Regulation of the Function of Eukaryotic DNA Topoisomerase I: Analysis of the Binding Step and of the Catalytic Constants of Topoisomerization as a Function of DNA Topology†

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Received August 23, 1989; Revised Manuscript Received May 8, 1990

ABSTRACT: It was previously observed that two steps of the reaction of eukaryotic DNA topoisomerase I (topoisomerization and cleavage) depend upon the conformation of the DNA substrate: in both instances the supercoiled form is a more efficient substrate than the relaxed one. This paper reports the analysis of two other steps of the reaction: the binding of DNA topoisomerase I to DNA and the catalytic constants (K_cs) of topoisomerization as a function of the topology of the substrate. *Binding.* Competition assays show that supercoiled DNA binds the enzyme with even slower kinetics than the relaxed form. Therefore, the preferential topoisomerization of supercoiled DNA is not due to the binding step. Additional evidence that the rate-limiting step of the topoisomerization reaction is not the binding of the enzyme to DNA is provided by the fact that the kinetics of relaxation is first order. *Catalysis.* The K_cs of the topoisomerization reaction have been calculated and it was shown that they do not vary as a function of the topology of the substrate or of its size. Taken together, the data on binding, cleavage, topoisomerization, and K_cs suggest that the preferential topoisomerization of torsionally strained DNA is due to the higher availability, on this topological form, of DNA sites that allow the onset of the reaction.

Eukaryotic DNA topoisomerase I is a ubiquitous and abundant enzyme. The mechanism of its reaction has been defined (Wang, 1985), its cellular localization has been determined in several systems (Javaherian & Liu, 1983; Gilmour & Elgin, 1987), and the gene has been cloned in various organisms (Wang, 1985; Brill et al., 1987). Although conditional mutants have been isolated and their behavior analyzed (Wang, 1985; Brill et al., 1987), the biological function of this enzyme remains unclear.

We have suggested that the basic role of eukaryotic DNA topoisomerase I is to keep the conformation of DNA constant (Camilloni et al., 1988, 1989) through the removal of the torsional strain and of the conformational alterations that may accumulate during various genetic processes. This would permit the cyclic repetitions of reactions that progressively modify the topology of DNA [i.e., transcription, which induces both negative and positive supercoiling during elongation (Liu & Wang, 1987) and unwinds the DNA at initiation (Gamper & Hearst, 1983; Camilloni et al., 1986)]. According to this model, DNA topoisomerase I would compensate the conformational modifications induced on DNA by removal or displacement of DNA binding proteins or protein complexes (i.e., nucleosomes).

To fulfil the function of topological sensor and regulator, eukaryotic DNA topoisomerases I could act according to one of the following alternatives: (1) continuously topoisomerize the DNA, independently of its functional and conformational state, or (2) sense the structure of its substrate and start the topoisomerization reaction only when DNA undergoes topological strain.

In eukaryotes a large mass of DNA is inert, not engaged in transcription or replication. In the absence of any regulatory mechanism, the abundance of DNA topoisomerase I and the diffuse availability of substrate DNA sites would cause the continuous topoisomerization of the inert sequences. This process would be characterized by gross futility. Reactive DNA sites are abundantly available, as shown by the correspondence of DNAse I hypersensitive sites with sites cleaved by eukaryotic DNA topoisomerase I (Bonven et al., 1985) and by the fact that eukaryotic DNA topoisomerase I has no stringent sequence requirement, with one exception (Bonven et al., 1985).

In favor of the second model, it has been shown that actively transcribed DNA regions are topologically perturbed (Zhang et al., 1987) and enriched in detectable DNA topoisomerase I cleavage sites (Stewart & Schutz, 1987). Supporting experimental evidence has also been obtained: supercoiled DNA is in vitro a more efficient substrate for eukaryotic DNA topoisomerase I than the relaxed forms, as shown by the analysis of both the topoisomerization (Camilloni et al., 1988) and cleavage reactions (Muller, 1985; Camilloni et al., 1989). Topology dependence could be caused by differential binding of the enzyme (E) to DNA and/or by different catalytic constants (K_cs) of the topoisomerization reaction for the various topological forms. We show that neither of these two steps of the reaction is responsible for the observed topology dependence and that the reactivity of target DNA sites is the same in relaxed and supercoiled DNAs, and we provide evidence which suggests that the difference in reactivity between different topological forms is caused by the different number of reactive sites per DNA molecule.

Materials and Methods

Restriction endonucleases and T4 DNA ligase were purchased from New England Biolabs and Boehringer. EtdBr was purchased from Sigma; radiochemicals were from NEN. Chicken erythrocyte DNA topoisomerase I was purified according to Martin et al. (1983). The purified enzyme had a

† This work was supported by Fondazione "Pasteur-Fondazione Cenci Bolognetti" (Università di Roma) and by Stimulation Programme CEE Contract ST2J-0372-C (A).

