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PROSTAGLANDIN SYNTHETASE DEPENDENT ACTIVATION OF 7,8-DIHYDRO-7,8-DIHYDROXY-BENZO(A)PYRENE TO MUTAGENIC DERIVATIVES

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SUMMARY

The combination of arachidonic acid and a Tween 20 solubilized enzyme preparation from sheep seminal vesicles converts 7,8-dihydro-7,8-dihydroxy-benzo(a)pyrene to derivatives strongly mutagenic to Salmonella typhimurium tester strain TA 98. Under similar conditions no activation of benzo(a)pyrene, 4,5-dihydro-4,5-dihydroxy-benzo(a)pyrene, or 9,10-dihydro-9,10-dihydroxy-benzo(a)pyrene occurs. The activation of 7,8-dihydro-7,8-dihydroxy-benzo(a)-pyrene is markedly reduced by the omission of arachidonic acid and is inhibited by the prostaglandin synthetase inhibitor indomethacin. 100 μM butylated hydroxyanisole is also an effective inhibitor. This is the first report of the metabolic activation of 7,8-dihydro-7,8-dihydroxy-benzo(a)pyrene by an enzyme system distinct from the mixed-function oxidases.

INTRODUCTION

Several lines of evidence suggest that the metabolic activation of the polycyclic aromatic hydrocarbon BP* to its ultimate carcinogenic form occurs by epoxidation, hydration, and epoxidation (eq 1) (1-7). The key feature

eq 1 $$\text{BP} \xrightarrow{\text{MFO}} \text{BP-7,8-epoxide} \xrightarrow{\text{E.H.}} \text{BP-7,8-diol} \xrightarrow{\text{MFO}} \text{BP-diolepoxide}$$

of this hypothesis is the requirement for two stages of oxidation for activation. The first converts BP to a 7,8-epoxide which is hydrated to BP-7,8-diol. The second converts the proximate carcinogen BP-7,8-diol to the highly reactive and strongly mutagenic BP-diolepoxide (2-4).

We have recently reported that prostaglandin synthetase catalyzes the

*Abbreviations: BP, Benzo(a)pyrene; MFO, mixed-function oxidase; BP-7,8-epoxide, 7,8-dihydro-7,8-epoxy-benzo(a)pyrene; BP-7,8-diol, 7,8-dihydro-7,8-dihydroxy-benzo(a)pyrene; BP-diolepoxide, 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydro-benzo(a)pyrene; EH, epoxide hydrase; BHA, butylated hydroxyanisole; BP-4,5-diol, 4,5-dihydro-4,5-dihydroxy-benzo(a)pyrene; BP-9,10-diol, 9,10-dihydro-9,10-dihydroxy-benzo(a)pyrene; [^{14}C]-BP, 7,10-[^{14}C]-benzo(a)pyrene; 20:4, arachidonic acid; Indo, indomethacin.

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cooxidation of BP to a mixture of BP-1,6-, 3,6-, and 6,12-quinones (8). The oxidation of BP to these quinones possibly produces mutagenic intermediates (9-10), but it is nonetheless distinct from the oxidation which produces the extremely mutagenic diolepoxides. However, the possibility exists that prostaglandin synthetase catalyzes the oxidation of the proximate carcinogen BP-7,8-diol to the BP-diolepoxide. We have, therefore, studied the prostaglandin synthetase dependent production of mutagenic derivatives from BP and its dihydrodiol metabolites using the Salmonella typhimurium tester strain TA 98. We would like to report that a Tween 20 solubilized enzyme preparation from sheep seminal vesicles selectively activates BP-7,8-diol to mutagenic derivatives when incubated with the prostaglandin synthetase substrate, arachidonic acid.

MATERIALS AND METHODS

Chemicals: BP, BHA, hemoglobin (bovine blood, Type 1), and indomethacin were obtained from Sigma. Arachidonic acid was generously provided by Dr. John Paulsrud of Hoffman-LaRoche. BP-4,5-, 7,8-, and 9,10-diols were obtained through the NCI Carcinogenesis Research Program.

Enzyme preparations: Solubilization of sheep vesicular gland microsomes with Tween 20 was carried out by the method of Miyamoto et al (11) as previously described (12). The final concentration of protein in the stock solution was 4 mg/ml.

