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Lipid Peroxidation and Benzo(a)pyrene Derivative Co-Oxygenation by Environmental Pollutants

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Several environmental pollutants initiate, promote or undergo one-electron oxidations. The occurrence of free-radical reaction under aerobic conditions of the lung, in the presence of lipids from the surfactant which lines the alveoli and airways, can initiate lipid peroxidation and cause damage to the surfactant system (Lachmann, 1989 and literature therein). Lipid peroxidation can also compromise the permeability of biomembranes and cause lung damage as a consequence of increased generation of superoxide, hydrogen peroxide, and other reactive oxygen species by sub-cellular organelles. This initial damage can attract and activate the polymorphonuclear cells which in turn discharge additional reactive oxygen species and exacerbate further damage (Crapo, 1986). Interstitial pulmonary fibrosis, a frequent result of many forms of lung injury, is also associated with the generation of oxygen-derived free radicals and their metabolites (Chvapil & Peng, 1975; Johnston et al. 1981). The pulmonary endothelium is particularly susceptible to injury by noxious agents that are inhaled and/or delivered to the lung by way of circulation. Recent evidence indicates that a variety of lipid-derived free radicals can cause structural derangement and loss of normal endothelial cell function. For instance, linoleate-derived free radicals can cause peroxidative cleavage of membrane lipids and subsequent derangements in the biomembrane function, similar to that seen in oxygen toxicity (Patel & Block, 1988). Lipid-derived peroxyl radicals can also interact with aromatic hydrocarbon pollutants, like benzo(a)pyrene or its 7,8-dihydrodiol, and activate them to reactive intermediates which bind to vital macromolecules. This co-oxygenation process was observed by Byczkowski & Gessner (1987, a, b, c) during the interaction of benzo(a)pyrene with asbestos and/or catalytically reactive iron in mouse liver microsomes. Recent experiments with isolated lipoxygenase plus linoleic acid

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suggested the involvement of lipid peroxyl radical in the activation of benzo(a)pyrene-7,8-dihydrodiol to ultimate muta- and carcinogenic epoxide (Byczkowski & Kulkarni, 1989). These observations may finally lead to understanding of the interaction of environmental pollutants with other co-pollutants which require oxidative activation to ultimate toxic or carcinogenic intermediates. Recently, Byczkowski & Kulkarni (1990) developed the model for testing the pro-oxidant properties of environmental pollutants in vitro. In the present study the peroxidative effects of major environmental pollutants: hydrated SO_2 (bisulfite), reduced vanadium (vanadyl) and asbestos fibers (Canadian chrysotile) on linoleic acid were investigated in vitro. The produced peroxyl radicals were trapped with benzo(a)pyrene derivative leading to its cooxygenation.

MATERIALS AND METHODS

Lipid peroxidation was followed by oxygen uptake using a Clark-type oxygen electrode at 30°C in a total volume of 2 ml in a medium containing 50 mM Tris-HCl with 15 mM KCl (pH 7.2). The lipid peroxidation was initiated by the indicated amount of vanadyl sulfate, sodium meta-bisulfite, or Canadian chrysotile, in the presence of different concentrations of linoleic acid. The system without linoleic acid served as a control. Freshly prepared linoleic acid was dissolved in a minimal volume of ethanol and diluted in the buffer with one drop of Tween 80. Partially peroxidized linoleic acid was prepared by shaking the aerated preparation until the concentration of hydroperoxylinoleate reached 2-3%. The hydroperoxylinoleate content was determined spectrophotometrically with the Aminco DW-2000 as conjugated dienes absorbing at 234 nm in a total volume of 3 ml at 25°C in 50 mM Tris-HCl buffer containing 15 mM KCl (pH 7.2). Co-oxygenation of benzo(a)pyrene and benzo(a)pyrene-7,8-dihydrodiol was studied in the presence of freshly prepared or partially autoperoxidized linoleic acid and analyzed by HPLC, essentially as described by Gessner & Byczkowski (1988).

