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INTERACTION OF (±)-7r,8t-DIHYDROXY-9t,10t-EPOXY-7,8,9,10-TETRAHYDRO-BENZO(a)PYRENE WITH RELAXED CIRCULAR pBR322 DNA

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The interaction of (±)-BPDE (1) with DNA at neutral pH was investigated by the application of relaxed circular pBR322 DNA. (±)-BPDE causes a rapid positive supercoiling of this DNA followed by a slower spontaneous relaxation. The results indicate that there are two clearly discernable types of chemical interactions between 1 and DNA, a rapid intercalative covalent binding and a slower strand breakage. The implications of these findings are discussed.

(+)-7r,8t-Dihydroxy-9t,10t-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene ((+)-BPDE, 1), the metabolically activated form of the ubiquitous precarcinogen benzo(a)pyrene (2), is carcinogenic and highly mutagenic.[1] It is known that BPDE will react chemically with DNA *in vitro* to yield stable covalent products, the major product being the 10r-adduct at the 2-amino group of guanine,[2] as well as to cause other chemical transformations, such as the single strand scission of the phosphodiester linkage. Calvin and his coworkers reported that supercoiled Col E1 DNA is unwound by (±)-BPDE and proposed that this relaxation was the result of strand scission induced by (±)-BPDE.[3] Drinkwater, Miller, Miller, and Yang subsequently demonstrated that intercalative covalent binding of aryloxiranes, including (±)-BPDE, caused the relaxation of SV 40 DNA; the abilities of oxiranes to relax supercoiled DNA were related to their mutagenicities against *S. typhimurium* TA-98.[4] Other investigators also demonstrated that supercoil unwinding and nicking occurred when DNA's were treated with (±)-BPDE.[5]

Recent investigation in our laboratory indicates that the intercalative covalent binding of (±)-BPDE to DNA is a rapid process which is complete

Abbreviations: BPDE, 7r,8t-dihydroxy-9t,10t-epoxy-7,8,9,10-tetrahydrobenzo(a)-pyrene; DNA, deoxyribonucleic acid.

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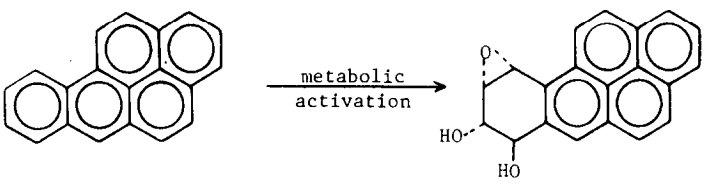

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within minutes, while strand scission at neutral pH is a process which occurs gradually over a period of several hours. Natural negatively supercoiled DNA is unwound by strand nicking as well as by intercalative covalent modification by BPDE, therefore it is not a suitable substrate for differentiating between these processes using electrophoretic techniques. Since the electrophoretic mobilities of positively supercoiled and nicked circular DNA's are different, the reaction of BPDE with relaxed circular DNA may be diagnostic for both processes. Rapid intercalative covalent binding of BPDE causes relaxed circular DNA to undergo positive supercoiling and to display an increased electrophoretic mobility, while the time dependent strand scission relaxes the positively supercoiled DNA and decreases its electrophoretic mobility. By studying the reaction of (±)-BPDE with relaxed circular pBR322 DNA, we were able to distinguish its strand-scission activity from its covalent intercalative binding.

EXPERIMENTAL

Supercoiled pBR322 DNA was isolated by the method of Clewell and Helinski.[6] A preparation of rat liver DNA-untwisting enzyme[7] was generously provided by Professor N. R. Cozzarelli. (±)-BPDE was prepared by the method of McCaustland and Engel.[8] All other chemical reagents and solvents used were of the highest purity available.

Relaxation of supercoiled pBR322 DNA was effected by treatment with the rat liver untwisting enzyme[7] at 37°C for 60 minutes. The reaction mixture was extracted with phenol/chloroform/isoamyl alcohol (49:49:2), and the DNA was ethanol-precipitated. Relaxed pBR322 DNA was purified by ethidium bromide-cesium chloride equilibrium density centrifugation according to the published method.[9] The purified relaxed pBR322 DNA was found to be contaminated with less than 5% of supercoiled DNA containing a few supercoils (1 to 3), but no detectable amount of nicked DNA. Relaxed pBR322 DNA was stored in a buffer containing 10 mM Tris-Cl (pH 7.4) and 1.0 mM disodium EDTA (buffer A).

