Published in final edited form as:

Protein Expr Purif. 2011 June ; 77(2): 214–219. doi:10.1016/j.pep.2011.02.002.

A Rapid Procedure to Purify *E. coli* DNA Topoisomerase I

Xiaozhou Xu and Fenfei Leng*

Department of Chemistry & Biochemistry, Florida International University, 11200 SW 8th Street, Miami, FL 33199

Abstract

On the basis of the asymmetrical charge distribution of *E. coli* DNA topoisomerase I, we developed a new procedure to purify *E. coli* DNA topoisomerase I in the milligram range. The new procedure includes using both cation- and anion-exchange columns, i.e., SP-Sepharose FF and Q-Sepharose FF columns. The *E. coli* DNA topoisomerase I purified here is free of DNase contamination. The kinetic constants of the DNA relaxation reaction of *E. coli* DNA topoisomerase I were also determined.

Keywords

E. coli DNA topoisomerase I; DNA relaxation; Q-Sepharose FF column; SP-Sepharose FF column

Introduction

DNA topoisomerases are essential enzymes of all biological organisms [1,2]. They specifically change DNA topology by cleaving and religating one or both strands of the DNA double helix to facilitate essential DNA transactions, such as DNA replication, recombination and transcription [1,3,4]. *E. coli* has four DNA topoisomerases: topoisomerase I, gyrase, topoisomerase III, and topoisomerase IV [1,2]. The DNA supercoiling level in *E. coli* is regulated through the actions of these four DNA topoisomerases [5,6].

E. coli DNA topoisomerase I (the ω protein) was discovered and purified by James C. Wang about 30 years ago [7]. It has 865 amino acid residues and contains three domains, i.e., the N-terminal transesterification domain, the Zn(II) domain, and the C-terminal DNA-binding domain [2]. The active tyrosine residue (Tyr319) is located in the N-terminal transesterification domain. Recently, accumulating evidence showed that bacterial DNA topoisomerase I is a potential target for the discovery of antibacterial compounds [8,9]. Historically, *E. coli* DNA topoisomerase I has been over-expressed and purified in a few different laboratories [7,10–13] and the purification procedure includes passing through a few columns, such as an anion-exchange column (DEAE-cellulose), a cation-exchange

© 2011 Elsevier Inc. All rights reserved.

*Corresponding Author: Fenfei Leng, Department of Chemistry & Biochemistry, Florida International University, 11200 SW 8th Street, FL 33199, Tel: 305-348-3277, Fax: 305-348-3772, lengf@fiu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Supplementary materials

Supplementary materials associated with this article can be found at Protein Expression and Purification Web page. The supplementary data comprises three Figures.

column (phosphocellulose), and a ssDNA agarose column. These procedures usually are quite time consuming and sometimes cannot completely remove the contamination of different nucleases (our observation). In this study, we found that *E. coli* DNA topoisomerase I binds to both cation and anion exchange columns at different experimental conditions. Using a combination of a strong cation-exchange column, i.e., SP-Sepharose FF column, and an anion-exchange column, i.e., Q-Sepharose FF column, we were able to purify 50 to 100 mg of *E. coli* DNA topoisomerase I without the contamination of DNases from 4 liters of *E. coli* cell culture over-expressing recombinant DNA topoisomerase I. We also determined the Michaelis-Menten kinetic constants of *E. coli* DNA topoisomerase I.

MATERIALS AND METHODS

Materials

Q-Sepharose FF, SP-Sepharose FF were purchased from GE Healthcare (Piscataway, NJ). Isopropyl-beta-D-thiogalactopyranoside (IPTG), dithiothreitol (DTT), phenylmethylsulfonyl fluoride (PMSF), Brij-58, Polymyxin P, and lysozyme were bought from Sigma-Aldrich Company (St. Louis, MO). Bradford reagent was a product of Bio-Rad (Hercules, CA). Bovine serum albumin (BSA) was obtained from New England Biolabs (Beverly, MA).

