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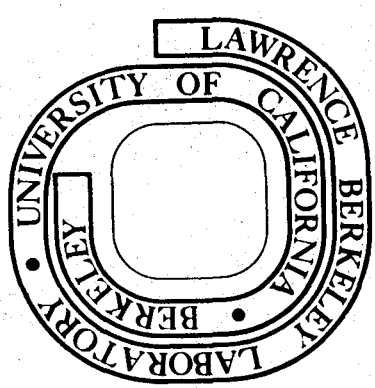

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Howard B. Gamper

DNA Strand Scission by Benzo[a]pyrene Diol Epoxides

ABSTRACT

Syn and anti-benzo[a]pyrene diol epoxides elicit a concentration dependent nicking of superhelical ColE1 DNA in an in vitro reaction monitored by agarose gel electrophoresis and electron microscopy. Since the kinetics of nicking appear too rapid for depurination strand scission, it is postulated that the diol epoxides form unstable phosphotriesters, hydrolysis of which nick the DNA.

Benzo[a]pyrene is a widespread environmental pollutant possessing potent mutagenic and carcinogenic activity. This activity is dependent upon metabolic activation of the hydrocarbon by microsomal monooxygenase. Recent evidence suggests that a 9,10-oxide of trans-7,8-dihydro-7,8-dihydroxybenzo[a]pyrene may be the ultimate carcinogenic metabolite (1). It appears that microsomal activation proceeds stereospecifically to give the anti-diol epoxide (2,3). Both diastereomers, however, react with nucleic acids and are highly mutagenic (3,4). Weinstein et al. (5), working with the anti isomer, and Koreeda et al. (6), employing the syn isomer, characterized the primary RNA adduct as a linkage between the N-2 amino group of guanine and the C-10 position of the hydrocarbon. Furthermore, Koreeda et al. (6) suggested that a minor, relatively labile RNA adduct they observed could have been a phosphate ester of the hydrocarbon. Reaction of diol epoxide with DNA has not been characterized. We describe here that both diastereomeric diol epoxides can cause nicking of ColE1 DNA and propose a mechanism for how this process might occur.

Covalently closed superhelical DNA, such as the E. coli plasmid ColE1, is a sensitive probe for detecting strand scission. Form I ColE1 DNA, with a MW of 4.2×10^6 daltons, contains over 7,000 phosphodiester linkages (7); cleavage of any one permits the DNA strands to unwind resulting in relaxed form II DNA. Introduction of another nick adjacent to the first but on the opposite strand gives linear form III DNA. All three forms are conveniently resolved by agarose gel electrophoresis. The ColE1 DNA used in this study contains 0.3% RNA as a discrete segment (7). Similar nicking, however, is seen with RNA free SV40 DNA.

When ColE1 DNA is reacted with either diastereomeric diol epoxide in tris-HCl buffer, pH 8.0, gel electrophoresis shows substantial nicking

(Fig. 1). A minimum diol epoxide to DNA mononucleotide ratio of 0.01 is required for detectable nicking. Above this value nicking increases, until at a ratio of 0.5 - 1.0 no form I DNA remains. The effects of strand scission are also observed by electron microscopy (Fig. 2). When form I DNA is reacted with the anti isomer, relaxed circles result from a ratio of 0.5 while linear segments of random length result from a ratio of 5. At even higher diol epoxide to mononucleotide ratios (i.e. 15) a significant fraction of the DNA (~ 20%) becomes acid soluble, presumably due to the release of small oligonucleotides. This increased fragmentation suggests that nicks are occurring at many sites around the molecule and are not restricted to the RNA segment mentioned above. When the reaction is carried out in 20 mM phosphate buffer, pH 7.3, nicking is detectable only at diol epoxide to mononucleotide ratios greater than 1.3. This probably reflects direct reaction of diol epoxi with inorganic phosphate (6,8). Hydrolysis of the diol epoxides resul in tetraols, which lack nicking activity (Fig. 1).