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0006-2960/90/0429-8152$02.50/0

specific activity of 4×10^3 units/mg; units are defined as the amount of enzyme that completely relaxes 1 μg of pBR322 DNA in 30 min at 37 °C in the low-salt buffer reported below [this unit definition is similar to that reported by Keller (1975)]. Wheat germ and calf thymus DNA topoisomerase I were purchased respectively from Promega Biotec, Madison, WI, and New England Biolabs. *Saccharomyces cerevisiae* DNA topoisomerase I was purified in this laboratory by R. Negri from a protease-deficient strain, according to Goto et al. (1983), and had a specific activity of 10^4 units/mg. The DNAs used in this study are the following: (1) The first is a segment of an immunoglobulin gene, the k-light chain (Lk) MPC11 2318-bp *Xba*I/*Xba*I fragment, which encompasses 439-bp upstream of the RNA initiation site, the leader exon, the V region, and part the second intron, including the three remaining J (recombinational signals) sequences. The MPC11 immunoglobulin gene is described by Chei et al. (1980) and Seidman and Leder (1980) and the sequence is in papers by Max et al. (1981) and Kelly et al. (1982). (2) The second is a 961-bp DNA subclone (the *Xba*I–*Hpa*II segment inserted in the *Sma*I site of the polylinker of pUC18M) (Venditti et al., 1988). (3) Next are pUC18 and pSP65, the standard plasmid vectors. (4) The fourth is the *S. cerevisiae* alcohol dehydrogenase gene (ADH II) obtained from the recombinant plasmid ADHII·BS·pBR322 (Russel et al., 1983). This plasmid contains a *Bam*HI/*Sau*3A 2.2-kb yeast fragment cloned in the *Bam*HI site of pBR322. This fragment emcompasses 1 kb of the upstream region of the complete coding sequence of the ADH II gene; the sequence is in the work of Russel et al. (1983). (5) The DNA microdomain, a 128-bp *EcoR*I–*EcoR*I fragment encompassing the sequence of *S. cerevisiae* alcohol dehydrogenase II gene that starts 19 nucleotides upstream of the TATA element and ends 5 nucleotides downstream of the major RNA initiation site, was cloned in the *Hind*II site of the pUC18M polylinker. A 185-bp DNA fragment (encompassing the 17 nucleotides of the modified polylinker and 128 bp of the insert) was obtained by *EcoR*I digestion of the chimaeric pUC18M.

Circularization of large DNA fragments was performed according to minor modifications [reported in Camilloni et al. (1986)] of the procedure developed by Shon et al. (1983).

Preparation of labeled 185-bp closed DNA microdomains was performed as described by Negri et al. (1989). This procedure allows the preparation of DNA molecules with three different linkage numbers. These isomers may be recovered uncontaminated from the gel and treated singly with DNA topoisomerase I. Detailed description of the conditions used for topoisomerization and for the analysis of the products is given by Negri et al. (1989). Low-salt buffer is 10 mM NaCl, 20 mM Tris-HCl, pH 7.9, and 10 mM $MgCl_2$; high salt is the same buffer made with 150 mM NaCl. Reactions were run at 20 °C with the amount of DNA topoisomerase I and for the time indicated where appropriate and were stopped by adding SDS and EDTA (final concentrations 1% and 10 mM, respectively).

Results

Binding of DNA Topoisomerase I on Topologically Different DNA Forms

Several factors hinder the study of the kinetics and thermodynamics of the binding of DNA topoisomerase I to different topological forms. No localized footprint [with the exception reported by Stevnsner et al. (1989)] or defined DNA band shift can be observed (not detailed), filter binding studies on different topological forms provide variable and unreproducible results due to variable non-protein-induced filter retention of different topoisomers, and the cleavage and topoisomerization reactions interfere with measurement of the binding. Reliable measurements of the differences in binding between various topological forms of DNA can be obtained by competition assays (i.e., DNAs with different topologies are tested for their competitory capacity toward a reference topoisomerization reaction).

Competitions Must Be Performed in Processive (=Irreversible Binding) Conditions. Competition assays are meaningful only if the reference topoisomerization reaction is performed in completely processive conditions (low salt) (Been et al., 1984). Figure 1 shows that our topoisomerization system fulfills this requirement. The figure shows the products of the topoisomerization of supercoiled pUC18 DNA by ct[1] DNA topoisomerase I as a function of the E/DNA ratio and of the reaction time. The amount of relaxed topoisomers produced does not change after the completion of the reaction (i.e., the relaxed topoisomers do not further increase as a function of time; Figure 1A,B). This shows that the enzyme remains bound to the topoisomerized substrate (as expected under the low-salt conditions used). Therefore the processive conditions in which the reaction is run are suitable for competition experiments. Complete or partial distributivity of the topoisomerization would prevent competition.