Plasmids

Plasmid pUNB was constructed by inserting the annealing product of oligomers 5'-CCGCTGAGGAG-3' and 5'-GGCCTCCTCAGCGGACGT-3', which contains a nicking restriction enzyme Nb. BbvCI site and a BglII site, into the AatII and Eco109 sites of pUC18. Plasmid pXXZ06 was created by the insertion of a 1870 bp BamHI-HindIII fragment of pLUC3, which contains the kanamycin resistant gene, into the BamHI-HindIII sites of pUNB. *E. coli* strain RLM1180, i.e., BL21(DE3)/pLysS containing plasmid pTI-1 to express *E. coli* DNA topoisomerase I [13] was kindly provided by Dr. R. McMacken (Johns Hopkins University).

E. coli DNA topoisomerase I purification

E. coli strain RLM180/pTI-1 was grown in LB medium containing 50 µg/ml of ampicillin overnight at 37 °C. The overnight cell culture was then transferred to 4 liters of terrific broth at a ratio of 1:100 (v/v). The cell growth at 37 °C was monitored at OD₅₉₅ using a Cary 50 Bio UV-Vis spectrometer. When the OD₅₉₅ reached to ~0.6, IPTG was added to 1 mM to induce the expression of *E. coli* DNA topoisomerase I. Cells were harvested after additional 3-hour incubation with centrifugation at 4,000 rpm at 4 °C for 30 min. The supernatant was discarded and the cells (~32 grams of wet weight cells) were resuspended in 32 ml of a cold Tris-HCl buffer (50 mM Tris-HCl, pH 8.0, 0.4 M KCl, 1 mM DTT and 1 mM PMSF). Then lysozyme was added to a final concentration of 1 mg/ml. The cells in the cold Tris-HCl buffer were incubated on ice for at least 1 hour, and then frozen in liquid nitrogen, and stored in -80 °C freezer. On the following day, the *E. coli* cells were thawed at room temperature and frozen in liquid nitrogen. After three freezing and thawing cycles, the solution was sonicated on ice 6 times at 30 W with 2 min interval between each sonication. Brij-58 was added to the sonicated solution to achieve a final concentration of 0.5% (w/v). After incubation at 4 °C for 15 min, the solution was centrifuged at 15,000 rpm at 4 °C for 30 min. 10% Polymyxin P (pH 7.5) was then added to the supernatant to reach a final concentration of 0.7% [14]. The sample was centrifuged again at 15,000 rpm at 4°C for 10 min and the pellet was discarded. In the next step, solid (NH₄)₂SO₄ was added to the supernatant to 30% and 70% saturation in steps and the protein samples were centrifuged at 12,000 rpm at 4°C for 25 min for each step. The pellet precipitated from 70% (NH₄)₂SO₄ saturation step was re-dissolved in Buffer I (20 mM potassium phosphate, pH 7.4, 10 % w/v glycerol, 1 mM EDTA) with the addition of freshly prepared DTT and PMSF to a final

concentration of 1 mM and dialyzed twice against 2 liters of Buffer I plus 0.1 M KCl at 4°C. After the dialysis, the protein solution was loaded onto a 30-ml SP-Sepharose FF cation-exchange column pre-equilibrated with Buffer I plus 0.1M KCl. The loaded column was washed with 150 ml Buffer I plus 0.2 M KCl. *E. coli* DNA topoisomerase I was then eluted with a 300 ml KCl gradient of 0.2– 0.5 M KCl in Buffer I. The DNA relaxation activity assays were used to monitor the activities of *E. coli* DNA topoisomerase I. Fractions with high DNA relaxation activities and low DNase activities were pooled and dialyzed against 2 liters of Buffer I plus 20 mM KCl twice at 4 °C. The dialyzed protein solution was loaded onto a 40-ml Q-Sepharose FF anion-exchange column pre-equilibrated with Buffer I plus 20 mM KCl. The loaded column was washed with 40 ml of Buffer I plus 20 mM KCl. *E. coli* DNA topoisomerase I was eluted with a 300 ml KCl gradient of 20– 300 mM KCl in Buffer I. The DNA relaxation activity and DNase activity assays were used to monitor the quality of the *E. coli* DNA topoisomerase I. The concentration of *E. coli* topoisomerase I was determined by using a molar extinction coefficient of 98,210 M⁻¹cm⁻¹ at 280 nm [15].