Reaction mixtures contained 2 µl (0.4 µg) relaxed pBR322 DNA, 6 µl buffer A, and 2 µl freshly prepared (±)-BPDE stock solution (in dry DMSO). Individual samples with final hydrocarbon concentrations ranging from 0-500 µM were incubated at 37°C, in the dark, for periods of 0.25, 2, 6, or 13 hours. Reaction samples were stained by the addition of 2 µl of a solution containing 0.1% bromophenol blue, 120 mM disodium EDTA, and 60% glycerol, and were applied immediately to horizontal slab gels (20x16x0.3 cm) of 1% agarose. A hydro-

carbon-free sample containing both supercoiled and nicked pBR322 DNA's was included for reference. Electrophoresis was carried out at 50 V at 21°C until the dye had migrated 10 cm. Gels were stained with ethidium bromide (1 μg/ml) to visualize DNA bands, illuminated on a uv light source, and photographed.

RESULTS AND DISCUSSION

The results displayed in Figures 1 and 2 are composites of two separate studies of the interaction of (±)-BPDE with relaxed circular pBR322 DNA as analyzed by gel electrophoresis. The products from the lower range of BPDE concentrations (5 μM to 100 μM) were examined after 0.25 and 6 hours, while those from the higher range of BPDE concentrations (50 μM to 500 μM) were examined after 0.25, 2, 6, and 13 hours.

After 0.25 hour, samples of relaxed DNA treated with (±)-BPDE show an increase in electrophoretic mobility relative to either untreated relaxed or nicked DNA. The increase in mobility is dependent upon the BPDE concentration, but there appears to be a saturation effect at concentrations higher than 50 μM (Figure 1, lane i). Although both uncoiling of supercoiled DNA and supercoiling of relaxed circular DNA may take place in the presence of an intercalating

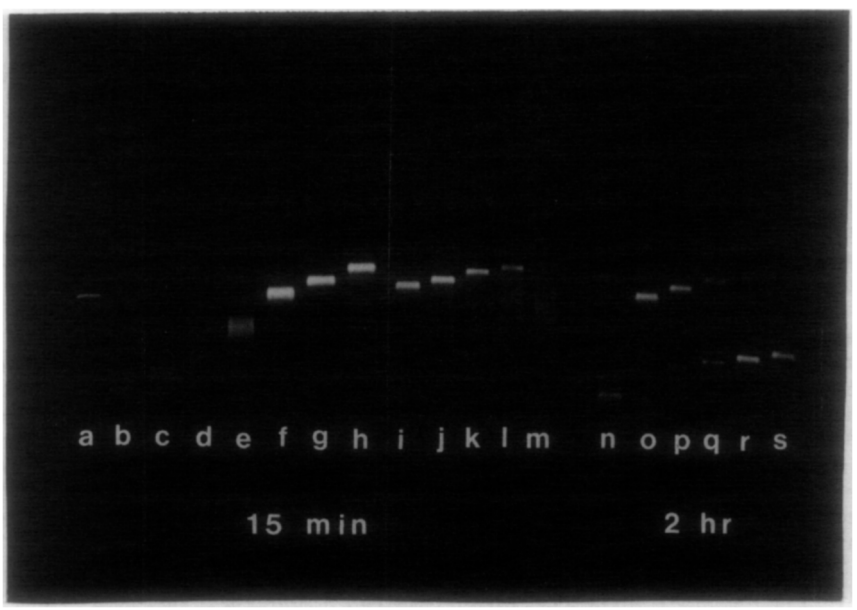

Figure 1. Results of agarose slab gel electrophoresis of relaxed pBR322 DNA treated with (±)-BPDE: [pBR322 DNA] = 120 μM; [(±)-BPDE] = 0 (b,n), 5 (c), 10 (d), 20 (e), 40 (f), 60 (g), 100 (h), 50 (i,o), 75 (j,p), 125 (k,q), 250 (l,r), and 500 μM (m,s). Reaction time: 0.25 hr (b-m) and 2 hr (n-s). A 3:1 mixture of supercoiled and nicked pBR322 DNA is included for reference (a). Migrating front is from bottom to top of the figure.