The amount of reaction products is a linear function of the E/DNA ratio (in the limiting-enzyme conditions used) (Figure 1C). The slope of the reaction (Figure 1C) is <1. This indicates that the binding of the enzyme to active DNA sites is partially in competition binding to unreactive sites present on the substrate and/or on the linear and/or nicked forms (whose presence in minor quantities is unavoidable).

Competitions. Competitions of the binding of DNA topoisomerase I to a supercoiled substrate were performed in the presence of a defined topological form of a differently sized DNA used as a competitor.

In this assay, topoisomerization of a supercoiled small DNA (961 bp) was used as a reference reaction. The competitive capacity by topologically different forms of a larger sized DNA was tested. The purpose of using differently sized DNAs is to allow easy identification of the various topological forms (superimposition in the gel of competitors and products of reaction would make the experiment uninterpretable). In order to minimize the possibility of sequence-dependent effects, a subclone of the competitor DNA was used for the reference reaction. Figure 2, panel A, shows the reference reaction in the absence of any competitor. Panels B–D show the competitions by supercoiled (B) and by relaxed DNAs (closed circles) [$\Delta Lk = 0$ (C), $\Delta Lk = +1$ (D)]. The higher competitive capacity of the relaxed forms (panels C and D) relative to the supercoiled DNA (panel B) is evident. Under distributive conditions (high salt), the differential competitive capacity is lost (not shown).

Quantitative evaluation of the reactions is shown in Figure 2, panels E and F. The competitions are described (panel E) by the appearance of the topoisomer that constitutes the major product of relaxation (open arrow in panel A) or by the decrease of unreacted supercoiled substrate (panel F). Panel G shows the competition by the linear form. It is evident that linear DNA is not a good competitor and its efficiency in binding DNA topoisomerase I resembles that of the supercoiled form (that is, low).

The same result was obtained with yeast DNA topoisomerase I (not detailed).

[1] Abbreviations: ct, calf thymus; wg, wheat germ; EtdBr, ethidium bromide.

