Casein Kinase II Stimulates *Xenopus laevis* DNA Topoisomerase I by Physical Association†

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Received April 26, 1994; Revised Manuscript Received August 26, 1994⊗

ABSTRACT: A *Xenopus laevis* casein kinase II-like activity copurified with *X. laevis* DNA topoisomerase I activity during chromatography on DEAE-cellulose, phosphocellulose, and hydroxylapatite, but the two activities were resolved by chromatography on DNA−agarose [Kaiserman, H. B., Ingebritsen, T. S., & Benbow, R. M. (1988) *Biochemistry 27,* 3216−3222]. Phosphorylation of the catalytic polypeptides of dephosphorylated *X. laevis* DNA topoisomerase I by the endogenous *X. laevis* casein kinase II-like activity apparently resulted in a severalfold increase in catalytic activity. In this study, we show that incubation of purified *X. laevis* DNA topoisomerase I with electrophoretically homogeneous bovine brain casein kinase II and ATP strongly stimulated catalytic activity. Surprisingly, purified bovine casein kinase II stimulated *X. laevis* DNA topoisomerase I activity by more than an order of magnitude in the absence of ATP, although ATP resulted in additional stimulation. Other basic proteins, such as histone H1 and HMG proteins, also stimulated *X. laevis* DNA topoisomerase I catalytic activity 2−3-fold in the absence of ATP. Modulation of catalytic activity by direct physical association (protein−protein interactions) must, therefore, be considered in addition to phosphorylation in assessing the physiological role of casein kinase II and other basic proteins during regulation of *X. laevis* DNA topoisomerase I activity *in vivo.*

Two major types of eukaryotic DNA topoisomerase activity, DNA topoisomerase I and DNA topoisomerase II, have been implicated in diverse essential cellular processes including transcription, genetic recombination, and chromosomal DNA replication (Wang, 1985, 1991; D'Arpa & Liu, 1989; Sternglanz, 1989; Kim & Wang, 1989; Hsieh, 1990; Kroeger & Rowe, 1992). DNA topoisomerase I has been shown to be essential for the growth and development of a multicellular organism, *Drosophila melanogaster* (Lee *et al.*, 1993). There is general agreement that the catalytic activity of DNA topoisomerase I, as well as DNA topoisomerase II, is modulated by posttranslational modifications such as phosphorylation/dephosphorylation and that these modifications may play a physiological role during cell growth (Higgins *et al.*, 1990).

Dephosphorylation of DNA topoisomerase I from Novikoff hepatoma (Durban *et al.*, 1983), *X. laevis* ovary (Kaiserman *et al.*, 1988), Chinese hamster ovary (Pommier *et al.*, 1990), or rat liver (Tournier *et al.*, 1992) greatly reduces or abolishes the ability of the enzyme to relax supercoiled DNA. Phosphorylation of dephosphorylated DNA topoisomerase I on serine residues by casein kinase II-like enzymes (Durban *et al.*, 1983; Mills *et al.*, 1982; Kaiserman *et al.*, 1988) or by protein kinase C (Samuels *et al.*, 1989; Samuels & Shimizu, 1992; Pommier *et al.*, 1990) enhances catalytic activity. In contrast, phosphorylation by a tyrosine protein kinase decreased the catalytic activity of calf thymus DNA topoisomerase I by more than 90% (Tse-Dinh *et al.*, 1984). DNA topoisomerase II activity is similarly modulated by phosphorylation/dephosphorylation in a variety of eukaryotic organisms (Kroll & Rowe, 1991; Rottman *et al.*, 1987; Ackerman *et al.*, 1988; Heck *et al.*, 1989; Saijo *et al.*, 1990).

Other posttranslational modifications can also modulate eukaryotic DNA topoisomerase I activity. Poly(ADP-ribosyl)ation of DNA topoisomerase I decreases or abolishes DNA-relaxing activity (Ferro *et al.*, 1983, 1984; Kasid *et al.*, 1989; Higgins *et al.*, 1990). In contrast, poly(ADP-ribose) polymerase stimulates the activity of another enzyme involved in chromosomal DNA replication and genetic recombination, DNA polymerase α, by 6−60-fold in a dose-dependent manner (Simbulan *et al.*, 1993). Interestingly, poly(ADP-ribosyl)ation of the DNA polymerase α enzyme is apparently not necessary: the stimulation is due to physical association of the poly(ADP-ribose) polymerase with DNA polymerase α.

Similar physical association may modulate the catalytic activity of DNA topoisomerase I, at least *in vitro.* Javaherian and Liu (1983) showed that HMG 17,[1] HMG 1, HMG 2, and histone H1 stimulated eukaryotic DNA topoisomerase I activity up to 64-fold. They also showed that labeled HMG 17 or H1 protein cosedimented with heterologous DNA

† This research was supported by Grants RO1GM29490 and GM10565 from the National Institutes of Health, Grant DIR9110258 from the National Science Foundation, Grant BE-94-E from the American Cancer Society, and Grant 480-21-31 and GM10565 from the Office of Biotechnology at Iowa State University. This is Journal Paper J-15783 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA (Projects 2974 and 0164).

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⊗ Abstract published in *Advance ACS Abstracts,* October 15, 1994.

0006-2960/94/0433-13484$04.50/0

topoisomerase I activities on sucrose gradients, suggesting that physical associations resulting in protein−protein complexes had formed. The implication was that protein−protein interactions caused the observed increases in DNA topoisomerase I catalytic activity. In apparent contradiction, however, Bina-Stein and Singer (1977) showed that histone H1 strongly inhibited mammalian DNA topoisomerase I activity. More recently, Richter and Kapitza (1991) also reported that histone H1 inhibited calf thymus and HeLa cell DNA topoisomerase I activity at molar ratios higher than 1 histone H1 molecule per 40 base pairs of DNA. In these studies, alteration of the conformation of the supercoiled DNA by histone H1 and competition for binding sites on the DNA were postulated to cause the observed decreases in DNA topoisomerase I activity.

