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EFFECT OF PURIFIED EPOXIDE HYDROLASE ON METABOLIC ACTIVA-TION AND BINDING OF BENZO(a)PYRENE TO EXOGENOUS DNA. SHIFT OF THE ACTIVATION PATHWAY

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SUMMARY

The effect of purified epoxide hydrolase (E.C. 3.3.2.3) on the binding of benzo(a)pyrene metabolites 9-hydroxybenzo(a)pyrene and 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene to DNA catalyzed by cytochrome P 448 from liver microsomes of methylcholanthrene pretreated rats has been investigated. The total binding and the major binding species derived from 9-hydroxybenzo (a)pyrene were strongly inhibited by the presence of purified epoxide hydrolase and the species derived from 7,8-dihydro-7,8-dihydroxybenzo(a)pyrene was slightly increased. By modifying the balance between cytochrome P 448 and epoxide hydrolase it is possible to shift quantitatively the binding of these two main reactive intermediates to DNA.

The carcinogenic and mutagenic effects of many polycyclic aromatic hydrocarbons are generally believed to be mediated through epoxides (1, 2, 3). The ultimate fate of these reactive intermediates depends upon their ability to react with critical sites on biomacromolecules and upon the rates of inactivation by competing enzyme reactions. Epoxide hydrolase is a detoxifing enzyme which inactivates numerous mutagenic epoxides. However at least for some particular polycyclic hydrocarbons, like benzo(a)pyrene, this enzyme catalyses an essential step in the formation of carcinogenic diolepoxides. It has been shown that purified epoxide hydrolase preparations obliterated the mutagenicity of several benzo(a)pyrene oxides (4, 5), but that certain of the resulting dihydrodiol yielded highly potent mutagens upon further oxidative activation (5).

Several studies using exogenous DNA and microsomes, rat liver nuclei, whole cells and perfused organs have shown that the main bound species

1 Abbreviations used are : AHH, aryl hydrocarbon hydroxylase ; EH, epoxide hydrolase ; UDPGA, UDP-glucuronic acid ; MC-microsomes, 3-methylcholanthrene induced rat liver microsomes : BP-7,8-diol, trans-7,8-dihydroxy-7,8-dihydroxybenzo(a)pyrene ; 9-OH-BP, 9-hydroxybenzo(a)pyrene ; MIC, microsomes ; BP, benzo(a)pyrene.

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of BP are derived from BP-7,8-diol[1] and the less carcinogenic 9-hydroxy-BP (6,7,8) through respectively the BP-7,8-diol-9,10-oxide (9) and most likely 9-OH-BP-4,5-oxide (6,7). In this study, we investigate the significance of purified epoxide hydrolase on the formation of BP-bound products to exogenous DNA catalyzed by the cytochrome P 448 monooxygenase and compare the binding of the bound products to DNA, both in absolute and relative terms. We provide the evidence that the addition of purified epoxide hydrolase strongly modifies the alkylation of DNA.

MATERIALS AND METHODS

^{3}H-benzo(a)pyrene (27 Ci/mmole) was purchased from Radiochemical Center, Amersham, Buchs, England. Calf thymus DNA, NADPH and enzymes for hydrolysis of DNA (10) were obtained from Boehringer, Mannheim, F.R.G. Sephadex LH-20 was obtained from Pharmacia, Uppsala, Sweden. Lumagel was obtained from Lumac System AG, Basel, Switzerland. All other chemicals were of analytical grade. Epoxide hydrolase was prepared from liver microsomes of phenobarbital treated adult male Sprague-Dawley rats essentially as described by Lu et al. (11) and had an activity of 450-500 units (nmole styrene diol/min/mg). The preparation of 3-methylcholanthrene induced rat liver microsomes was done as previously described (10). The incubation mixture (1 ml) for DNA binding contained 0.05 potassium phosphate buffer (pH = 8.0), 3 mM $MgCl_2$, 2 mM EDTA, 0.6 mM NADPH, rat liver microsomes (0.2-0.4 mg), ^{3}H-BP (20 nmoles, 20 uCi) and native calf thymus DNA (2.0 mg). When needed, 5 x 10^{-5}M trichloropropene-oxide (TCPO) or 300 units of purified epoxide hydrolase were added. DNA was isolated, purified and hydrolysed to deoxyribonucleosides by treatment with DNAse followed by the addition of phosphodiesterase and alkaline phosphatase as described previously (10). BP-modified deoxyribonucleosides were separated (essentially as described (12)) on 20 x 1.0 cm columns by Sephadex LH-20 chromatography. Samples were applied to the column and eluted with a 30 % to 100 % linear gradient of methanol in water. 2.0 ml fractions were collected with an LKB Redirac fractions collector and each fraction was counted in Lumagel scintillation cocktail. The column was washed in methanol and re-equilibrated with 30 % methanol.

