MUTAGENICITY OF METABOLICALLY ACTIVATED BENZO[a]ANTHRACENE 3,4-DIHYDRODIOL: EVIDENCE FOR BAY REGION ACTIVATION OF CARCINOGENIC POLYCYCLIC HYDROCARBONS

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SUMMARY: Benzo[a]anthracene and the 5 metabolically possible trans dihydrodiols of benzo[a]anthracene were metabolized, in the presence of S. typhimurium strain TA100, by a highly purified hepatic microsomal monooxygenase system. The metabolic product(s) of benzo[a]anthracene 3,4-dihydrodiol was nearly 10 times as mutagenic to the bacteria as were the metabolites of benzo[a]anthracene and the other four dihydrodiols. The marked activation of benzo[a]anthracene 3,4-dihydrodiol, presumably to the 3,4-diol-1,2-epoxide is consistent with and supports the hypothesis that bay region epoxides of unsubstituted polycyclic hydrocarbons are ultimately reactive forms of these carcinogenic compounds.

Interest in diol epoxides as potential ultimate carcinogens of benzo[a]-pyrene (BP)[1] was stimulated by observations that BP 7,8-dihydrodiol was bound more extensively to DNA on metabolic activation than was BP or several other metabolites of BP (1) and that a 7,8-diol-9,10-epoxide of BP accounted for much of the chemical binding to DNA in vivo (2). Since our report of the remarkably high mutagenicity of one of the stereosiomeric trans-7,8-diol-9,10-epoxides of BP at the International Pharmacology Congress (3) in the summer of 1975, several papers have appeared on the high mutagenicity of these compounds toward bacterial (4-7) and mammalian cells (4,6-9). In contrast, all of the known primary oxidative metabolites of BP are relatively weak or inactive as mutagens when compared to the 7,8-diol-9,10-epoxides (3,4,6-11).

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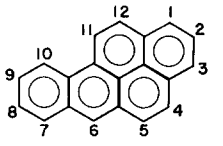
[1] Abbreviations used are BP, benzo[a]pyrene; BA, benzo[a]anthracene; BA 3,4-dihydrodiol, trans-3,4-dihydroxy-3,4-dihydro BA; BA 1,2-, 5,6-, 8,9- and 10,11-dihydrodiols, other trans dihydrodiols of BA.

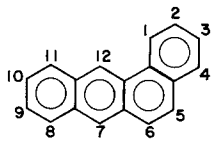
In an attempt to correlate structure with carcinogenic activity, we have surveyed the literature to ascertain the effects of substituents on the carcinogenic activity of polycyclic aromatic hydrocarbons and have found that substitutents on angular benzo rings of polycyclic hydrocarbons markedly reduce carcinogenic activity (12), presumably through blocking metabolism at that portion of the molecule. Furthermore, we have calculated that benzylic carbonium ions such as would be derived from the epoxides of diol epoxides on tetrahydrobenzo rings of carcinogenic hydrocarbons should have an unusual ease of formation when the carbonium ion is located in the "bay region"[2] of the hydrocarbon (12,13). In accord with these calculations 7,8,9,10-tetrahydro BP 9, 10-epoxide, with the benzylic carbon atom of the oxirane ring in the bay region of the hydrocarbon, is far more mutagenic than the isomeric 7,8,9,10-tetrahydro BP 7,8-epoxide in which the oxirane ring is remote from the bay region (7). Thus, a consistent theory emerges; the carcinogenicity of polycyclic aromatic hydrocarbons results from metabolic formation of extremely reactive benzo ring diol epoxides in which the epoxide ring is situated near the requisite bay region of the hydrocarbon. To test this concept, the present study evaluates the metabolic activation of the five possible trans, vicinal dihydrodiols of the hydrocarbon benzo[a]anthracene (BA). Our prediction that among the several dihydrodiols of BA the BA 3,4-dihydrodiol should be metabolically activated to the most mutagenic metabolite, presumably isomeric 3,4-diol-1,2-epoxides based on prior studies of the metabolism of BP 7,8 dihydrodiol to one (9) or both (14) of the isomeric 7,8-diol-9,10-epoxides, has proved to be valid and supports our prior quantum mechanical calculations (13) which predict biological activity or the lack of it for the unsubstituted polycyclic hydrocarbons.