Bacterial preparations: Salmonella typhimurium tester strain TA 98 was kindly provided by Prof. Bruce Ames, University of California, Berkeley. The properties of this strain have been described (13). Standard methodology for the preparation of bacterial suspensions was used (14) with the following modifications described by Wood et al (15). An overnight nutrient broth culture containing 25 μg/ml ampicillin was centrifuged at 2000xg for 15 min and the bacterial pellet was resuspended in 0.9% sodium chloride/5mM potassium phosphate (pH 7.0). The final cell density was 10^9 bacteria/ml.

Assay procedure: All operations were carried out in diffuse light. The solubilized enzyme preparation and the buffer (100mM potassium phosphate, pH 7.8) were sterilized by passage through a Swinex filter. Solutions of BP and BP-diols in dimethylsulfoxide, indomethacin and arachidonic acid in ethanol, and BHA in acetone were sterile.

Incubations were carried out in a total volume of 0.5 ml and contained 10^8 bacteria, 50 μl solubilized enzyme, hemoglobin (1μM), and the other additives in the concentrations stated in the Tables. After a three min preincubation at 37°, arachidonic acid was added and the incubations were allowed to proceed for 30 min. Two ml top agar (1.0% Difco agar, 0.5% NaCl) containing 50 μM histidine and 50 μM biotin was added and the mixed contents of the tube were poured onto Petri dishes containing 30 ml of minimal-glucose agar medium. After 15 min at room temperature the plates were incubated for 44 hr at 37°

and the revertant colonies scored. All determinations were performed in quadruplicate.

Control experiments using 7,10-[^{14}C]-BP (Amersham/Searle) were performed as before (8) with the exception that 10^8 bacteria were added to the incubation mixtures. Workup and quantitation were exactly as described previously (8).

RESULTS

The data in Table 1 reveal that there is no increase in the reversion rate of Salmonella typhimurium tester strain TA 98 following incubation with BP in the presence of prostaglandin synthetase and arachidonic acid. The presence of the bacteria have no effect on the oxidation of BP to quinones. Incubations performed with [^{14}C]-BP followed by standard workup and analysis reveal no change in the amounts or patterns of quinones formed. Therefore, the lack of mutagenicity is not due to an inhibition of PG synthetase dependent BP oxidation by the bacterial preparation.

In contrast to the results obtained with BP, the addition of BP-7,8-diol to the assay system results in a greater than 10-fold increase in the number of revertants over controls. The activation of BP-7,8-diol is markedly reduced by the omission of arachidonic acid, is inhibited by the prostaglandin synthetase inhibitor indomethacin, and exhibits dose-response with respect to the diol (Fig 1). Table 1 also shows that the inclusion of 100 μM of the antioxidant BHA in the assay mixture inhibits by 89% the increase in the number of revertants induced by BP-7,8-diol. We have shown that BHA and other antioxidants inhibit prostaglandin synthetase dependent BP oxidation without inhibiting prostaglandin biosynthesis (8). The activation process appears to be specific for BP-7,8-diol. Table 2 shows that no increase in the number of revertants occurs at equivalent concentrations of either the BP-4,5-diol or the BP-9,10-diol.

DISCUSSION

Previous studies have shown that the NADPH dependent mixed-function oxidases are capable of the metabolic activation of both BP and BP-7,8-diol

TABLE 1. Differential Effect of Prostaglandin Synthetase on Mutagenicity of BP and BP-7,8-Diol.

Conditions	Revertants/plate[a]
TA 98 alone	31 ± 2
Complete system[b]	39 ± 9
" " + BP[c]	40 ± 5
" " + BP[d]	38 ± 3
TA 98 + BP-7,8-Diol[e]	45 ± 11
Complete system + BP-7,8-Diol[e]	735 ± 134
" " - 20:4 + BP-7,8-Diol[e]	108 ± 17
" " + Indo[f] + BP-7,8-Diol[e]	142 ± 20
" " + BHA[g] + BP-7,8-Diol[e]	142 ± 19

a. Mean ± std. dev; b. Complete system contained TA 98, enzyme, and [20:4]= 265μM ; c. [BP]=20μM; d. [BP]=100μM; e. [BP-7,8-Diol]=20μM; f. [Indo]=100μM; g. [BHA]=100μM.

to derivatives mutagenic to Salmonella typhimurium tester strain TA 98 (4,15). In the present work, we have found that the arachidonic acid dependent prostaglandin synthetase selectively activates BP-7,8-diol but not the parent hydrocarbon BP. The inability to activate BP could indicate that in spite of the substantial arachidonic acid dependent oxidation which occurs, no mutagenic derivatives are produced. Alternatively, it could indicate that intermediates in the formation of the BP quinones are too unstable under the assay conditions to be detected as mutagens by this method.

