-terminal truncation containing residues 1–1202, a 92-kDa fragment spanning residues 410–1202, and an A′-fragment spanning residues 660–1202, were examined by transmission electron microscopy. Analysis of rotary-shadowed images of these molecules shows that the full-length enzyme assumes a tripartite structure, in which a large globular core comprising the carboxyl parts of the dimeric enzyme is connected to a pair of smaller spherical masses comprising the ATPase domains of the enzyme. The linkers bridging the large globular structure and each of the smaller spheres are not visible in most of the images but appear to be sufficiently stiff to keep the relative positions of the connected parts. The angle extended by the pair of spherical masses is variable and falls in a range of 50–100° for the majority of the images. On binding of a nonhydrolyzable ATP analog to the enzyme, this angle is significantly reduced as the two spherical masses swing into contact. These observations, together with results from previous biochemical and x-ray crystallographic studies of the enzyme, provide a sketch of the molecular architecture and conformational states of a catalytically active type II DNA topoisomerase.

Type II DNA topoisomerases are ubiquitous enzymes that catalyze the ATP-dependent transport of one double-stranded DNA segment through another. In this reaction, a dimeric enzyme cleaves a double-stranded DNA segment to create an opening or gate in it for the enzyme-mediated passage of a second double-stranded DNA segment; the enzyme rejoins the severed DNA strands following the passage of the second DNA segment to complete a reaction cycle. Together with the type I DNA topoisomerases, which transiently cleave one DNA strand at a time for the passage of another strand, these enzymes participate in a number of vital cellular processes that require disentanglement of DNA, for example, in the segregation of newly replicated pairs of intertwined chromosomes (1).

The complex set of reactions in the manipulation of DNA duplexes by a type II DNA topoisomerase is coupled to the binding and hydrolysis of ATP at a specific site in each half of a dimeric enzyme. Studies using nonhydrolyzable ATP analogs, such as the β,γ-imido derivative AMPPNP,[1] implicate that the binding of ATP to a DNA-bound type II topoisomerase is sufficient to trigger a cascade of events leading to the transport of one DNA duplex through another; hydrolysis of ATP and release of the resulting ADP and orthophosphate are required only for enzyme turnover (2).

Extensive studies have been carried out in the past two decades to deduce how a type II DNA topoisomerase catalyzes these intricate reactions. In our laboratories, most of the mechanistic studies were carried out with DNA topoisomerase II of the budding yeast *Saccharomyces cerevisiae*, a protein containing two identical polypeptides with 1429 amino acid residues in each. Because all type II DNA topoisomerases are closely related, the yeast enzyme serves as a model for all other members of this subfamily of topoisomerases. Based mainly on the sequence homologies between the yeast enzyme and the two subunits GyrA and GyrB of *Escherichia coli* gyrase, various segments of the yeast enzyme have been termed the "GyrA" region (residues 660–1429), the "GyrB" region (residues 1–660), the ATPase domain (residues 1–410), the B′-fragment (residues 410–660), and the A′-fragment (residues 660–1200), as outlined in Fig. 1. It has been shown that the C-terminal 260 amino acid residues of the yeast enzyme is dispensable for activity, and hence a GyrB fragment joined to an A′-fragment constitutes a fully active enzyme *in vitro* (1).

A molecular picture of an active yeast DNA topoisomerase II has emerged from a combination of biochemical and x-ray crystallographic studies of the enzyme (3–5). The enzyme can be viewed as an ATP-modulated clamp with two sets of jaws at opposite ends of a homodimeric protein. The crystal structure of a 92-kDa yeast enzyme fragment spanning amino acids 410–1202 shows that the two A′-fragments form a V-shaped dimer through contacts between residues 1030–1130 of the two protomers (4). This dimer interface represents contacts between one set of the jaws, which form the "C gate" of the enzyme, so named because residues forming this interface are close to the C terminus of the A′-fragments. There is a pair of symmetry-related semicircular grooves near the top of the V-shaped A'_2-dimer, which have been postulated to bind the DNA segment within which a transient opening or gate is to be introduced for the passage of a second DNA double helix. The DNA segment containing the gate has been termed the G segment, and the second DNA segment has been termed the T segment.

In the 92-kDa crystal, a pair of B′-fragments form a dimeric arch to cap the V-shaped A'_2-dimer, enclosing a large hole (4). The ATPase domain is absent in the fragment. The structure of this domain is most likely to resemble, however, that of its counterpart in *E. coli* gyrase, which has been determined to a

* The work was supported in part by grants from the Progetto Finalizzato, Consiglio Nazionale delle Ricerche, ACRO, and Associazione Italiana Ricerca sul Cancro and by National Institutes of Health USPHS Grants GM24544 and CA47958. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ Recipient of a fellowship from the Fondazione A. Buzzati-Traverso.
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[1] The abbreviations used are: AMPPNP, adenylyl-imidophosphate; Gyr, gyrase; Top, topoisomerase.

