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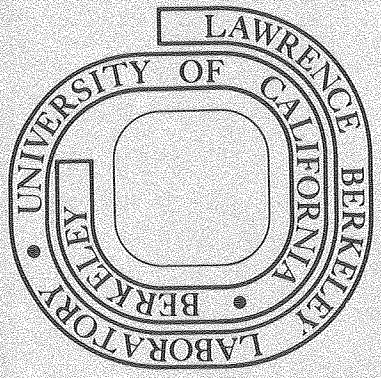

THE COVALENT BINDING OF ENANTIOMERIC BENZO[A]PYRENE DIOL EPOXIDES TO DOUBLE STRANDED DNA IS STEREOSELECTIVE

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ABSTRACT

Reaction of optically pure (+) and (-) 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene with DNA in vitro yielded diastereomeric covalent adducts with the exocyclic amino groups of deoxyguanosine and deoxyadenosine. The ratio of two deoxyguanosine diastereomers derived by reacting the (+) and (-) hydrocarbons with native calf thymus and double stranded ØX174 DNA was 20:1 while reaction of the enantiomers with heat denatured calf thymus and single stranded ØX174 DNA resulted in a ratio near 1:1. In contrast, deoxyadenosine diastereomer pairs were approximately 1:1 in all cases studied. The (+) and (-) enantiomers of the benzo[a]pyrene diol epoxide, therefore, interact asymmetrically with the guanine binding sites of double stranded but not single stranded polydeoxynucleotides. In contrast, reaction of the enantiomers with adenine is not stereoselective.

Benzo[a]pyrene (BaP) is a common environmental contaminant and a potent carcinogen and mutagen. The hydrocarbon belongs to a class of compounds which require metabolic activation prior to exerting their mutagenic and carcinogenic activities[1]. Based primarily on correlative evidence[2] it is widely held that the most probable initiating event which results in the biological properties of these hydrocarbons is covalent binding to DNA. The predominant activated form of BaP is a diol epoxide derivative[3]. Four possible isomers of the BaP diol epoxide are: (+) and (-) enantiomers of 7β,8α-dihydroxy9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (anti-BaP diol epoxide) and (+) and (-) enantiomers of 7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (syn-BaP diol epoxide). Although the principal microsomal diol epoxide metabolite has the (+) anti configuration[4] a recent report provides evidence that the (+) syn isomer is also formed in vivo[5].

As a model system for carcinogen DNA interactions we have been studying the covalent adducts formed as a result of chemical reaction between racemic (±) anti-BaP diol epoxide and DNA in vitro. We have reported that (±) anti-BaP diol epoxide forms two stable covalent adducts each with the exocyclic amino groups of deoxyguanosine and deoxyadenosine and two with deoxycytidine. In the latter case, the base position of the hydrocarbon was equivocal. Based on spectroscopic evidence we proposed that the pairs of adducts with deoxyguanosine and deoxyadenosine were diastereomers[6]. By resolving the (+) and (-) enantiomers of anti-BaP diol epoxide, and analyzing the covalent adducts formed by reaction of the optically pure compounds with DNA we have now demonstrated that 1) the (+) enantiomer reacts stereoselectively with deoxyguanosine in double stranded DNA, 2) the (+) and (-) enantiomers react equally with deoxyguanosine in single stranded DNA 3) the (+) and

(-) enantiomers react equally with deoxyadenosine in both single and double stranded DNA and 4) the proportion of deoxyadenosine adducts increases significantly in single stranded or denatured versus native DNA.