The DNA relaxation activity assay

The DNA relaxation activity assay was used to monitor the activities of *E. coli* DNA topoisomerase I during purification. Briefly, in a 25 µl of reaction mixture that contains 20 mM Tris-acetate, pH 7.9, 50 mM KOAc, 6 mM Mg(OAc)₂, 1 mM DTT, and 0.25 µg/µl BSA, 0.5 or 1 µg of supercoiled plasmid DNA template pXXZ06 was relaxed by using different amount of *E. coli* DNA topoisomerase I at 37 °C for 15 min. The reaction was then stopped by phenol extraction. The topological state of each DNA sample was analyzed by electrophoresis in 1×TAE buffer, pH 7.8, in 1% agarose gels. One unit of *E. coli* DNA topoisomerase I was defined as the amount of enzyme that catalyzes the relaxation of 0.5 µg of supercoiled plasmid DNA template pXXZ06 in 25 µl at 37 °C for 15 min.

The nonspecific DNase activity assay

The nonspecific DNase activity assays were used to monitor the quality of *E. coli* DNA topoisomerase I. In this assay, 1 µg of the supercoiled plasmid DNA template pXXZ06 was incubated with 1 µg of *E. coli* DNA topoisomerase I in 25 µl of Tris-acetate buffer containing 20 mM Tris-acetate, pH 7.9, 50 mM KOAc, 6 mM Mg(OAc)₂, 1 mM DTT, and 0.25 µg/ml BSA for 2 hours at 37 °C. The reaction was then stopped by phenol extraction. The DNA samples were analyzed by electrophoresis in 1×TAE buffer, pH 7.8, in 1% agarose gels in the presence or absence of 1 µg/ml of chloroquine.

The determination of the initial DNA relaxation velocity of *E. coli* DNA topoisomerase I

To determine the initial DNA relaxation velocity of *E. coli* DNA topoisomerase I, different concentrations of the negatively supercoiled plasmid DNA template pXXZ06 were relaxed with 3.5 units of *E. coli* DNA topoisomerase I (8 nM) in 200 µl of the reaction mixture containing 20 mM Tris-acetate, pH 7.9, 50 mM KOAc, 6 mM Mg(OAc)₂, 1 mM DTT, and 0.1 mg/ml BSA. The reactions were stopped by extraction with an equal volume of phenol. The topological state of each DNA sample was determined by electrophoresis in 1×TAE buffer, pH 7.8, in 1% agarose gels and analyzed by using KODAK 1D Image Analysis Software. The initial DNA relaxation velocities (V_0) of *E. coli* DNA topoisomerase I were calculated by linear fitting the following equation:

$$V_0 = [\text{scDNA}] \frac{1 - r}{t}$$

where [scDNA] is the initial concentration of supercoiled plasmid DNA template pXXZ06, r is the ratio of the area of the supercoiled DNA band at time zero to the area of the

supercoiled DNA band at time t . The steady state kinetic constants K_M , V_{max} , and k_{cat} were determined by the analysis of the Michaelis-Menten equation using the double-reciprocal or Lineweaver-Burk plot.

