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Inhibition of *Escherichia coli* DNA topoisomerase I activity by phospholipids

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The DNA relaxation activity of *Escherichia coli* DNA topoisomerase I *in vitro* was greatly inhibited by cardiolipin. Inhibition also occurred to some extent with phosphatidylglycerol from egg yolk. Analysis with synthetic phospholipid revealed that phosphatidylglycerol containing unsaturated fatty acids exhibited a strong inhibitory effect, whereas inhibition by phosphatidylglycerol containing saturated fatty acids was weak. Phosphatidylethanolamine showed no inhibitory effect. Chlorpromazine, which interacts with phospholipids, suppressed the inhibitory effect of cardiolipin. Cardiolipin and phosphatidylglycerol with unsaturated fatty acid precipitated topoisomerase I even at low concentrations, whereas phosphatidylglycerol from egg yolk and a synthetic phosphatidylglycerol containing saturated fatty acids precipitated this enzyme only at high concentrations. One-third of the total topoisomerase I in *E. coli* was found in the membrane fraction. Treatment of *E. coli* cells with chlorpromazine resulted in relaxation of plasmid DNA. This DNA relaxation was not observed in a *topA* mutant, suggesting that this relaxation by chlorpromazine *in vivo* is catalysed by topoisomerase I.

INTRODUCTION

DNA topology is an important factor in regulating transcription, replication and recombination [1–4]. *Escherichia coli* cells possess two major topoisomerases, which are responsible for regulation of DNA topology: topoisomerase I, encoded by the *topA* gene, and DNA gyrase, encoded by the *gyrA* and *gyrB* genes [1,2]. Various factors, such as temperature [5], aerobiosis and growth phase [6] and osmolarity [7], affect DNA topology in cells, but the mechanisms involved remain to be elucidated. Previously, the activity of topoisomerase I from mouse cells was shown to be inhibited by acidic phospholipids, which are usual constituents of biological membranes [8,9]. This finding suggested to us that DNA topology might be regulated *in vivo* by the interaction of topoisomerase I with membranes.

Psychotropic drugs and local anaesthetics interact with phospholipids in biological membranes and affect various biological processes [10,11]. They also counteract the actions of phospholipids on some enzymes [12–14]. In the present study we examined the effects of phospholipids on the activity of purified *E. coli* topoisomerase I. The results showed that cardiolipin (CL; diphosphatidylglycerol) and phosphatidylglycerol (PG) inhibited the activity of this enzyme, and that chlorpromazine (CPZ), a psychotropic drug, suppressed the inhibition. We also showed that CPZ caused the topoisomerase I-dependent relaxation of plasmid DNA in *E. coli* cells.

MATERIALS AND METHODS

Chemicals

CPZ hydrochloride was provided by Shionogi Co., Osaka, Japan. CL (bovine heart), PG (an enzymic digestion product of egg lecithin), phosphatidylethanolamine (PE; from *E. coli*), and synthetic PG ($C_{18:0}$ and $C_{18:2}$ fatty acids) were purchased from Lipid Products, Redhill, Surrey, U.K., and Avanti, Pelham, AL, U.S.A. Liposomes were prepared from dried phospholipids on the bottom of glass tubes by vigorous vortex mixing in water. PE was suspended in 0.05% Triton X-100. The amount of phosphorus in the phospholipid fraction was determined by the method of Chen *et al.* [15], and the concentration of phospholipids was calculated. Phospholipid liposomes were stocked at −20 °C in the tube filled with N_2.

Assay of topoisomerase I

The standard reaction mixture (50 µl) contained 50 mM-Tris/HCl, pH 7.9 at 25 °C, 50 mM-NaCl, 10% (v/v) glycerol, BSA (50 µg/ml), 0.5 µg of form I DNA pUC118 and 20 ng of purified topoisomerase I. The reaction was carried out at 37 °C for 10 min. Then samples were subjected to 1%-(w/v)-agarose-gel electrophoresis in TAE buffer (100 mM-Tris/acetate/2 mM-EDTA), gels were stained with ethidium bromide and photographs were taken in an apparatus from Polaroid.

