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XANTHINE OXIDASE-CATALYZED DNA BINDING OF DIHYDRODIOL DERIVATIVES OF NITRO-POLYCYCLIC AROMATIC HYDROCARBONS

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SUMMARY: Xanthine oxidase, a mammalian nitroreductase, catalyzed the covalent binding of a series of nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) trans-dihydrodiols to DNA. Some of the trans-dihydrodiols bound to DNA to a greater extent than their parent nitro-PAHs; however, when the dihydrodiol moiety was peri to the nitro substituent low levels of binding were observed. These data illustrate that ring-oxidation and hydrolysis of nitro-PAHs to their trans-dihydrodiols followed by nitroreduction is a potential metabolic pathway leading to DNA adducts in mammals.

Nitro-polycyclic aromatic hydrocarbons (nitro-PAHs) are genotoxic environmental pollutants formed from the incomplete combustion of organic material in the presence of nitrogen and from the reactions of PAHs with atmospheric nitrogen oxides (1,2). The biological effects of these compounds are thought to arise from metabolic activation to reactive electrophiles by nitroreduction and/or by ring-oxidation (1-7). Although some ring-oxidized metabolites of nitro-PAHs are direct acting mutagens in Salmonella typhimurium strain TA98, many of these compounds exhibit lower mutagenic activity in strain TA98NR, which lacks a major nitroreductase (1,2,8,9). This suggests that ring-oxidized metabolites of nitro-PAHs must undergo reduction of the nitro group to exert their full mutagenicity.

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Abbreviations: Nitro-PAHs, nitropolycyclic aromatic hydrocarbons; BaP, benzo[a]pyrene; BA, benz[a]anthracene; 1-nitro-BaP trans-7,8-dihydrodiol, trans-7,8-dihydroxy-7,8-dihydro-1-nitrobenzo[a]pyrene; other dihydrodiols are similarly designated; DMSO, dimethylsufoxide.

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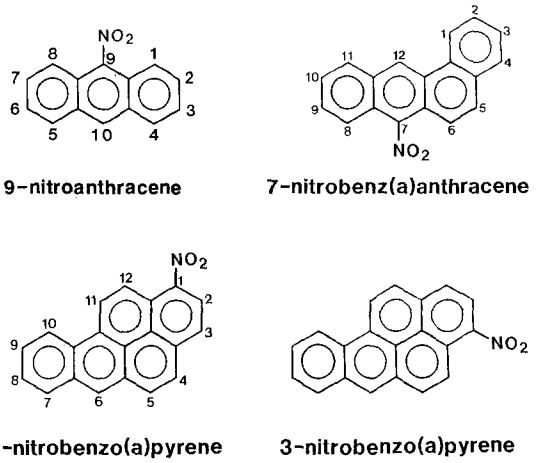

Figure 1. Structures of the parent nitro-polycyclic aromatic hydrocarbons.

It is important to determine whether or not ring oxidation followed by nitroreduction is also a metabolic activation pathway in mammals. We have recently studied the aerobic metabolism of a number of nitro PAHs commonly detected in the environment. These included 1- and 3-nitro-BaP, 7-nitro-BA and 9-nitroanthracene (Figure 1). Upon incubation with rat liver microsomes each nitro-PAH yielded primarily two trans-dihydrodiol metabolites (8,10-12). In this study we have used a mammalian nitroreductase, xanthine oxidase, to catalyze the binding to DNA of these four nitro-PAHs and their trans-dihydrodiol metabolites. The extent of binding of each compound was compared to assess the potential for ring-oxidation followed by nitroreduction as an activation pathway in mammals.