In this study, we show that highly purified bovine brain casein kinase II stimulates *X. laevis* DNA topoisomerase I activity, both in the presence and in the absence of ATP. Stimulation was enhanced when ATP was present, but dramatic stimulation was observed in the absence of ATP. Moderate stimulation of *X. laevis* DNA topoisomerase I catalytic activity by mixed HMG proteins and by histone H1 was also observed at low protein concentrations, in possible agreement with Javaherian and Liu (1983). At higher protein concentrations, however, inhibition of DNA topoisomerase I by histone H1 (but not by bovine casein kinase II) was observed in agreement with Bina-Stein and Singer (1977) and Richter and Kapitza (1991). In addition, we confirmed that *X. laevis* casein kinase II activity copurified with *X. laevis* DNA topoisomerase I as reported by Kaiserman *et al.* (1988) and was physically associated with partially purified enzyme as has been shown very recently for rat liver casein kinase II and DNA topoisomerase I (Turman & Douvas, 1993). We propose, therefore, that regulation of *X. laevis* DNA topoisomerase I catalytic activity by *X. laevis* casein kinase II occurs by a complex mechanism that involves direct physical association (protein−protein interactions) as well as phosphorylation/dephosphorylation.

EXPERIMENTAL PROCEDURES

Materials

Frogs and Cows. Adult *X. laevis* females were purchased from the South African Snake Farm (Fish Hoek, Cape Providence, South Africa), from Xenopus I (Ann Arbor, MI), or from Charles D. Sullivan Co., Inc. (Nashville, TN). Intact brains from freshly slaughtered cows were obtained from Amends Packing Co. (Des Moines, IA).

Chemicals. Tris, glycerol, other enzyme-grade buffer components, bromophenol blue, Ficoll, and calf intestinal alkaline phosphatase immobilized on agarose were from Sigma. Bovine serum albumin (nuclease-free), SDS, and DTT were from Boehringer Mannheim. Xylene cyanol and ethidium bromide were from International Biotechnologies Inc. (New Haven, CT). pUC19 DNA was a gift of Howard Kaiserman. [γ-^{32}P]ATP (700−1000 Ci/mol) was prepared according to the method of Johnson and Walseth (1979). [γ-^{32}P]P_i was from Dupont−NEN.

Enzymes and Proteins. X. laevis ovarian DNA topoisomerase I was purified from *X. laevis* ovaries as described previously (Kaiserman *et al.*, 1988). The purified enzyme had a specific activity, as defined by Kaiserman *et al.* (1988), of 43 750 units/mg of protein. In this study, 1 unit of DNA topoisomerase I is defined as the amount of enzyme that can relax 250 ng of supercoiled pUC19 DNA in 30 min. Calf thymus histone H1 and HMG proteins were purified by the method of Sanders (1977). The HMG proteins are present in the mixture in the approximate percentages as follows: HMG 1, 44.1%; HMG 2, 28.7%; HMG 14, 5.1%; and HMG 17, 22.1%.

Bovine brain casein kinase II was prepared as described by Hathaway and Traugh (1979, 1983) for rabbit reticulocyte enzyme with the modifications described below. Brains from two freshly killed heifers were placed immediately on ice. All subsequent steps were carried out at 2 °C. Brains (796 g) were immersed in 2388 mL of homogenization buffer (50 mM Tris-HCl, pH 7.0, 5 mM 2-mercaptoethanol, 1 mM EGTA, 1 mM PMSF, and 1 mM benzamidine), homogenized in a Waring blender for 4 min, and centrifuged at 10000*g* for 60 min. The pellet was resuspended in 1800 mL of homogenization buffer and centrifuged at 10000*g* for 60 min, and supernatants were pooled. Ammonium sulfate was added to 40% (w/v) with stirring. The slurry was centrifuged at 18000*g* for 30 min and the pellet resuspended in buffer A (20 mM Tris-HCl, pH 7.4, 10 mM 2-mercaptoethanol, 1 mM EDTA, 1 mM PMSF, 1 mM benzamidine, and 0.02% sodium azide) and dialyzed overnight in 5 L of buffer A. All subsequent steps were exactly as described by Hathaway and Traugh (1979). The specific activity of the purified enzyme was 89 000 units/mg of protein. Analysis of the purified enzyme by SDS−PAGE showed major bands at 44 kDa (α subunit) and 25 kDa (β subunit) and a minor band at 38 kDa (α' subunit). A trace amount of a protein of 42 kDa was also present [see Hathaway and Traugh (1979) and Figure 1 (top panel)]. The subunit composition of the bovine brain casein kinase II was similar to the rabbit reticulocyte enzyme except that the amount of α' subunit recovered from brain was lower (Hathaway & Traugh, 1979, 1983).

Protein concentrations were determined by the method of Bradford (1976) with bovine serum albumin as standard.

SDS−PAGE. SDS−PAGE was carried out according to Laemmli (1970). Silver staining was performed with a Bio-Rad silver stain kit as described by the manufacturer. Molecular mass markers were phosphorylase *b*, 97.4 kDa; bovine serum albumin, 68 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 29.5 kDa; and trypsinogen, 24 kDa.

Chromatography Media. DEAE-cellulose (DE52) and cellulose phosphate (P11) were from Whatman. Hydroxylapatite−agarose (HA-Ultrogel) was from IBF Biotechnics Inc. Sephacryl S300 and CL-Sepharose were from Pharmacia LKB.