Purification of topoisomerase I

E. coli topoisomerase I was purified by the method of Wang (J. C. Wang, personal communication; details are available from Dr. Wang on request). Briefly, *E. coli* W3110 was transfected with plasmid pJW312-Sal, which contains the *topA* gene under the control of the *lac* promoter. This plasmid differs from pJW312 described in Zumstein & Wang [16] only by an inconsequential change of a *Bgl*II site upstream of the *lac* promoter to a *Sal*I site by linker insertion. Topoisomerase I was purified from a cell lysate by $(NH_4)_2SO_4$ fractionation and phosphocellulose column chromatography. The purity of the final fraction was greater than 80% as judged by PAGE.

Analysis of binding of topoisomerase I to phospholipid vesicles

Purified topoisomerase I (0.1 µg) was incubated with phospholipids (10–333 nmol) at 25 °C for 5 min in a mixture (300 µl) containing 50 mM-Tris/HCl (pH 7.9 at 25 °C), 50 mM-NaCl and BSA (50 µg/ml). Samples were centrifuged at 89000 *g* in a Beckman TL100.2 rotor for 30 min, and the precipitates were dissolved in SDS buffer consisting of 2% SDS, 2% 2-mercaptoethanol, 50 mM-Tris/HCl (pH 7.9 at 25 °C) and Bromophenol Blue. The samples were then heated at 95 °C for 3 min, and subjected to SDS/PAGE. Gels were stained with

Abbreviations used: CL, cardiolipin (diphosphatidylglycerol); PG, phosphatidylglycerol; PE, phosphatidylethanolamine; CPZ, chlorpromazine; LB, Luria–Bertani.

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Coomassie Brilliant Blue R-250, and the amount of precipitated topoisomerase I was determined by densitometric scanning.

Subcellular fractionation

The membrane fraction was prepared as described in [17]. W3110 cells were cultured in 1 litre of Luria–Bertani (LB) medium at 37 °C until the A_{650} reached 0.5. Cells treated with lysozyme, EDTA and DNAase I were lysed in a French pressure cell, homogenized, and centrifuged at 150000 g for 2 h. The amounts of topoisomerase I in the supernatant (cytosol) and the precipitate (membrane) were determined by immunoblot analysis with anti-(topoisomerase I) antiserum raised against purified topoisomerase I in a rabbit. The activity of β-galactosidase was measured as described [18] as a marker of the cytosol enzyme. One unit of β-galactosidase was defined as the amount catalysing hydrolysis of 1 μmol of *o*-nitrophenyl β-D-galactoside/min at 37 °C in a 0.4 ml reaction mixture.

Bacterial strains

The following strains of *E. coli* were used: W3110 was from our laboratory stock. DM800 Δ (*cysB-topA*) [19] and DM4100, *cysB*, *topA*$^+$ were provided by Dr. H. Ikeda (Institute of Medical Sciences of this University).

Analysis of DNA topology in *E. coli* cells

E. coli cells habouring pUC118 were grown in 5 ml of LB medium at 37 °C until A_{650} reached 0.5. Plasmid DNA was extracted by the alkaline method and analysed by 1 %-agarose-gel electrophoresis in the presence of chloroquine (5 μg/ml). The gels were stained with ethidium bromide, and photographs were taken in an apparatus from Polaroid.

RESULTS

Inhibition of *E. coli* topoisomerase I activity by phospholipids and its suppression by CPZ

The effects of various phospholipids on the activity of *E. coli* topoisomerase I *in vitro* were examined by monitoring the relaxation of form I DNA of pUC118 to form II (Fig. 1). CL strongly inhibited topoisomerase I activity, most of the substrate DNA remained as form I. PG prepared from egg lecithin exhibited weaker inhibition. PE, the most abundant phospholipid in the *E. coli* cell membranes, was not inhibitory.

Experiments with synthetic PG, which is commercially available, revealed that unsaturation of the acyl moiety of PG affects its interaction with DNA-binding proteins, such as DnaA protein [20] and simian-virus-40 T antigen [21]. Therefore, we examined the effect of unsaturation of the acyl moiety of PG on the inhibition of *E. coli* topoisomerase I (Fig. 2). PG containing unsaturated fatty acids inhibited the activity as strongly as CL did, whereas PG containing saturated fatty acids was much less inhibitory.