Excluding photochemical and free radical processes, chemically induced strand scission is generally assumed to occur by two possible mechanisms (9). Depurination strand scission is unique to DNA. Reaction of an electrophile with a purine ring nitrogen (primarily guanine N-7 and adenine N-3) introduces a formal positive charge into the π system. This labilizes the glycosidic bond and leads to loss of the modified purine through β-elimination. The depurination reaction has an estimated half-life of 150 h (10). The second mechanism involves reaction of an electrophile with the sugar-phosphate backbone to give a phosphotriester. Subsequent hydrolysis of the triester occurs with strand scission when the electrophilic adduct is retained. At neutrality phosphotriesters are normally quite stable, but properly oriented β-

hydroxyl groups can catalyze their hydrolysis. In RNA this catalysis is mediated by the 2'-hydroxyl. While it is clear that strand scission does arise from ribophosphotriester hydrolysis (11), recent work by Singer et al. (12) indicates the half-life of such esters may be several hours. In DNA the 2'-hydroxyl group is lacking and therefore deoxyribophosphotriesters are stable species (13). However, the presence of a β-hydroxyl group on the electrophile could catalyze phosphotriester hydrolysis and concommitant DNA strand scission (14).

If diol epoxide forms phosphotriesters in DNA, the triesters should be labilized by the C-9 hydroxyl of the hydrocarbon. The nicking activity of diol epoxide could therefore represent depurination strand scission or phosphotriester hydrolysis. If the former mechanism is operative then one might expect the kinetics of nicking to be comparable between diol epoxide and alkylating agents which react with the purine ring nitrogens. Dimethyl sulfate is one such agent, reacting primarily with guanine N-7 (15). In aqueous solution at pH 7.0 it has a half-life of 10 min (15). By comparison, we found the half-life of anti-diol epoxide in tris-HCl buffer, pH 8.0, to be 21 min at 37° (16). At μM concentrations of 16.5 and 530, respectively, anti-diol epoxide and dimethyl sulfate give similar percentages of form II ColE1 DNA after 24 h reaction. While the absolute number of nicks per relaxed DNA molecule is similar for each electrophile, the kinetics of nicking differ markedly (Fig. 3). Dimethyl sulfate gives a slow nicking rate characteristic of depurination strand scission. Anti-diol epoxide gives rapid nicking, indicative of the formation and hydrolysis of an unstable phosphotriester.

A model for diol epoxide induced DNA strand scission is presented

in Fig. 4. Since DNA phosphate does not readily displace electrophiles, ester formation at the C-10 position of the hydrocarbon most likely proceeds through an S_N1 mechanism (17). The C-9 hydroxyl group can then displace one of the sugars thereby breaking the DNA backbone and forming a cyclic triester. Tertiary cyclic phosphates, like the one proposed, hydrolyze rapidly to relieve ring strain (18). Upon hydrolysis the hydrocarbon remains attached to the phosphate. Since the β-hydroxyl group is on the hydrocarbon and not the sugar, each triester hydrolysis gives a nick.

The role of strand scission in regard to <u>in vivo</u> mutagenesis and carcinogenesis is unknown. However, there is a correlation between the extent of phosphate reaction and the oncogenic activity for a limited number of simple alkylating agents (12). In RNA phage both triesters and strand nicks are inactivating lesions (17,19). In DNA stable ethyl triesters do not appear to be lethal (20). The nicks induced by diol epoxide are likely to be inactivating since the bulky hydrocarbon adduct is retained. Repair of such nicks would be contingent upon loss of the hydrocarbon by hydrolysis of the C-10 phosphate linkage or by nucleolytic excision of the modified mononucleotide.

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2. Syn and anti-diol epoxide refer, respectively, to (±)-7α,8β-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene and (±)-7α,8β-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene.

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16. The hydrolysis of anti-diol epoxide was followed by an indirect assay based on the ability of diol epoxide to nick supercoiled ColE1 DNA. The half-life reported here is substantially longer than values obtained by Wood et al. using mutagenesis and cytotoxicity assays [A.W. Wood, P.G. Wislocki, R.L. Chang, W. Levin, A.Y.H. Lu, H. Yagi, O. Hernandez, D.M. Jerina, A.H. Conney, Cancer Res. 36, 3358 (1976)].