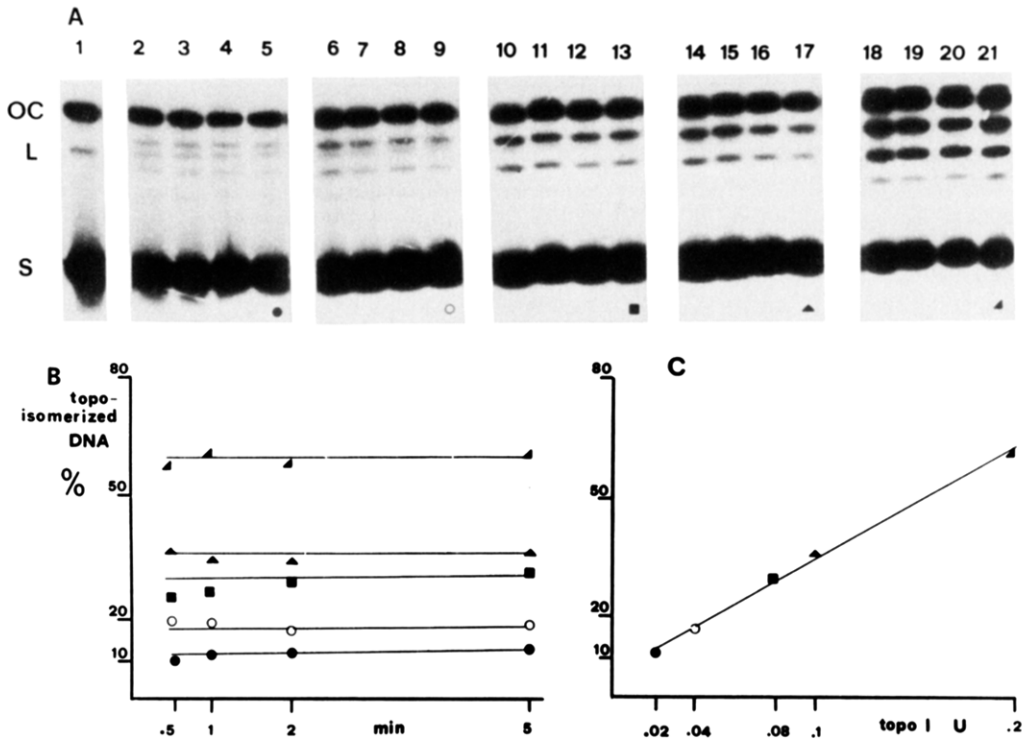

FIGURE 1: Topoisomerization under processive conditions. Irreversibility of binding. Panel A: 2000 cpm (=10 ng) of internally labeled purified supercoiled pUC18 DNA ($\Delta Lk = -30$) was reacted with 0.02 (lanes 2–5), 0.04 (lanes 6–9), 0.08 (lanes 10–13), 0.1 (lanes 14–17), and 0.2 (lanes 18–21) units of ct DNA topoisomerase I for 0.5, 1, 2, and 5 min. Lane 1: untreated. S = supercoiled, OC = open circle, L = linear. Panel B: quantitative evaluation of the topoisomerization shown in panel A. Ordinate: topoisomerized DNA (calculated as percent of the total) for each dose of DNA topoisomerase I (from top: 0.02, 0.04, 0.08, 0.1, and 0.2 unit). Data obtained by scanning densitometry. Abscissa: reaction time. Panel C: percent of topoisomerized DNA (ordinate) as a function of the amount of DNA topoisomerase I. Under the limiting-enzyme conditions used, the amount of the products of the reaction is a linear function of the E/DNA ratio. The slope of the reaction is <1, showing that the binding of the enzyme on active DNA sites is partially competed by unreactive sites present on the substrate and/or the unavoidable minor quantities of linear and/or nicked forms.

Supercoiled DNA Binds DNA Topoisomerase I Slower Than the Closed Relaxed Forms. At low salt (i.e., in processive conditions) the distribution of the binding binary complexes depends only upon the association kinetics, not upon thermodynamic properties (i.e., stability), because stability is so high as to yield an essentially irreversible complex. In high salt (i.e., distributive conditions) the differences in competitive capacity displayed by the different topological forms are neglectable (not shown). This indicates that the differences in the equilibrium constants for the binding of topoisomerase among the different topological forms are also negligible and that therefore the differences in binding among the various forms are predominantly due to kinetics rather than to thermodynamic equilibrium. In accordance with this observation, Ellison and Pulleyblank (1982) have shown that under conditions of reversible binding the association equilibrium constants between supercoiled and relaxed forms are very similar.

In conclusion, the competition assays do not allow measurement of binding rate constants but clearly show that relaxed DNAs bind the enzyme faster than the supercoiled form. Evidently, the preferential topoisomerization previously observed for torsionally strained molecules is not due to preferential binding to this form.

Analysis of the Kinetics of Relaxation

(i) Determination of the Catalytic Constants for Supercoiled Large DNA Domains. The possibility that the differential topoisomerization of the various topological forms could be caused by different catalytic constants has been explored. Topoisomerizations of various DNAs (pUC18, pSP65, 2.3-kb IgK) by yeast, ct, and wg DNA topoisomerase I have been analyzed; all the enzyme/DNA combinations have produced similar results. Data on the determination of the K_c of the reaction of ct DNA topoisomerase I on supercoiled (Figure 3) and relaxed (Figure 4) pSP65 DNA are reported. Supercoiled DNA was reacted for various times with limiting amounts of DNA topoisomerase I (Figure 3, panels A, C, and E). Determination of the K_c is shown in the same figure (panels B, D, and F). The graphs are semilog plots of the unreacted residual substrate (subtracted of the unreacting base line; calculated as shown in panel G) (ordinate) as a function of the reaction time (abscissa). See legend to Figure 3 for details. The existance of base lines shows that the reactions are completely processive. Determination of the base line is important for the calculation of the K_cs (see below).

The plots for the determination of the K_c (Panels B, D, and F) reveal that the reaction is biphasic, suggesting the existence of two partially distinguishable classes of sites (see Discussion). The higher K_c is 0.035 s^{-1}, as shown by the first part of the topoisomerization reaction at the intermediate amount of DNA topoisomerase I (0.05 unit, panel D) (in this case, the slope has been calculated by subtracting the slower component from the total curve) and by the slope of the reaction at the higher concentration of the enzyme (0.1 unit, panel F). At this latter higher concentration, the curve is monophasic, with slope of 0.035 s^{-1}.

The lower K_c is 0.007 s^{-1}, as observed at the low concentration of DNA topoisomerase I (0.03 unit, panel B) and in the lower part of the curve at 0.05 unit (panel D). K_cs variable between 0.007^{-1} and 0.01 s^{-1} have been observed also in the other DNA/DNA topoisomerase I systems analyzed (not detailed).

Figure 3 panel H is the scanning densitometry of selected lanes of the topoisomerization shown in panels C and E.

