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Acidic phospholipids directly inhibit DNA binding of mammalian DNA topoisomerase I

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Inhibition of mammalian DNA topoisomerase I by phospholipids was investigated using purified enzyme. Acidic phospholipids inhibited the DNA relaxation activity of topoisomerase I whereas neutral phospholipid, phosphatidylethanolamine, did not. Accumulation of a protein-DNA cleavable complex, an intermediate which is known to accumulate upon inhibition by a specific inhibitor camptothecin, did not occur. The filter binding assay revealed that the DNA binding activity of the enzyme was inhibited by acidic phospholipids. Moreoever, direct binding of phosphatidylglycerol to topoisomerase I was demonstrated. These results indicated that the inhibitory effect of acidic phospholipids on topoisomerase I was due to the loss of the DNA binding of the enzyme as a result of direct interaction between phospholipids and the enzyme.

Topoisomerase I; Phospholipid

1. INTRODUCTION

Recently eukaryotic DNA topoisomerase I has been considered to have an important function in replication, transcription and recombination [1–7]. The enzyme catalyzes topological alterations in DNA by introducing a transient single-strand break through which another DNA strand can pass [1].

Several lines of evidence suggest that active chromatin may exist in a dynamic, tortionally stressed state [1,2]. The degree of DNA supercoiling in chromatin probably controlled by balancing the relaxation activity of DNA topoisomerase I and the recently discovered supercoiling activity of DNA topoisomerase II assisted by 'supercoiling factor' [8,9]. A correlation between the proliferation state of eukaryotic cells and DNA superhelicity has been observed by several investigators [10–12]. From the point of view described above, regulation of topoisomerase I activity may represent one possible mechanism of regulating cellular proliferation. Regulation of topoisomerase I activity in vivo is still unclear although post-translational modifications such as poly(ADP-ribosylation) [13,14] and phosphorylation [15,16] were found to modulate topoisomerase I activity in vitro.

Recently, Umekawa et al. reported that phospholipids inhibit in vitro replication of an autonomous replicating sequence presumably due to the inhibition of topoisomerase I activity in the nuclear extract [17]. Using purified topoisomerase I, we report here that the inhibition of topoisomerase I by acidic phospholipids is due to direct binding of phospholipids to the enzyme resulting in the inhibition of the DNA binding of topoisomerase I.

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Abbreviations: CL, cardiolipin; PS, phosphatidylserine; PI, phosphatidylinositol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; CPT, camptothecin

2. MATERIALS AND METHODS

2.1. *Materials*

Cardiolipin (CL; bovine heart), phosphatidylserine (PS; bovine spinal cord), phosphatidylinositol (PI; wheat germ), phosphatidylglycerol (PG; egg lecithin) and phosphatidylethanolamine (PE; egg yolk) were purchased from Lipid Products (England). Phospholipids were vacuum-dried to form films on the bottom of glass tubes and suspended in distilled water by vigorous vortex mixing. PE was suspended in 0.05% Triton X-100. Camptothecin (CPT) was obtained from Yakult Honsha Co.

Topoisomerase I was purified from mouse Ehrlich ascites tumor cells as described [18] with some modifications. 3'-End labeling of *Eco*RI-restricted ColE1 DNA with [α-^{32}P]dATP was performed as described [19].

2.2. *DNA relaxation and cleavable complex formation assays*

DNA relaxation activity of topoisomerase I was assayed essentially as described previously [18]. The reaction mixture contained in a total volume of 40 μl 0.3 μg supercoiled ColE1 DNA, 50 mM NaCl, 50 mM Tris-HCl (pH 7.5), 10% glycerol, 50 μg/ml BSA, 1 mM EDTA and topoisomerase I (3 units, 10 ng) with or without phospholipid or CPT. The mixture was incubated at 37°C for 15 min. One unit of the enzyme is defined as the minimum amount of enzyme giving complete relaxation of 0.3 μg supercoiled ColE1