Results and Discussion

Purification of *E. coli* DNA topoisomerase I

Fig. S1 shows the amino acid sequence of the *E. coli* DNA topoisomerase I. It contains 132 positively charged amino acid residues, i.e. Lys and Arg and 122 negatively charged residues, i.e., Glu and Asp. After a careful analysis, we found that *E. coli* DNA topoisomerase I has a region (residues 524 to 598, the region between the the N-terminal transesterification domain and the Zn(II) domain) that is highly negatively charged (18 negative charged amino acids versus 6 positively charged amino acids). We therefore reasoned that *E. coli* DNA topoisomerase I should bind to the anion-exchange column, i.e., Q-Sepharose FF ion-exchange column at low salt buffer conditions. Indeed, our results in this article showed that *E. coli* DNA topoisomerase I bound to both cation- and anion-exchange columns, such as SP-Sepharose FF and Q-Sepharose FF columns. The Q-Sepharose FF column is particular important because it removed most of the DNase activities in the purification procedure (see below for details). The basic purification steps include cell lysis, nucleic acid removal by Polymin P precipitation, $(NH_4)_2SO_4$ precipitation, SP-Sepharose FF, and Q-Sepharose FF ion-exchange chromatography. The purification can be completed in five days and produces 50 to 100 mg of *E. coli* DNA topoisomerase I from 4 liters of cell culture (~2 mg per g wet weight cell pellet).

(i) Cell growth and lysis. Here, we used BL21(DE3)/pLysS/pTI-1 for over-expressing the *E. coli* DNA topoisomerase I with IPTG induction. The addition of 1 mM of IPTG did not significantly affect the cell growth (data not shown). After additional 3-hour incubation, the cells were harvested by centrifugation at 4 °C. In this procedure, we utilized a combination of lysozyme digestion of the cell wall and the freezing-thawing cycle to break the cell membrane. After the freezing-thawing cycles, the cell lysate became very viscous and sonication was used to reduce viscosity. Cell debris were then removed by centrifugation at 15,000 rpm at 4 °C. This relatively mild lysis procedure ensured that we obtained highly active *E. coli* DNA topoisomerase I. Polymin P was added to remove nucleic acids and the ammonium sulfate precipitation steps were used to remove unwanted proteins and concentrate *E. coli* DNA topoisomerase I for the following step.

(ii) SP-Sepharose FF chromatography. After dialysis with Buffer I plus 0.1 M KCl, the protein sample was loaded onto a 30-ml SP-Sepharose FF column, a strong cation-exchange column. The column was equilibrated with the dialysis buffer to ensure an identical ionic environment. After loading the sample, we washed the column using 2 to 3 column volumes of buffer I plus 0.2 M KCl. From the SDS-PAGE gels, we found that a large portion of cellular proteins with none or very weak interaction with SP-Sepharose resin were removed by the washing buffer. No detectable *E. coli* DNA topoisomerase I was eluted at this washing step. *E. coli* DNA topoisomerase I was then eluted with a 0.2–0.5 M KCl gradient. Fig. 1A shows the elution profile where *E. coli* DNA topoisomerase I was eluted in buffer I from 275 to 375 mM of KCl. After this SP-Sepharose FF column, we performed DNA relaxation assays for each fraction as described under Materials and Methods using 1 µg of *E. coli* DNA topoisomerase I (~412 nM). Our results are shown in Fig. 2A. Although we incubated our DNA samples for only 15 minutes, we still observed significant nonspecific DNase activities for all fractions of *E. coli* DNA topoisomerase I, especially those fractions eluted with high salt concentrations (fractions 46 and up, Fig. 2A). These results suggest that *E. coli* DNA topoisomerase I after this column cannot be used for certain experiments especially for those biophysical assays requiring a large amount of the enzyme. Regardless,

when we reduced the enzyme concentration to about 5 to 20 nM, we did not observe much nonspecific DNase activities, which allow us to determine the specific activities of *E. coli* DNA topoisomerase I purified at this stage (Fig. 2B). Because of the nonspecific DNase activities, we decided to pool those fractions with less nonspecific DNase activities, i.e., fractions 25 to 42 (from elution volume 70 to 125 ml and the total volume is about 55 ml) for the next step. In this case, about 70 mg of *E. coli* DNA topoisomerase I (~50%) were discarded. This is the reason why we lost ~50% of the *E. coli* DNA topoisomerase I after the Q-Sepharose FF chromatography (Table 1).

