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Metabolism of benzo[a]pyrene in peroxynitrite/Fe(III) porphyrin system

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Abstract

The peroxynitrite/porphyrin biomimetic system was established to investigate the effects of peroxynitrite on benzo[a]pyrene (B[a]P) metabolism. Three model systems consisting of different iron porphyrins were compared, and the results showed that the peroxynitrite/T(*p*-Cl)PPFeCl system was the highest catalytic efficiency in the metabolism of B[a]P. We analyzed the B[a]P metabolites produced from this system by RP-HPLC method and firstly identified the formation of nitrobenzo[a]pyrenes which are the special metabolites of B[a]P induced by peroxynitrite.

Key words: peroxynitrite; benzo[a]pyrene metabolism; nitrobenzo[a]pyrenes

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants that widely present in incomplete combustion of organic materials (Perera *et al*., 2004). It was reported that the enhancement of cytotoxicity of PAHs was related with peroxynitrite (PN) (Bai *et al*., 2001). However, the role of peroxynitrite on PAHs metabolism is unknown. Peroxynitrite is a strong oxidation and nitration agent *in vivo* that can be produced under the pathological conditions in human body (Beckman and Koppenol, 1996; Szabo and Ohshima, 1997). A metalloporphyrin/oxidant model system has been employed to study on the mutagenicity of some carcinogens (Keilko and Masataka, 2002). Benzo[a]pyrene (B[a]P), the representative compound of PAHs, is the typical promutagen relying on metabolic activation to exhibit its cytotoxicity (Conney *et al*., 1994; Gao *et al*., 2005). We took peroxynitrite as the oxidant to establish a peroxynitrite/porphyrin biomimetic system to investigate the effects of peroxynitrite on metabolism of B[a]P.

All porphyrins were newly synthesized by our group (Wang *et al*., 2006). B[a]P was purchased from Sigma (USA) and B[a]P metabolite standards were purchased from America National Cancer Institute. Peroxynitrite was synthesized according to the reaction of hydroperoxide anion and isoamyl nitrite (Uppu and Pryor, 1996). Then peroxynitrite was stored at –20°C. Prior to experiments, the concentration of stored peroxynitrite was determined spectraphotometrically at 302 nm ($\epsilon_{302\,nm}$= 1670 $mol^{-1}cm^{-1}$) (Radi *et al*., 1991). All the other reagents were analytical reagent grade, and deionized water was used throughout the experiment.

Phosphate buffer solution (0.1 mol/L, pH 7.4) containing 100 μl porphyrin (5 mmol/L) and 50 μl alcoholic solution of B[a]P (the initial concentration was 2 mmol/L) was vigorously stirred at 37°C. Then peroxynitrite was slowly dropped into the reaction solution with the rate of 10 μmol/10s to keep the concentration of peroxynitrite at 1 mmol/L in the chemical system, and the final volume of solution was 10 ml. After 1 h reaction, the reaction solution was extracted with 2.0 ml ethyl acetate and the extract solution was separated by centrifugation. The supernatant was evaporated under vacuum to remove ethyl acetate, and then dissolved in 0.2 ml methanol for HPLC analysis.

HPLC was performed on a reverse phase chromatographic system (Waters models 600 system) consisting of Waters 600 HPLC pump and Waters 2487 model UV-Vis detector and Millennium32 workstation. The analytical column is Eclipse XDB-C18 reverse phase column (4.6 mm×250 mm, 5 μm, Agilent Company). Methanol 50% in water was used to equilibrate the column before injection. Then the column was eluted with a convex gradient of 50% methanol to 90% methanol during 35 min and 90% methanol maintained for 20 min. The eluent was monitored by ultraviolet absorption at 280 nm. The identification of metabolites was ascertained by coinjection of the samples with standards one at a time. Peak-height comparison was based on the results of samples with and without the standard. The system without peroxynitrite or porphyrin was used as the control experiment.

To obtain an effective model system, the relationship between iron porphyrin and conversion ratio of B[a]P was investigated. The effect of porphyrin on the conversion of B[a]P was in the order: T(*p*-Cl)PPFeCl (20.71%) > T(*p*-CH_3)PPFeCl (15.12%) > T(*p*-OCH_3)PPFeCl (13.15%).

