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Asbestos-Catalyzed Oxidation of Benzo(a)pyrene by Superoxide-Peroxidized Microsomes

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Asbestos and benzo(a)pyrene [B(a)P] are ubiquitous in our environment and both are recognized as causal factors for cancer in man and animals. Epidemiological evidence suggests that asbestos fibers can increase the health risk from exposure to carcinogens present e.g. in cigarette smoke (Selikoff et al. 1968; Hammond et al. 1979). In vitro studies showed a synergism in morphological transformation of mammalian cells treated with asbestos and B(a)P (DiPaolo et al. 1983). The mechanism for this interaction may involve an increased dispersal and membrane transport of B(a)P by asbestos fibers (Lakowicz and Hylden 1978) as well as effects of metal ions, present in asbestos, on B(a)P metabolic activation and/or deactivation process (Thomson et al. 1974).

Weitzman and Weitberg (1985) showed that asbestos can mediate lipid peroxidation and that iron cations might be involved in the catalytic activity of asbestos fibers. Our previous study of B(a)P metabolism by microsomes showed that peroxidative conditions change the balance between activation and deactivation of B(a)P and demonstrated that catalytically active iron can play a role in this process (Byczkowski and Gessner 1987). The present investigation examines the effect of asbestos on oxidation of B(a)P by superoxide - peroxidized microsomes in vitro.

MATERIALS AND METHODS

Mouse liver microsomes were prepared by the routine method as described previously by Byczkowski and Gessner (1987). The final, washed microsomal preparation was suspended in a buffered medium containing: 130 mM NaCl, 5.2 mM KCl, 1.3 mM KH_2PO_4, 10 mM Na_2HPO_4, 1.3 mM $MgSO_4$ (pH 7.4) to yield a protein concentration of 10 mg/ml. $O_2^{\cdot}$ -peroxidized microsomes were prepared by incubating 1 ml of the fresh microsomal suspension

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with 1 ml of the buffered medium (pH 7.4) containing 0.1 mM $Fe_2(SO_4)_3$ plus 0.1 mM Fe·EDTA with addition of 142 µg of KO_2, equivalent to a final concentration of 1 mM of KO_2. After addition, with rapid stirring, of the preweighed solid KO_2, the sample was incubated at 37°C for 10 min, the incubation was terminated by adding 23 ml of ice-cold Tris-HCl buffer (pH 7.2), the microsomes were reisolated and diluted to 10 mg protein per ml.

The incubations at 37°C for 10 min were done in triplicate in the buffered medium (pH 7.4) listed above. Where indicated, the following additions were done (to the final volume of 0.2 ml): 1 mg of asbestos per sample, 50 µM B(a)P (58.5 mCi of ^{14}C per mmole), 0.1 mM $Fe_2(SO_4)_3$ plus 0.1 mM Fe·EDTA, 5 mM NADPH and 0.5 mg of native or peroxidized microsomal protein. The reaction was started by addition of 5 µl of B(a)P dissolved in acetone and terminated by adding 0.3 ml of chilled methanol. Insoluble precipitate was centrifuged and the supernatant directly used for HPLC analysis. The precipitated pellet was washed with methanol until washable radioactivity was gone and analysed for remaining non-extractable radioactivity as described elsewhere by Byczkowski and Gessner (1987). Aliquots of 200 µl of methanolic extracts were injected into an HPLC Perkin-Elmer series 400 liquid chromotrograph, equipped with 200 µl loop Rheodyne injector, connected to Waters "µBondapak" C18 guard-pak and analytical column. Water/methanol separation program from 46% to 96% for 55 min at 1 ml per min was used as described earlier (Byczkowski and Gessner 1987). The column eluent was continuously monitored with a Waters 490 programmable multiwavelength detector set at 276 nm and connected with a Perkin-Elmer R-100A recorder, and with Flo-one β Radiomatic Instruments radioactivity flow meter, equipped with Micromate PMC computer, Qume Co. monitor and C. Itoh Electonic Inc. printer. ^{14}C was counted and converted into DPM using a quench-curve. All chemicals purchased from Fisher Scientific were of highest purity available or HPLC-grade. [7,10-^{14}C] B(a)P was purchased from Amersham; KO_2 from Sigma. Asbestos-Canadian Chrysotile UICC Reference Standard (Timbrell and Rendall 1971/72) was purchased from MRC Epidemiology Unit, Cardiff, U.K. B(a)P metabolites were kindly donated by the Chemical and Physical Carcinogenesis Branch of the National Cancer Institute, through procurement from its Chemical Repository at IIT Research Institute, Chicago.

