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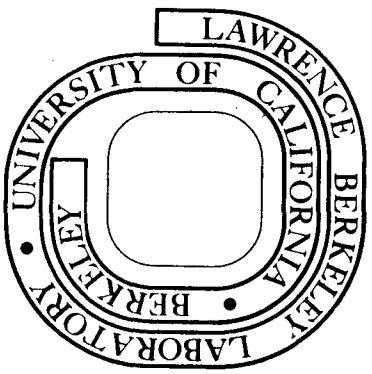

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DISCLAIMER

BENZO[A]PYRENE DIOL EPOXIDE COVALENTLY BINDS TO DEOXYGUANOSINE AND DEOXYADENOSINE IN DNA

Chemical carcinogens, for example benzo[a]pyrene (BaP), are metabolised to reactive intermediates which bind covalently to cellular macromolecules[1-3] and the extent of binding appears to correlate with the carcinogenic potency of the hydrocarbons[1]. Through a variety of mutagenicity, metabolism and binding studies, the intermediate which undergoes formation of a stable covalent complex with DNA has been identified as 7β,8α-dihydroxy-9α, 10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BaP diol epoxide)[4-9]. The structure of a covalent adduct formed between this hydrocarbon and poly(G) has also been published[6,9].

We now report the identity of several adducts obtained by reacting (±)BaP diol epoxide with tritium labelled DNA. Four differently labelled lots of DNA were synthesized in vitro with DNA polymerase I by incorporating in each case three unlabelled and one tritium labelled nucleoside triphosphate. Through the use of this unambiguous labelling technique we have demonstrated that activated BaP forms two adducts with deoxyguanosine, two with deoxyadenosine and possibly one with deoxycytidine, while reaction with deoxythymidine was not detected. This approach also allowed the relative percent of each adduct to be calculated. The deoxyguanosine adducts predominated and constituted 92% of the total stable covalent adducts formed.

(±)BaP diol epoxide was synthesized as previously described[10,11]. Covalent binding of adducts to calf thymus DNA was carried out at neutral pH by enzymatic activation of [^{3}H]BaP or reaction of unlabelled or [^{14}C](±)BaP diol epoxide directly with the nucleic acid. The phosphodiester backbone

of the DNA was enzymatically hydrolysed and the products were chromatographed on Sephadex LH-20. Hydrophobic material eluting from the column was concentrated and further analyzed by high pressure liquid chromatography (HPLC).

Figure Ia presents a HPLC profile of hydrolysis products resulting from a co-injection of unlabelled DNA reacted with 1) [^{3}H]BaP plus microsomes and 2) unlabelled (±)BaP diol epoxide. Chromatography of DNA-BaP diol epoxide adducts alone results in a fluorescence trace identical with that of Figure Ia. However, chromatography of the microsome catalysed adducts alone results in detection of peaks 1, 4, 5 and 7 only. Therefore, the reaction between (±)BaP diol epoxide and DNA yields 3 more adducts (peaks 2, 3 and 6) than BaP plus microsomes and DNA.

The bases to which (±)BaP diol epoxide binds were identified by reacting [^{14}C](±)BaP diol epoxide with each of four samples of [^{3}H] labelled DNA. The DNA samples were labelled unambiguously with [^{3}H] in either deoxyguanine, deoxyadenine, deoxycytosine or deoxythymine. The four samples of labelled DNA were prepared by incorporating three unlabelled triphosphates and one [^{3}H] labelled triphosphate into calf thymus DNA by Escherichia coli DNA polymerase I. The reaction conditions were essentially as previously described[12], except that the DNA template was untreated and the tritiated nucleoside triphospate was not diluted with cold material. The labelled DNA was ethanol-precipitated, dialysed, reacted with [^{14}C](±)BaP diol epoxide and the covalent adducts were isolated after hydrolysis and Sephadex LH-20 chromatography. The results of three of the four double label experiments are presented in figures Ib, IIa and IIb. HPLC of these samples resolved five [^{14}C] labelled adducts. Figure Ib represents reaction of [^{14}C](±)BaP diol epoxide with [^{3}H] deoxyguanine labelled DNA. Peaks 3 and 5 contain both [^{3}H] and [^{14}C] and therefore represent hydrocarbon-deoxyguanosine adducts. From the