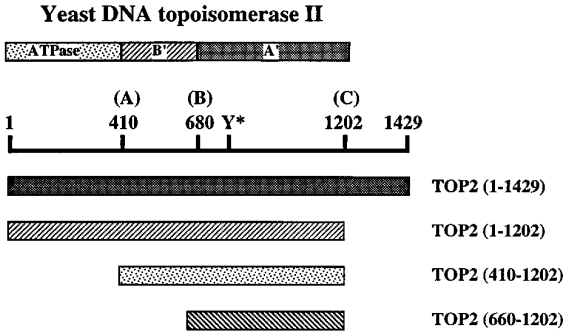

FIG. 1. **Schematic drawing illustrating the various regions of yeast DNA topoisomerase II and the polypeptides used in this work.** *Top bar,* ATPase domain and the B′- and A′-fragments of the enzyme; *scale* below, positions of amino acid residues at the ends and domain boundaries (*A–C,* proteolytic sites described by Lindsley and Wang (9); *Y**, position of the active tyrosine Tyr-783). The regions spanned by the four proteins used in this work are illustrated by the four lower *bars*. Top2(1–1429) is the full-length yeast enzyme.

resolution of 2.5 Å by x-ray crystallography (6). Significantly, in the presence of AMPPNP the 43-kDa *E. coli* GyrB N-terminal fragment is dimeric in the crystal (6) as well as in solution (7). In the absence of the nucleotide, the fragment was found to be monomeric in solution (7). The dimer contacts in the crystal structure of the 43-kDa *E. coli* GyrB fragment are made primarily between a 15-amino acid arm of each monomer and the opposite monomer (6). Because AMPPNP binding is known to convert yeast DNA topoisomerase II to the closed clamp form inaccessible to a DNA ring (3), the AMPPNP-modulated dimerization between the N-terminal domains of *E. coli* GyrB suggests strongly that the N-terminal ATPase domains of yeast DNA topoisomerase II form the entrance gate of the enzyme. This gate has been termed the N-terminal gate or N gate (8).

According to the current model (4, 8), in the reaction catalyzed by a type II DNA topoisomerase a T segment first enters through the N gate of a G segment-bound enzyme. ATP binding triggers the closure of the N gate, and the trapped T segment is forced through a transient opening in the G segment and moved into the large hole seen in the 92-kDa yeast DNA topoisomerase II fragment structure. Following this event, the two halves of the enzyme retract toward each other to rejoin the G segment. This retraction in turn reduces the size of the central hole containing the T segment and forces the expulsion of the T segment through the C gate. The enzyme is set for another round of reaction when the N gate reopens after ATP hydrolysis and product release. Recently, site-directed mutagenesis based on the crystal structure of the 92-kDa fragment of the yeast enzyme has made it possible to form reversibly a pair of disulfide bonds across the C gate (5). It was shown that locking the C gate by disulfide cross-links interferes with neither the entrance of the T segment into the enzyme nor the transport of the T segment through the G segment; exit of the T segment from the enzyme is blocked, however, in agreement with the prediction of the model (4, 8).

Despite several attempts, crystallization of an active type II DNA topoisomerase has been unsuccessful. Thus how the complementary GyrB and A′ parts of a functional enzyme are positioned in an active enzyme remains uncertain. This uncertainty is compounded by the very large conformational changes of the enzyme as it undergoes the various steps of a reaction cycle (3, 9, 10). Because of these large conformational changes, the known crystal structure of an enzyme fragment is likely to represent its structure in a particular conformation of the enzyme, and it is difficult to establish which state of the enzyme this particular structure corresponds to. We and others have therefore resorted to electron microscopy to complement the x-ray crystallographic results (11–13). We report here electron microscopic examination of the 1429-amino acid full-length yeast DNA topoisomerase II and three of its deletion derivatives: a C-terminal truncation containing amino acids 1–1202 of the enzyme, a 92-kDa fragment spanning amino acids 410–1202, which was used in the earlier crystallographic work (4), and an A′-fragment spanning amino acid residues 660–1202.

EXPERIMENTAL PROCEDURES

Materials—Yeast DNA topoisomerase II and its truncation derivatives were purified from cells overexpressing these proteins, as described previously (14). Working stocks of the proteins were typically 1–3 mg/ml in 50 mM Hepes buffer, pH 8, 1 mM EDTA, 1 mM EGTA, 150 mM KCl, 4 mM dithiothreitol, and 20% glycerol by volume. Samples were kept at −70 °C and thawed before use. Mica was purchased from Polysciences, and AMPPNP was purchased from Sigma.