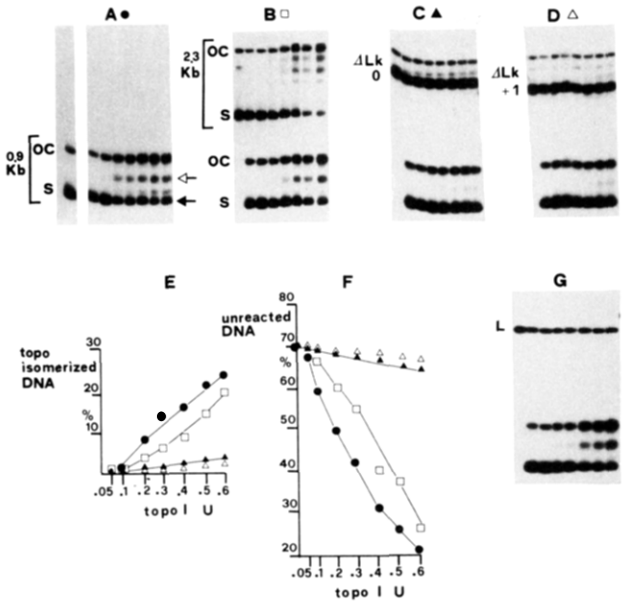

FIGURE 2: Competition of topoisomerization by different forms of DNA. Panel A: reference topoisomerization (●). The leftmost lane is the unreacted 0.9-kb Igk supercoiled DNA. Topoisomerization (from left to right) with 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 unit of ct DNA topoisomerase I. Panel B: as in panel A, in the presence of an equal number of molecules of supercoiled 2.3-kbp IgK DNA. Unreacted DNA is shown in the leftmost lane of this panel. The number of DNA molecules was evaluated through measurement of radioactivity. The large and the small DNA fragments were labeled in the same reaction. Reactions were started by addition of enzyme to premixed DNAs. The other added competitors are, panel C (▲), ΔLk = 0 and, Panel D (△), ΔLk = +1. Panel E quantitates the appearance of the major product of relaxation (empty arrow in panel A) in the absence (●) or in the presence of competitors (symbols as above). Panel F shows the decrease of the reference supercoiled DNA. Panel G shows the competition by linear DNA (L).

Analysis of the pattern of the topoisomers shows two relevant points: (1) the existance of stable intermediates of the reaction (Lk from −10 to −3), evident both at 5 (panel H2) and 20 min (panel H3); (2) the stability of the patterns of the intermediate products as a function of the reaction time, at limiting E/DNA. Panel H4 shows the control pattern: the Gaussian distribution of the products of complete relaxation obtained only with excess DNA topoisomerase I. The meaning of the formation of intermediate products is discussed in section iv.

(*ii*) *Determination of the Catalytic Constants for Relaxed Large DNA Domains.* Relaxed pSP65 DNA (ΔLk = 0) was treated (as for the supercoiled form detailed in section i) with various amounts of DNA topoisomerase I for increasing times. The results of three kinetic analyses are reported in Figure 4. The fast reactivity of these forms is evident. The catalytic constant of the topoisomerization for these DNAs was evaluated by scanning densitometries and was calculated to be ≥ 0.01 s^{-1}. Similar values were observed for the relaxed 2.3-kb ADHII yeast domain and the pUC18 DNA (not detailed). The fact that topoisomerization of relaxed forms requires a larger amount of DNA topoisomerase I has already been observed and discussed (Camilloni et al., 1988, 1989).

(*iii*) *Determination of the Catalytic Constants for Linking-Deficient DNA Microdomains.* The analysis of the topoisomerization of supercoiled (i) and relaxed (ii) DNAs has shown that the reaction is fast and that the K_cs of the topoisomerization of the different topological forms are first order and do not vary. Therefore, the rate-limiting step of topoisomerization is not the association of the enzyme with DNA. The DNAs used for these analyses were 2–3 kb in size, large enough to offer a high number of potential binding and nicking–closing sites. We have performed the same analysis in a superhelically strained 185-bp DNA microdomain. Circles made with DNA of this size are unable to establish titrable superhelical turns [τ, as defined by Wang et al. (1983)] upon linkage reduction at the moment of closure by ligase: this inability is our operational definition of a *micro*domain. Using the standard procedure of ligation in the presence of various concentrations of EtdBr, DNA domains with three different linking numbers were obtained (ΔLk = 0, −1, and −2) (Negri et al., 1989). The torsionally strained ΔLk = −2 was topoisomerized for various times with different concentrations of ct or yeast DNA topoisomerase I. The results are reported in Figure 5. Panel A shows the disappearance of the substrate as a function of three concentrations of enzyme. As for the larger domains, the existence of differential base lines of unreacted substrate as a function of the E/DNA ratio (panel A) shows processivity also for this highly strained domain. Panel B is the graphical representation of the kinetics of the reaction reported as ln of the percent of the unreacted substrate as a function of the reaction time. The plot shows that (1) the reaction kinetics and the catalytic constant are the same as for the larger DNAs and (2) the K_c is ≥ 0.01 s^{-1} [as observed for larger DNAs (sections i and II)]. Similar K_cs were observed on ΔLk = −1 DNA and on both ΔLk = −2 and −1 with yeast DNA topoisomerase I (not shown).