Psychotropic drugs and local anaesthetics interact with phospholipids and inhibit various biochemical reactions [11–13]. We found that 1 mM-CPZ partly restored the topoisomerase I activity which had been inhibited by 1 mM-CL (Fig. 3). In the presence of CL, the topoisomerase I-catalysed DNA relaxation took place in a CPZ-dependent manner. CPZ alone did not stimulate topoisomerase I activity (results not shown).

Binding of topoisomerase I to phospholipid vesicles

The above results suggested that topoisomerase I has affinity for CL and PG. We examined the binding of topoisomerase I to various phospholipids. For this, topoisomerase I was incubated with phospholipid vesicles, and the binding complex was collected

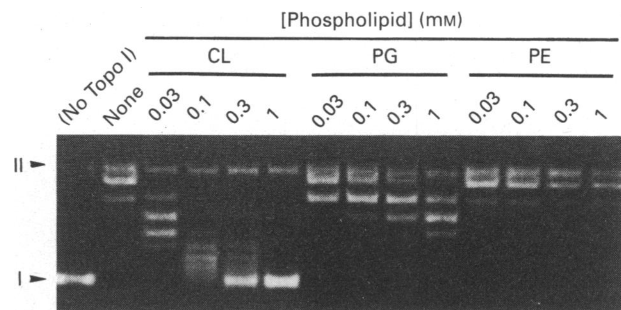

Fig 1. Influence of phospholipids on the activity of purified DNA topoisomerase I (Topo I)

Purified *E. coli* DNA topoisomerase I (20 ng) was preincubated with various phospholipids in standard reaction mixture without substrate DNA at 37 °C for 10 min. Then pUC118 form I DNA (0.5 μg) was added and incubation was continued at 37 °C for 10 min. DNA was analysed by 1 %-agarose-gel electrophoresis. The positions of form I DNA and form II DNA are shown by arrowheads. The pattern of substrate pUC118 DNA is shown in the far left lane.

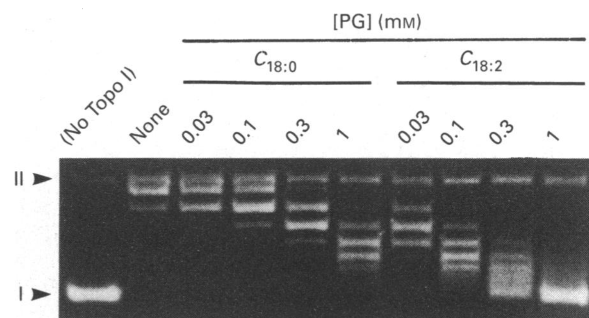

Fig 2. Influence of unsaturation of the acyl moiety of PG on its inhibition of topoisomerase I (Topo I)

Purified topoisomerase I (20 ng) was preincubated with synthetic PG possessing an unsaturated acyl moiety ($C_{18:2}$ fatty acid) or saturated acyl moiety ($C_{18:0}$ fatty acid) at 37 °C for 10 min. Then pUC118 form I DNA (0.5 μg) was added and the activity of topoisomerase I was determined as described in the legend to Fig. 1.

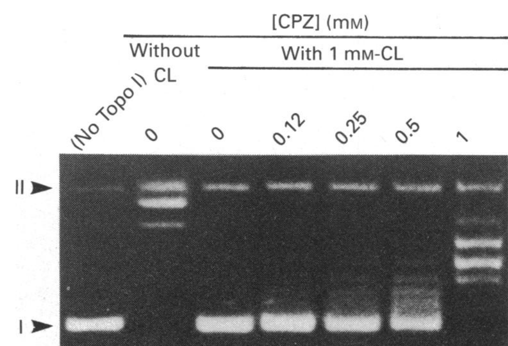

Fig 3. Restoration by CPZ of topoisomerase I (Topo I) activity inhibited by CL

Purified topoisomerase I (20 ng) was preincubated with CL (1 mM) in the presence of various concentrations of CPZ at 37 °C for 10 min. Then pUC118 form I DNA (0.5 μg) was added and the activity of topoisomerase I was determined as described in the legend to Fig. 1.