Electron Microscopy—Each 10-μl sample contained approximately 500 ng of yeast DNA topoisomerase II or its truncation derivative in 50 mM Hepes, pH 8, 1 mM EDTA, 1 mM EGTA, 150 mM KCl, 4 mM dithiothreitol, and 20% glycerol. Samples were preincubated at 30 °C for 5 min. For samples containing AMPPNP, the nucleotide was added to a final concentration of 2 mM, and incubation was continued for 10 min after nucleotide addition. Samples were then diluted 10-fold into 100 mM ammonium acetate and 66% glycerol, and the mixture was immediately sprayed onto freshly cleaved mica by the use of a modified airbrush (15). After drying at reduced pressure in an Edwards 306 evaporator, samples were rotary-shadowed with platinum at an angle of 7°. A thin carbon film was then deposited at an angle of 90° onto the metal-shadowed surface, and the carbon-coated replicas were floated off mica and mounted on 400-mesh copper grids (11, 12, 16). Samples were observed in a Philips CM 12 electron microscope at 80 kV. For samples examined by negative staining, the protein was adsorbed onto freshly glow-discharged carbon-coated grids and stained with 1% uranyl acetate. For size measurements, some of the images on enlarged electron micrographs were scanned in a scanner (Apple) and analyzed with NIH Image 1.55 software. Tropomyosin paracrystals were used as a magnification standard.

RESULTS

Images of Full-length Yeast DNA Topoisomerase II—Fig. 2 shows a typical field of full-length yeast topoisomerase II molecules, which had been sprayed onto mica and rotary-shadowed with platinum (11, 12). Most of the molecules appear as tripartite structures with a 170-Å diameter globular core and two 80-Å diameter spherical masses (unless stated otherwise, all sizes refer to direct measurements of images without corrections for platinum grains). In the majority of the images, the two spherical masses of each structure appear to be symmetrically oriented, and a line bisecting them and the 170-Å globular core can be drawn. This line is likely to represent a molecular dyad, as the enzyme is known to be made of two identical halves. The distance between the external edges of the 170-Å core and each of the 80-Å masses is about 240–270 Å, with a distinct gap but no discernible linker between the globular bodies in most of the images.

The angle extended by the symmetrically oriented 80-Å spheres from the center of the 170-Å globular core varies from near 0 to about 180°, and the spacing between the external edges of the pair of 80-Å spheres varies accordingly from as small as 160 to as large as 400 Å. The images of the full-length enzyme molecules can be grouped into several categories, and representative images of each category are shown in Fig. 3, *1–5*. The angles extended by the pair of smaller spherical masses of images shown in Fig. 3, *1*, are in the range of 160–180°, and those in Fig. 3, *2*, are in the range of 50–100°. In the images shown in Fig. 3, *3*, the pair of smaller masses are in contact; in those shown in Fig. 3, *4*, they are unresolved. Fig. 3, *5*, shows images in which the dimeric enzyme appears to have come apart during sample treatment. Molecules represented by images shown in Fig. 3, *2*, were the most abundant, whereas those