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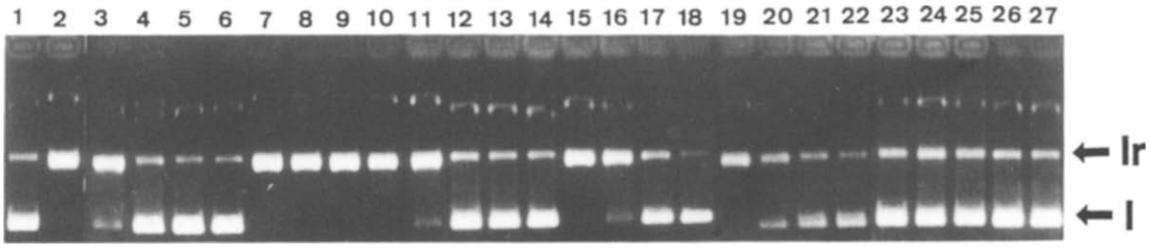

Fig.1. Inhibition of DNA relaxation activity of topoisomerase I by phospholipids. Phospholipids used were CL (lanes 3–6), PE (lanes 7–10), PG (lanes 11–14), PS (lanes 15–18), PI (lanes 19–22). Phospholipids were added at concentrations of 2 μM (lanes 3, 7, 11, 15 and 19), 10 μM (lanes 4, 8, 12, 16 and 20), 50 μM (lanes 5, 9, 13, 17 and 21) and 100 μM (lanes 6, 10, 14, 18 and 22). (Lanes 23–27) Reactions without topoisomerase I in the presence of phospholipids (lane 23, CL; lane 24, PE; lane 25, PG; lane 26, PS; lane 27, PI). (Lane 1) ColE1 DNA only; and (lane 2) ColE1 DNA and topoisomerase I. I and Ir represent supercoiled form I DNA and relaxed form I DNA, respectively.

DNA under the conditions used. Cleavable complex formation between DNA and topoisomerase I in the presence of CPT or phospholipids was assayed as described by Hsiang et al. [20] except that 30 units (100 ng) topoisomerase I and 0.3 μg DNA were used.

2.3. *Filter binding assay*

[^{32}P]-ColE1 DNA (1 ng) and 25 ng topoisomerase I were mixed in 40 μl binding buffer (10 mM Tris-HCl, pH 7.5, 20 μg/ml BSA and 0.1 mM EDTA) with or without phospholipids and incubated for 10 min at 0°C and then filtered through a nitrocellulose membrane (Toyo Roshi, 0.2 μm). The DNA-protein complexes trapped were washed with 3 ml binding buffer. The bound radioactivity was measured by a liquid scintillation counter.

2.4. *Binding analysis of topoisomerase I to phospholipids*

Phospholipid (100 μg) and topoisomerase I (0.5 μg) were mixed in 40 μl buffer (10 mM Tris-HCl, pH 7.5, 150 μg/ml BSA and 100 mM NaCl) and then centrifuged at 1000 × *g* for 10 min at 4°C. The precipitate was then dissolved in 10 μl SDS-sample buffer (0.1 M dithiothreitol, 2% SDS, 0.08 M Tris-HCl, pH 6.8, 15% glycerol and 0.006% bromophenol blue) and analyzed by SDS-polyacrylamide gel electrophoresis as described [21].

3. RESULTS AND DISCUSSION

Fig.1 shows the effect of various phospholipids on the relaxation activity of purified mouse topoisomerase I. Relaxation assays were carried out as described in section 2. From the extent of relaxed molecules, the doses giving 50% inhibition (ID_{50}) were estimated as follows: 4 μM for CL and PG, 10 μM for PI, 30 μM for PS and >100 μM for PE. CL and PG showed stronger inhibitory effect than PI and PS did. On the contrary, PE showed no effect. The inhibitory concentrations of phospholipids were surprisingly similar to that of CPT (5 μM), a specific inhibitor of mammalian topoisomerase I (data not shown). These results indicate that the inhibition of topoisomerase I by phospholipids is quite potent and significant. The inhibitory effect of phospholipids may well be due to the interaction with the enzyme rather than with the substrate, since phospholipid itself had no effect on the supercoiled DNA judged from their mobilities (fig.1, lanes 23–27). Inhibitory concentrations of phospholipids were well consistent with those of Umekawa et al. [17], although they used crude nuclear extracts as a source of topoisomerase I.