Methods

DNA topoisomerase I activity was quantitated by agarose gel electrophoresis to monitor the relaxation of supercoiled (form I) pUC19 plasmid DNA. The procedure was based on that used by Liu and Miller (1981). The assay mixture contained 50 mM Tris-HCl, pH 7.5, 120 mM KCl, 10 mM

[1] Abbreviations: HMG, high mobility group; Tris, tris(hydroxymethyl)aminomethane; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; EDTA, ethylenediaminetetraacetic acid; DTT, dithiothreitol; polybrene, hexadimethrine bromide; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

$MgCl_2$, 1.0 mM DTT, 0.4 nM bovine serum albumin, and the indicated concentrations of DNA and enzyme in a total volume of 30 μL except where noted. Reactions were carried out for 30 min at 30 °C, except where noted, and were terminated by addition of 1.5 μL of 20% sodium dodecyl sulfate.

Agarose gel electrophoresis was performed on 1.1% agarose gels at 30 V with Ficoll-based loading buffer and Tris−borate electrophoresis buffer according to Maniatis *et al.* (1989). Supercoiled and relaxed pUC19 DNA was quantitated by densitometry using a GS300 transmittance/reflectance scanning densitometer (Hoefer Scientific Instruments) as described previously (Kaiserman *et al.*, 1988).

Dephosphorylation of DNA Topoisomerase I by Alkaline Phosphatase. X. laevis DNA topoisomerase I (51.0 units) was incubated with 780 milliunits of immobilized calf thymus intestinal alkaline phosphatase at 2 °C for 5 h with rocking in a final volume of 288 μL, followed by centrifugation at 10000*g* for 30 s.

Casein kinase II assays were performed using a modification of the method of Glover and colleagues (Glover *et al.*, 1983). Assay mixtures contained 57 mM Tris-HCl, pH 8.5, 167 mM NaCl, 10 mM $MgCl_2$, 1 mg/mL casein, 0.17 mM EDTA, 0.017% Triton X-100, 3.3% glycerol, 0.17 mM 2-mercaptoethanol, 100 μM [γ-^{32}P]ATP (1 μCi), and the indicated amount of bovine casein kinase II in a total volume of 60 μL. Reactions were started by adding [γ-^{32}P]ATP and incubated at 30 °C for 10 min (Glover *et al.*, 1983). Phosphorylated products were evaluated according to the method of Glass (Glass *et al.*, 1978). Briefly, 30 μL of reaction mixture was applied to a 2 × 2 cm square of Whatman P81 paper, which was immersed in 5 mL of 0.5% phosphoric acid with stirring for 15 min, washed 3 times, transferred to 95% ethanol for 5 min, dried, and placed in scintillant to determine radioactivity. One unit of activity is the amount of enzyme that catalyzes the transfer of 1 pmol of [^{32}P]P_i per minute to casein.

Time Course of Casein Kinase II Stimulation in the Presence of ATP. Dephosphorylated *X. laevis* DNA topoisomerase I (30 nM) was incubated with bovine casein kinase II (35 nM) followed by addition of [γ-^{32}P]ATP to a final concentration of 10 μM in a total volume of 135 μL at 30 °C for the indicated time. For each time point, 25 μL was assayed for *X. laevis* DNA topoisomerase I activity.

Concentration Dependence of Effects of Histone H1 and Casein Kinase II on X. laevis DNA Topoisomerase I Activity. The indicated concentrations of histone H1 or bovine casein kinase II were preincubated at 36 °C for 5 min with 125 ng of pUC19 plasmid DNA in a total volume of 25 μL. Reactions contained 1.9 nM *X. laevis* DNA topoisomerase I. In order to ensure adequate substrate DNA was present, reactions were terminated as follows: Controls containing DNA topoisomerase I without histone H1 and casein kinase II without DNA topoisomerase I and reactions containing histone H1 at concentrations of 2.22 and 434 nM and casein kinase II at a concentration of 0.52 nM were stopped at 10 min. Reactions containing histone H1 concentrations of 4.44, 8.88, and 222.2 nM and casein kinase II at a concentration of 1.56 nM were stopped at 5 min. Reactions containing histone H1 at concentrations of 44.4 and 88.8 nM and casein kinase II at concentrations of 5.20, 15.60, 52.00, 156.00, and 521.00 nM were stopped at 3 min.

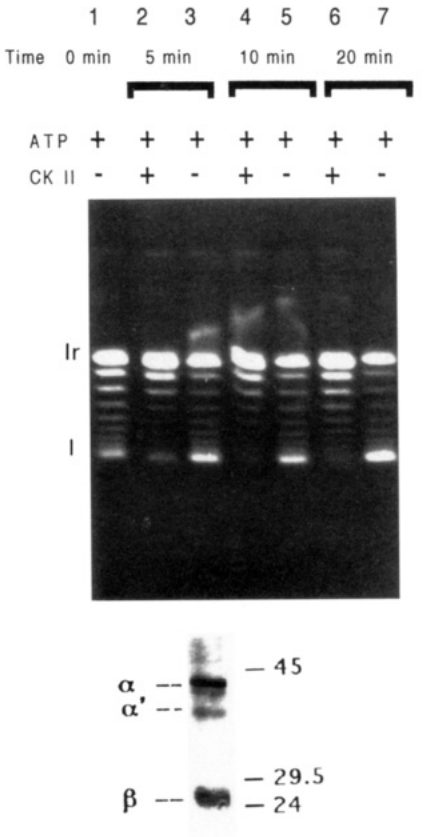

FIGURE 1: Time course of stimulation of dephosphorylated *X. laevis* DNA topoisomerase I by bovine casein kinase II in the presence of ATP. DNA topoisomerase I (30 nM) was preincubated for the indicated time with ATP (10 μM) and, where indicated, casein kinase II (35 nM), or equivalent buffer lacking casein kinase II, in a total volume of 25 μL. At the end of preincubation, 5 μL of pUC19 (1.3 μg) in DNA topoisomerase assay buffer was added, and the mixture was assayed for DNA topoisomerase activity (10 min assay). (Top) Lane 1, no casein kinase II, 0 min; lane 2, casein kinase II, 5 min; lane 3, no casein kinase II, 5 min; lane 4, casein kinase II, 10 min; lane 5, no casein kinase II, 10 min; lane 6, casein kinase II, 20 min; lane 7, no casein kinase II, 20 min. I denotes the position of migration of supercoiled DNA; Ir, relaxed DNA. (Bottom) SDS−polyacrylamide gel electrophoresis of purified bovine casein kinase II. 25 ng of casein kinase II was analyzed by SDS−PAGE as described under Experimental Procedures.