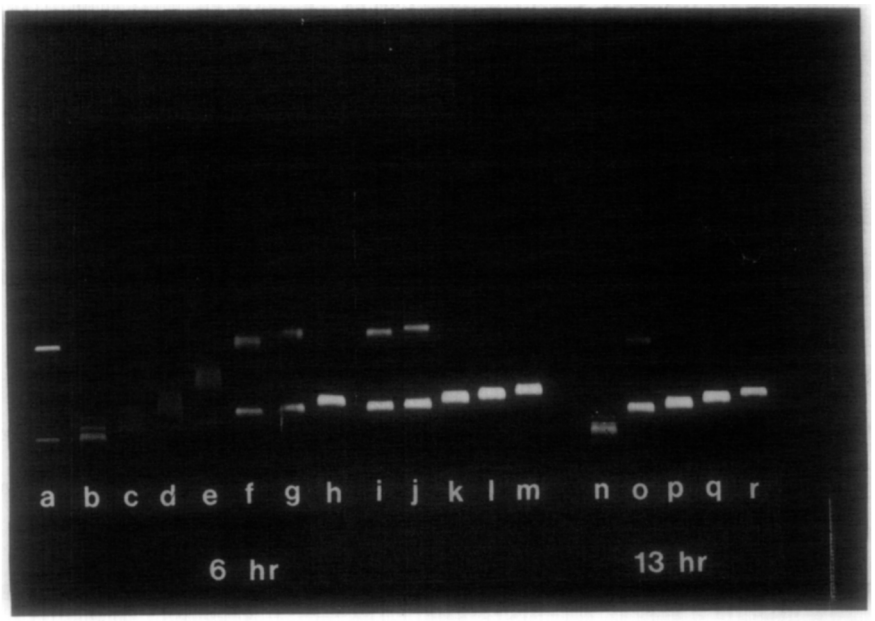

Figure 2. Results of agarose slab gel electrophoresis of relaxed pBR322 DNA treated with (±)-BPDE: [pBR322 DNA] = 120 μM; [(±)-BPDE] = 0 (b,n), 5 (c), 10 (d), 20 (e), 40 (f), 60 (g), 100 (h), 50 (i,o), 75 (j,p), 125 (k,q), 250 (l,r), and 500 μM (m). Reaction time: 6 hr (b-m) and 13 hr (n-r). A 3:1 mixture of supercoiled and nicked pBR322 DNA is included for reference (a). Migrating front is from bottom to top of Figure.

agent, the latter process is thermodynamically unfavorable in the absence of an intercalating agent. Since noncovalent intercalating agents, such as ethidium bromide or hydrolysis products of BPDE, do not alter the electrophoretic mobility of circular DNA under these experimental conditions, the changes observed are caused by covalently-bound BPDE.

Gel electrophoresis of products after 2 hour reaction (Figure 1) shows the appearance of a second band with a mobility similar to unmodified relaxed or nicked circular DNA. The mobility of this new band, like that of nicked DNA, increased slightly as more BPDE was incorporated. The extent of nicking was dependent upon the concentration of (±)-BPDE, and it also increased as the reaction time was lengthened from 2 to 13 hours. Strand scission began to occur at 10 μM (±)-BPDE, and it was essentially complete at 100 μM (±)-BPDE after a 6 hour incubation period (Figure 2, lane h).

Although alkylation at the 7-nitrogen of guanine followed by apurination and β-elimination is a possible mechanism for the nicking, alternative mecha-

nisms cannot be excluded at this moment.[3,10-13] Previously, other investigators have reported independently that (±)-BPDE caused the relaxation of supercoiled circular DNA either by nicking or by intercalative covalent binding.[3,4,11-13] Our current investigation established unambiguously that both processes take place. Rapid covalent intercalative binding of BPDE and related compounds is complete within minutes and is followed by a slower nicking process which occurs over a period of several hours at neutral pH. These two processes may readily be delineated by choosing relaxed circular DNA as the substrate for time dependent reactions with BPDE. Furthermore, the results obtained are reproducible (Figure 1, lanes f-h and i-k and Figure 2, lanes f-h and i-k) and may be quantitated.

Drinkwater, Miller, Miller, and Yang correlated the abilities of BPDE and related aryloxiranes to uncoil supercoiled DNA via covalent intercalative binding with their mutagenicities against S. typhimurium TA-98.[4] Boutwell and coworkers found, however, that this intercalation ability alone was not related to the carcinogenicities of these compounds.[14] The possible relationship between DNA strand scission activities of BPDE and related oxiranes and their carcinogenic activities is currently being investigated.

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