specific activities of both the hydrocarbon and base a one to one ratio of the two components was calculated. Similar inspection of Figures IIa and b identifies peaks 6 and 7 as deoxyadenosine adducts while peak 4 may be a deoxycytidine complex. Assignment of this adduct must remain tentative because of the lack of complete symmetry and coincidence of the [^{3}H] and [^{14}C]. Reaction of deoxythymidine was not observed despite our ability to detect approximately 1 adduct per 2.5 x 10^{7} base pairs. The relative percent of each adduct formed is shown in Table 1. Binding occurred to the extent of 92% deoxyguanosine, 5% deoxyadenosine, 3% deoxycytidine and 0% deoxythymidine. These results are not in accord with those obtained by reaction of (±)BaP diol epoxide with model nucleic acids[13], where the hydrocarbon reacted with poly(G), poly(A) and poly(C) to a similar extent. The reactions with model systems were carried out in 50% acetone which would have denatured the nucleic acids[13], while the conditions used here were more favorable to maintaining helical conformation. The differences in reaction conditions may explain the relative lack of binding asymmetry found with the model systems.

Two adducts were obtained for both deoxyguanosine and deoxyadenosine and this could also be the case for deoxycytidine since peak 2 was not identified and could represent a second deoxycytidine adduct. The formation of two adducts with each base could have resulted either from the reaction with both stereoisomers of (±)BaP diol epoxide or alternatively a single isomer forming cis and trans addition products. Reaction of (±)BaP diol epoxide with poly(G) results in predominately trans addition of equal amounts of both stereoisomeric hydrocarbons[6]. We also analyzed the adducts formed between (±)BaP diol epoxide and poly(G) by HPLC and CD spectroscopy. In confirmation of the results of Weinstein et al[6], we found that two major adducts were formed in about equal amounts and correspond to reaction

of (+) and (-)BaP diol epoxide. In addition two minor adducts were found which could represent (+) and (-)BaP diol epoxide reacting by cis addition of the guanine base.

Only one deoxyguanosine adduct was formed with the microsome system. This probably represents trans addition of only one enantiomer since enzymatic formation of BaP diol epoxide is stereoselective[14]. Two adducts were were formed by reaction of (±)BaP diol epoxide with DNA and by analogy with the poly(G) system correspond to adduct formation with the (+) and (-) enantiomers of the hydrocarbon. In contrast to the poly(G) system where diastereomers are formed in approximately equal amounts, the DNA adducts occur at 90 and 10% for deoxyguanosine and 80 and 20% for deoxyadenosine. The amounts of the two adducts with deoxyguanosine and deoxyadenosine tend to equalise upon binding of the hydrocarbon to heat denatured DNA. Also, there is an increase in the overall proportion of deoxyadenosine adducts. These results suggest that the assymmetric binding of the two stereoisomers of (±)BaP diol epoxide is less with single stranded DNA than with double stranded DNA. The preferential reaction of one enantiomer with DNA may be related to the dissymmetry of the right handed helix, while the increase in deoxyadenosine adducts may result from topological changes in DNA conformation. Thus, the rates, sites and amounts of products obtained by reacting activated BaP with nucleic acids are probably controlled to a large degree by the amount of helical form and the conformation of the polymer. Of the adducts which have not been characterised the identity of peak 2 remains unknown while peak 1 co-chromatographs by HPLC with a hydrolysis product of (±)BaP diol epoxide. The high resolution mass spectrum of the main deoxyguanosine adduct (peak 5) is consistent with a structure in which the 10-position of the hydrocarbon is attached to the N^2-exocyclic amine of the base. This structure is analogous to that obtained

by reacting (±)BaP diol epoxide with poly(G)[6]. Quantitatively the most important adduct is binding to the exocyclic amine of guanine. Since (±)BaP diol epoxide bound only to those bases with a free amino group it is possible that this is the preferred binding site of the hydrocarbon.