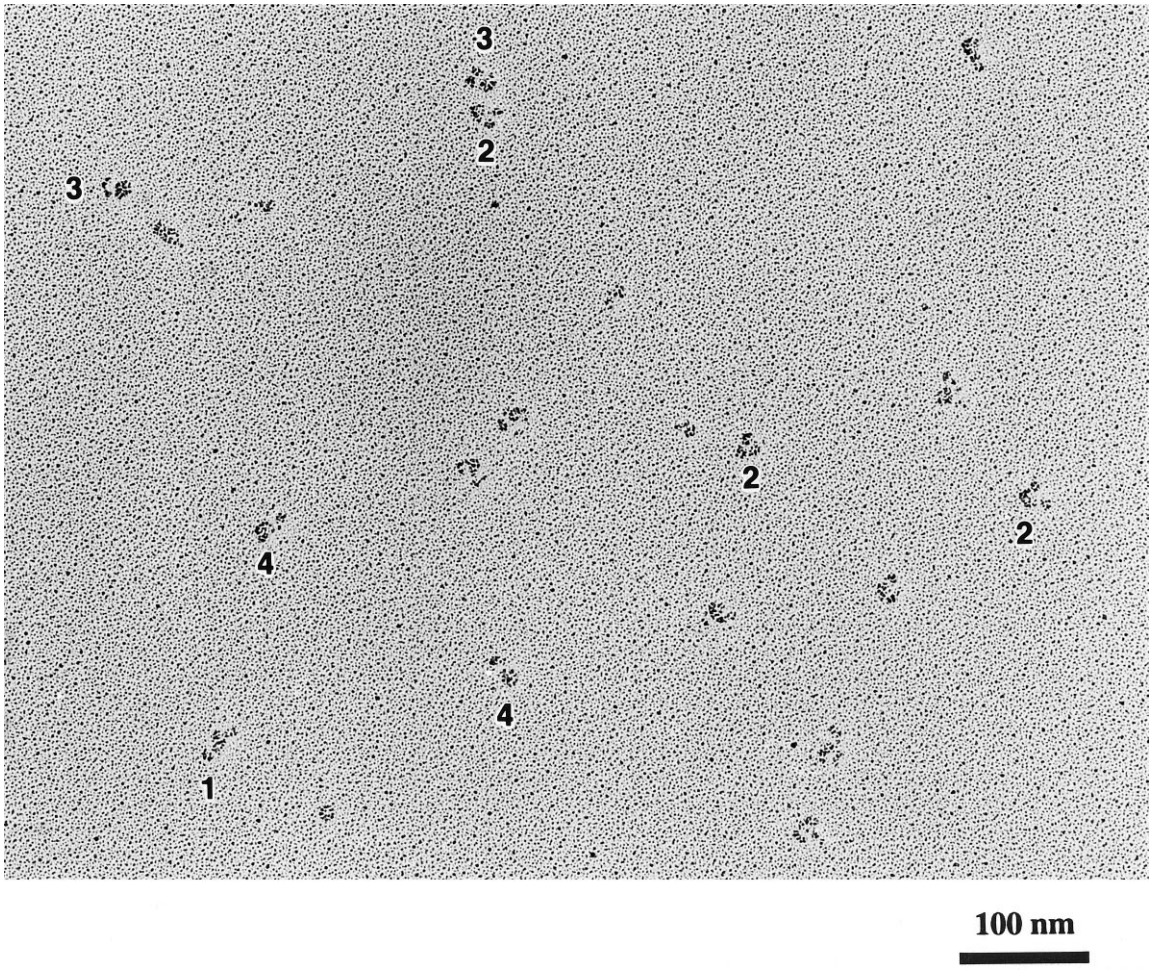

FIG. 2. **Representative field of rotary-shadowed full-length yeast DNA topoisomerase II molecules sprayed onto mica surface.** *Numbers* under individual molecules mark those corresponding to different classes of shapes illustrated in Fig. 3. Overall magnification, approximately × 160 000 (see scale at *lower right*).

represented by images shown in Fig. 3, *4* and *5*, constituted only several percent of the population.

Images of Truncation Derivatives of Yeast DNA Topoisomerase II—To identify the protein domains in the structures seen in the electron micrographs described above, we examined three truncation derivatives of yeast DNA topoisomerase II in addition to the full-length enzyme: a C-terminal truncation containing amino acid residues 1–1202, a 92-kDa fragment spanning amino acid residues 410–1202, which was used in the determination of the crystal structure (4), and an A′-fragment spanning amino acid residues 660–1202. These truncation derivatives are designated Top2(1–1202), Top2(410–1202), and Top2 (660–1202), respectively. Panels of representative micrographs of the proteins are shown in Fig. 4.

A comparison of images of the Top2(410–1202) molecules (Fig. 4, *3*) and those of the intact enzyme and the C-terminal truncation Top2(1–1202) (Fig. 4, *1* and *2*, respectively) shows that the diameter of the 80-Å spherical masses is much reduced by the removal of the N-terminal 409 amino acids of yeast DNA topoisomerase II. From these observations, it is clear that the two 80-Å spherical bodies in the images of intact yeast DNA topoisomerase II represent the N-terminal domains of the dimeric enzyme. Each 80-Å sphere probably contains the bulk of the N-terminal half of the intact enzyme (the GyrB half). In the Top2(410–1202) images, the 80-Å spherical masses are largely absent, and instead two minute dots appear to extend from the globular core. Removal of the C-terminal 230 amino acids of the intact enzyme, on the other hand, results in no major change in the dimension of either the 170-Å globular core or the 80-Å spherical masses (Fig. 4, *1* and *2*). Thus the C-terminal 230 residues of the full-length enzyme are likely to be within the general confines of the 170-Å core and do not form protruding appendages.