In summary, the K_cs of topoisomerization are equal or very similar for both large and small domains, for every tested DNA topoisomerase or DNA, independently of its topological state.

(*iv*) *The Existence of Stable Intermediate Products in the Topoisomerization of Larger Domains Suggests a Topology-Dependent Inactivation of DNA Reactive Sites.* The topoisomerization reaction of 2–3-kb DNA domains by ct DNA topoisomerase I has catalytic constants of 0.035–0.007/s^{-1}. This means (as shown in Figure 3, panel G) that the final product of the reaction (final Gaussian relaxed family plus blocked intermediates) receives no further contribution from the supercoiled substrate after 5 min. At this time the reaction is complete; the intermediates remain stable (Figure 3, panel H) and are not further topoisomerized. The intermediates disappear at excess DNA topoisomerase I (see Figure 3, panel C). In a reaction run under conditions in which the binding of the enzyme is irreversible, the formation of stable intermediates is presumably due to inactivation of the process of nicking–closing (not to the interruption of a rebinding process). The interruption of the topoisomerization process, which induces stable intermediates to accumulate, can only be caused by the progressive inactivation of the DNA substrate due to its relaxation [see also Camilloni et al. (1988, 1989)]. The fact that the relative amount of the inactive intermediates increases while approaching the relaxed state shows that the inactivation is progressive. Figure 3, panels H2 and H3, shows that the blocked intermediates that are not part of the final Gaussian distribution progressively increase (namely: ΔLk = −10 < −9 < −8, ..., < −3).

The alternative explanation (inactivation of the enzyme during catalysis) is ruled out by the observation that DNA topoisomerase I is stable and active for long times, as shown by the kinetics of the reaction performed at limiting E/DNA under distributive conditions [not detailed; see also Camilloni et al. (1988)].

Discussion

The Topology-Dependent Steps in the Eukaryotic DNA Topoisomerase I Reaction. Previous analyses had shown that topologically strained DNA molecules are preferentially cleaved (Muller, 1985; Camilloni et al., 1989) and topoisom-