RESULTS AND DISCUSSION

It has been well established in several biosystems, that B(a)P diones can be formed by one-electron oxidation in the presence of enzymes with peroxidatic activity (for review see Cavalieri and Rogan 1984). It was also demonstrated in microsomes that lipid peroxidation can lead to the formation of B(a)P diones (Morgenstern et al. 1981). Gower and Wills (1984) showed that

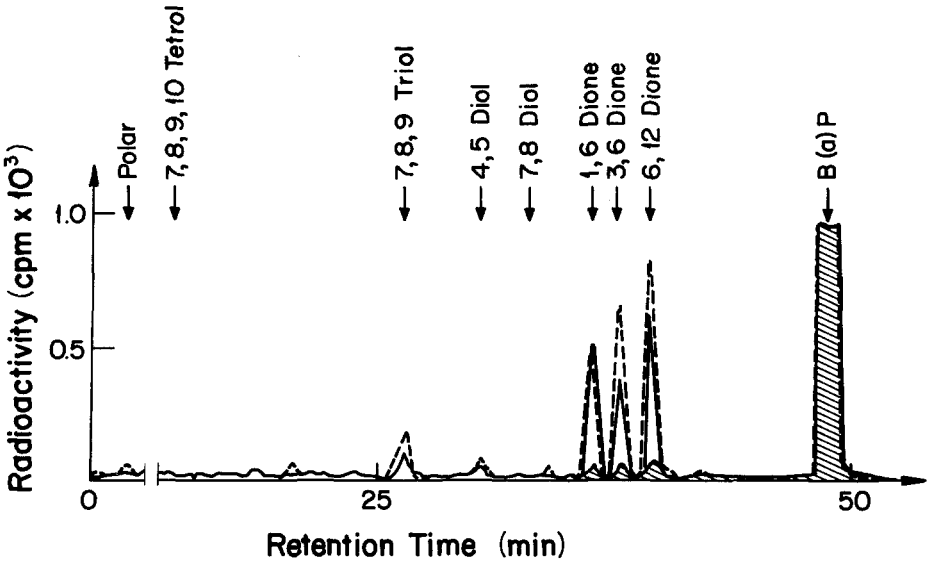

Figure 1. HPLC analysis of products of benzo(a)pyrene oxidation by $O_2^{\cdot -}$-peroxidized microsomes, without NADPH, in the presence of asbestos (solid line) and asbestos plus Fe^{3+}·FeEDTA (broken line). The shaded areas represent control without asbestos. Peaks were identified by comparison of their retention time to standards. For experimental details see Materials and Methods

some oxidation products of lipid peroxidation are able to react directly with B(a)P, producing large amounts of B(a)P diones, in the absence of enzyme. Moreover, in our previous study (Byczkowski and Gessner 1987), B(a)P oxidation was achieved by generating superoxide anion-radicals in the presence of catalytically active iron. The above observations suggest that lipid peroxides, hydroperoxides, and/or $O_2^{\cdot -}$, or other active oxygen species may activate B(a)P through a one-electron pathway, with B(a)P radical-cation as an intermediate (Jeftic and Adams 1970) and/or through the singlet oxygen reaction with B(a)P dioxetan or perepoxide as an intermediate (Lee-Ruff et al. 1986).