MATERIALS AND METHODS

Materials: [4,5,9,10-^{3}H]1-Nitropyrene, [G-^{3}H]1-nitro-BaP, [G-^{3}H]3-nitro-BaP, and [10,11-^{3}H]BA were obtained from R. Roth of Midwest Research Institute, Kansas City, MO. [10,11-^{3}H]7-Nitro-BA was synthesized from [10,11-^{3}H]BA using a previously described procedure (13). [1-8-^{3}H]9-Nitroanthracene was synthesized as reported (12). The trans-7,8- and 9,10-dihydrodiols of 1-nitro-BaP and 3-nitro-BaP, the trans-3,4- and 8,9-dihydrodiols of 7-nitro-BA, and the trans-1,2- and 3,4-dihydrodiols of 9-nitroanthracene were obtained by incubation of the respective nitro PAHs with liver microsomes of rats pretreated with 3-methylcholanthrene and were purified as previously described (8,10-12)

Incubations (1 ml) were conducted as described by Howard et al. (5). Solutions containing 50 mM potassium phosphate buffer (pH 5.8 or pH 7.4), 3.7 mM hypoxanthine, calf thymus DNA (2 mg/ml) and 20 μM tritiated substrate (4 mM

in DMSO) were purged with argon for 15 min. Xanthine oxidase was added to the desired concentration and the solutions incubated for 1 hr at 37°C. The headspace above the solutions before and after incubation contained <2% O_2 as determined by mass spectrometry. Reactions were terminated by the addition of an equal volume of phenol. DNA was isolated and purified after the method of Djurić et al. (6). After isolation, the DNA was redissolved in 5 mM Bis-Tris buffer/0.1 mM EDTA (pH 7.1) and its concentration was determined spectrophotometrically with a Cary 219 recording spectrophotometer. Aliquots were treated with DNase and the bound radioactivity was determined with a Serle Mark III scintillation counter. Results are expressed as the mean ± standard deviation for at least three separate incubations.

RESULTS AND DISCUSSION

Tritium-labeled 1-nitro-BaP, 3-nitro-BaP, 7-nitro-BA, 9-nitroanthracene and their trans-dihydrodiol metabolites were incubated with xanthine oxidase and hypoxanthine in the presence of calf thymus DNA. Covalent binding of the reductive metabolites to DNA was indicated for the four parent nitro-PAHs and for six of the eight trans-dihydrodiols (Table 1). In the absence of xanthine oxidase, very low levels of non-enzymatic binding of nitro-PAHs to DNA were observed. Very low binding was also observed in the absence of hypoxanthine, which indicates that, while required as a cofactor, hypoxanthine is incapable of reducing nitro-PAHs and the trans-dihydrodiol metabolites. The binding was dependent upon enzyme concentration as illustrated by a two-fold increase in binding of 3-nitro-BaP trans-9,10-dihydrodiol observed upon increasing xanthine oxidase concentration from 0.1 U/ml to 0.5 U/ml. When allopurinol, a specific inhibitor of xanthine oxidase (14,15), was included in concentrations up to 4.8 μM, the extent of DNA binding decreased by as much as 80%. These data confirm that the observed binding was enzymatically mediated by xanthine oxidase.

The DNA binding of all compounds assayed, except 9-nitroanthracene trans 1,2-dihydrodiol and 7-nitro-BA trans-8,9-dihydrodiol, exhibited a pH dependence similar to that reported by Howard et al. (5) for 1-nitropyrene. Thus, as shown in Table 1, the extent of DNA binding was higher at pH 5.8 than at pH 7.4. A proposed mechanism for DNA adduct formation from nitro-PAHs involves the reduction to N-hydroxylamines, subsequent protonation, and then elimination of water to form highly electrophilic nitrenium ions (1,2,16). The higher binding observed at pH 5.8 is consistent with increased

Table 1. Xanthine Oxidase Catalyzed Binding of Nitro-PAHs and trans-Dihydrodiol Metabolites to DNA[1]