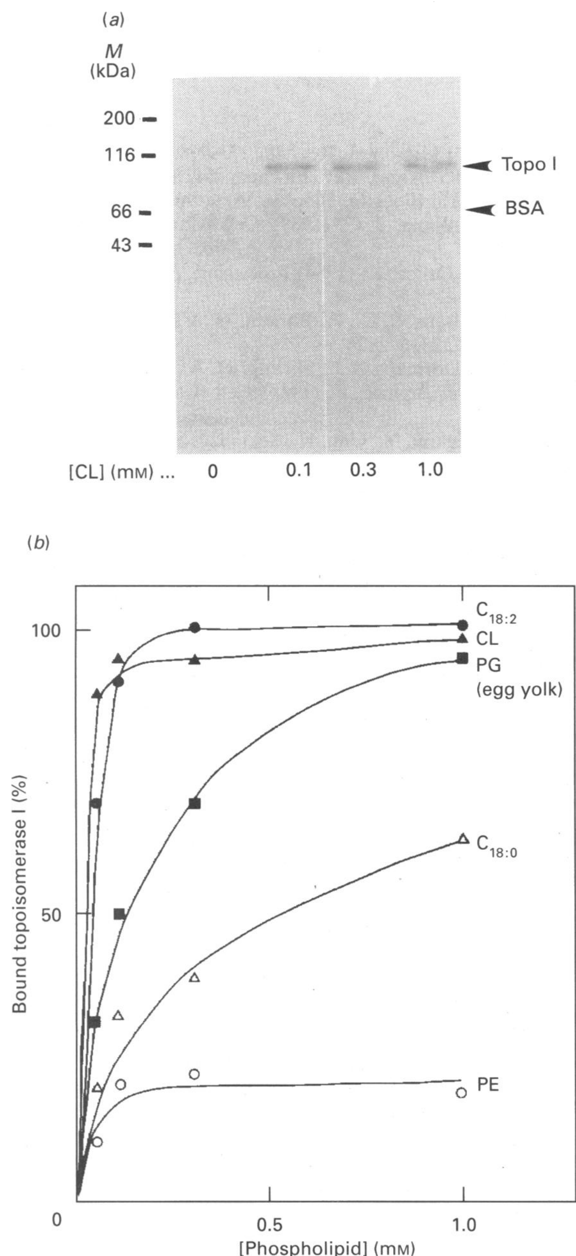

Fig 4. Precipitation of topoisomerase I (Topo I) with various phospholipids

Purified topoisomerase I was incubated with phospholipids, and precipitated protein was analysed by PAGE (*a*). The amount of topoisomerase I precipitated in a phospholipid-dependent manner was determined with densitometric scanning of the stained gels (*b*). Values are corrected for the amount of topoisomerase I precipitated in the absence of phospholipid, which was less than 3%. ●, Synthetic PG ($C_{18:2}$); ▲, CL; ■, PG from egg yolk; △, synthetic PG ($C_{18:0}$); ○, PE.

by centrifugation. Fig. 4(*a*) shows electrophoretic patterns of the precipitates with cardiolipin. The amounts of topoisomerase I precipitated with phospholipid vesicles were determined with densitometric scanning of the stained gels (Fig. 4*b*). CL and PG containing unsaturated fatty acids ($C_{18:2}$), both of which strongly inhibited topoisomerase I activity, precipitating the enzyme almost completely at concentrations of 0.05 mM. PG prepared from egg lecithin and the synthetic PG with saturated fatty acids ($C_{18:0}$), both of which showed weaker inhibition, precipitated the enzyme only at higher concentrations. PE, which did not inhibit the topoisomerase I activity, precipitated only a small amount of the enzyme, even at higher concentrations. Since the reaction mixture contains 50 μg of BSA/ml and no BSA was detected in the precipitate, this precipitation was thought to be specific for topoisomerase I.