RESULTS AND DISCUSSION

The results on the binding of BP to exogenous DNA in the presence of liver microsomes from MC-pretreated rats, E.H. inhibitor TCPO and purified epoxide hydrolase are shown on Figure 1 and Table I respectively. The chromatographic analysis of the ^{3}H-BP-conjugated DNA (Figure 1a) revealed significant quantities of products eluted with peak A and a dominating late running product (peak D). Small quantities of products B and E are also observed. When TCPO is present product C appears. The chromatographic pattern corresponds well to the one obtained by other laboratories (8,12,13). Peak A has been shown (8,9) to contain mostly products derived from binding of the isomers of 7,8-dihydroxy-BP-9,10-oxide to DNA (8,9). Product C (Figure 1b) has the same retention as the product obtained by reaction of BP-4,5-oxide with DNA (8,12). Peak D has been described as resulting from the

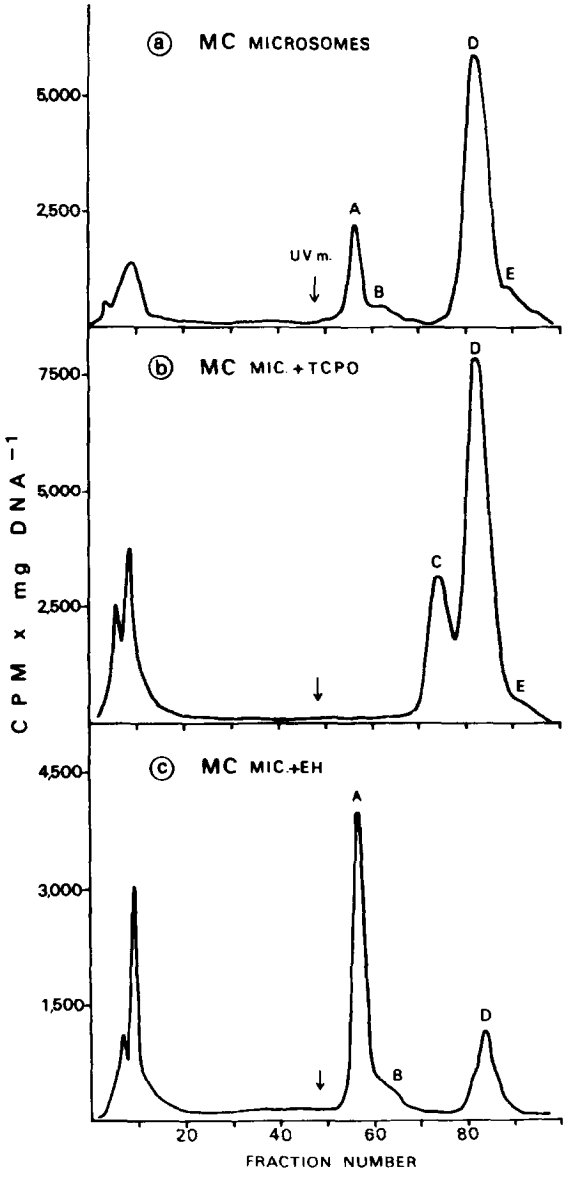

Fig. 1 Sephadex LH-20 column elution profiles of calf thymus DNA after exposure to ^{3}H-BP in presence of NADPH and MC microsomes. a) Control incubation contained ^{3}H-BP, DNA, NADPH and microsomes; b) microsomes and TCPO (5×10^{-5}M); c) microsomes and purified epoxide hydrolase (300 units).

reaction with DNA of a reactive species derived through further metabolism of benzo(a)pyrene phenols (6,8,10,20) and good evidence has suggested that its major constituent should most likely be 9-OH-BP-4,5-oxide (7). Products B and E include products of benzo(a)pyrene quinones that are further metabolized (8). The E.H. inhibitor TCPO added in vitro at concentration 5×10^{-5}M enhanced the formation of peak C and peak D (Figure 1b) with the

TABLE 1 Effect of E.H. inhibitor TCPO and purified epoxide hydrolase on benzo(a)pyrene metabolite-DNA nucleoside adducts formed during incubation with liver microsomes from MC pretreated rats.