The (±)-BaP diol epoxide was synthesized as described[7]. [^{3}H](±)anti-BaP diol epoxide was synthesized by an adaptation of the method employed by McCaustland et al.[8]. The (+) and (-) enantiomers of trans-7,8-dihydroxy-7,8-dihydroBaP were resolved by published methods[9] and converted to (-) and (+) anti-BaP diol epoxides, respectively. Either racemic (±) anti-BaP diol epoxide or the optically pure (>96%) (+) or (-) enantiomers were reacted with calf thymus or ØX174 DNA. After removal of unreacted hydrocarbon the DNA was enzymatically hydrolyzed to hydrocarbon-deoxynucleosides and the stable covalent adducts isolated by Sephadex LH-20 column chromatography. The adducts were then separated by reverse phase high pressure liquid chromatography. Of the stable covalent adducts formed, 95% were recovered as determined by radioactivity measurements[10]. Fig. 1A shows the HPLC profile obtained by reacting racemic (±) anti-BaP diol epoxide with native calf thymus DNA after hydrolysis and Sephadex LH-20 column chromatography. We have previously identified adducts numbered 1-5, 7 and 8[6]. Peak number 1 is a tetraol hydrolysis product of the anti-BaP diol epoxide while adducts 2 and 4 represented deoxycytidine derived products. The contribution of peak 4 to total adduct formation was variable since it cochromatographed with one of the tetraol hydrolysis products of the diol epoxide. Adducts 3 and 5 were reaction products with deoxyguanosine while numbers 7, 8 and 9 were deoxyadenosine products. With native calf thymus DNA the deoxyguanosine adducts represented 90% of those formed. However, the distribution of adducts obtained depended upon the reaction conditions employed and was primarily a function of DNA secondary structure, i.e., the more denatured the polymer the higher the

proportion of adenine adducts obtained.

We had previously reported[6] that the pair of deoxyguanosine adducts (peaks 3 and 5) from reaction of racemic (±) anti-BaP diol epoxide with native calf thymus DNA were probably diastereomers, since their high resolution mass spectra were identical and their circular dichroism spectra were of similar shape but inverted. We have now demonstrated this result for both the deoxyguanosine and deoxyadenosine products by resolving the enantiomeric diol epoxides and carrying out adduct formation individually with DNA. The results of these reactions are shown in Figs. 1B and C. These profiles indicate that the (+) enantiomer reacted to form deoxyguanosine adduct 5 and deoxyadenosine adducts 7 and 9 while the (-) enantiomer resulted in deoxyguanosine adducts 3 and 6 and deoxyadenosine adduct 8. Peaks 6 and 9, although not previously identified, corresponded to deoxyguanosine and deoxyadenosine adducts respectively, as indicated by field desorption mass spectrometry[11].

Reaction of nucleophiles with anti-BaP diol epoxide has been shown to occur exclusively at the benzylic C-10 position, with a preference for trans addition[12]. It is possible, however, that DNA secondary structure could influence the stereochemistry of the epoxide ring opening to give cis addition products as well. Since the D-ribose moiety of each nucleoside unit within the DNA polymer contains an asymmetric center at C-1 , the reaction of a racemic mixture of (±) BaP diol epoxide would be expected to yield a maximum of four diastereomeric products per alkylation site ((+) diol epoxide/D-ribose and (-) diol epoxide/D-ribose, each with cis or trans addition at C-10 of the hydrocarbon)[13]. We obtained two resolvable deoxyadenosine diasteromers by reaction of the (+) anti-BaP diol epoxide with DNA (adducts 7 and 9), which were probably the result of cis and trans addition of the adenine exocyclic amino group. Reaction of the (-) diol

epoxide yielded peak 8 which by recycle chromatography consisted of two partially resolvable components. Each had virtually identical mass spectra[11] indicating they were deoxyadenosine exocyclic amino adducts. Thus, the (-) enantiomer reacted with deoxyadenosine to give two adducts in agreement with the above predictions.

Three hydrocarbon-deoxyguanosine adducts were observed in the reaction of racemic (±) anti-BaP diol epoxide with DNA - peaks 3, 5 and 6. Peaks 3 and 5 corresponded to the major and, presumably, trans addition products of (-) and (+) diol epoxide with N^2-deoxyguanosine, respectively. Peak 6, derived from the (-) enantiomer, was a relatively minor component and may correspond to either an exocyclic amino cis addition product or to a different guanine adduct with alkylation at some other site. For deoxycytidine only two adducts (peaks 2 and 4) were observed. The relative contributions of (+) and (-) anti-BaP diol epoxide to these two peaks was difficult to assess, since peak 4 also contained varying amounts of a tetraol hydrolysis product.