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22. ColE1 DNA was obtained from Prof. J. Hearst. We thank Dr. T. Meehan for critical reading of the manuscript. This study was supported by National Cancer Institute Contract YO1-CP-50203 and the Division of Biomedical and Environmental Research of the U. S. Energy Research and Development Administration.

FIGURE LEGENDS

Fig. 1. Nicking of superhelical ColE1 DNA by benzo[a]pyrene diol epoxides. Superhelical ColE1 DNA (mononucleotide conc. 32.5 μM) in 20 mM tris-HCl, pH 8.0, containing 0.5 mM EDTA and 5% (v/v) DMSO was incubated at 37° with the indicated concs. of diol epoxide (closed circles) or tetraol (open circles). After 24 h 40 μl aliquots were loaded onto a 1.4% agarose slab gel and electrophoresed at 50 V for 18 h. The gel was stained with ethidium bromide and the DNA bands quantified with a Schoeffel model SD3000 spectrodensitometer in the reflectance mode. Fig. 1a refers to anti-diol epoxide and its tetraol hydrolysis product; Fig. 1b refers to syn-diol epoxide and its tetraol hydrolysis product. Syn and anti-diol epoxides were synthesized according to published procedures (4,21).

Fig. 2. Electron micrographs of ColE1 DNA (mononucleotide conc. 32.5 μM) reacted with (A) 0, (B) 16.5, (C) 165 μM anti-diol epoxide. The DNA was visualized with the Kleinschmidt technique using an unidirectional Pt/Pd shadow.

Fig. 3. Kinetics of ColE1 DNA relaxation by anti-diol epoxide and dimethyl sulfate. Superhelical ColE1 DNA (mononucleotide conc. 32.5 μM) was reacted with 16.5 μM anti-diol epoxide (closed circles) or 530 μM dimethyl sulfate (open circles) in 20 mM tris-HCl, pH 8.0, containing 0.5 mM EDTA and 5% (v/v) DMSO at 37°. Aliquots were taken at various times for analysis by agarose gel electrophoresis.

Fig. 4. Postulated mechanism of DNA strand scission by benzo[a]pyrene diol epoxides.