Compound	pmoles bound/mg DNA			
	pH 5.8		pH 7.4	
	- xanthine oxidase	+ xanthine oxidase[2]	- xanthine oxidase	+ xanthine oxidase[2]
1-Nitro-BaP	9 ± 3	300 ± 120	5 ± 1	60 ± 10
1-Nitro-BaP trans-7,8-dihydrodiol	20 ± 12	720 ± 30	34 ± 6	80 ± 5
1-Nitro-BaP trans-9,10-dihydrodiol	32 ± 2	1410 ± 25	17 ± 3	350 ± 140
3-Nitro-BaP	13 ± 12	295 ± 135	2 ± 1	50 ± 15
3-Nitro-BaP trans-7,8-dihydrodiol	13 ± 3	670 ± 75	15 ± 4	335 ± 70
3-Nitro-BaP trans-9,10-dihydrodiol	15 ± 4	875 ± 145	8 ± 2	230 + 60
7-Nitro-BA	6 ± 1	85 ± 40	6 ± 1	50 ± 15
7-Nitro-BA trans-3,4-dihydrodiol	32 ± 4	255 ± 85	26 ± 4	115 ± 50
7-Nitro-BA trans-8,9-dihydrodiol	2 ± 1	9 ± 5	3 ± 2	8 ± 5
9-Nitroanthracene	6 ± 4	210 ± 85	6 ± 4	160 ± 40
9-Nitroanthracene trans-1,2-dihydrodiol	7 ± 3	9 ± 1	8 ± 2	11 ± 4
9-Nitroanthracene trans-3,4-dihydrodiol	13 ± 2	65 ± 30	10 ± 1	45 ± 15
1-Nitropyrene	5 ± 2	1525 ± 175	6 ± 2	370 ± 80

[1]Binding assays were performed in triplicate, using 0.1 U/ml of xanthine oxidase in all experiments. See Materials and Methods for details.
[2]With the exception of 7-nitro-BA trans 8,9-dihydrodiol and 9-nitroanthracene trans-1,2-dihydrodiol the binding was significantly different ($p<0.05$) in the presence of xanthine oxidase.

N-hydroxylamine protonation under acidic conditions. 9-Nitroanthracene trans-1,2-dihydrodiol and 7-nitro-BA trans-8,9-dihydrodiol gave only very low binding at both pHs. The seeming inability of xanthine oxidase to activate these latter two compounds to DNA-binding species may be associated with their common geometrical feature of a dihydrodiol group peri to the nitro

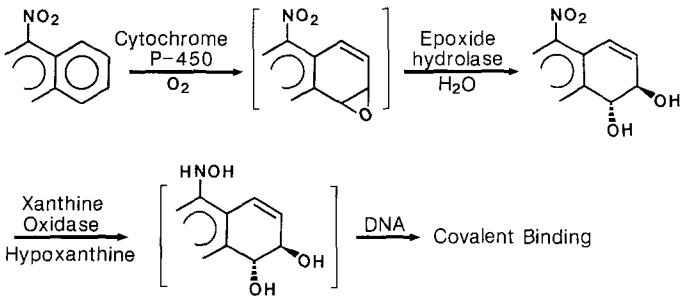

Figure 2. The metabolic activation pathway proposed for nitro-PAHs involving ring oxidation and nitroreduction.

substituent. It has been shown, however, that peri-epoxides of 1-nitropyrene can serve as substrates for xanthine oxidase (7). Factors other than simply having a peri functional group would therefore seem to be involved.

The binding levels of the trans-7,8- and trans-9,10-dihydrodiols of 1- and 3-nitro-BaP were 2-5 fold higher than those of their corresponding parent compounds ($p<0.05$). In the case of 7-nitro-BA and its derivatives, the trans-3,4-dihydrodiol ($p<0.1$), but not the trans-8,9-dihydrodiol ($p<0.05$), exhibited higher binding than the parent compound. However, both 9-nitroanthracene trans-dihydrodiols bound to a lesser degree than 9-nitroanthracene ($p<0.05$). Thus, comparison of the extent of binding to DNA between the trans-dihydrodiols and their parent nitro-PAHs indicates that ring-oxidation and hydrolysis to trans-dihydrodiol metabolites followed by enzymatic nitroreduction can lead to covalent binding to DNA in mammals, although the specific effect of oxidation prior to nitroreduction on binding appears to be compound-dependent (Figure 2).

Recently, Djurić et al. (7) reported that in the presence of xanthine oxidase the three in vitro metabolites of 1-nitropyrene, 3-hydroxy-, 4,5-epoxy- and 9,10-epoxy-1-nitropyrene, exhibited higher binding than 1-nitropyrene. Our results, together with theirs, clearly illustrate that ring-oxidation followed by nitroreduction is a general metabolic activation pathway in mammals. The importance of this pathway in vivo is not known; however, with some nitro-PAHs it has been demonstrated that, in vivo, oxidative metabolism predominants over reduction (17-22). Ring-oxidation, as

the most likely first step, followed then by nitroreduction may thus play a significant role in the metabolic activation of nitro-PAHs.

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