Determination of Stokes Radii. Stokes radii were measured by gel filtration (Siegel & Monty, 1966) on Sephacryl S300 relative to calibration standards exactly as described (Nelson *et al.*, 1983) using the calibration standards described in that study.

Coimmunoprecipitation of DNA Topoisomerase I and Casein Kinase II. X. laevis DNA topoisomerase I and the copurifying endogenous casein kinase II were immunoprecipitated at 4 °C overnight with polyclonal anti-*X. laevis* DNA topoisomerase I antibody (HKTOP-I, the generous gift of H. B. Kaiserman) conjugated to CnBr-activated Sepharose 4-B (Pharmacia) and centrifuged for 15 min at 15 000 rpm [essentially as described by Simbulan *et al.* (1993)]. Control precipitations were done with preimmune serum and with Sepharose 4-B beads alone. Supernatants were assayed for DNA topoisomerase activity and casein kinase II activity as described above.

RESULTS

Purified Bovine Casein Kinase II Stimulates X. laevis DNA Topoisomerase I in the Absence of ATP. In a previous study,

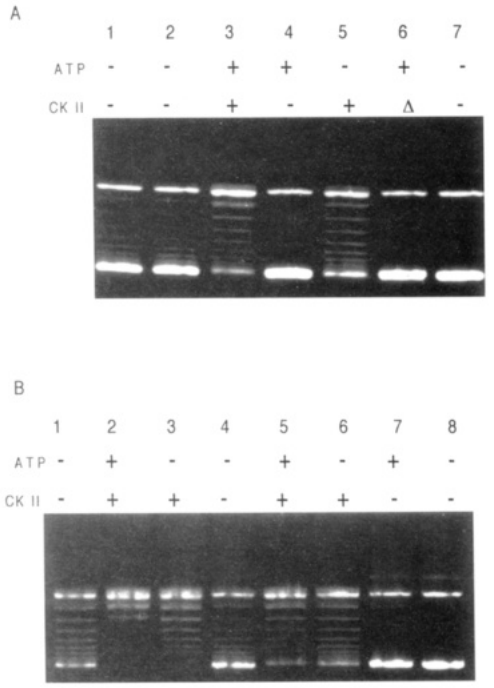

FIGURE 2: Stimulation of DNA topoisomerase I by casein kinase II in the absence of ATP. *X. laevis* DNA topoisomerase I (40 nM) was preincubated for 5 min with casein kinase II (28 nM) and, where indicated, 10 μM ATP. DNA topoisomerase assays were then started by addition of 1.3 μg of pUC19 DNA and stopped after 30 min at 36 °C. The *X. laevis* DNA topoisomerase I used in (A) was dephosphorylated. The *X. laevis* DNA topoisomerase I used in (B), lanes 4, 5, and 6, had been mock-dephosphorylated for 50 min at 30 °C. (A) Lane 1, no casein kinase II; lane 2, no casein kinase II; lane 3, casein kinase II, ATP; lane 4, no casein kinase II, ATP; lane 5, casein kinase II, no ATP; lane 6, boiled casein kinase II, ATP; lane 7, input DNA. (B) Lane 1, no casein kinase II; lane 2, casein kinase II, ATP; lane 3, casein kinase II, no ATP; lane 4, no casein kinase II; lane 5, casein kinase II, ATP; lane 6, casein kinase II, no ATP; lane 7, no casein kinase, ATP; lane 8, input DNA.

we showed that a *X. laevis* casein kinase II-like activity reactivated dephosphorylated *X. laevis* DNA topoisomerase I and stimulated the activity to a level greater than that observed prior to dephosphorylation (Kaiserman *et al.*, 1988). A time course in which dephosphorylated *X. laevis* DNA topoisomerase I was preincubated with purified bovine casein kinase II for the indicated time in the presence of ATP showed strong stimulation of catalytic activity at 5 min, and maximal stimulation by 10 min (Figure 1, top panel). In the absence of bovine casein kinase II, no stimulation was observed (Figure 1, top, compare lanes 1, 3, and 5), establishing that the casein kinase II-like activity that copurified with *X. laevis* DNA topoisomerase I during chromatography on DEAE-cellulose, phosphocellulose, and hydroxylapatite had been completely removed from the purified *X. laevis* DNA topoisomerase I fraction VIII enzyme used in this study. After 20 min preincubation, slight inactivation of DNA topoisomerase I activity was apparent in either the presence or the absence of casein kinase II (Figure 1, top, compare lanes 6 and 7 with lanes 4 and 5).

Surprisingly, reactions which were intended as controls that contained purified casein kinase II but lacked ATP showed stimulation nearly equivalent to that resulting from preincubation with casein kinase II in the presence of ATP (Figure 2A, compare lanes 3 and 5; and Figure 2B, compare lanes 2 and 3, and lanes 5 and 6). In the absence of casein kinase II, slight inhibition of *X. laevis* DNA topoisomerase

