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NOTE

Inhibition Mode of DNA Topoisomerase by Dibutyl Phthalate

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Dibutyl phthalate induced topoisomerase I mediated DNA relaxation comparable to that of camptothecin, and topoisomerase II mediated DNA relaxation equipotent to that of 4'-(9-acridinylamino) methanesulfon-m-anisidide (m-AMSA). The relaxation activities of dibutyl phthalate were dose-dependent and nearly as potent as those of camptothecin and m-AMSA.

DNA topoisomerase I and topoisomerase II are now viewed as important cellular targets of a number of antitumor drugs, which include topoisomerase I poisons such as camptothecin and its derivatives and topoisomerase II poisons such as acridines, ellipticines, anthracyclines, and epipodophyllotoxins (1, 7). These drugs have been used to establish a relationship between drug-induced cleavable complex formation and cytotoxicity.

Over the past several years, the mechanisms and actions of DNA topoisomerases and their poisons have been studied intensively (7-11). As for topoisomerase I, it is reported that the catalytic process is composed of the following 4 steps (10): (1) DNA binding, (2) single strand cleavage, (3) strand passage, and (4) DNA rejoining. Camptothecin, one of the most potent topoisomerase I inhibitors, is known to inhibit the step (4) and the topoisomerase I-mediated DNA strand breaks are induced as a result of inhibition by camptothecin (3, 4).

We have reported that dibutyl phthalate produced by *Streptomyces melanosporofaciens* 7489 is a poison of calf thymus DNA topoisomerase I (5, 6). To examine whether dibutyl phthalate stimulates the enzyme-linked DNA breakage as camptothecin does, supercoiled pBR 322 DNA was incubated with topoisomerase I in the presence of dibutyl phthalate.

In the DNA relaxation assay using topoisomerase I, dibutyl phthalate induced the formation of nicked circular DNA which resulted from topoisomerase I-mediated single-strand cleavage (Fig. 1). The activity of dibutyl phthalate (Fig. 1, lanes c to g) in inducing relaxation was similar to that of camptothecin (Fig. 1, lanes h to l).

To examine whether dibutyl phthalate inhibits topoisomerase I by interacting with the enzyme or the substrate, each of these was increased in the reaction mixture containing a constant amount of an inhibitor. As shown in Fig. 2, the inhibitory effect of 25 μM dibutyl phthalate was observed with 1 unit of enzyme (lane c), and was completely concealed when the amount of enzyme was increased to 6 units (lanes d to f). In contrast, no recovery of enzyme activity was observed when the amount of substrate DNA was increased to 0.2 μg (lanes g to i). This result showing that dibutyl phthalate doesn't interact with DNA was also supported by the fact that the migration of substrate DNA on agarose gel was not affected by dibutyl phthalate (100 μM), even at high concentrations, although many compounds known to interact with DNA and affect the mobility of DNA on the gel at high concentrations (data not shown). Dibutyl phthalate inhibited topoisomerase I activity in a dose-dependent manner (Fig. 2).

Dibutyl phthalate induced DNA relaxation *in vitro* in

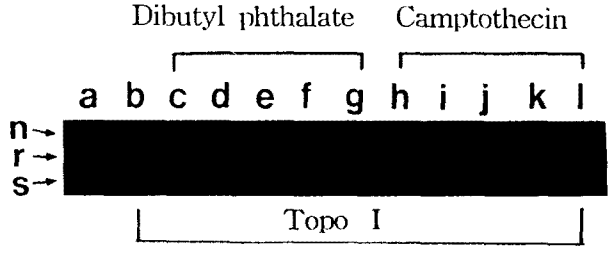

Fig. 1. Effect of dibutyl phthalate on relaxation activity of DNA topoisomerase I.

Plasmid DNA (pBR322, 0.2 μg) was treated with 1 unit of topoisomerase I in the presence of dibutyl phthalate (lanes c to g), and then analyzed on an agarose gel (1%): lane a, pBR322 DNA control; lane b, no drug; lanes c to g, dibutyl phthalate, lanes h to l, camptothecin. Drug concentrations were as follows: lanes c and h, 25 μM; lanes d and i, 10 μM; lanes e and j, 5 μM; lanes f and k, 1 μM; lanes g and l, 0.5 μM. "n", "r", and "s" denote nicked, relaxed, and supercoiled DNA, respectively.