Whereas the 170-Å core in the images of intact yeast DNA topoisomerase II and its truncation derivatives Top2(1–1202) and Top2(410–1202) showed no distinctive classes of shapes, two major classes of images of Top2(660–1202) are discernible. Fig. 5 depicts a representative field of rotary-shadowed molecules of the protein. In about one-quarter of the images, the molecules show distinct V-shaped forms with varying angles between the arms (the top two and lower left images of the quartet shown in Fig. 4). These forms are in accordance with that of the A'_2-dimer in the crystal structure (4) and that of rotary-shadowed *E. coli* GyrA dimers in earlier electron micrographs (11). Whereas differences in these images could be caused by molecules of the same shape but oriented differently on the mica surface, it is more likely that they correspond to molecules with different angles between the two arms of the V; the latter interpretation is more consistent with the observation that in images of this class the height of the V correlates inversely with its width. In the remaining three-quarters of the images, the expected pair of arms of each dimer are not resolved, and many of the molecules appear to be wedge shaped with a central indentation at the top. These images have a width of about 170 Å at the top and a height of about 150 Å (Fig. 4, *lower right panels*). The dimensions of the Top2(660–1202)

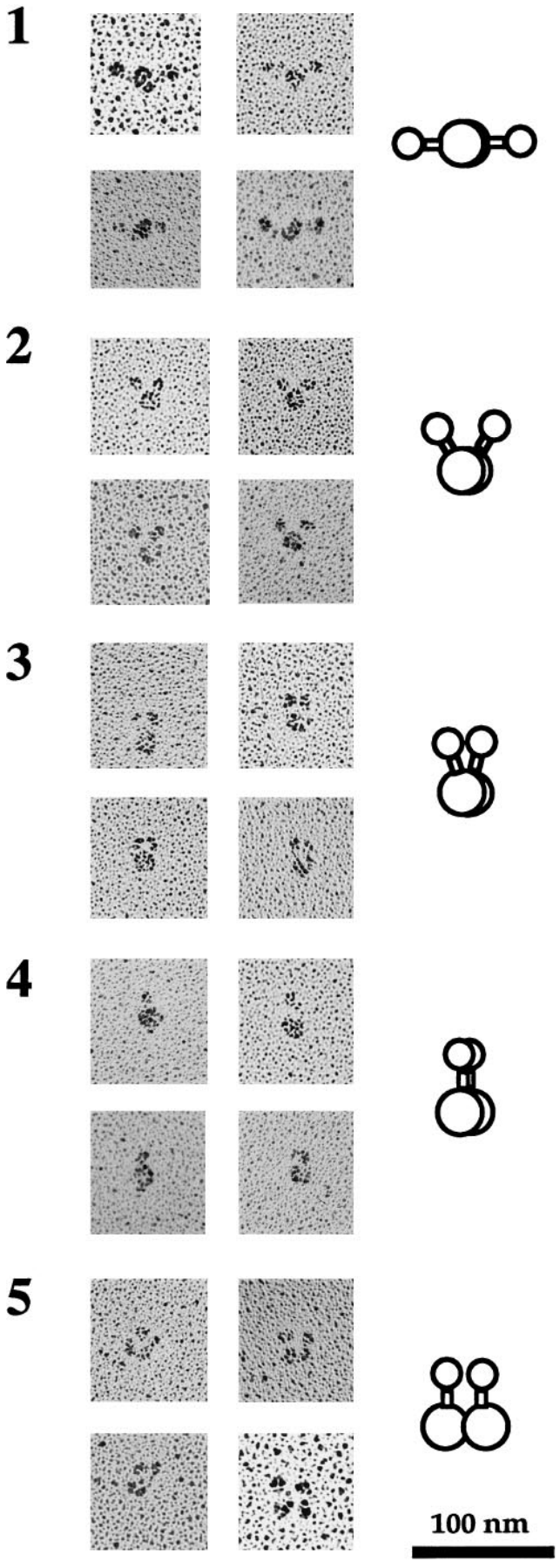

FIG. 3. **Selected images of molecules of different shapes.** Each panel shows images of four molecules of the category illustrated at the *right*. See text for details.

molecules are consistent with the assignment of the 170-Å globular core of the tripartite structures of full-length yeast DNA topoisomerase II and its truncation derivatives Top2(1–1202) and Top2(410–1202) as the A'_2-part of the enzyme.

Conformational Changes Induced by the Binding of AMP-PNP—In the absence of AMPPNP, the majority of the rotary-shadowed images is of the category shown in Fig. 3, *2*. The two 80-Å spheres extend from the larger globular core with an angular displacement between 50 and 100°. On incubation of the enzyme with AMPPNP, the major population seen is that represented by the images shown in Fig. 3, *3*, in which the two 80-Å spherical masses appear to contact each other. The presence of AMPPNP also reduces the abundance of images represented by those shown in Fig. 3, *1*, in which the pairs of 80-Å satellites in the tripartite structures are nearly 180° apart, and increases that represented by images shown in Fig. 3, *4*, in which the satellite pairs are unresolved. Results from quantitating the distributions of yeast topoisomerase II molecules in the absence and presence of the nucleotide are summarized in Fig. 6.