Fig. III shows the structures of the trans-addition adducts and their percent distribution obtained by reaction of racemic (±) anti-BaP diol epoxide and native calf thymus DNA. (These percentages also represent any cis-addition products.) With the identification of adduct 9, we found that reaction of racemic (±) anti diol epoxide with DNA resulted in nearly equal distribution of deoxyadenosine diastereomer products, i.e., adducts 7 and 9 from the (+) enantiomer of the hydrocarbon were about equal to adduct 8 from the (-) enantiomer. By contrast, deoxyguanosine in DNA reacted stereoselectively with racemic anti-BaP diol epoxide, i.e., the ratio of adduct 5 derived from the (+) hydrocarbon to that of adduct 3 from the (-) enantiomer was 20:1. The same ratio of diastereomers was obtained by reaction of the optically resolved hydrocarbons and native calf thymus

(Figs. 1B and C). We initially postulated that the stereoselective binding could be a result of the hydrocarbons interacting asymmetrically with the secondary structure of DNA[6]. This postulate was tested by reacting racemic (±) anti-BaP diol epoxide with double (Fig. IIA) and single (Fig. IIB) stranded ØX174 DNA. The same distribution of stereoselective guanine adducts were obtained with native calf thymus DNA (Fig. IA) and double stranded ØX174 DNA (Fig. IIA). Reaction of racemic (±) anti-BaP diol epoxide with single stranded ØX174, however, resulted in nearly equal distribution of hydrocarbon-deoxyguanosine diastereomers (peaks 3 and 5). In addition, reaction with the single stranded polydeoxynucleotide resulted in a significantly increased proportion of deoxyadenosine adducts. These same results were obtained with native and heat or base denatured calf thymus DNA. The ratio of deoxyguanosine diasteriomers in the single stranded calf thymus DNA's was approximately 1:1 and the proportion of deoxyadenosine adducts was 40% compared to 10% in the double stranded polydeoxynucleotide. These results demonstrated that the asymmetric binding of the two enantiomeric anti-BaP diol epoxides to the exocyclic amino group of guanine in double stranded DNA was dependent on the secondary structure of the polymer. One possible mechanism for the ability of DNA secondary structure to distinguish two enantiomeric molecules is by stereoselective physical interactions (possibly intercalation) prior to covalent binding. Intercalation or some other physical interaction may readily occur with the (+) enantiomer but not with the mirror image hydrocarbon. Alternatively, the non-covalent interactions could "fix" the hydrocarbon so that only one configuration is in a favorable position to react with the exocyclic amino group of deoxyguanosine. The following observations suggest that intercalation or some physical association between the BaP diol epoxide and DNA takes place prior to covalent binding:

1) as with intercalation of BaP[14] or actinomycin[15] into DNA, binding of the diol epoxide occurs with a preference for guanine sites and 2) reaction with denatured DNA "randomizes" adduct formation (loss of stereoselectivity and more adenine binding).

Although these results do not prove that physical association or intercalation is involved, we propose such a model to account for the stereoselective interactions between BaP diol epoxide and DNA. The lack of any stereoselectivity in the adenine covalent binding sites with either double or single stranded DNA suggests that the reaction mechanism may be different from that of guanine binding. In the case of adenine the hydrocarbon may react without any physical interactions modulating the ratio of diastereomers obtained.