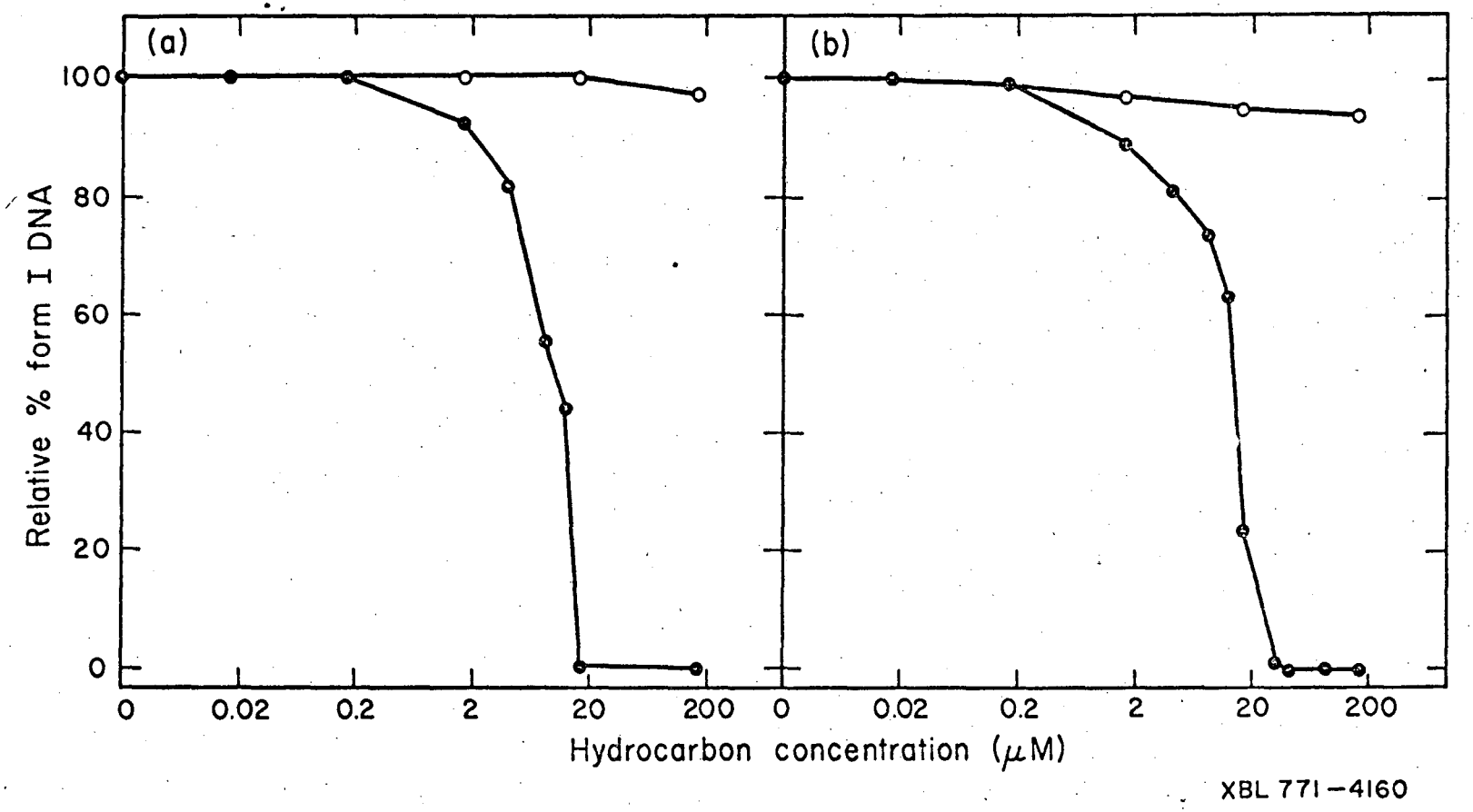