DISCUSSION

From results presented above, it is clear that the large globular structure in the tripartite images of full-length yeast DNA topoisomerase II molecules corresponds to the C-terminal or GyrA part of the enzyme. Similarly, each of the 80-Å satellites in a tripartite structure must contain the N-terminal or GyrB part of the yeast DNA topoisomerase II polypeptide.

The precise polypeptide stretches that form the 80- and 170-Å bodies are less certain. Because the images of the Top2(410–1202) but not the Top2(660–1202) molecules show clearly a pair of small dots extending out of the large globular core, the 80-Å sphere must contain at least the N-terminal 410 amino acid residues and probably a significantly longer stretch. By the same reasoning, the 170-Å globular core is likely to contain the bulk of the C-terminal region past residue 660 of the enzyme but probably not the B′-polypeptide spanning residues 410–660. Otherwise, it would be difficult to interpret the presence of the pair of minute satellites in the tripartite images of Top2(410–1202). In the crystal structure of yeast Top2(410–1202), each dimer has overall dimensions of 120 × 120 × 50 Å, and the B′-fragments (residues 410–660) form a compact dimeric arch to cap the opening of the V-shaped A'_2-dimer. Whereas the B′-fragments form an integral part of the heart-shaped Top2(410–1202) dimer in the crystal structure, the images of Top2(410–1202) molecules in the electron micrographs suggest that they may extend away from the A'_2-dimer through a linker. It is likely that the differences in structures seen in the crystal and in the electron microscope reflect differences in molecular conformations of the enzyme.

The actual dimension of the 170-Å globular core in images of the full-length yeast enzyme or Top2(410–1202), after correction for the estimated average platinum grain size of about 20 Å, is around 130 Å. This size suggests that the 120 × 120 × 50-Å disclike core of yeast DNA topoisomerase II is more likely to lie flat rather than on its side on the mica surface. Whereas the linker between the 80- and 170-Å masses of the tripartite structures of full-length yeast DNA topoisomerase II and Top2(1–1202) is invisible in the electron micrographs, which suggests a low mass/length ratio, it nevertheless appears to be sufficiently stiff to keep the general shape of the tripartite structures; the two 80-Å spheres are usually symmetrically positioned relative to the globular core.

There is a remarkable change in the appearance of the rotary-shadowed yeast DNA topoisomerase II molecules on AMP-PNP binding to the enzyme prior to electron microscopy (Fig. 6). In the majority of the images of the enzyme in the presence