RESULTS AND DISCUSSION

Both sodium-metabisulfite and vanadyl sulfate were found to trigger lipid peroxidation of linoleic acid under the experimental conditions employed. Fig. 1a shows effects of sodium meta-bisulfite on peroxidation of linoleic acid as measured by oxygen consumption in comparison to the effect of vanadyl sulfate (Fig. 1b). Fig. 2a shows the effects of different concentrations of sodium meta-bisulfite on peroxidation of linoleic acid (1 and 5 mM). The velocity of linoleate oxidation was measured as a linear rate, about 1 min after the initial drop of oxygen concentration caused by addition

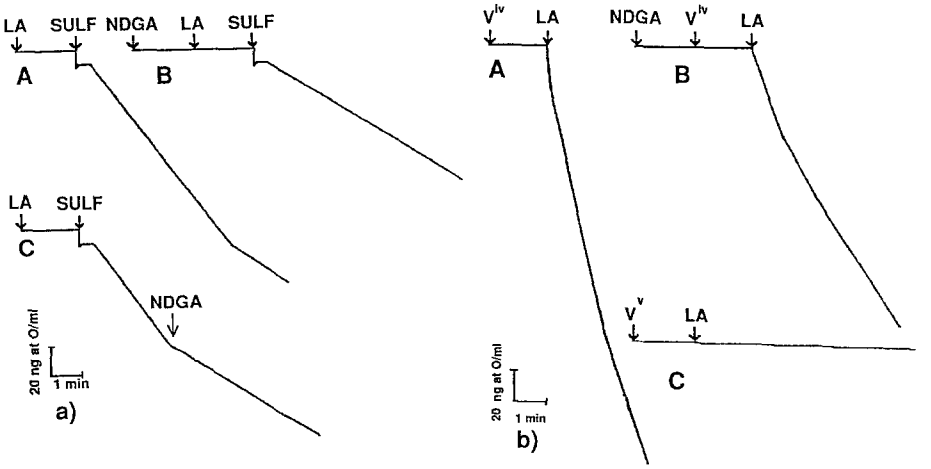

Figure 1. a) Effects of 80 μM sodium meta-bisulfite (SULF) on oxygen uptake during peroxidation of 1 mM linoleic acid (LA). NDGA - 10 μM nor-dihydroguaiaretic acid. b) Effects of 0.5 mM vanadyl sulfate (V^{IV}) on oxygen uptake during peroxidation of 1 mM linoleic acid (LA). V^{V} - 0.5 mM sodium orto-vanadate; NDGA - 10 μM.

of anaerobic solution of meta-bisulfite to the medium (Fig. 1 a). The effects of increasing concentrations of linoleic acid on the bisulfite-initiated peroxidation are shown in Fig. 2b. The lipid peroxidation was accompanied by the production of conjugated dienes (data not shown). The bisulfite-initiated peroxidation of linoleate was inhibited by NDGA at concentrations ≥10 μM (Fig. 1a). At these concentrations NDGA acts as a free-radical quencher and an antioxidant. Two free-radical pathways have been postulated to explain the mechanisms responsible for SO_2 toxicity: i. a one-electron auto-oxidation of sulfite and bisulfite, and ii. an enzymatic one-electron oxidation by peroxidases. The univalent oxidation of sulfite and bisulfite initiates a free-radical chain oxidation (Kaplan et al. 1975), in which $SO_3^{-\cdot}$ and $SO_5^{-\cdot}$ serve as chain propagating intermediates (Neta & Huie, 1985). Earlier, the free-radical oxidation of bisulfite was demonstrated with the prostaglandin synthetase system (Mottley et al. 1982) and it was proposed that sulfur peroxyl free-radical, formed as an intermediate of bisulfite autooxidation, co-oxygenates benzo(a)pyrene-7,8-dihydrodiol (Reed et al. 1986). This co-oxygenation may explain the co-carcinogenic effect of sulfur dioxide for benzo(a)pyrene-induced pulmonary carcinoma (Laskin et al. 1976; Pauluhn et al. 1985).

The results presented in Fig. 3A indicate, that only a limited amount of trans-anti-tetrahydrotetrol was produced in the control experiment in the presence of 80μM

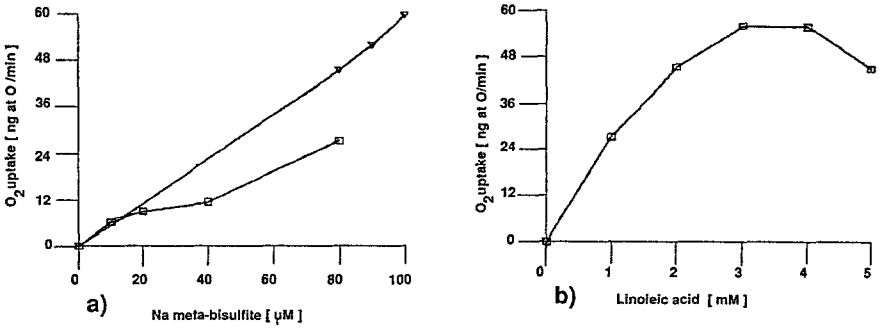

Figure 2. a) Kinetics of initiation by sodium meta-bisulfite of 1 mM (□) and 5 mM (▽) linoleic acid peroxidation. b) Kinetics of linoleic acid peroxidation initiated by 80 µM sodium meta-bisulfite.