The catalytic process by DNA topoisomerase I might be divided into 4 steps: (i) DNA binding, (ii) single-strand cleavage, (iii) strand passage, and (iv) DNA resealing [1]. To know which step(s) is inhibited by phospholipids, we performed an analysis to detect an intermediate cleavable complex which is known to be accumulated upon inhibition by camptothecin (CPT) [20]. In this experiment a 10 times greater amount of enzyme was used in order to detect the intermediates clearly, so the inhibitory effect of phospholipids was relatively weak as shown in fig.2 compared to that

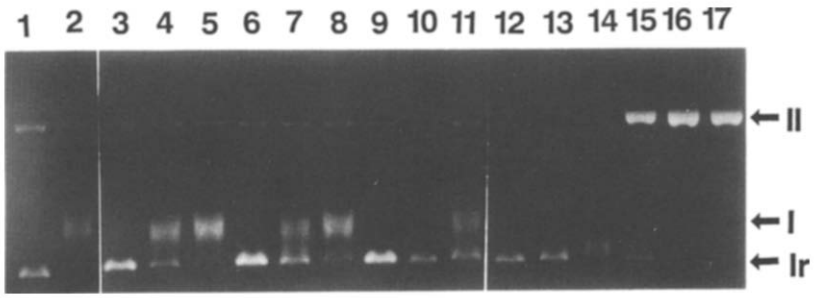

Fig.2. Detection of cleavable complexes accumulated by phospholipids and CPT. Phospholipids or CPT were added at doses of 10 μM (lanes 3, 6, 9, 12 and 15), 50 μM (lanes 4, 7, 10, 13 and 16) and 150 μM (lanes 5, 8, 11, 14 and 17). (Lanes 3–5) CL; (lanes 6–8) PG; (lanes 9–11) PS; (lanes 12–14) PI; (lanes 15–17) CPT. (Lane 1) ColE1 DNA and topoisomerase I. (Lane 2) ColE1 DNA only. I and Ir are described in fig.1. II indicates the position of nicked circular DNA (form II).

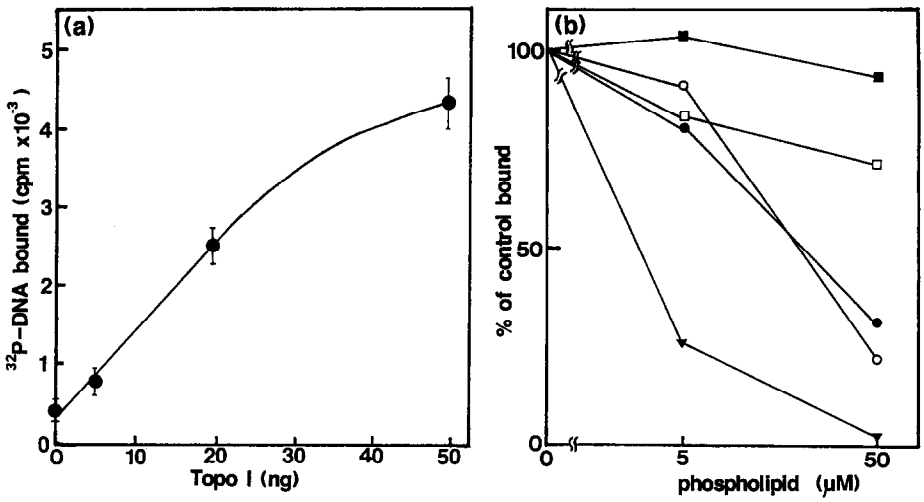

Fig.3. (a) Nitrocellulose filter assay for binding of enzyme to DNA. Each point shows the average of triplicate reactions. Bars indicate SD. The input count of DNA was about 10000 cpm (~1 ng). (b) Effect of phospholipids on DNA binding of topoisomerase I. Each point represents the average of two assays. CL (○), PG (●), PS (□), PE (■), PI (▼).

shown in fig.1. Cleavable complexes formed in in vitro reactions with phospholipids were analyzed by an agarose gel electrophoresis containing 0.25 μg/ml ethidium bromide where relaxed form I DNA is distinguished from nicked circular form II DNA. There were no significant accumulations of intermediate form II DNA for all phospholipids tested (fig.2, lanes 3–14) whereas CPT caused the accumulation (fig.2, lanes 15–17). PE did not show any effect on the intermediate accumulation as well as on the DNA relaxation (data not shown). These results indicate that phospholipids inhibit topoisomerase I reaction by some other mechanism distinct from that of CPT which stabilizes intermediates.