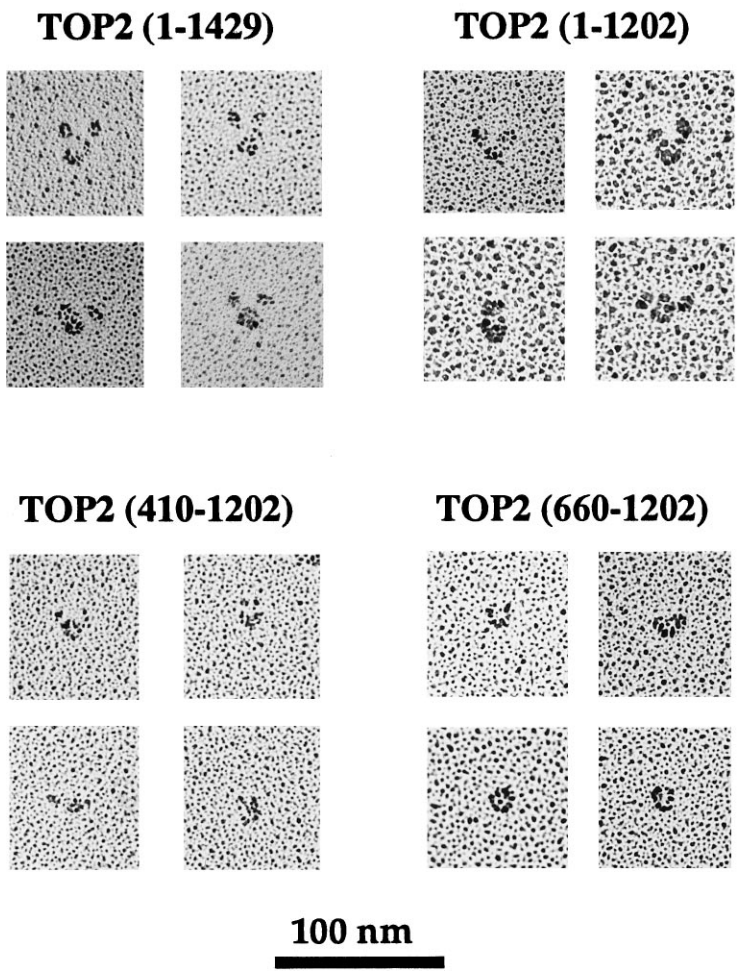

FIG. 4. **Images of full-length and truncation derivatives of yeast DNA topoisomerase II.** Representative rotary-shadowed images of full-length yeast DNA topoisomerase II (*upper left panels*) and three of its truncation derivatives, Top2(1–1202), Top2(410–1202), and Top2(660–1202). All images were obtained at the same magnification.

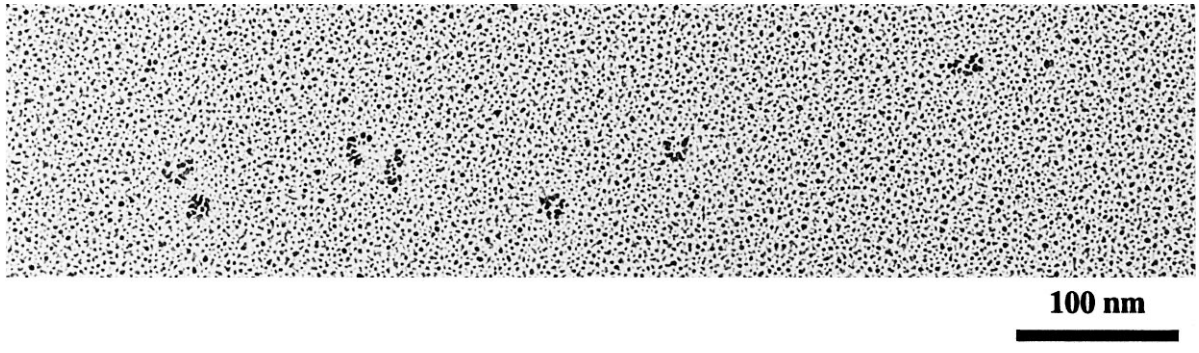

FIG. 5. **A field of rotary-shadowed images of yeast Top2(660–1202).** Both V-shaped and wedge-shaped images are present. See Fig. 4, *lower right panels,* and the text for further details.

of the nucleotide, the two 80-Å spheres came into contact. This AMPPNP-modulated structural change is consistent with the known dimerization of the N-terminal domains of *E. coli* GyrB protein on AMPPNP binding (6, 7) and the conversion of the open clamp form of yeast DNA topoisomerase II to the closed clamp form by AMPPNP (3). In a previous examination of rotary-shadowed bacterial gyrase, no major change in molecular shape was observed after addition of AMPPNP (11). This discrepancy was probably due to the presence of a large fraction of inactive enzyme molecules in the sample used in the earlier work. The large global conformational change accompanying the binding of AMPPNP to the yeast enzyme requires the presence of hinges and/or flexible linkers in the dimeric protein and may involve significant structural changes within particular regions of the polypeptide. It was previously observed that AMPPNP binding to the yeast enzyme changes the sites of cleavage by endoproteinase SV8: a strong cleavage site after Glu-480 disappears and a new cleavage site after Glu-680 appears (9). There is probably sufficient flexibility in the dimeric enzyme to permit the two N-terminal ATPase domains to swing into contact in the absence of ATP, and the binding of ATP to the enzyme presumably triggers a conformational change that stabilizes the closed clamp form of the enzyme.

The present findings are generally in agreement with those of Schultz *et al.* (13). Their examination of negatively stained human and yeast DNA topoisomerase II by scanning transmis-

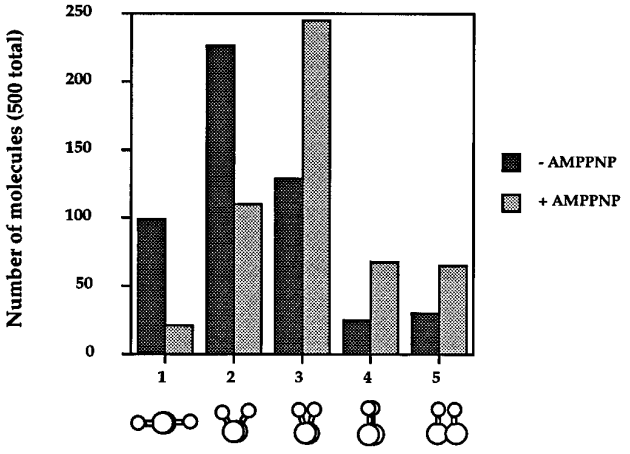

FIG. 6. **Distribution of images of different classes 1–5, diagrammatically illustrated by the drawings shown, in the absence and presence of AMPPNP.** Five hundred molecules in the absence or presence of AMPPNP were scored in the calculations.