bisulfite alone. The production of this metabolite dramatically increased when freshly prepared linoleic acid was included in the incubation mixture (Fig. 3 B). The trans-anti-benzo(a)pyrene- 7, 8, 9, 10-tetrahydrotetrol (peak # 1) is the product of ultimate carcinogenic benzo(a)pyrene-7, 8-dihydrodiolepoxide hydrolysis, and is diagnostic for peroxidative co-oxygenation of (+) benzo(a)pyrene-7,8-dihydrodiol (Dix & Marnett, 1984). The co-oxygenation of benzo(a)pyrene in the same system also leads to the formation of diones (results not shown).

Fig. 1 b shows the effects of vanadyl sulfate on peroxidation of linoleic acid as measured by oxygen consumption. NDGA ≥10 µM was inhibitory. The oxidized form of vanadium, vanadate(V), was without effect when tested under identical conditions (Fig. 1b. C) but in the presence of NAD(P)H it is first reduced to vanadyl and then undergoes redox cycling (Zychlinski et al. 1990). Vanadium circulates in the oxygenated blood as a polyvanadate (V^{V}, isopolyanions containing vanadium in the +5 oxidation state), whereas inside the tissues vanadium stays mainly as vanadyl (V^{IV}, cationic form of vanadium in the +4 oxidation state) due to the reduction effected by endogenous compounds such as reduced glutathione (Erdmann et al. 1984).

Our experiments performed on microsomes from human tissue showed that both vanadyl and vanadate trigger lipid peroxidation, due to redox cycling in the presence of NAD(P)H and the formation of reactive peroxy-vanadyl complex with superoxide (Byczkowski et al 1988). The proposed mechanism of lipid peroxidation initiation by peroxy-vanadyl complex predicts that superoxide anion-

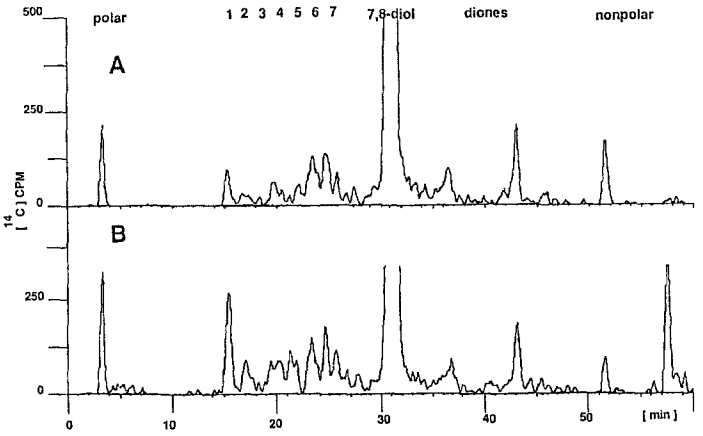

Figure 3. HPLC profile of 50 μM [^{14}C]benzo(a)pyrene-7,8-dihydrodiol co-oxygenation by peroxyl radicals produced by peroxidation of 1 mM linoleic acid with 80 μM sodium meta-bisulfite (B) compared to the control without linoleic acid (A). Peak # 1 was identified as trans-anti-benzo(a)pyrene-7,8,9,10-tetrahydrotetrol.

radical is essential for vanadium redox cycling. The superoxide can be generated during vanadyl autooxidation. However, in the system containing vanadyl and benzo(a)pyrene-7,8-dihydrodiol very little of trans-anti-tetrahydrotetrol was produced (Fig. 4 A), unless freshly prepared linoleic acid was added (Fig. 4 B). The co-oxygenation of benzo(a)pyrene under identical conditions produced mainly diones (results not shown). These results suggested that the autooxidation of vanadyl triggered free-radical peroxidation of linoleic acid, analogous to bisulfite, and linoleate peroxyl radical was trapped by benzo(a)pyrene-7,8-dihydrodiol and the process generated the ultimate carcinogenic dihydrodiolepoxide.