(iii) Q-Sepharose FF chromatography. The pooled fractions from SP-Sepharose FF column were dialyzed against Buffer I plus 20 mM KCl. The dialyzed protein sample was then loaded onto a 40-ml Q-Sepharose FF column equilibrated with Buffer I plus 20 mM KCl and washed with 40 ml of the same buffer. *E. coli* DNA topoisomerase I was not detectable at this washing step, indicating that *E. coli* DNA topoisomerase I indeed bound to the Q-Sepharose FF column at the low salt concentration. The *E. coli* DNA topoisomerase I was eluted in Buffer I plus a 20 to 300 mM KCl gradient. Fig. 1B shows the elution profile where *E. coli* DNA topoisomerase I was eluted in Buffer I from 50–100 mM KCl. We also performed a DNA relaxation assay and a nonspecific DNase activity assay for all fractions purified with the Q-Sepharose FF column. For the 15-min DNA relaxation assay, the enzyme from all fractions relaxed the supercoiled DNA sample and no DNase activities were detected (Fig. 3A). For the nonspecific DNase activity assays where the incubation time was 2 hours, we observed some nonspecific DNase activities for the early fraction eluted with very low salt concentrations, i.e., fractions 22 to 27 (data not shown). For most fractions, we did not observe nonspecific DNase activities (Fig. 3B), indicating that the Q-Sepharose FF column was able to remove most nonspecific DNase activities from the *E. coli* DNA topoisomerase I samples. The *E. coli* DNA topoisomerase I samples are particularly useful for biochemical and biophysical assays that require a large amount of the enzyme and/or incubate for a long period of time. We also determined the specific activities of the enzyme after the Q-Sepharose FF column (Fig. 3C). After the Q-Sepharose FF column, *E. coli* DNA topoisomerase I is essentially pure as judged from SDS-PAGE gels. Fig. 4 is a 10% SDS-PAGE gel of the protein samples at different stages of the purification procedure. Fig. S2 and Table 1 summarize the purification procedure of *E. coli* DNA topoisomerase I.

The determination of $V_{\max}$ and K_M of the *E. coli* DNA topoisomerase I for the relaxation of the supercoiled plasmid DNA template pXXZ06

Previous studies from Tse-Dinh's laboratory demonstrated that Mg^{2+} is required for the relaxation of the negatively supercoiled DNA templates and the optimal Mg^{2+} concentration was determined to be ~ 6 mM [16]. In this study, we also performed a similar study and our results are shown in Fig. S3. Our results clearly demonstrated that 6 mM of Mg^{2+} is the optimal concentration for the relaxation activities of *E. coli* DNA topoisomerase I (compare lanes 1 to 8 of Fig. S3). Next, we carried out a time course for the relaxation of different concentrations of negatively supercoiled plasmid pXXZ06 by using 3.5 units of *E. coli* DNA topoisomerase I. Our results are shown in Fig. 5. The velocities for these experiments are linear for the first two minutes. In this case, we may use them to calculate the initial velocities for the relaxation of the negatively supercoiled plasmid pXXZ06 (Fig. 6A). These kinetic analysis results are displayed in a double-reciprocal or Lineweaver-Burk plot (Fig. 6B). The K_M and $V_{\max}$ were determined to be 7.52 ± 2.17 nM and 7.98 ± 0.95 nM/min, respectively (Table 2). The turnover number (k_{cat}) was calculated to be 1.20 ± 0.47 per min. These kinetic parameters can serve as an initial point for a comprehensive kinetic study and for drug discovery.