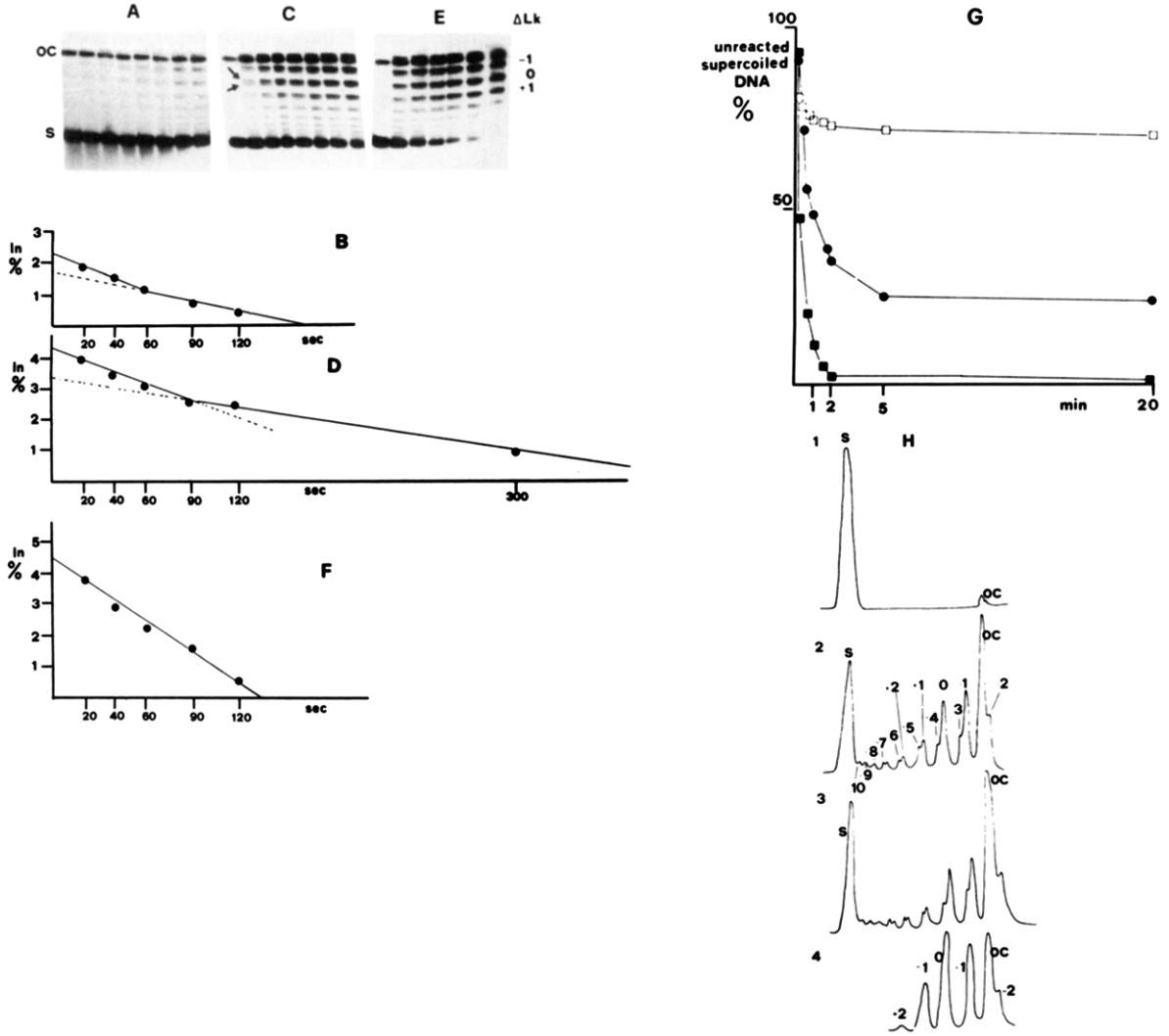

FIGURE 3: Determination of the catalytic constants for the topoisomerization of pSP65 supercoiled DNA. Reaction of 0.03 (panel A), 0.05 (panel C), and 0.1 unit (panel E) of ct DNA topoisomerase I with 10 ng of labeled supercoiled pSP65 for 20, 40, 60, 90, 120, 300, and 1200 s. (from left to right). In each panel the leftmost lane is the untreated control. In panel E the 5-min time point is missing. Panels B, D, and F are the corresponding plots [unreacted substrate (ln, %) versus the reaction time] used to evaluate the K_c (see text). Panel G: determination of the amount of unreacted substrate (base lines of the reactions). Data were obtained by densitometric scanning of underexposed autoradiograms of the experiment reported in panels A (□), C (●), and E (■). The percent refers to the unreacted material relative to the total material of each lane. This automatically corrects possible errors deriving from loading variations. Panel H: densitometry of (1) supercoiled unreacted DNA and (2) DNA topoisomerization by 0.05 unit for 5 min, (3) by 0.05 unit for 20 min, and (4) by 0.1 unit for 20 min. The numbers from −10 to +2 in panel H3 indicate the writhe of the DNA molecule in topoisomerase buffer [corrections of the writhing number according to Camilloni et al. (1986)]. The stability of the pattern of the products shows that the reaction is processive (if it were even partially distributive, the pattern would change as a function of time).

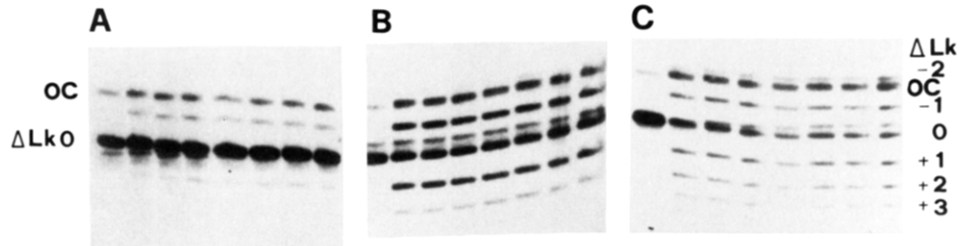

FIGURE 4: Topoisomerization of relaxed (ΔLk = 0) pSP65 DNA by 0.1 (panel A), 0.5 (B), and 1.5 units (C) of ct DNA topoisomerase I. Reaction conditions as described in Figure 3. At high E/DNA ratios (see panel C) the distribution of the final relaxed topoisomers becomes increasingly positive. This phenomenon appears to be associated with the high concentration of DNA topoisomerase I necessary to topoisomerize the relaxed form and has not been analyzed further.