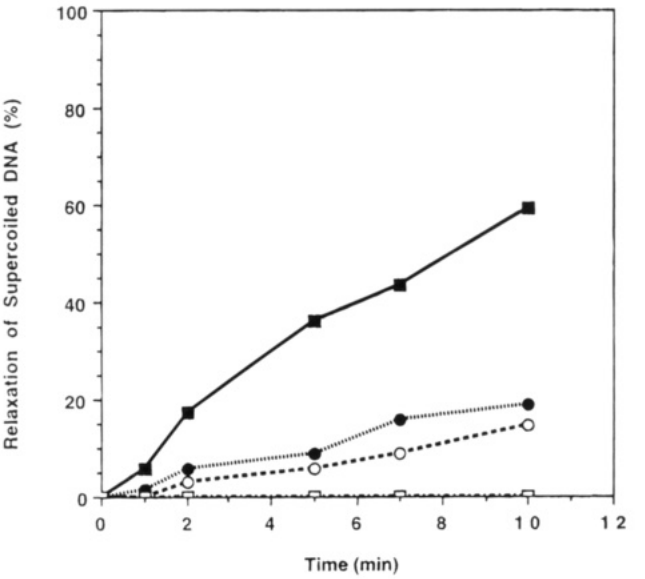

FIGURE 3: Time course of relaxation of supercoiled DNA by *X. laevis* DNA topoisomerase I in the presence of casein kinase II and absence of ATP. 1.05 μg of pUC19 plasmid DNA and DNA topoisomerase I was incubated with or without casein kinase II at 36 °C in a total starting volume of 210 μL. At the indicated times, a 30 μL reaction mixture was removed and added to 8.75 μL of loading buffer containing 2% sodium dodecyl sulfate. Reaction mixtures contained 11 nM casein kinase II (open squares), 1.8 nM DNA topoisomerase I (open circles), 1.1 nM casein kinase II and 1.8 nM DNA topoisomerase I (closed circles), and 11 nM casein kinase II and 1.8 nM DNA topoisomerase I (closed squares).

I activity by ATP was noted (Figure 2A, lanes 4 and 6; Figure 2B, lane 7) as reported previously [see Tournier *et al.* (1992) and references cited therein]. ATP has been shown previously to inhibit mammalian DNA topoisomerase I, but 60-fold higher ATP concentrations were required for 50% inhibition (Castora & Kelly, 1986). Stimulation was not observed with casein kinase II that had been boiled for 5 min (Figure 2A, lane 6), but was always observed with native casein kinase II in the absence of ATP using various incubation protocols (Figure 2 and data not shown).

While the possibility exists that the bovine casein kinase II preparation might be contaminated with some other substance which is stimulatory to *X. laevis* DNA topoisomerase I, this seems unlikely. The bovine casein kinase II was highly purified and contained polypeptides that were identical to those comprising casein kinase II from other organisms (Figure 1B). Heat-treated enzyme failed to stimulate activity. Attempts to show that the stimulation was specific to casein kinase II using the casein kinase II-specific inhibitors heparin and pyridoxal 5′-phosphate were unsuccessful, since these inhibitors also inhibited the *X. laevis* DNA topoisomerase I at the required concentrations (data not shown).

Stimulation of X. laevis DNA Topoisomerase I Activity by Casein Kinase II in the Absence of ATP Is Independent of Time. A time course of relaxation of DNA by *X. laevis* DNA topoisomerase I incubated with casein kinase II in the absence of ATP proceeded linearly (Figure 3). This implies that maximal stimulation of *X. laevis* DNA topoisomerase I by casein kinase II in the absence of ATP was obtained within 1 min. Moreover, the amount of stimulation was dependent on the concentration of casein kinase II (Figure 3). This time course and dose-dependence suggest that a

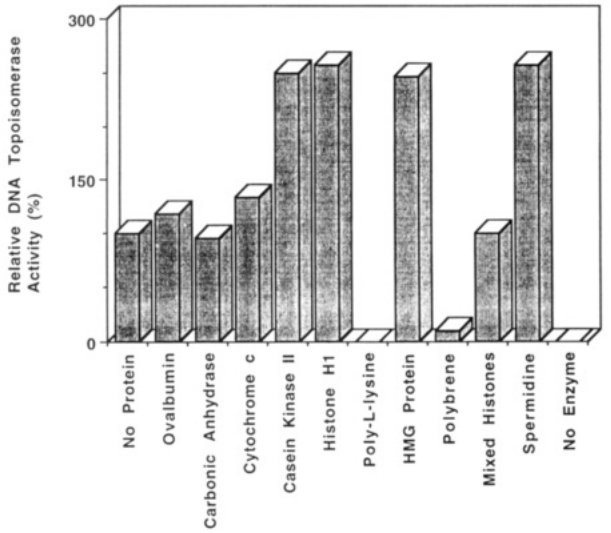

FIGURE 4: Stimulation of *X. laevis* DNA topoisomerase I by macromolecules in the absence of ATP. DNA topoisomerase I (3.8 nM) was assayed in the presence of a 3 μg/mL aliquot of the following macromolecules: ovalbumin (67 nM), carbonic anhydrase (102 nM), cytochrome *c* (240 nM), casein kinase II (22.7 nM), histone H1 (130 nM), HMG proteins (ca. 200 nM), mixed histones (ca. 150 nM), poly(L-lysine) (140 nM), polybrene (ca. 400 nM), or spermidine (100 μM). Assays also contained 125 ng of pUC19 DNA and were carried out for 30 min at 36 °C in a total volume of 30 μL. Lane 1, no macromolecule; lane 2, ovalbumin; lane 3, carbonic anhydrase; lane 4, cytochrome *c*; lane 5, casein kinase II; lane 6, histone H1; lane 7, HMG proteins; lane 8, mixed histones; lane 9, spermidine; lane 10, input DNA, no DNA topoisomerase I. The fold stimulation of *X. laevis* DNA topoisomerase I by casein kinase II and histone H1 in this experiment is underestimated because of the limited linear range of the DNA topoisomerase I assay (see Figure 5).