Conclusion

In this paper, we developed a rapid procedure to purify *E. coli* DNA topoisomerase I on a large scale for biochemical and biophysical studies that require a large amount of the enzyme, such as isothermal titration calorimetry. Our purification procedure is based on our analysis in which we found that the positive and negative charges are asymmetrical distributed over the primary structure of the enzyme. In this case, the enzyme binds to both cation- and anion-exchange columns. Using a combination of the SP-Sepharose FF and Q-Sepharose FF columns, we can purify more than 50 mg of *E. coli* DNA topoisomerase I in five days from 4 liters of *E. coli* cell culture. More importantly, this preparation of *E. coli* DNA topoisomerase I is free of nonspecific DNase and nucleic acids contamination. We also performed a preliminary kinetic analysis of *E. coli* DNA topoisomerase I, which may be useful for drug discovery studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Dr. Roger McMacken at Johns Hopkins University for providing *E. coli* strain RLM1180, i.e., BL21 (DE3)/pLysS containing plasmid pTI-1 to express *E. coli* DNA topoisomerase I. We also thank members in our laboratory for critically reading the manuscript before submission. This work was supported by National Institutes of Health Grant 1SC1HD063059-01A1 (to FL).

References

1. Wang JC. DNA topoisomerases. Annu. Rev. Biochem. 1996; 65:635–692. [PubMed: 8811192]
2. Champoux JJ. DNA topoisomerases: structure, function, and mechanism. Annu. Rev. Biochem. 2001; 70:369–413. [PubMed: 11395412]
3. Bates, AD.; Maxwell, A. DNA Topology. Oxford, UK: Oxford University Press; 2005.
4. Wang, James C. Untangling the Double Helix: DNA Entanglement and the Action of the DNA Topoisomerases. Cold Spring Harbor Laboratory Press; 2009.
5. Pruss GJ, Drlica K. DNA supercoiling and prokaryotic transcription. Cell. 1989; 56:521–523. [PubMed: 2645054]
6. Zechiedrich EL, Khodursky AB, Bachellier S, Schneider R, Chen D, Lilley DM, Cozzarelli NR. Roles of topoisomerases in maintaining steady-state DNA supercoiling in *Escherichia coli*. J. Biol. Chem. 2000; 275:8103–8113. [PubMed: 10713132]
7. Wang JC. Interaction between DNA and an *Escherichia coli* protein omega. J. Mol. Biol. 1971; 55:523–533. [PubMed: 4927945]
8. Tse-Dinh YC. Exploring DNA topoisomerases as targets of novel therapeutic agents in the treatment of infectious diseases. Infect. Disord. Drug Targets. 2007; 7:3–9. [PubMed: 17346206]
9. Tse-Dinh YC. Bacterial topoisomerase I as a target for discovery of antibacterial compounds. Nucleic Acids Res. 2009; 37:731–737. [PubMed: 19042977]
10. Depew RE, Liu LF, Wang JC. Interaction between DNA and *Escherichia coli* protein omega. Formation of a complex between single-stranded DNA and omega protein. J. Biol. Chem. 1978; 253:511–518. [PubMed: 338610]
11. Bhaduri T, Bagui TK, Sikder D, Nagaraja V. DNA topoisomerase I from *Mycobacterium smegmatis*. An enzyme with distinct features. J. Biol. Chem. 1998; 273:13925–13932. [PubMed: 9593741]
12. Zhu CX, Tse-Dinh YC. Overexpression and purification of bacterial DNA topoisomerase I. Methods Mol. Biol. 1999; 94:145–151. [PubMed: 12844870]

13. Hiasa H, DiGate RJ, Marians KJ. Decatenating activity of *Escherichia coli* DNA gyrase and topoisomerases I and III during oriC and pBR322 DNA replication in vitro. *J. Biol. Chem.* 1994; 269:2093–2099. [PubMed: 8294462]
14. Burgess RR. Use of polyethyleneimine in purification of DNA-binding proteins. *Methods Enzymol.* 1991; 208:3–10. [PubMed: 1779840]
15. Gill SC, von Hippel PH. Calculation of protein extinction coefficients from amino acid sequence data. *Anal. Biochem.* 1989; 182:319–326. [PubMed: 2610349]
16. Zhu CX, Roche CJ, Tse-Dinh YC. Effect of Mg(II) binding on the structure and activity of *Escherichia coli* DNA topoisomerase I. *J. Biol. Chem.* 1997; 272:16206–16210. [PubMed: 9195920]

Fig. 1.