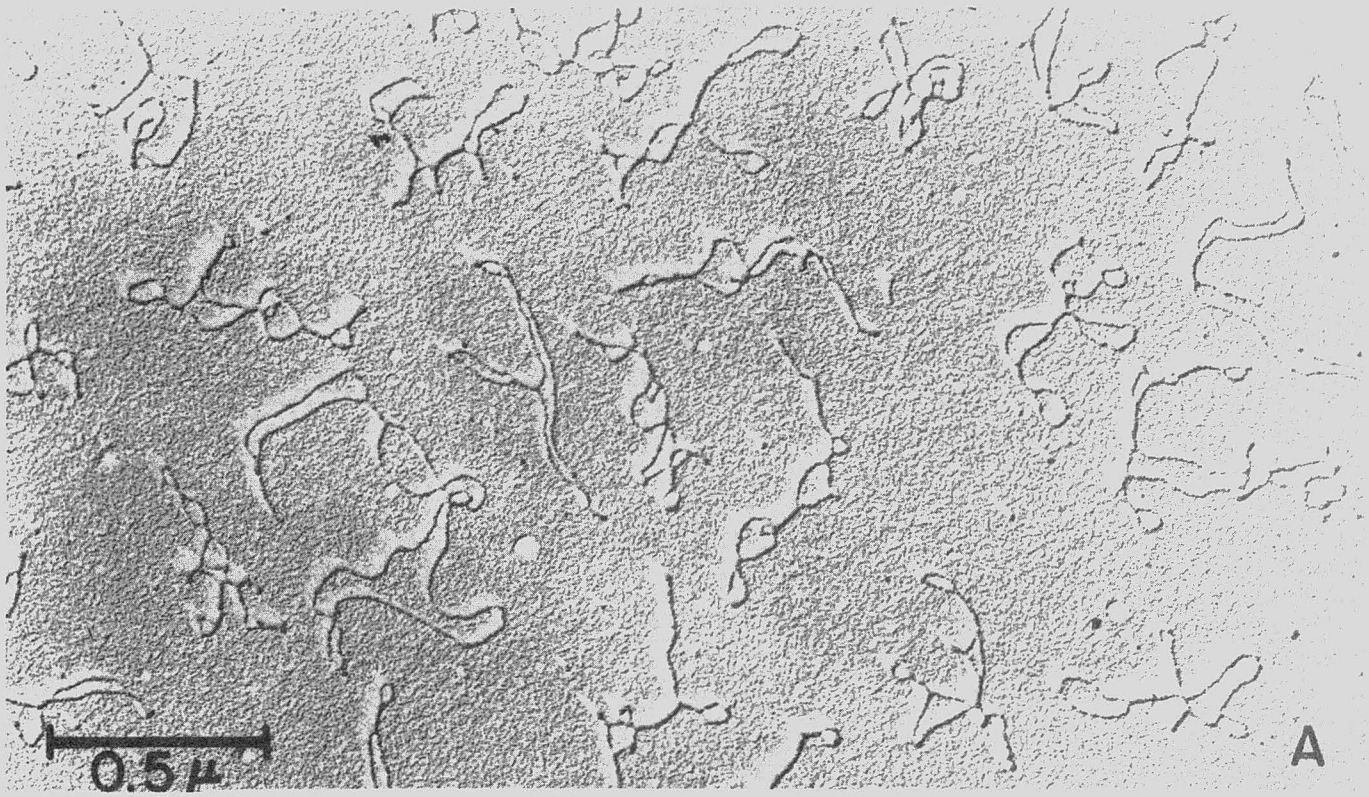