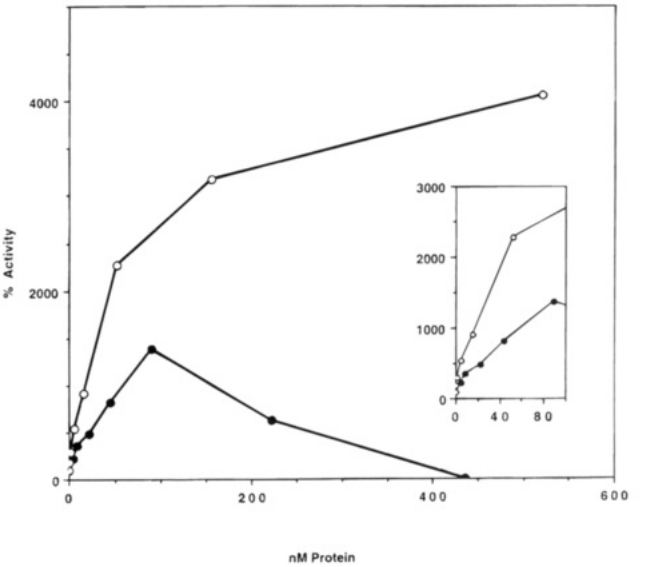

FIGURE 5: Comparison of effects of bovine casein kinase II versus calf thymus histone H1 on *X. laevis* DNA topoisomerase I activity. Assays contained DNA topoisomerase I (1.9 nM), 125 ng of pUC19 DNA, and the indicated concentrations of histone H1 (closed circles) or casein kinase II (open circles) in a total volume of 30 μL. Assay times varied from 3 to 10 min depending on the level of DNA topoisomerase activity. Reactions were stopped by the addition of 8.75 μL of loading buffer. The inset shows an expanded plot at the lower concentrations. These results were replicated 5 times using different incubation times and concentrations. The profile shown is representative of these results over the entire concentration range examined.

rapid process (physical association), rather than a time-dependent enzyme-catalyzed reaction, was responsible for the ATP-independent stimulation. The additional stimulation observed in the top panel of Figure 1 from 5 to 10 min in the presence of ATP may be ATP-dependent, i.e., phosphorylation (compare Figures 1, top panel, and Figure 3).

Histone H1 and HMG Proteins Stimulate X. laevis DNA Topoisomerase I in the Absence of ATP. In addition to casein kinase II, histone H1, HMG proteins, and spermidine also stimulated *X. laevis* DNA topoisomerase I severalfold in the absence of ATP (Figure 4). The level of stimulation by histone H1 and mixed HMG proteins, as well as by the polyamine spermidine, was similar to the level of stimulation by low concentrations of bovine casein kinase II. Other proteins tested, including ovalbumin, carbonic anhydrase, and cytochrome *c*, did not stimulate *X. laevis* DNA topoisomerase I. A mixture of histones consisting primarily of core histones, which are less lysine-rich than histone H1, also failed to stimulate *X. laevis* DNA topoisomerase I activity (Figure 4). Polybrene and poly(L-lysine) both inhibited *X. laevis* DNA topoisomerase I catalytic activity. These experiments were repeated at least 3 times with similar results.

Comparison of X. laevis DNA Topoisomerase I Stimulation by Histone H1 and Casein Kinase II. The magnitude of stimulation of *X. laevis* DNA topoisomerase I catalytic activity by incubation with casein kinase II was much greater than the stimulation by histone H1 (Figure 5). Stimulation of *X. laevis* DNA topoisomerase I activity was maximal at 88.8 nM histone H1, which corresponds to one histone H1 molecule per 7100 bp DNA. Strong inhibition of DNA topoisomerase I activity was observed at 434 nM histone H1 (one histone H1 per 1470 bp DNA). Bovine casein kinase II stimulated *X. laevis* DNA topoisomerase I activity at concentrations of 521 nM (or higher, data not shown). Furthermore, at the optimal concentration for histone H1, stimulation was approximately 6-fold, whereas maximal stimulation by casein kinase II was more than 3-fold greater than the maximal histone H1 stimulation (i.e., more than 20-fold). It should be noted that the stimulation observed in Figure 5 for histone H1 and casein kinase II is greater than that shown in Figure 4 because the stimulation in Figure 4 exceeded the linear range of the 30 min DNA topoisomerase I assay.

Physical Association of X. laevis Casein Kinase II and X. laevis DNA Topoisomerase I. Physical association of rat liver casein kinase II and DNA topoisomerase I activities has recently been reported (Turman & Douvas, 1993). Since physical association between bovine casein kinase II and *X. laevis* DNA topoisomerase I may be weaker than associations between these enzymes from the same species, we first confirmed that endogenous *X. laevis* casein kinase II-like activity copurified with *X. laevis* DNA topoisomerase I activity through DEAE-cellulose, phosphocellulose, and hydroxylapatite column chromatography [data not shown, see Table 1 of Kaiserman *et al.* (1988)]. The copurifying *X. laevis* casein kinase II and *X. laevis* DNA topoisomerase I activities were only partially separated when sedimented in glycerol gradients (data not shown) or chromatographed on Sephacryl S300: overlapping activities were seen at *s* values or Stokes radii greater than exhibited by either enzyme alone. Partially purified (fraction IV, V, or VI) *X. laevis* DNA topoisomerase I was chromatographed on Sephacryl S300 with similar results: *X. laevis* DNA topoisomerase I

activity eluted as three overlapping peaks at 35 ± 2, 48 ± 3, and 63 ± 3 Å, whereas the endogenous copurifying casein kinase II-like activity eluted at 54 ± 3 and 63 ± 3 Å. When the pooled 63 Å peak was rechromatographed, DNA topoisomerase I activity was detected predominantly at 48 ± 3 Å, and casein kinase II-like activity at 54 ± 3 Å; less than 20% of the activities rechromatographed to 63 ± 3 Å. *X. laevis* DNA topoisomerase I and *X. laevis* casein kinase II-like activities also coimmunoprecipitated from fraction VI of Kaiserman *et al.* (1988) using a polyclonal antibody to *X. laevis* DNA topoisomerase I, HKTOP-I. The supernatant contained no detectable (<1%) DNA topoisomerase I activity and 42% of the copurifying casein kinase II activity. These results were repeated twice, and essentially similar results were also obtained with fractions IV and V (data not shown). Controls (preimmune serum or Sepharose 4 beads alone) contained essentially all of both the DNA topoisomerase I (98%, 96%) and casein kinase II (105%, 100%) activities. None of the casein kinase II activity remaining in the supernatant, and none of the endogenous casein kinase II activity separated from DNA topoisomerase I by chromatography on DNA–cellulose [step VII of Kaiserman *et al.* (1988)], was depleted by precipitation with HKTOP-I. This establishes that the *X. laevis* casein kinase II-like and DNA topoisomerase I activities are physically associated.