sion electron microscopy led to the conclusion that a type II topoisomerase is made of a larger core comprising the C-terminal parts of the pair of polypeptides and two smaller, symmetrically located bodies comprising the N-terminal portions of the polypeptides. Furthermore, the binding of AMPPNP was found to move the two smaller bodies toward each other. There are, however, some quantitative differences between their results and ours. The dimensions of the negatively stained molecules in their work are in general smaller than those of the rotary-shadowed molecules in our work. These differences can be attributed to a combination of two factors: the finite grain size in shadowing, which exaggerates molecular sizes, and the negative and positive staining occurring with uranyl acetate penetration into the protein domains, which may reduce their apparent sizes. We had also examined yeast DNA topoisomerase II adsorbed to glow-discharged carbon grids and stained with 1% uranyl acetate. In our negatively stained images, the majority of the molecules appeared as a four-dot structure with a large central hole (not shown), suggesting that the two halves of the homodimeric protein had come apart during staining. Human DNA topoisomerase IIa is apparently more resistant to the staining treatment, and fewer molecules showed a split large globular core in the micrographs of Schultz *et al*. (13).

In conclusion, the electron microscopic images of yeast DNA topoisomerase II reveal a remarkable tripartite structure with a large dimeric core and a pair of smaller spherical masses comprising the ATPase domains of the enzyme. The two globular bodies are bridged to each other by a pair of linkers, which, although invisible in the micrographs, appear to be sufficiently stiff to keep the globular bodies properly oriented and yet flexible enough to accommodate large conformational changes of the enzyme during its reaction cycle. These results, when combined with results obtained by biochemical and x-ray crystallographic studies, provide new insights on the molecular architecture and conformational states of a type II DNA topoisomerase.

Acknowledgments—We thank James Berger, Ryo Hanai, Wei Li, Quiyong Liu, Emma Roberts, and Susan Gasser for protein samples and helpful discussions, Mary-Ann Bjornsti and Jim Amatruda for suggestions and careful reading of the manuscript, Loriana Castellani and E. L. Benedetti for advice on electron microscopy, and Giuseppe Di Franco for excellent technical assistance.

REFERENCES

1. Wang, J. C. (1996) *Annu. Rev. Biochem.* **65,** 635–692
2. Sugino, A., Higgins, N. P., Brown, P. O., Peebles, C. L., and Cozzarelli, N. R. (1978) *Proc. Natl. Acad. Sci. U. S. A.* **75,** 4838–4842
3. Roca, J., and Wang, J. (1992) *Cell* **71,** 833–840
4. Berger, J. M., Gamblin, S. J., Harrison, S. C., and Wang, J. C. (1996) *Nature* **379,** 225–232
5. Roca, J., Berger J. M., Harrison S. C., and Wang, J. C. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 4057–4062
6. Wigley, D. B., Davies, G. J., Dodson, E. J., Maxwell, A., and Dodson, G. (1991) *Nature* **351,** 624–629
7. Ali, J. A., Orphanides, G., and Maxwell, A. (1995) *Biochemistry* **34,** 9801–9808
8. Roca, J., and Wang, J. C. (1994) *Cell* **77,** 609–616
9. Lindsley, J. E., and Wang, J. C. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 10485–10489
10. Lindsley, J. E., and Wang, J. C. (1993) *J. Biol. Chem.* **268,** 8096–8104
11. Kirchhausen, T., Wang, J. C., and Harrison, S. H. (1985) *Cell* **41,** 933–943
12. Vassetzky, Y. S., Dang, Q., Benedetti, P., and Gasser, S. M. (1994) *Mol. Cell. Biol.* **14,** 6962–6974
13. Schultz, P., Olland, S., Oudet, P., and Hancok, R. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 5936–5940
14. Worland, S. T., and Wang, J. C. (1989) *J. Biol. Chem.* **264,** 4412–4416
15. Tyler, J. M., and Branton, D. (1980) *J. Ultrastruct. Res.* **71,** 95–102
16. Fowler, W. E., and Erikson, H. P. (1979) *J. Mol. Biol.* **134,** 241–249

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J. Biol. Chem. 1997, 272:12132-12137.
doi: 10.1074/jbc.272.18.12132