MATERIALS AND METHODS: Dilauroyl phosphatidylcholine was obtained from Serdary Res. Labs, Ontario, Canada, while BA and other commercially available biochemicals were obtained from Sigma Chemical Co., Inc., St. Louis, Mo. The five possible trans dihydrodiols of BA were synthesized by unequivocal chemical procedures and their structures were confirmed by nuclear magnetic resonance spectrometry (15). The analytically pure dihydrodiols were stored at -90° and dissolved immediately before use in acetone/NH_4OH (1000:1) under subdued light. Oxidative metabolism of BA and BA dihydrodiols was catalyzed by a highly purified and reconstituted hepatic microsomal monooxygenase system consisting of cytochrome P-448 from 3-methylcholanthrene-treated rats, NADPH-

[2] A bay region in a polycyclic aromatic hydrocarbon exists when two non-adjacent benzene rings, one of which is a benzo-ring, are in close proximity. The prototype for a bay region is the sterically hindered area between the 4- and 5-positions of the phenanthrene molecule. Thus, the region between 10- and 11-positions of BP and the 1- and 12-positions of BA (Figure 1) are bay regions.



BENZO[a]PYRENE



BENZO[a]ANTHRACENE

Figure 1. Structure of benzo[a]pyrene and benzo[a]anthracene. The region between the 10 and 11 positions of BP and the 1 and 12 positions of BA are bay regions.

cytochrome c reductase (150 units), dilauroyl phosphatidylcholine (0.08 μmol) and NADPH (0.1 μmol) (16). Reaction mixtures of 0.5 ml, contained 2 x 10^8 histidine requiring bacteria of strain TA100 of S. typhimurium (17) and were as previously described for the metabolic activation of benzo[a]pyrene and benzo[a]pyrene derivatives (18). Mutations were quantified two days after treatment by counting the number of macroscopic colonies of bacteria which were present on a histidine deficient agar medium. All experiments were performed in triplicate and in those cases where standard deviations of the mean were omitted for clarity, the coefficients of variation averaged 13 percent.

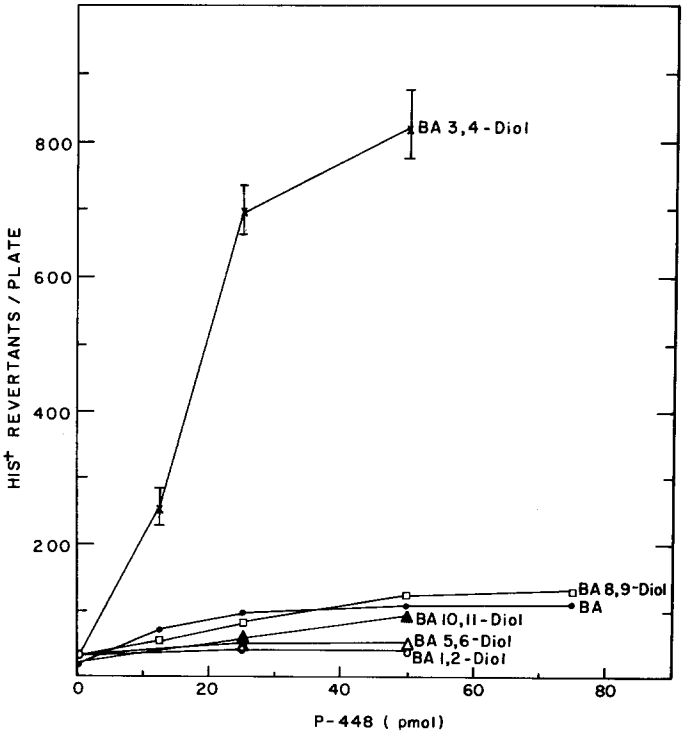


Figure 2. Effect of cytochrome P-448 concentration on the metabolic activation of BA and BA diols. Incubation time was 15 minutes at 37° and the concentration of each hydrocarbon was 25 μM.