Next, we tested an inhibitory effect of phospholipids on the DNA binding activity of topoisomerase I by the filter binding assay [23]. Fig.3a shows a typical profile of an effect of increasing concentrations of enzyme on the binding. Fig.3b shows the effect of phospholipids on this assay. Twenty-five ng of topoisomerase I and ^{32}P-labeled ColE1 DNA (1 ng) were mixed with or without phospholipids and then DNA-protein complexes were trapped on the filter. All phospholipids, except PE, inhibited the binding of topoisomerase I to DNA. PI was the most effective, and CL and PG were more effective than PS. PE showed no effect as it had done on the relaxation assay. The extent of inhibitory activities of phospholipids on the DNA binding of topoisomerase I correlated fairly well with those on the relaxation activity. This strongly suggests that acidic phospholipids inhibit the DNA binding step of topoisomerase I.

To demonstrate more directly whether phospholipids interact with topoisomerase I, we carried out the experiment as follows. Purified topoisomerase I (0.5 μg) was mixed with PG or PE vesicles (100 μg each) and then the phospholipid vesicles were recovered by centrifugation. Topoisomerase I bound to phospholipid vesicles was analyzed by SDS-polyacrylamide gel electrophoresis. Fig.4 shows that only in the case of PG vesicles, topoisomerase I was recovered in the precipitate (fig.4, lane 2). This result indicates that PG interacts with topoisomerase I directly resulting in inhibition of DNA binding, presumably due to masking of DNA binding site(s) of the enzyme. Although we have not carried out the assay for other acidic phospholipids, the situation might be the same.

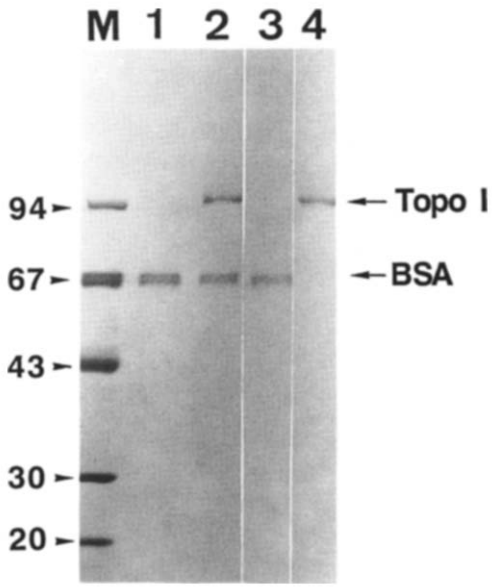

Fig.4. Binding of topoisomerase I to phospholipid vesicles. (Lane 1) Without phospholipids; (lane 2) phosphatidylglycerol vesicles; (lane 3) phosphatidylethanolamine vesicles; (lane 4) 0.5 μg purified mouse topoisomerase I; (lane M) marker proteins with molecular masses in kilodaltons.

The present report demonstrates that inhibition of eukaryotic topoisomerase I by acidic phospholipids is mediated by direct interaction between the enzyme and phospholipids. This direct interaction may well be due to an electrostatic interaction between acidic phospholipid and basic protein topoisomerase I with a p*I* of 8.4 [24]. It is of relevance to refer to the inhibition of the enzyme by acidic polysaccharide-like heparin [22]. Although it is not known whether phospholipids interact with topoisomerase I in vivo, it is tempting to speculate that the activity of topoisomerase I localized in nuclei [25] is modulated by interacting with phospholipids, one of the components of nuclear membrane [26]. If this was the case, changes of nuclear phospholipid composition coupled with cellular physiological changes might control the activity of the enzyme. This may then lead to the alteration of the activity of chromatin, i.e. transcription, replication, etc. This might, in turn, affect the cellular proliferation and differentiation.

Recently involvement of phospholipids in DNA replication and transcription of both eukaryotic and prokaryotic systems have been suggested [27–30] (Hirai et al., unpublished). Regulation of nuclear enzymes by phospholipids might be ubiquitous and important as well as transmembrane signaling that is known to be regulated by phospholipid metabolites [31].

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