In addition to the base adducts reported here, (±)BaP diol epoxide catalyses DNA and RNA strand scission[15]. Despite the fact that 250-300 base adducts are formed for each strand scission[15] the relative importance, and indeed physiologic significance, of each of these processes are unknown. Ethyl nitrosourea forms alkyl phosphotriesters and strand scission which are lethal[16]. Since (±)BaP diol epoxide undergoes similar reactions this could account for some of the cytotoxic properties of BaP[17]. Furthermore, the excision repair of adenine carcinogen adducts in a number of cases is known to occur at a greater rate than those of guanine[18]. The N^2-exocyclic amine adduct of deoxyguanine-BaP diol epoxide may therefore have a relatively long biological half life. Since cancer induction occurs over a long period of time this may be an important adduct in the biological transformation caused by BaP.

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Table 1 Relative distribution of (±) BaP diol epoxide adducts with the bases of DNA

Peak #*	1≠	2	3	4	5	6	7
Base	-	?	dG_1	dC[1≠]	dG_2	dA_1	dA_2
Percent total based on§							
[^{3}H]	-	-	10	2.4	86	0.1	1.4
[^{14}C]	-	-	10	3	82	1	4

* Peak # refers to Figure Ia

≠ Peak #1 is probably a hydrolysis product of (±)BaP diol epoxide

≠ Assignment of this adduct is tentative. See text.

§ Computed from Figure Ib.

Figure 1. HPLC profiles of BaP and (±)BaP diol epoxide-DNA adducts were carried out on a Varian model 8500 fitted with two columns (Waters μBONDAPAK C_{18}, 4 mm by 30 cm) in series and eluted with 50% methanol water. BaP covalent adducts were isolated after reaction of [G-^{3}H]BaP with DNA in the presence of NADPH and rat liver microsomes from animals pre-treated with 3-methylcholanthrene. Alternatively, unlabelled or [^{14}C](±)BaP diol epoxide was reacted directly with unlabelled or [^{3}H] labelled DNA under the same conditions as the enzyme activation of the hydrocarbon. The unlabelled or [^{14}C](±)BaP diol epoxide was used at the same concentration as the [^{3}H]BaP. The [^{3}H] labelled DNA's were enzymatically made by incorporation of three unlabelled and one [^{3}H] labelled nucleoside triphosphate with DNA polymerase from Escherichia coli. Calf thymus DNA, untreated, was used as template. The labelled nucleoside triphosphate was used undiluted and this procedure resulted in DNA with a specific activity of 14 μCi/mg. Four DNA samples were synthesized with [^{3}H] located in either 1) deoxyguanine, 2) deoxyadenine, 3) deoxycytosine or 4) deoxythymine. Following reaction of the hydrocarbon with DNA any protein was removed by phenol extraction. The DNA was precipitated with ethanol and the precipitate heated to remove intercalated hydrocarbon. The DNA was enzymatically hydrolysed and hydrophobic material isolated by Sephadex LH-20 chromatography. The HPLC profiles represent adducts obtained from LH-20 columns. a) Co-injections of [^{3}H]BaP plus microsomes reacted with unlabelled DNA and unlabelled (±)BaP diol epoxide reacted with unlabelled DNA and b) [^{14}C](±)BaP diol epoxide reacted with [^{3}H] deoxyguanine labelled DNA.

Figure II. HPLC profile of LH-20 samples isolated as described in Figure I after reaction of [^{14}C](±)BaP diol epoxide with a) [^{3}H] deoxyadenine labelled DNA and b) [^{3}H] deoxycytosine labelled DNA.