RESULTS: BA 3,4-dihydrodiol is metabolically activated to a product(s) which is highly mutagenic to strain TA100 of S. typhimurium. Reversion to histidine autotrophy is proportional to the amount of cytochrome P-448 present in the reaction mixture (Figure 2) and as little as 25 pmol of the hemoprotein induces a 30 fold increase in mutation frequency. In marked contrast to these results BA and the next most active dihydrodiol, BA 8,9-dihydrodiol, are metabolized to products which induce less than a 5-fold increase in mutation frequency in the presence of 75 pmol of cytochrome P-448. The BA 1,2-, 5,6- and 10,11-dihydrodiols undergo little or no metabolic activation to mutagenic products. Since the number of histidine revertants induced in the absence of cytochrome P-448 (ordinate intercept, Figure 2) are within spontaneous background levels, BA and the BA dihydrodiols are without intrinsic mutagenic activity in strain TA100.

The number of mutations induced by the metabolite(s) of BA 3,4-dihydrodiol is proportional to the duration of the monooxygenase reaction (Figure 3A).

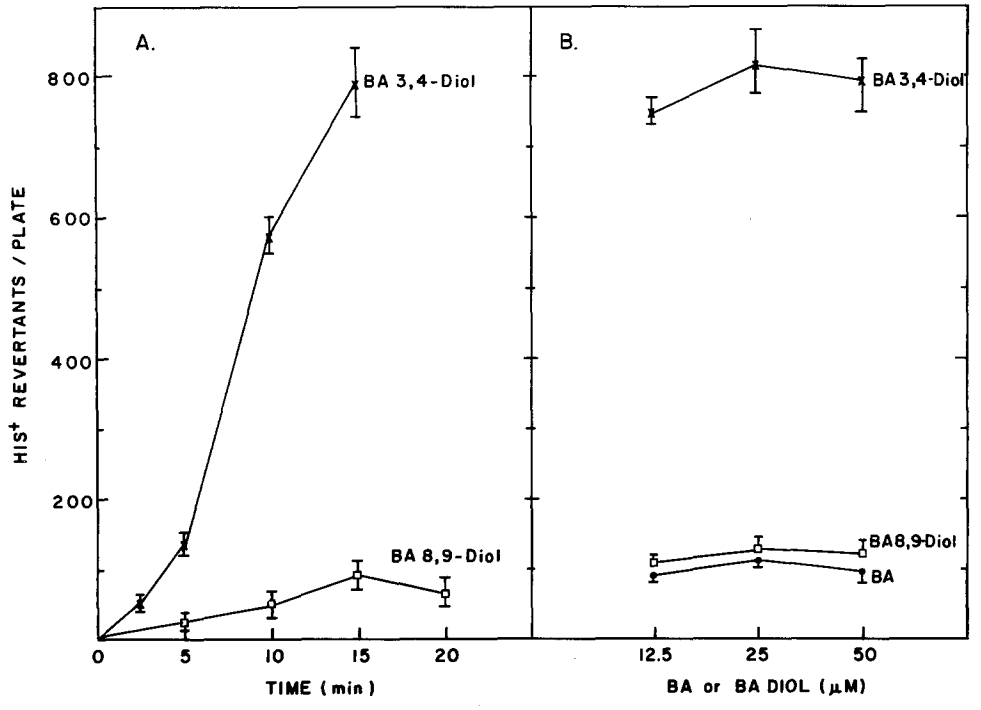


Figure 3. Effect of incubation time (A) and hydrocarbon concentration (B) on mutations induced by the metabolism of BA or BA dihydrodiol. Dihydrodiol concentration was 25 μM in (A), incubation time was 15 minutes in (B) and 50 pmol of cytochrome P-448 was used in both experiments.