Adducts	Addition					
	No		+ TCPO		+ EH	
Peak A	9.3	(15.4)	-	-	10.7	(58.2)
Peak B	0.8	(1.4)	-	-	0.6	(3.5)
Peak C	-	-	10.2	(10.6)	-	-
Peak D	41	(68.2)	78.6	(81.5)	2.6	(14.3)
Peak E	1.9	(3.2)	4.0	(4.2)	-	-
TOTAL BINDING	60.2	(100)	96.4	(100)	18.4	(100)

The quantity of protein was 0.2 to 0.4 mg/ml of medium. ^{3}H-BP (25 ug/ml, specific activity 1 Ci/mmole) was incubated with the calf thymus DNA (1 mg/ml) in the presence of 5 x 10^{-5}M TCPO or purified epoxide hydrolase (300 units) in 4.5 ml total. DNA was enzymatically hydrolyzed and resolved by LH-20 chromatography as described in Materials and Methods. Each result is the mean for duplicate flasks, which varied by not more than 10 %. The radioactivity excluded in the void volume of the column is not given in the table. Binding is expressed in pmole per mg of DNA, number in parenthesis show the percentage.

elimination of products A and B (Figure 1 and Table I). The enhanced binding of BP to DNA in the presence of TCPO is presumably due to the inhibition of epoxide hydrolase and thus increased formation of phenols through oxide rearrangement. Similar results have been previously reported (13,20). We present them here as a negative control to the effect of addition of epoxide hydrolase. The addition of purified epoxide hydrolase (300 units) to the medium decreases the total binding of ^{3}H-BP to DNA by a factor of three (Table I). The same binding species are seen (Figure 1a and 1c), but both the absolute amounts bound and their ratio to each other differ from the control group (without epoxide hydrolase). The results show that peak D is reduced strongly while peak A is increased in absolute amount and almost four times in relative amount.

There is much evidence that the mutagenic and carcinogenic effects ob benzo(a)pyrene are caused by the epoxide metabolites of this hydrocarbon. An increase of epoxide hydrolase activity may under certain circumstances protect against or under other, enhance carcinogenic effects : a) Liver microsomes with induced epoxide hydrolase from rats fed the antioxydant

ethoxyquin, an inhibitor of BP tumorigenesis increased DNA adduct from benzo(a)pyrene 7,8-diol-9,10-epoxide and decreased DNA adduct from 9-OH-BP-4,5-oxide (14). A similar pattern for these two main peaks was observed for DNA from rat liver nuclei with elevated epoxide hydrolase activity following treatment with phenobarbital (10). b) Very modest amounts of pure epoxide hydrolase are sufficient to prevent the mutagenesis of metabolically activated benzo(a)pyrene by microsomes from PB induced rats (cytochrome P 450) but have a more complicated effect on the mutagenesisof BP activation by microsomes from MC induced rats (cytochrome P 448)(5). All DNA adducts with BP metabolites except BP-4,5-oxide appear to be predominantly associated with BP metabolism mediated by cytochrome P 448 (15). c) Addition of highly purified epoxide hydrolase decreases the mutagenicity of benzo(a)pyrene in the Ames test (4). Epoxide hydrolase changes the metabolic pattern of benzo(a) pyrene by catalyzing the formation of dihydrodiols from the 4,5-, 7,8- and 9,10-benzo(a)pyrene oxides with decrease in the amount of phenols formed by rearrangement of the oxides (16).

Wiebel (17) reported that 3-, 6- and 9-OH-BP are metabolized by rat liver microsomes and it was demonstrated that 3-OH- and 9-OH-BP can be metabolized to product (s) which covalently binds to DNA (19, 20, 21). The major DNA adduct formed is most likely derived from 9-hydroxy-BP via epoxidation of the 4,5-bound (6, 7). However a recent report shows that TCPO has little effect on 9-hydroxy-BP binding and suggests that the reactive intermediate is probably not 9-hydroxy-BP-4,5-oxide and is not a substrate for the epoxide hydrolase (20). Our paper demonstrates that the major BP-metabolite-nucleoside product formed via microsomal activation of the parent hydrocarbon and derived from either 9-OH-BP-4,5-oxide (7) or another 9-OH-BP-intermediate (20) is strongly inhibited in presence of purified epoxide hydrolase. This inhibition could proceed by two ways : either the formation of 9-OH-BP is strongly inhibited by transformation of its precursor BP-9, 10-oxide to BP-9,10-diol (16) or the supposed further metabolite of 9-OH-BP is a good substrate for epoxide hydrolase. The fact that it is not possible to completely suppress this peak means that other phenol derivatives which are poor substrates for epoxide hydrolase may be formed. Attempts to identify these components are presently in progress in our laboratory. Recently Guenthner (21) has reported a similar effect of the cytosol on BP-DNA binding in rat liver nuclei. Similarly, Owens et al. (19) have shown that UDP glucuronic acid reduced all DNA adducts derived from 9-OH-BP and 3-OH-BP, and Fall et al. (22) that UDPGA increased BP-diol epoxide adducts from BP.

In conclusion, the present study shows that the purified epoxide hydrolase increased slighly the formation of 7,8-dihydro-7,8-dihydroxy-BP-

9,10-oxide adducts to DNA. Modifying the balance between P 448 and epoxide hydrolase influences markedly the quality and quantity of reactive intermediates of BP that bind to DNA. It should be pointed out that although the role of "Bay region diol-epoxides" of BP seems to be clearly established, other activated metabolites may be involved in the tumor induction process (23).

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