erized (Camilloni et al., 1988). We show here that the catalytic constant of the topoisomerization reaction is not topology dependent and that the binding step is not rate limiting or is faster for supercoiled forms. The differential reactivity observed for relaxed (R) and supercoiled (S) DNAs may thus be described and explained as follows. The reactivity of each single active DNA site is not topology related and is constant in S and R DNAs (at least approximatively, as shown by the constancy of the K_cs at limiting E/DNA) (experiments reported in Figures 3–5) (i). What supposedly changes between the S and R states is the number of reactive sites per molecule. A consequence of the relaxation-dependent inactivation of reactive DNA sites would be the progressive switch-off of the topoisomerization reaction; this would result in the production

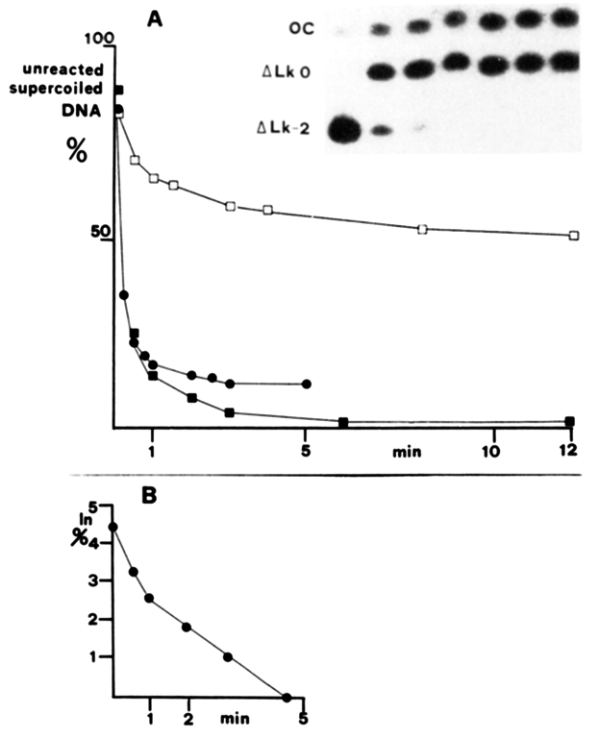

FIGURE 5: Determination of the catalytic constant for the 185-bp microdomain. Labeled purified ΔLk = −2 (10 ng) was reacted with ct DNA topoisomerase I under the described low-salt conditions. Panel A: determination of the amount of unreacted substrate, as described in Figure 3. Treatment with 0.05 (□), 0.15 (●), and 0.3 (■) unit of DNA topoisomerase I for the indicated time. The picture shows the relaxation of the ΔLk = −2 as a function of time (0, 0.5, 1, 2, 3, 6, and 12 min) (0.3 unit of DNA topoisomerase I). Panel B: determination of K_c. Data from the reaction with 0.3 unit of DNA topoisomerase I. Unreacted substrate (ln %, ordinate) versus reaction time (min, abscissa).

of kinetically stable intermediates (ii), in spite of the fact that the reaction occurs in completely processive conditions. Under the conditions of irreversible association used, every reacting supercoiled substrate molecule should be transformed—in the absence of inactivation caused by the progressive relaxation—to the final Gaussian family of relaxed topoisomers. The hypothetical mechanism (that is, active DNA sites switched off by relaxation or switched on by strain) implies that the patterns of cleavage sites differ between R and S DNAs (iii). The decrease of the number of the cleaved sites from S to R has been reported (Camilloni et al., 1989).

Support for our hypothesis is provided by the unambiguous experimental evidence obtained for the points i, ii, and iii.

In conclusion, both binding and the rate of catalysis can be ruled out as causes of the preferential reaction of DNA topoisomerase I with torsionally strained DNA. Evidence is provided [in agreement with the analysis of the cleaved sites previously reported by Camilloni et al. (1989)] that the number of potentially reactive sites on supercoiled DNA is higher than on the relaxed form.

Taken together, the data in this paper and in previous work suggest the following overall mechanism of eukaryotic DNA topoisomerase I: when a molecule of DNA topoisomerase I binds to topologically strained supercoiled DNA, fast topoisomerization occurs. The resulting relaxed DNA becomes unreactive; the enzyme remains stably bound on potentially active but actually inert DNA sites. Activation of the cleavage reaction occurs when the DNA molecule undergoes even minor changes of its conformation (Camilloni et al., 1989).

DNA topoisomerase I appears to be an enzyme that sits inactive on potentially active DNA sites and that starts its reaction when DNA—deformed by any environmental or functional factor—changes its conformation on target sites. In addition to the relevance in in vivo topological phenomena, this behavior of DNA topoisomerase I makes this enzyme an important tool for the study of topology-dependent changes of DNA conformation and of DNA conformational dynamics.

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