A correlation between DNA binding and carcinogenic or mutagenic potency for a number of polycyclic aromatic hydrocarbons has been reported[2]. Wood et al.[16] has determined the difference in the mutagenic activities of the (+) and (-) anti-BaP diol epoxides utilizing a mammalian tissue culture cell line and two bacterial tester strains. The difference in the mutagenic activity between the (+) and (-) anti diol epoxides in the mammalian cell line was of the same order as we found for the difference in the level of covalent binding of the two isomers to the N^2-guanine site in native DNA. Therefore, the extent to which these activated hydrocarbons chemically react with DNA in vitro correlates with their biological activities. These results suggest that, in addition to metabolic factors, chemical and physical interactions with DNA may be important in evaluating the biological potency of carcinogenic and mutagenic polycyclic aromatic hydrocarbons.

The regulation of gene expression usually involves protein-nucleic acid interactions[17]. Since we have demonstrated highly selective binding between small organic molecules and DNA, the possibility exists that this type of interaction may also play a regulatory role in the normal functioning cell.

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FIGURE LEGENDS

Fig. 1: Tritium labeled racemic (±) anti-BaP diol epoxide or the resolved enantiomers were incubated with 1 mg deproteinized native calf thymus DNA at an initial concentration of 1 hydrocarbon/250 nucleotides in 0.01 M sodium phosphate, pH 7.5, for 1 hr at 37°C. Unbound hydrocarbon was removed by ethyl acetate extraction and repeated isopropanol precipitation of the DNA until zero time blanks contained <3% of the tritium of incubated samples. Routinely, incubated samples resulted in a modified DNA of 1 hydrocarbon/8000 base pairs. The diol epoxide treated DNA was converted to hydrocarbon-deoxynucleosides and deoxynucleosides by treatment with DNase II, spleen phosphodiesterase and alkaline phosphatase. The hydrolyzed sample was applied to a Sephadex LH-20 column and the free deoxynucleosides and enzymes eluted with water. The hydrocarbon-deoxynucleosides were quantitatively recovered with methanol and analyzed by high pressure liquid chromatography (6). The elution profiles represent DNA reacted with (A) racemic (±) anti-BaP diol epoxide (B) (+) anti-BaP diol epoxide (C) (-) anti-BaP diol epoxide. The identity of adducts 1-9 are discussed in the text.

Fig. II: Racemic (±) anti-BaP diol epoxide was reacted with (A) double stranded (replicative) ØX174 DNA and (B) single stranded (viral) ØX174 DNA. Adducts were formed and isolated as described in Fig. 1.

Fig. III: The structure and percent distribution of the principal guanine and adenine trans addition products obtained by reacting racemic (±) anti-BaP diol epoxide with native calf thymus DNA. The distribution also includes any cis addition products (see text).