(a)
(b)
100
80
60
40
20
0
Relative % form I DNA
0
0.02
0.2
2
20
200
Hydrocarbon concentration (μM)
XBL 771-4160

Fig. 1



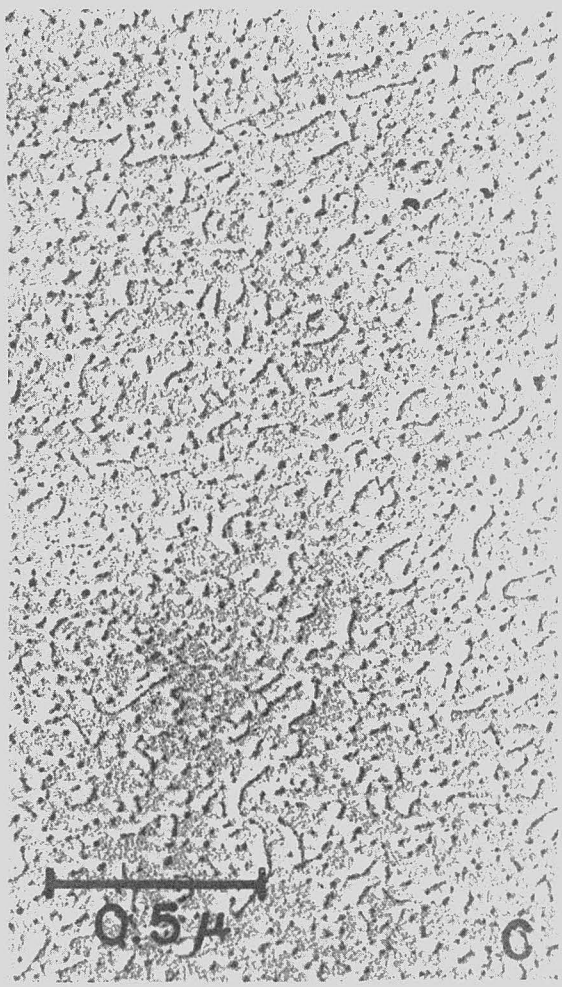


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Fig. 2