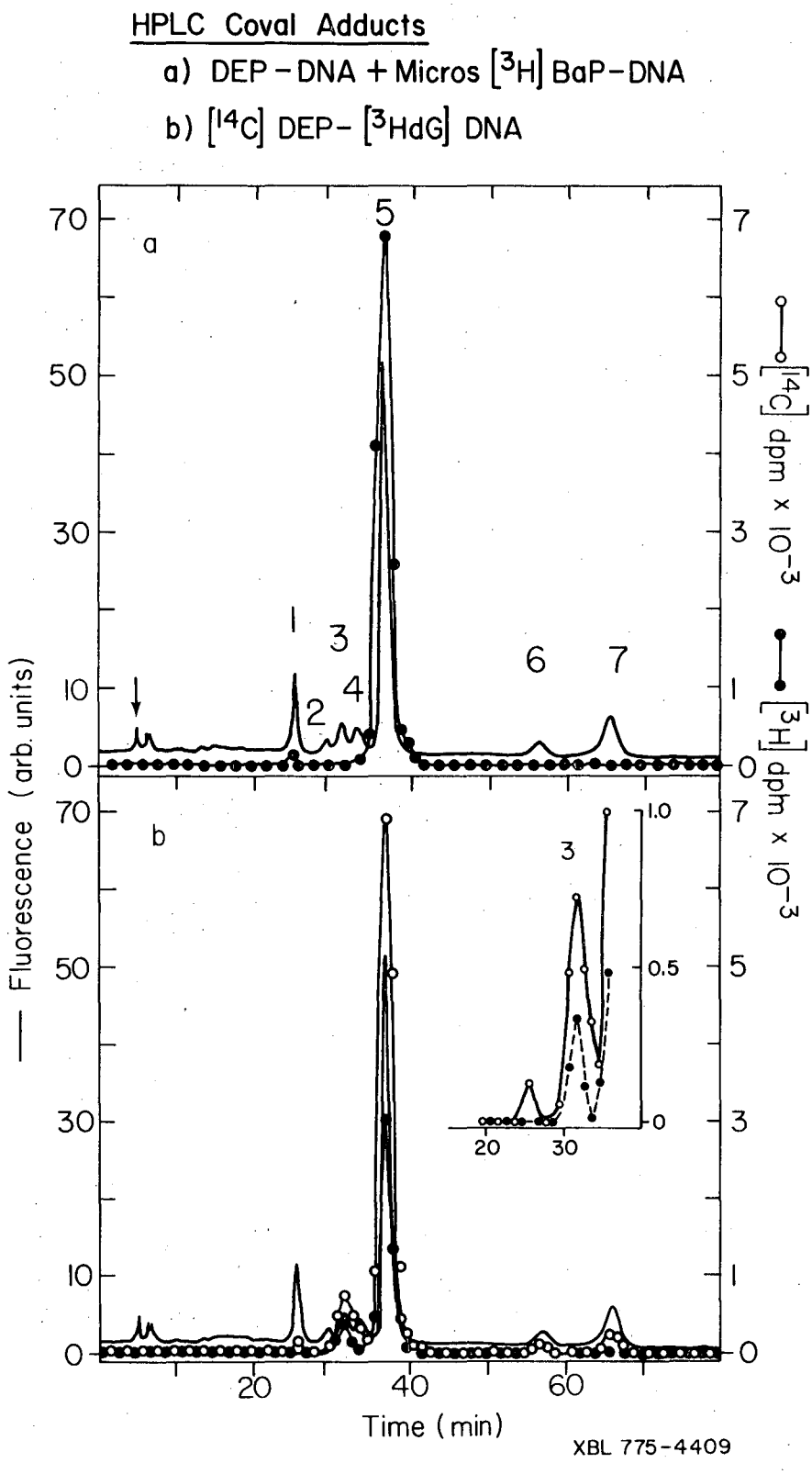

Fig. 1

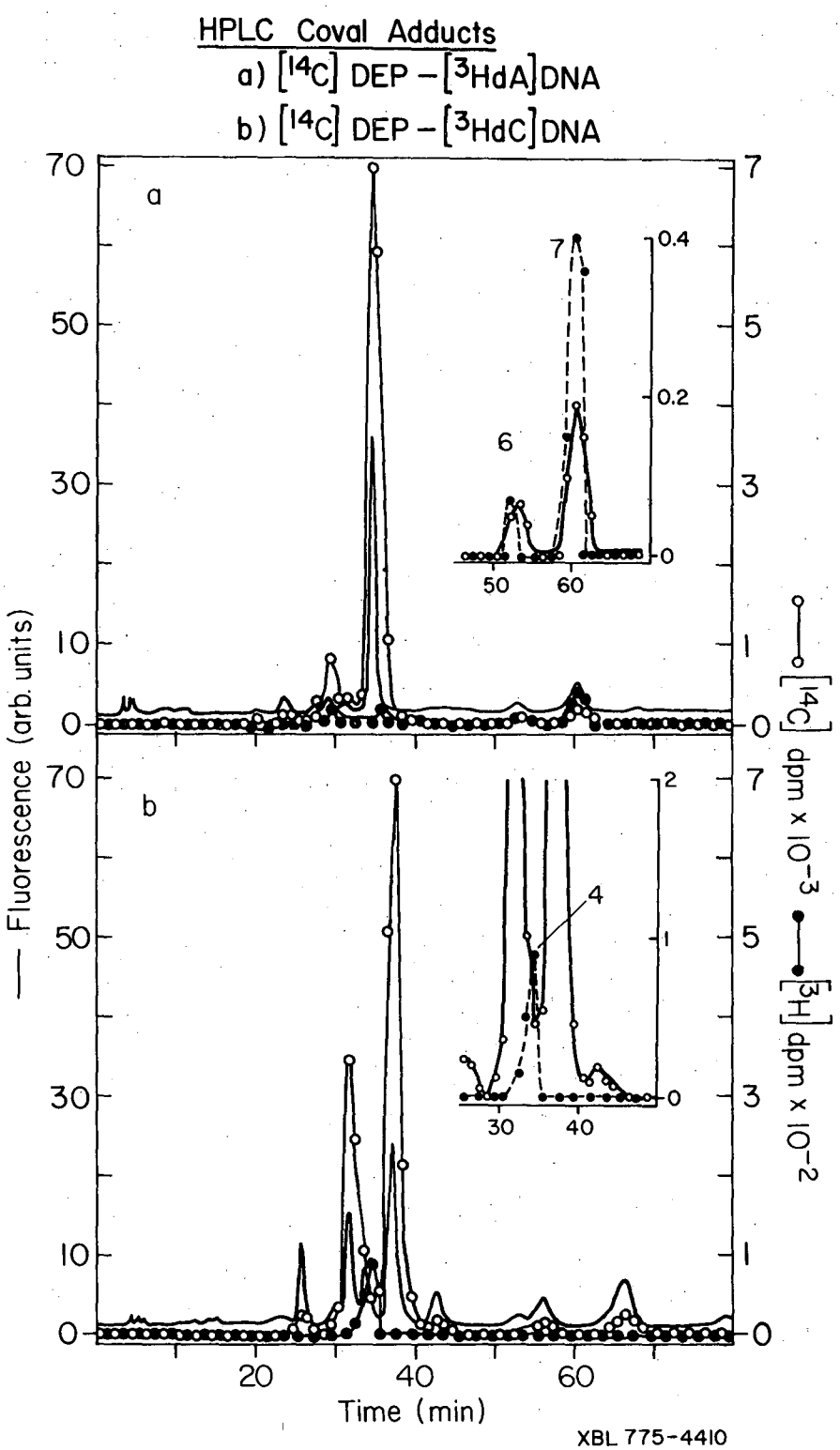
HPLC Coval Adducts
a) [14C] DEP – [3HdA]DNA
b) [14C] DEP – [3HdC]DNA
a
b
6
7
4
— Fluorescence (arb. units)
[14C] dpm x 10-3
[3H] dpm x 10-2
Time (min)
XBL 775-4410

Fig. 2

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