After a 15 minute incubation with 50 pmol of cytochrome P-448, metabolites of BA 3,4-dihydrodiol induce 9 times as many histidine revertants as do the metabolic products of BA 8,9-dihydrodiol. The marked activation of BA 3,4-dihydrodiol relative to BA or BA 8,9-dihydrodiol is not due to suboptimal concentrations of the hydrocarbons in the incubation mixture since all three compounds show maximal activation at a 25 μM concentration (Figure 3B).

DISCUSSION: Of the five possible trans dihydrodiols of BA, only BA 3,4-dihydrodiol is metabolized to a product(s) highly mutagenic to S. typhimurium strain TA100. Similarly, recent studies with four trans dihydrodiols of BP (BP 4,5-, 7,8-, 9,10- and 11,12-dihydrodiols) have shown that only BP 7,8-dihydrodiol is metabolized to a product(s) with high mutagenicity (5,9,18) and DNA binding capacity (1). In addition, BP 7,8-dihydrodiol (19) and its immediate metabolic precursor, BP 7,8-oxide (20), are both highly carcinogenic on mouse skin. The high chemical reactivity (including binding to DNA), cytotoxicity and mutagenicity of the two stereoisomers of BP 7,8-diol-9,10-epoxide (2-9) strongly implicate these metabolites as reactive ultimate carcinogens obtained on metabolism of BP 7,8-dihydrodiol. Likewise, our "bay region" concept (12) predicts that the isomeric BA 3,4-diol-1,2-epoxides should be the most mutagenic metabolites of BA. In support of this hypothesis is the present observation that only BA 3,4-dihydrodiol is metabolically activated to potent mutagens when BA and the 5 metabolically possible trans dihydrodiols of BA are utilized as substrates. By analogy to the metabolism of BP 7,8-dihydrodiol (14), the 2 stereoisomeric 3,4-diol-1,2-epoxides in which the benzylic 4-hydroxy group is either syn or anti to the epoxide ring are expected to be highly mutagenic metabolites of BA 3,4-dihydrodiol.

The 1,2-position of BA 3,4-dihydrodiol and the 9,10-position of BP 7,8-dihydrodiol are structurally similar in that these are the only positions of the hydrocarbons at which a bay region epoxide of a saturated, angular benzo-ring can be metabolically formed. Perturbational molecular orbital calculations (21) indicate unusually low energy requirements for the opening of these diol epoxides to benzylic carbonium ions (13). These easily formed carbonium ions may account for the high mutagenic activity of the bay region diol epoxides of BP. An essential feature of this mechanism is the formation of a diol epoxide as opposed to an arene oxide at the bay region. Direct epoxidation of the BP or BA molecule in the 9,10 or 1,2-region respectively would yield the corresponding arene oxides, which still possess a double bond in the benzo-ring containing the epoxide. These arene oxides are chemically distinct from their corresponding epoxides and are much less susceptible to

nucleophilic attack (22). In contrast to BP 7,8-diol-9,10-epoxide and 9,10-epoxy-7,8,9,10-tetrahydro BP, BP 9,10-oxide is only weakly mutagenic in bacterial and mammalian cells (7,10,11). Thus the metabolic importance of dihydrodiol formation is to saturate the double bond adjacent to the subsequent site of epoxidation. Lack of mutagenic activity for the stereoisomeric diol epoxides from trans-1,2-dihydroxy-1,2-dihydronaphthalene (4) and the weak mutagenic activity of a 8,9-diol-10,11-epoxide from BA (5) further support our concept since the benzylic carbonium ions which result on opening of these diol epoxides are not in a bay region of these hydrocarbons. Present research is directed toward the synthesis and evaluation of biological activity of selected diol epoxides of BA with the anticipation that the 3,4-diol-1,2-epoxides will be the most mutagenic. In addition, the appropriate dihydrodiols of other polycyclic hydrocarbons are being synthesized and evaluated for biologic activity in order to further establish that epoxidations in the bay region of dihydrodiols are key reactions in the activation of unsubstituted carcinogenic hydrocarbons.

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