(A) Cation-exchange chromatography of *E. coli* DNA topoisomerase I on SP-Sepharose FF column. (B) Anion-exchange chromatography of *E. coli* DNA topoisomerase I on Q-Sepharose FF column.

Fig. 2.

(A) The DNA relaxation activity assays of *E. coli* DNA topoisomerase I from SP-Sepharose FF column as described under Materials and Methods. Briefly, 1 μ g of supercoiled plasmid DNA pXXZ06 was relaxed by 1 μ g of *E. coli* DNA topoisomerase I (~412 nM) from different fractions of the SP-Sepharose FF column at 37 °C for 15 min. Lane 1–11 are DNA samples isolated from the DNA relaxation activity assays by using the *E. coli* DNA topoisomerase I samples of fractions 11, 16, 21, 26, 30, 34, 38, 42, 46, 50, and 54, respectively. Lane 12 is the negatively supercoiled plasmid pXXZ06. **(B)** The specific DNA relaxation activity assays of *E. coli* DNA topoisomerase I from the SP-Sepharose FF column as described under Materials and Methods. In these assays, 0.5 μ g of supercoiled plasmid DNA pXXZ06 was relaxed by using different concentrations of *E. coli* DNA topoisomerase I. Lane 1 to 7 represent the DNA samples isolated from the DNA specific relaxation activity assays of *E. coli* DNA topoisomerase I by using 0, 5, 10, 12.5, 15, 17.5, and 20 nM of *E. coli* DNA topoisomerase I, respectively. Symbols R and S represent the relaxed and supercoiled DNA, respectively.

Fig. 3.

(A) The DNA relaxation activity assays of *E. coli* DNA topoisomerase I from the Q-Sepharose FF column as described under Materials and Methods. Briefly, 1 μ g of supercoiled plasmid DNA pXXZ06 was relaxed by 1 μ g of *E. coli* DNA topoisomerase I (412 nM) from different fractions of the Q-Sepharose FF column at 37 °C for 15 min. Lanes 1–10 are DNA samples isolated from the DNA relaxation activity assays by using the *E. coli* DNA topoisomerase I samples of fractions 24, 26, 28, 30, 32, 34, 36, 38, and 40, respectively. Lane 11 is the DNA sample isolated from the DNA relaxation assay by using the protein sample of the pass-through fraction when loading the Q-Sepharose FF column; Lane 12 is the negatively supercoiled plasmid pXXZ06. **(B)** The nonspecific DNase activity assays of *E. coli* DNA topoisomerase I from the Q-Sepharose FF column as described under Materials and Methods. Here, 1 μ g of supercoiled plasmid DNA pXXZ06 was relaxed by 1 μ g of *E. coli* DNA topoisomerase I (~412 nM) from different fractions of the Q-Sepharose FF column at 37 °C for 2 hrs. Lanes 1–10 are DNA samples isolated from the DNA relaxation activity assays by using the *E. coli* DNA topoisomerase I samples of fractions 24, 26, 28, 30, 32, 34, 36, 38, and 40, respectively. Lane 11 is the DNA sample isolated from the DNA relaxation assay by using the protein sample of the pass-through fraction when loading the Q-Sepharose FF column; Lane 12 is the negatively supercoiled plasmid pXXZ06. **(C)** The specific DNA relaxation activity assays of *E. coli* DNA topoisomerase I from Q-Sepharose FF column as described under Materials and Methods. Briefly, 0.5 μ g of supercoiled plasmid DNA pXXZ06 was relaxed by using different concentrations of *E. coli* DNA topoisomerase I. Lane 1 to 8 represent the DNA samples isolated from the DNA specific relaxation activity assays of *E. coli* DNA topoisomerase I by using 0, 10, 12.5, 15, 17.5, 20, and 22.5 nM of *E. coli* DNA topoisomerase I, respectively. Symbols R and S represent the relaxed and supercoiled DNA, respectively.