DISCUSSION

There appears to be general agreement that the catalytic activity of DNA topoisomerase I is modulated by phosphorylation/dephosphorylation (Durban *et al.*, 1983, 1985; Mills *et al.*, 1982; Kaiserman *et al.*, 1988; Pommier *et al.*, 1990). Dephosphorylation of rat liver DNA topoisomerase I by alkaline phosphatase resulted in the disappearance of phosphorylated variants of the enzyme and inactivation of the catalytic activity (Tournier *et al.*, 1992). In this study, *X. laevis* DNA topoisomerase I activity was greatly reduced by incubation with alkaline phosphatase (see Experimental Procedures). Nevertheless, residual catalytic activity was observed even after 5 h dephosphorylation (Figure 1, lane 1). This suggests that the apparently complete inactivation of DNA topoisomerase I by alkaline phosphatase reported previously (Kaiserman *et al.*, 1988; Pommier *et al.*, 1990) may have reflected the detection threshold of the DNA topoisomerase I assay used rather than complete inactivation of the enzyme. Consistent with this possibility, yeast DNA topoisomerase I cloned and expressed in bacterial cells is catalytically active, suggesting that eukaryotic DNA topoisomerase I does not absolutely require phosphorylation to be active (Bjornisti & Wang, 1987).

Using the conditions in Figure 1, we confirmed that both the 110 kDa and 62 kDa catalytic polypeptides of *X. laevis* DNA topoisomerase I were phosphorylated by bovine casein kinase II in the presence of ATP (data not shown), as shown previously using endogenous *X. laevis* casein kinase II-like activity [see Figure 4 in Kaiserman (1988)]. These data support the suggestion of Durban *et al.* (1983, 1985), Mills *et al.* (1982), and Kaiserman *et al.* (1988) that casein kinase II-like activities can modulate DNA topoisomerase I activity by phosphorylation. Catalytic activity of dephosphorylated DNA topoisomerase I can also be restored or enhanced by phosphorylation mediated by protein kinase C activities (Samuels *et al.*, 1989; Samuels & Shimizu, 1992; Pommier *et al.*, 1990).

The data presented in this study strongly suggest that, in addition to modulation of DNA topoisomerase I activity by phosphorylation/dephosphorylation, regulation of *X. laevis* DNA topoisomerase I activity can be mediated by physical association with casein kinase II. Bovine casein kinase II stimulated *X. laevis* DNA topoisomerase I activity more than 20-fold in the absence of ATP (Figure 5). Stimulation of DNA topoisomerase I catalytic activity was dose-dependent. Endogenous casein kinase II activity coimmunoprecipitated with DNA topoisomerase I activity from rat liver (Turman & Douvas, 1993) and *X. laevis* ovary (see Results), strongly suggesting physical association between these proteins.

Other basic proteins also stimulated *X. laevis* DNA topoisomerase I activity in the absence of ATP, but there were fundamental differences in the concentration dependence of the stimulation. Histone H1 stimulated *X. laevis* DNA topoisomerase I, but only moderately, and only at relatively low concentrations. Similar results were obtained with mixed HMG proteins. In contrast, histone H1 strongly inhibited DNA topoisomerase I activity at higher concentrations as reported previously (Richter & Kapitza, 1991; Bina-Stein & Singer, 1977). This is very different from the stimulation of DNA topoisomerase I activity by casein kinase II: the latter stimulation was much greater and was observed up to the highest concentrations of casein kinase tested.

The positively charged polyamines spermidine and spermine enhance the formation of stable noncovalent complexes between mammalian DNA topoisomerase II and DNA, and stimulate DNA topoisomerase activity (Pommier *et al.*, 1989). *X. laevis* DNA topoisomerase I activity was similarly stimulated by spermidine (Figure 4). Since the HMG proteins are approximately 20–25% lysine and histone H1 is 29% lysine (Sanders, 1977), it might be envisioned that positively charged residues stimulate DNA topoisomerase I activity. Moreover, the casein kinase II β subunit has a basic region in the carboxyl-terminal sequence (Hu & Rubin, 1991), and also might stimulate on the basis of charge. The cause of the stimulation must be more complex than this, however, since poly(L-lysine) strongly inhibited *X. laevis* DNA topoisomerase I activity. Moreover, when polybrene (hexadimethrine bromide), a positively charged polymer, is substituted in the reaction, it also strongly inhibited *X. laevis* DNA topoisomerase I activity. Thus, positively charged residues are not sufficient to stimulate *X. laevis* DNA topoisomerase I activity.