The conversion of BP-7,8-diol to mutagenic derivatives following incubation with a prostaglandin synthetase preparation and arachidonic acid is the first example of the metabolic activation of this compound by an enzyme system distinct from the mixed-function oxidases. The involvement of prostaglandin

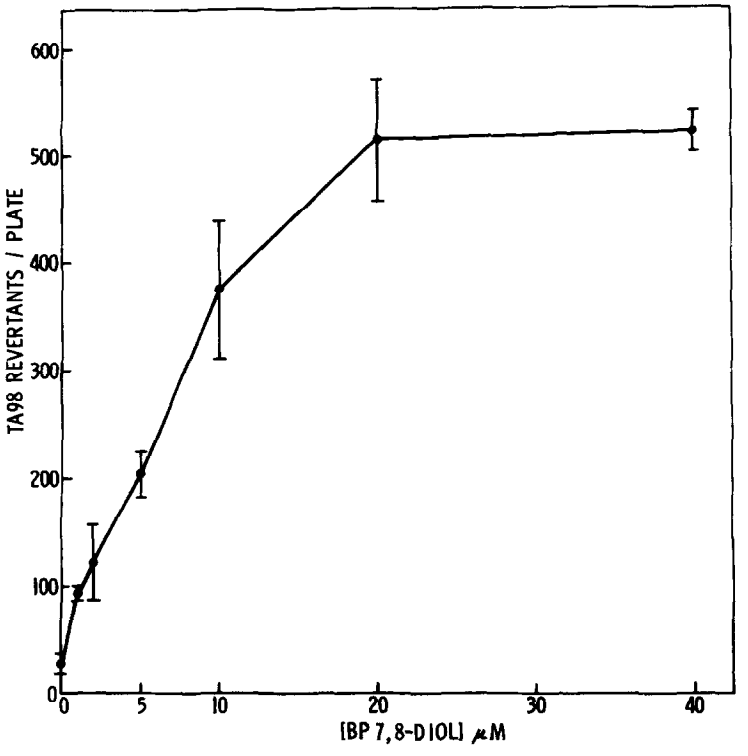

Fig. 1. Effect of Concentration of BP-7,8-Diol on Prostaglandin Synthetase Dependent Mutagenicity. Conditions as described in Table 1 for Complete System.

TABLE 2. Comparison of Effect of Prostaglandin Synthetase on Mutagenicity of Dihydrodiol Metabolites of BP.

Diol[a]	Revertants/plate[b]
None - Control	67 ± 16
BP-4,5-Diol[c]	53 ± 14
BP-7,8-Diol[c]	764 ± 42
BP-9,10-Diol[c]	79 ± 13

a. Conditions described as Complete System in Table 1; b. Mean ± std. dev.; c. [Diol]=20μM.

synthetase is suggested by the dependence on arachidonic acid and by the inhibition by indomethacin. The most obvious interpretation is that during prostaglandin biosynthesis BP-7,8-diol is cooxidized to a BP-diolepoxide. Bay region diolepoxides of BP (4,16,17) and benz(a)anthracene (18) have been found to be potent direct acting mutagens and the formation of bay region diolepoxides of 7-methylbenzanthracene (19) and chrysene (20) has been suggested to explain the high microsome mediated mutagenicity of the precursor dihydrodiols. The activation of BP-7,8-diol in the present case but not BP-4,5-diol or BP-9,10-diol (both of which cannot form bay region diolepoxides) is consistent with this hypothesis. However, we cannot rule out the possibility that BP-7,8-diol is activated by a unique pathway to strongly mutagenic derivatives which are not diolepoxides. Studies of the prostaglandin synthetase dependent metabolism of BP-7,8-diol are, therefore, in progress.

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