Fig. 4. 10% SDS-PAGE of protein samples from different purification stages. Lane 1. LMW-SDS Marker (Amersham Biosciences). Lane 2. Cell extracts prepared from BL21/DE3/pLysS/pTI-1 in the absence of IPTG. Lane 3. Cells extracts prepared from BL21/DE3/pLysS/pTI-1 after 3-hour induction with 1mM IPTG. Lane 4. The protein sample after the Bij-58 treatment. Lane 5. The protein sample after Polymyxin P precipitation to remove nucleic acids. Lane 6. The protein sample after $(\text{NH}_4)_2\text{SO}_4$ precipitation before dialysis. Lane 7. The protein sample prior to loading onto SP-Sepharose FF column after dialysis. Lane 8. The *E. coli* DNA topoisomerase I sample prior to loading onto Q-Sepharose FF column. Lane 9. *E. coli* DNA topoisomerase I after elution from Q-Sepharose FF column.

Fig. 5. Time courses of the relaxation of the negatively supercoiled plasmid pXXZ06 by *E. coli* DNA topoisomerase I. The DNA relaxation assays using 3.5 units of *E. coli* DNA topoisomerase I and different concentrations of pXXZ06 (6.1 nM (**A**), 9.2 nM (**B**), 12.2 nM (**C**), and 18.3 nM (**D**)) in 20 mM Tris-acetate, pH 7.9, 50 mM KOAc, 6 mM Mg(OAc)₂, 1 mM DTT, and 0.1 mg/ml BSA were described under Materials and Methods. Lanes 1–7 contained, respectively, DNA samples isolated after incubation at 37 °C for 0, 0.5, 1, 2, 5, 15 min. DNA topoisomers were resolved by electrophoresis in 1% agarose gels. Symbols R and S represent the relaxed and supercoiled DNA, respectively.

Fig. 6. (A) The quantification of the time courses of the relaxation of the negatively supercoiled plasmid pXXZ06 by *E. coli* DNA topoisomerase I. The method of quantification was described under Materials and Methods. The data shown are obtained from the analysis of Fig. 5A. (B) The Lineweaver-Burk analysis of the kinetics of the relaxation of the negatively supercoiled plasmid pXXZ06 as described in Figure 5. Assay condition is described under Materials and Methods.

Table 1Summary of *E. coli* topoisomerase I purification

Steps	Total protein^a (mg)	Target protein^b (mg)	Total activities^c (units)	Specific activities^d (units)
Homogenate after Brij-58 precipitation	1,470	440	N/A	N/A
Polymin P Precipitation	1,170	410	N/A	N/A
(NH ₄) ₂ SO ₄ precipitation	686	274	N/A	N/A
SP-Sepharose FF column	155	155	4,560,000	29,400
Q-Sepharose FF column	68	68	2,150,000	31,700

^a Total amount of protein at different purification stages was calculated according to the Bradford assays by using BSA as a protein standard.

^b The amount of target protein was estimated according to the SDS-PAGE gel shown in Fig. 4.

^c Total activities represent the total DNA relaxation activities of *E. coli* DNA topoisomerase I calculated as described under Materials and Methods.

^d Specific activities represents Specific relaxation activity of *E. coli* DNA topoisomerase I.

Table 2Kinetic parameters of *E. coli* DNA topoisomerase I

K_M	V_{max}	k_{cat}
(nM)	(nM/min)	(min ⁻¹)
7.52±2.17	7.98±0.95	1.20±0.47