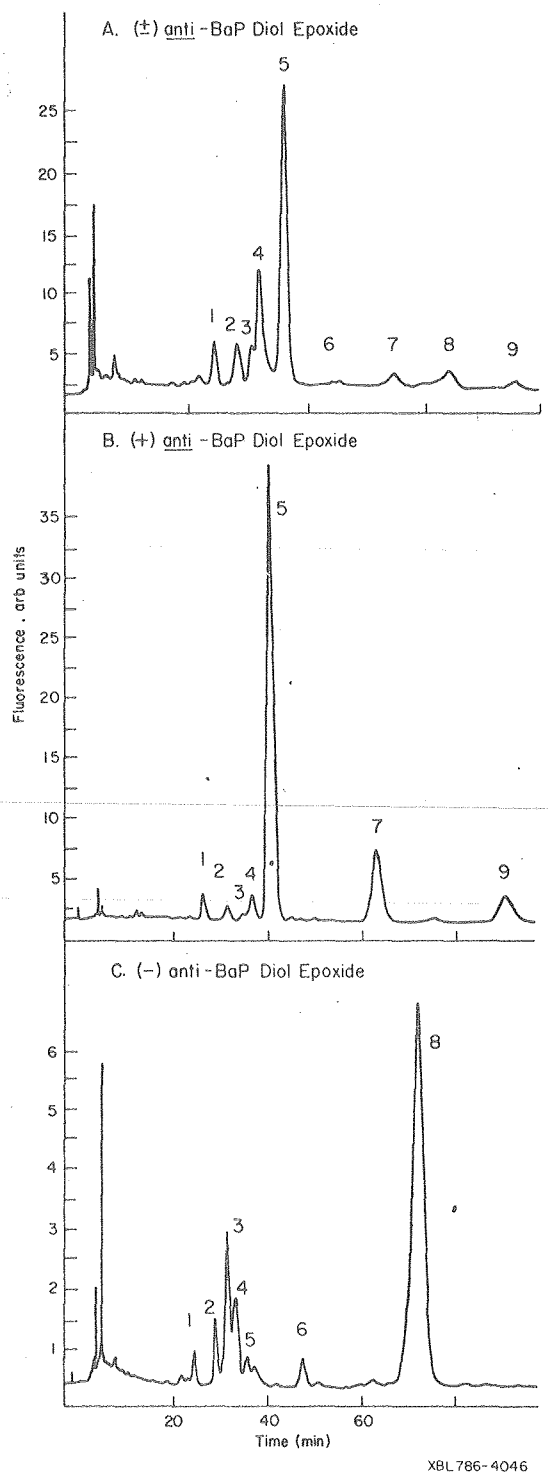
A. (±) anti-BaP Diol Epoxide
B. (+) anti-BaP Diol Epoxide
C. (-) anti-BaP Diol Epoxide
Fluorescence, arb units
Time (min)
XBL786-4046