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Among them, the peroxynitrite/T(*p*-Cl)PPFeCl system showed the best efficiency that the conversion ratio of B[a]P reached 20.71%, which was similar with our previous results (Wang *et al*., 2006). This is because the existence of electron-withdrawing group which enhances the catalytic activity of metalloporphyrin in the reaction of *p*-nitrotoluene to *p*-nitrobenzoic acid. So peroxynitrite/T(*p*-Cl)PPFeCl system was chosen for the following studies.

Eight of B[a]P metabolites induced by the peroxynitrite/T(*p*-Cl)PPFeCl system (Table 1) was identified as shown in Fig.1. From Table 1, it was found that while six

Table 1 Identified metabolites of B[a]P formed in peroxynitrite/T(*p*-Cl)PPFeCl system

Number of peak in Fig.1	1	2	3	4
Metabolites of B[a]P	B[a]P- 9, 10-diol	B[a]P-4, 5-diol	B[a]P-7, 8-diol	B[a]P-1, 6-quinone
Number of peak	5	6	8	9
Metabolites of B[a]P	B[a]P-3, 6-quinone	B[a]P-6, 12-quinone	6-nitro-B[a]P	1-nitro-B[a]P and 3-nitro-B[a]P

B[a]P metabolites were also formed from the metabolism of benzo[a]pyrene induced by microsome or cytochrome P450 (Selkirk *et al*., 1974; Datta and Samanta, 1988), 6-nitro-B[a]P, 3-nitro-B[a]P and 1-nitro-B[a]P were first identified in peroxynitrite system. It should be noted that nitrobenzo[a]pyrenes are the most potent direct-acting

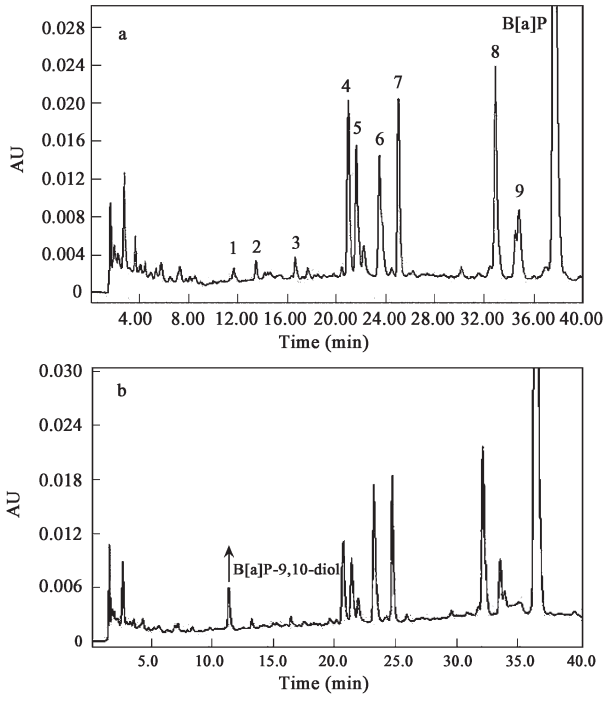

Fig. 1 Chromatogram of products from B[a]P induced by *p*-ClFeTPPCl/PN system. The column was eluted at room temperature with a convex gradient (curvature setting 5) of 50% methanol to 90% methanol in 35 min and then held at 90% methanol for 20 min, and the flow rate was 1.5 ml/min. (a) B[a]P metabolism induced by T(*p*-Cl)PPFeCl/peroxynitrite system; (b) addition of standard B[a]P-9,10-diol.

mutagens that can not only induce base-pair substitution mutations in bacterial assay (Watanabe *et al*., 1997) but also bring health risks to humans even at lower content levels (Schauer *et al*., 2004).

As a conclusion, we used peroxynitrite that exists not only in the human body but also in the environment (Pollack *et al*., 2003) as an oxidant to induce the metabolism of B[a]P. The activation of B[a]P by peroxynitrite/Fe porphyrin system can form a more powerful carcinogen, nitro-B[a]P. The identification of B[a]P metabolites induced by this system is helpful for the understanding of the effect of the peroxynitrite on PAHs. The biomimetic system established above can also be used to study the metabolism of other PAHs.

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