In this study, we confirmed, at least in part, the controversial claim of stimulation of mammalian DNA topoisomerase I activities by histone H1 and HMG 17 reported by Javaharian and Liu (1983). However, stimulation of *X. laevis* DNA topoisomerase I was observed only at very low protein:DNA ratios (maximal at less than 1 histone H1 to 7100 bp DNA, and still observed at 1 histone per 2800 bp DNA), and was lower than reported for the mammalian enzymes. Direct comparison of our study with the results of Javaherian and Liu cannot be carried out, however, since it was not possible to calculate the histone H1:DNA ratios used in their experiments. At high histone:DNA ratios, histones compete for binding sites on the DNA (Richter & Kapitza, 1991; Bina-Stein & Singer, 1977). At a histone H1 to DNA ratio of 1:1470 bp DNA, nearly complete inhibition of DNA topoisomerase I activity was observed in our study, consistent with reports of inhibition of mammalian DNA topoisomerase I activities by histone H1 at higher

histone:DNA ratios (Richter & Kapitza, 1991; Bina-Stein & Singer, 1977). The bimodal response of *X. laevis* DNA topoisomerase I to histone H1 may be due to the cooperativity of association of histone H1 with DNA or to changes in the structural conformation of the DNA (Bina-Stein & Singer, 1977), as well as the ability of histone H1 to interact directly by physical association with DNA topoisomerase I (Watanabe, 1986). In any event, the complex interactions of histone H1 with eukaryotic DNA topoisomerase I activities and DNA are clearly different than the interaction of casein kinase II with *X. laevis* DNA topoisomerase I.

Our laboratory has described an endogenous casein kinase II-like activity that copurified from extracts of ovaries with *X. laevis* DNA topoisomerase I (Kaiserman *et al.*, 1988). The major casein kinase II enzyme in *X. laevis* ovaries has subsequently been purified and characterized (Leiva *et al.*, 1987; Mullner-Lorillon *et al.*, 1988). Comparison of the properties of the purified *X. laevis* casein kinase II with our previously described endogenous casein kinase II-like activity (both are highly sensitive to heparin, phosphorylate casein and phosvitin, utilize both ATP and GTP as donor molecules, and autophosphorylate a 28 kDa presumptive β subunit), coupled with the demonstration that there is only one major protein phosphorylating protein with these properties found in *X. laevis* oocytes, makes it virtually certain that the endogenous activity we described previously was the *bona fide X. laevis* casein kinase II.

X. laevis casein kinase II was found within full grown oocytes at at least 2−6 nM concentrations (Mullner-Lorillon *et al.*, 1988). In our study, we showed that this concentration is sufficient to induce large changes in *X. laevis* DNA topoisomerase I activity in the absence of ATP (Figures 5 and 3). Moreover, since casein kinase II is a predominantly nuclear enzyme (Krek *et al.*, 1992), and most, if not all, of the endogenous casein kinase II activity in *X. laevis* oocytes is localized within the germinal vesicles (nuclei) (Leiva *et al.*, 1987), the effective concentration of casein kinase II in the nucleus should be 1−2 orders of magnitude higher. This concentration was shown to stimulate *X. laevis* DNA topoisomerase I by more than an order of magnitude in the absence of phosphorylation. *X. laevis* DNA topoisomerase I was found within full grown oocytes at about 30 nM concentrations, calculated from the purification of *X. laevis* DNA topoisomerase I by Kaiserman and colleagues (Kaiserman *et al.*, 1988). Since high levels of *X. laevis* DNA topoisomerase I were found in enucleated oocytes and in mitochondria (Kaiserman *et al.*, 1988), the nuclear concentration of *X. laevis* DNA topoisomerase I is likely to be somewhat lower than, but of the same order of magnitude as, the endogenous nuclear casein kinase II concentration.

The *X. laevis* casein kinase II enzyme inhibits progesterone-induced maturation and can participate in meiotic prophase arrest by phosphorylating Mp protein (Ozon *et al.*, 1987). In addition, casein kinase facilitates MPF-induced maturation, presumably because one of its substrates is involved in the MPF-associated phosphorylation cascade (Mullner-Lorillon *et al.*, 1988). These effects are presumably modulated by the phosphorylation events, and there seems little question that casein kinase II plays a major role in the phosphorylation of transcriptional factors and cell-cycle-dependent proteins (Meisner & Czech, 1991).

In addition to the direct role of the phosphorylation events, however, the results of this study raise the possibility that physical association of casein kinase II and DNA topoisomerase I may also play a critical role in regulating DNA topoisomerase I activity. Casein kinase II copurifies with, and was shown by coimmunoprecipitation with scleroderma autoimmune anti-DNA topoisomerase I antibodies to be physically associated with, DNA topoisomerase I from rat liver (Turman & Douvas, 1993). Copurification and coimmunoprecipitation of the two activities from *X. laevis* ovary were similarly observed (Kaiserman *et al.*, 1988; and this study). Both enzymes are found at the appropriate concentrations for stimulation by physical association to be physiologically relevant. Moreover, casein kinase II copurifies with DNA topoisomerase II from yeast (Cardenas *et al.*, 1993). It seems possible, therefore, that physical association of casein kinase II with potential substrate proteins may be a general phenomenon. Direct physical association rather than posttranslational modifications have recently been shown to result in stimulation of DNA polymerase α by poly(ADP-ribose)polymerase (Simbulan *et al.*, 1993).

Zhao and Benbow have recently identified and purified to homogeneity a *X. laevis* ovarian protein that inhibits *X. laevis* DNA topoisomerase I activity, apparently by also interacting directly with the enzyme (Zhao & Benbow, 1993). Taken together with the results of this study showing direct stimulation of *X. laevis* DNA topoisomerase I by physical association with casein kinase II, these findings suggest that direct physical association and the accompanying protein−protein interactions may play a larger role than previously envisioned in the regulation of DNA topoisomerase I activity. We speculate that physical association with proteins not only plays a crucial role in down-regulating (inhibitor) or up-regulating (casein kinase II) DNA topoisomerase I activity, but also facilitates subsequent modulation of DNA topoisomerase I activity by ensuring that casein kinase II and its substrate protein remain in close proximity during the cell cycle.

ACKNOWLEDGMENT

We are grateful to H. B. Kaiserman and E. H. A. Poll for assistance during this work. We thank Nick Marini, Charisse Buising, and members of the DNA Replication Society for thoughtful comments and discussions. We thank Jiyong Zhao and, especially, Drena L. Dobbs for critical reading of the manuscript.

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