Fig. 1

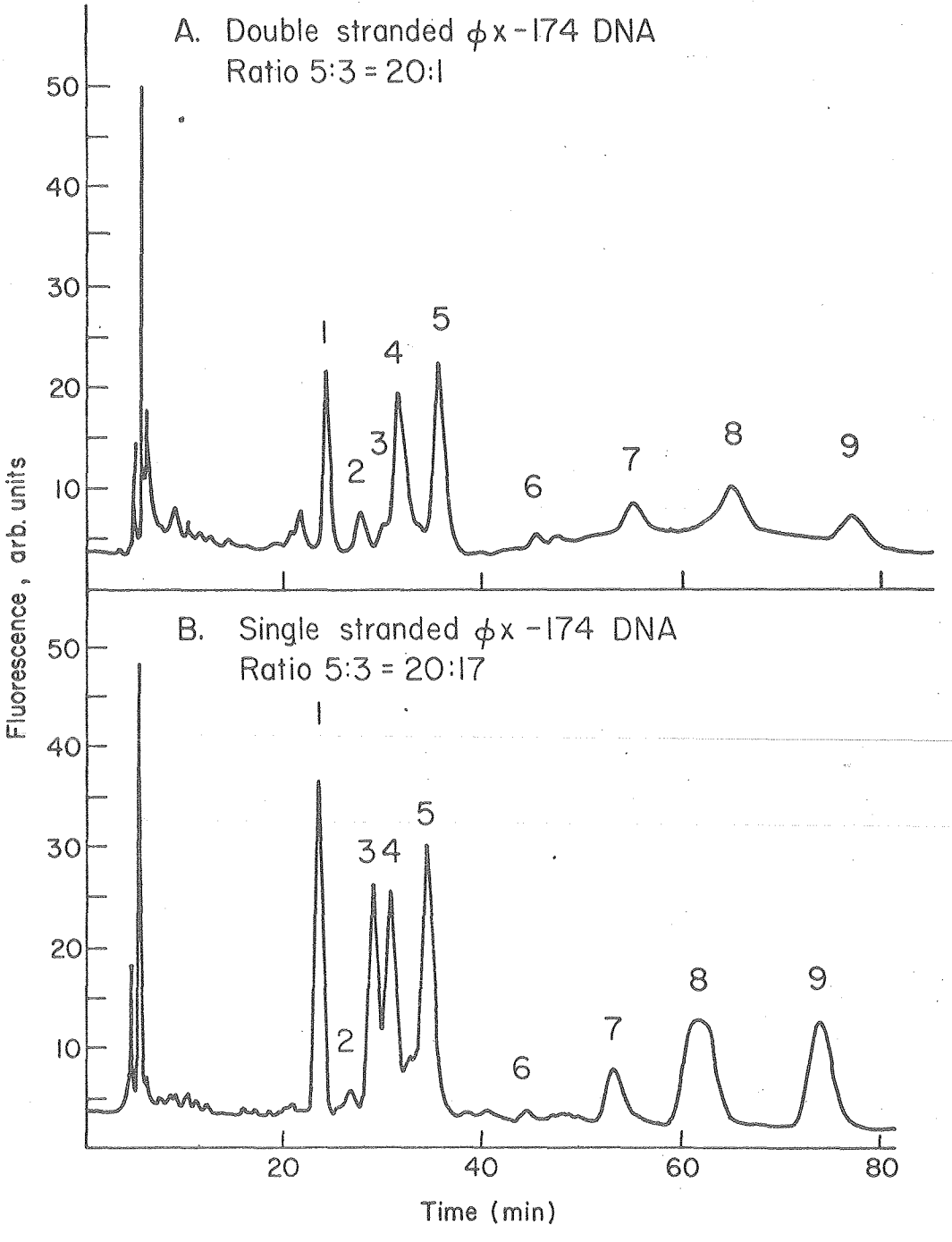

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Fig. 2

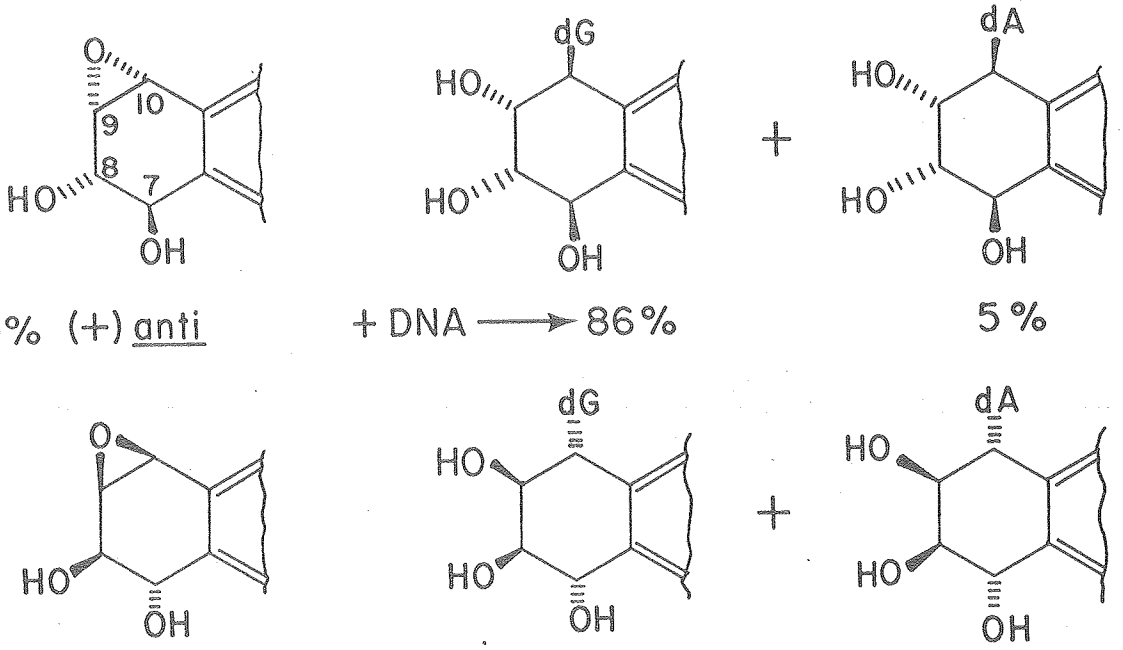

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Fig. 3

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