As apparent from Fig. 1, addition of asbestos to the incubation system containing $O_2^{\cdot -}$-peroxidized microsomes, without NADPH, resulted in a tremendous increase of B(a)P oxidaton, as evidenced by more than ten-fold increase in the amount of B(a)P diones produced (Fig. 1). Even more B(a)P diones were produced when Fe^{3+}·FeEDTA was present in the system, in addition to asbestos. In both cases B(a)P-6,12-dione was the major product

Table 1. Effect of asbestos on oxidation of benzo(a)pyrene by $O_2^{\cdot}$ -peroxidized mouse liver microsomes

Metabolites	B(a)P products formation (pmoles per ml)						
	Asbestos		Native Microsomes	Peroxidized Microsomes			
		$+Fe^{3+}$.FeEDTA			+Asbestos	+ Asbestos $+Fe^{3+}$.FeEDTA	+ NADPH
Polar	n.d.	n.d.	n.d.	n.d.	n.d.	18± 5	40± 3
Tetrols/triols	n.d.	n.d.	n.d.	18± 3	103± 8	185± 13	138±13
Diols	n.d.	n.d.	n.d.	n.d	18± 3	60± 8	70±18
Diones	95±18	143±23	80±11	145±23	1253±135	1635±235	520±23
4.5-Epoxides	n.d.	n.d.	n.d.	n.d.	n.d.	20± 5	90±25
Monohydroxy	n.d.	13± 5	n.d.	n.d.	n.d.	n.d.	148±15
Non-extractable	10± 3	10± 3	25± 7	53±10	553± 58	490± 53	325±48
Total products	105±21	166±25	105±19	216±20	1927±190	2408±193	1330±90

Mean values ± S.D. from three experiments (n = 3) are presented. n.d. - not detected. For experimental details see Materials and Methods.

(Fig. 1) which apparently is indicative of a singlet oxygen mechanism of the reaction (Lee-Ruff et al. 1986). From data summarized in Table 1 it can be seen that, even without asbestos, the $O_2^{\cdot -}$-peroxidized microsomes oxidized B(a)P, producing diones and small amounts of an unidentified product, which co-chromotographed with B(a)P-triols in our system. Addition of asbestos increased the extent of oxidation almost ten-fold and this effect was further enhanced by Fe^{3+}·FeEDTA. The extent of asbestos plus Fe^{3+}.FeEDTA-catalyzed oxidation even exceeded the NADPH-driven metabolism of B(a)P by $O_2^{\cdot -}$-peroxidized microsomes (Table 1). The relative amount of diones, as compared with overall B(a)P products, more than 60% for system with asbestos versus 39% for NADPH-driven metabolism, suggests the involvement of one-electron oxidation of B(a)P in the presence of asbestos (Cavalieri and Rogan 1984). On the other hand, the presence of unstable epoxides, which cochromatographed in our system with B(a)P-4,5-epoxide, and the presence of B(a)P-6,12-dione as a major product may indicate involvement of a singlet oxygen reaction, leading to B(a)P dioxetan or perepoxide as a precursor to diones (Lee-Ruff et al. 1986).

It is unlikely that any free oxygen radicals might survive the reisolation procedure of microsomes after peroxidation but they may be formed during secondary reactions between the products of lipid peroxidation and catalytically active iron. Moreover, Fe^{3+} is known to stabilize active oxygen species in complexes, as well as to catalyze singlet oxygen release from hydroperoxides (Naqui et al. 1986). It seems, therefore, that catalytically active iron is essential for asbestos-catalyzed oxidation of B(a)P by peroxidized microsomes. The phenomenon, found in vitro and described in this paper, may be of significance for environmental carcinogenesis because it has already been demonstrated that, in the presence of iron, peroxide not only increased binding of B(a)P intermediates to DNA in vitro (Umans et al. 1969; Morreal et al. 1968) but also enhanced B(a)P carcinogenicity for mice (Nagata et al. 1973). It is postulated, therefore, that asbestos can supply catalytically active iron able to activate B(a)P in peroxidative conditions.

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