This mechanism, however, does not appear to be applicable to asbestos. Canadian chrysotile, which contains only traces of iron, did not initiate the peroxidation of freshly prepared linoleic acid, perhaps due to the lack of redox cycling mechanism. On the other hand, as shown by Byczkowski & Gessner (1987 b, c) the same asbestos catalyzed co-oxygenation of benzo(a)pyrene to diones in superoxide-peroxidized microsomes, especially, when loaded with partially chelated iron. These results suggested that co-oxygenation by asbestos required the presence of lipid hydroperoxides. The data presented in Fig. 5 A show that indeed, a very little of trans-anti-benzo(a)pyrene-7,8,9,10-tetrahydrotetrol

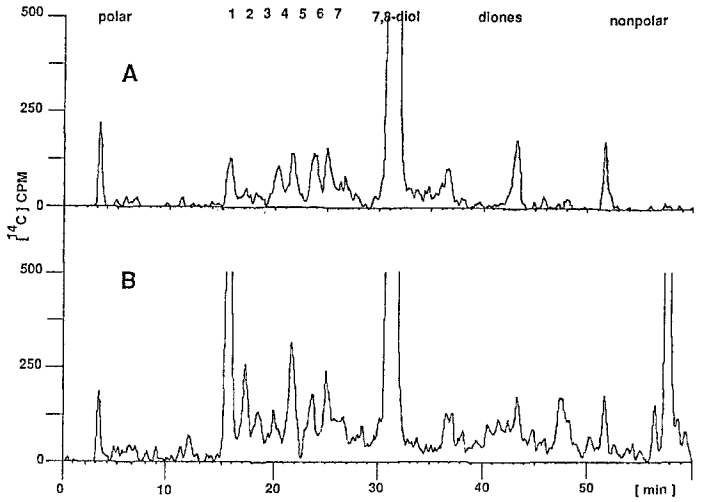

Figure 4. HPLC profile of 50 μM [^{14}C]benzo(a)pyrene-7, 8-dihydrodiol co-oxygenation by peroxyl radicals produced by peroxidation of 1 mM linoleic acid with 0.5 mM vanadyl sulfate (B) compared to the control without linoleic acid (A).

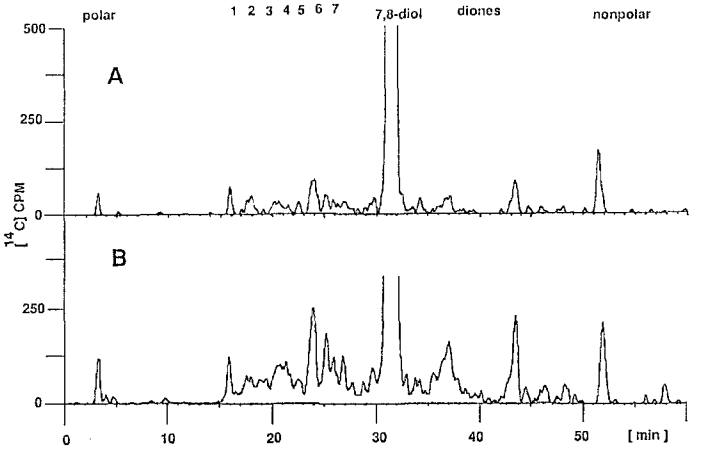

Figure 5. HPLC profile of 50 μM [^{14}C]benzo(a)pyrene-7, 8-dihydrodiol co-oxygenation by peroxyl radicals produced by 5 mg/ml of Canadian chrysotile from hydroperoxides present in partially autoperoxidized 1 mM linoleic acid (B) compared to the control containing freshly prepared 1 mM linoleic acid without hydroperoxides (A).

is produced from dihydrodiol by asbestos incubated with freshly prepared linoleic acid. There was, however, a

significant production of trans-anti-tetrahydrotetrol and diones when partially peroxidized linoleic acid was used (Fig. 5 B). These results suggest that the trace amounts of transition metals, like iron in asbestos can catalyze generation of peroxyl radical via Russel mechanism from the pre-formed linoleate hydroperoxide, present in partially peroxidized preparation.

It seems, therefore, that under the experimental conditions employed hydrated SO_2 and reduced vanadium are capable of causing lipid peroxidation and that lipid peroxyl radical generated in this process co-oxygenates benzo(a)pyrene-7,8-dihydrodiol to the ultimate carcinogen. Similarly, in the presence of asbestos fibers, peroxyl radical generation from lipid hydroperoxide occurs that mediates the co-oxygenation of the proximate carcinogen. The postulated mechanism may play an important role in pulmonary toxicity by these major environmental pollutants. We believe that the peroxidative pathway of xenobiotic bioactivation may be crucial for co-carcinogenic interaction between major pollutants, such as SO_2, reduced vanadyl and asbestos, and the ubiquitos co-pollutant, benzo(a)pyrene. Further in-depth studies are needed to solve its molecular mechanism.

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