Table 1. Localization of topoisomerase I in *E. coli* cells

Cytosol and membrane fractions were prepared from W3110 cells as described in the Materials and methods section. Protein was determined by the method of Lowry *et al.* [23], with BSA as a standard. The amount of topoisomerase I was determined by immunoblot analysis. The activity of β-galactosidase was determined as described in [18].

Fraction	Protein (mg)	Topoisomerase I (μg)	β-Galactosidase (units)
Total	80	5.8	8800
Cytosol	64	3.9	7200
Membrane	12	2.0	800

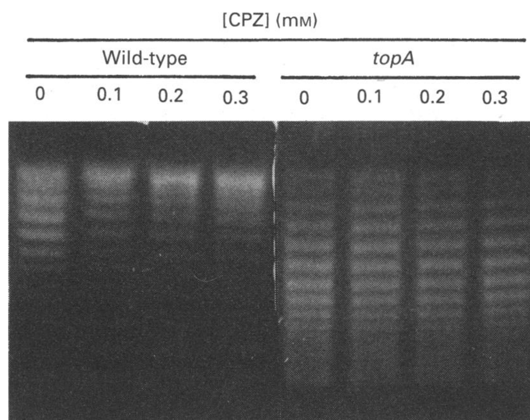

Fig 5. *topA*-dependent relaxation of pUC118 DNA in *E. coli* by CPZ

E. coli strains HI557 (wild-type) and HI558 (*topA*) habouring pUC118 DNA were cultured in 5 ml of LB medium at 37 °C until the A_{650} reached 0.5. CPZ was added and incubation was continued at 37 °C for 10 min. Plasmid DNA was extracted and analysed by agarose-gel electrophoresis in the presence of chloroquine (5 μg/ml).

Localization of topoisomerase I in *E. coli* cells

The above results suggested that topoisomerase I binds to the cytoplasmic membrane *in vivo*, so we studied the localization of topoisomerase I in *E. coli* cells without overproducing plasmid. W3110 cells were harvested and disrupted in a French pressure cell, the membrane and cytosolic fractions were separated by centrifugation, and the amounts of topoisomerase I in the two fractions were determined (Table 1).

Immunoblot analysis showed that 34% of total topoisomerase I was recovered in the membrane fraction. As only 9% of the total β-galactosidase activity was recovered in the membrane fraction, the recovery of topoisomerase I in the membrane fraction cannot be explained by the contamination of this fraction with the cytosol. Therefore, we concluded that an appreciable part of the total topoisomerase I exists as a membrane-bound form in *E. coli* cells.

Relaxation of plasmid DNA in *E. coli* cells by chlorpromazine

To find a physiological explanation for the inhibitory effects of phospholipids on topoisomerase I and suppression of these

inhibitions by CPZ (Figs. 1 and 3) we examined the influence of CPZ on the topology of DNA in cells. Exponentially growing wild-type *E. coli* cells habouring pUC118 DNA were treated with CPZ, and plasmid DNA was extracted and analysed by agarose-gel electrophoresis in the presence of chloroquine. As Fig. 5 shows, CPZ caused relaxation of plasmid DNA in cells at a concentration of 0.1 mM. Furthermore, the DNA topology of a *topA* mutant was not changed by CPZ, suggesting that the relaxation was catalysed by topoisomerase I encoded by the *topA* gene.

DISCUSSION

In the present paper we show that vesicles of CL and PG bound to purified *E. coli* topoisomerase I and inhibited its activity. We also showed that CPZ suppressed the inhibition of topoisomerase I by phospholipids *in vitro*. Moreover, we found that this drug induced relaxation of DNA in cells in a *topA*-dependent manner. Immunoblot analysis revealed that approximately one-third of the topoisomerase I was recovered in the membrane fraction. We assume that the enzyme in the membrane fraction is repressed by acidic phospholipids *in vivo* and that it is activated by CPZ, causing DNA relaxation in the cells. The inhibitory effect of PG on topoisomerase I was observed only when the acyl moiety was unsaturated. Increase in the extent of unsaturation of the acyl moiety in phospholipids causes increase of membrane fluidity [22]. We therefore propose that changes in membrane fluidity in response to various environmental stresses may affect the activity of DNA topoisomerase I, which in turn will change the topology of DNA in the cells.

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