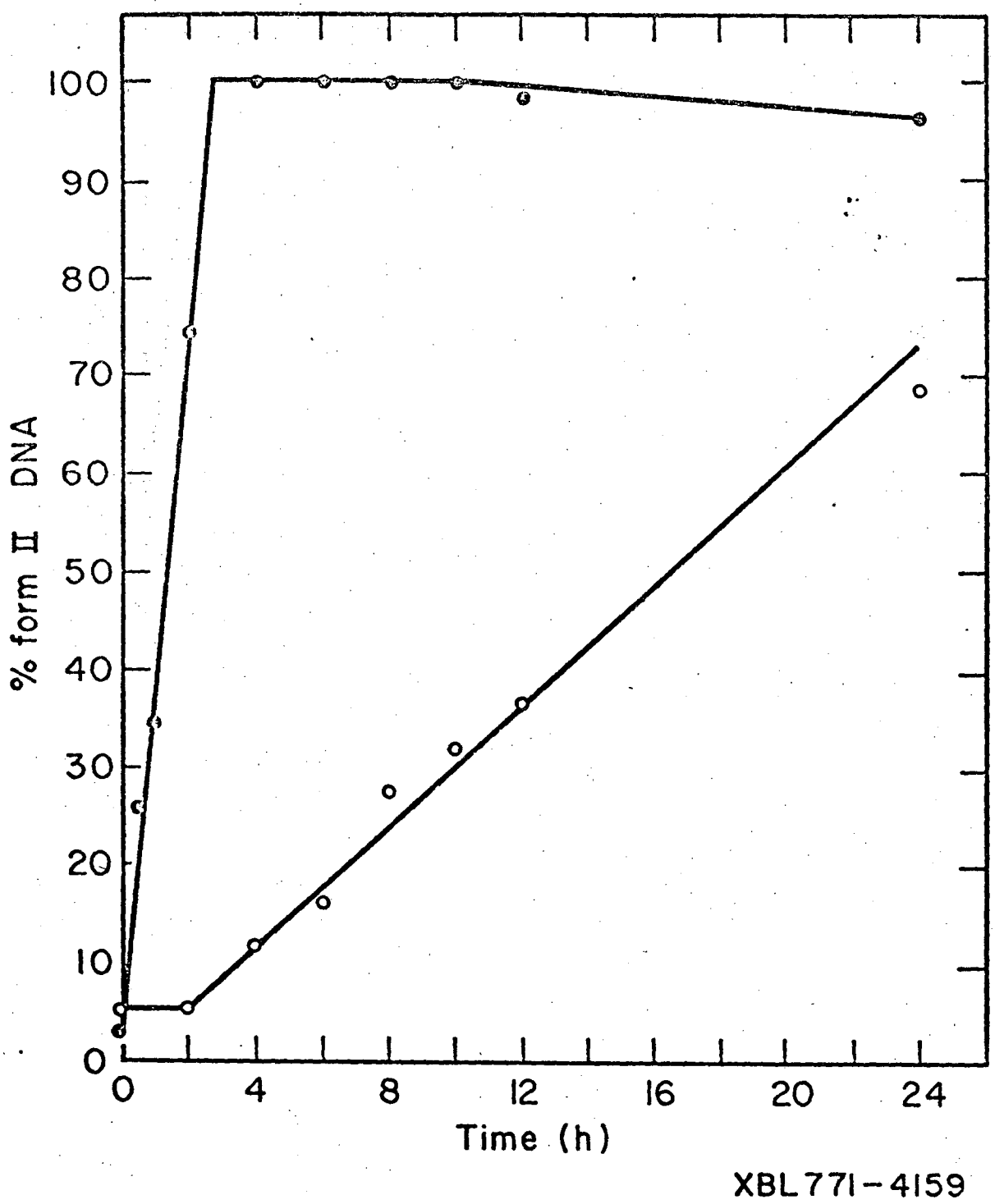
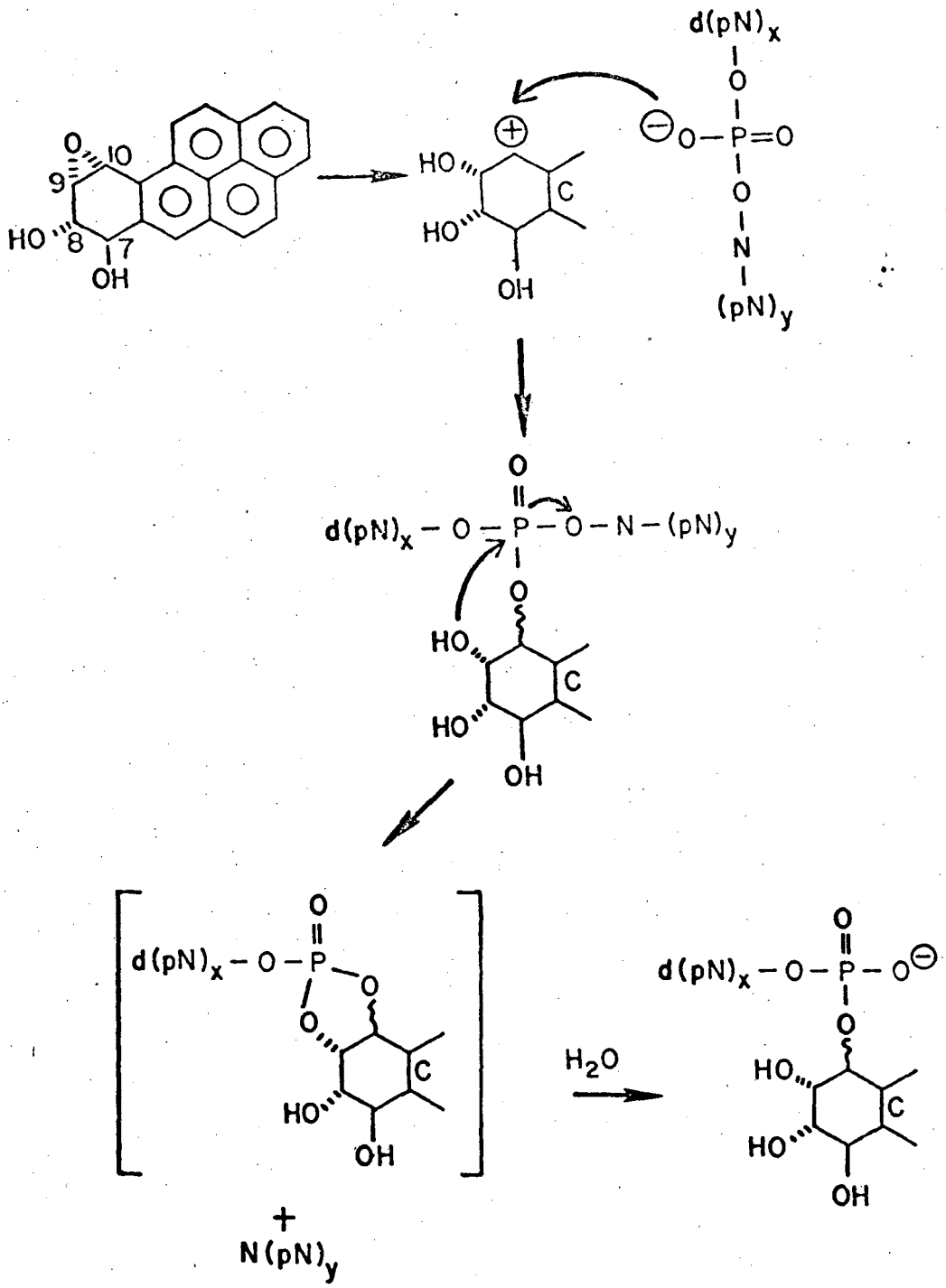

100
90
80
70
60
50
40
30
20
10
0
% form II DNA
0
4
8
12
16
20
24
Time (h)
XBL771-4159

Fig. 3



XBL771-4115A

Fig. 4

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