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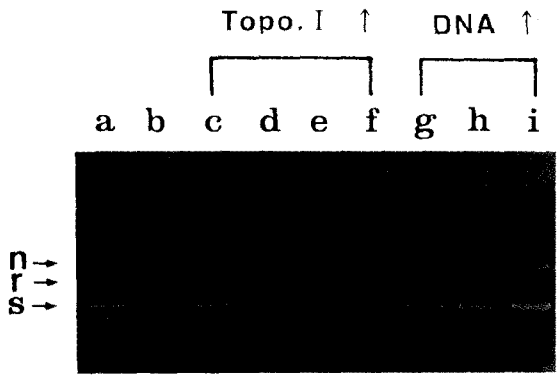

Fig. 2. Effect of the enzyme and substrate DNA concentrations on inhibition of topoisomerase I action by dibutyl phthalate.
Lane a, 0.1 μg pBR322 DNA alone; lane b, pBR322 DNA was incubated with 1 unit of topoisomerase I in 20 μl of the reaction mixture; lane c, 25 μM of dibutyl phthalate was added to the reaction mixture; lanes d to f, enzyme concentration was increased to 2, 4, and 6 units in the same reaction mixture as lane c; lanes g to j, DNA concentration was increased to 0.1, 0.15, and 0.2 μg in the same reaction mixture as lane c. "n", "r", and "s" denote nicked, relaxed, and supercoiled DNA, respectively.

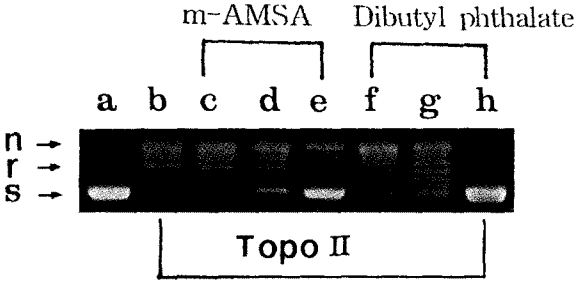

Fig. 3. Effect of dibutyl phthalate on relaxation activity of DNA topoisomerase II.
Plasmid DNA (pBR322, 0.3 μg) was treated with 1 unit of topoisomerase II in the presence of the drug (lanes c to h) and then analyzed on an agarose gel (1%): lane a, pBR322 DNA control; lane b, no drug; lanes c to e, m-AMSA, lanes f to h, dibutyl phthalate. Drug concentrations were as follows: lanes c and f, 5 μM; lanes d and g, 10 μM; lanes e and h, 25 μM. "n", "r", and "s" denote nicked, relaxed, and supercoiled DNA, respectively.

the assay using calf thymus DNA topoisomerase II and supercoiled pBR322 DNA (Fig. 3). As the concentration of dibutyl phthalate was increased from 5 μM (Fig. 3, lane f) to 25 μM (Fig. 3, lane h), the supercoiled DNA appeared in a dose-dependent manner. The DNA relaxation activity of dibutyl phthalate (Fig. 3, lanes f to h) was stronger than that of m-AMSA (Fig. 3, lanes c to e).

Other studies with anthracyclines or ellipticine derivatives indicated that topoisomerase II mediated DNA relaxation by these strong intercalators was suppressed at higher drug concentration due to the change of DNA conformation which blocks topoisomerase II access to the DNA (2). In the case of dibutyl phthalate, however, topoisomerase II mediated DNA relaxation increased in a dose-dependent manner, and no suppression of DNA relaxation was observed at the concentration of 100 μM (data not shown).

In this study, we present data showing that dibutyl phthalate is a posion of topoisomerase I and II like saintopin (12). The relaxation activities of dibutyl phthalate were dose-dependent and nearly as potent as those of camptothecin and m-AMSA (8) (Fig. 1 and 3).

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