Biotransformation of Mycotoxins in the Reconstituted Cytochrome P-450 System

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再構成チトクローム P-450 系によるマイコトキシンの生転換

Summary

Metabolic transformation of ochratoxin A (OA) and emodin (EM) was investigated in the microsomal and reconstituted cytochrome P-450 systems of the rat liver. In the microsomal system, OA was transformed into 4(R)- and 4(S)-4-hydroxy-OAs, and 3-methylcholanthrene (3MC) as well as PCB enhanced the microsomal hydroxylation of OA to 4(R)-4-hydroxy-OA. In the reconstituted cytochrome P-450 system, cytochrome P-450 II-a (maximal CO-differential spectrum at 448.0 nm and high-spin form), fractionated from PCB-microsomes, selectively catalyzed the hydroxylation of OA and EM into 4(R)-4-hydroxy-OA and 2-hydroxy-EM, respectively.

In the preceding papers^{1,2)}, we reported that AFB₁ was biotransformed into DNA-binding form (s) and a hydroxylated metabolite, AFM₁, in the reconstituted cytochrome P-450 system composed of cytochrome P-450 s, NADPH-cytochrome C reductase (fpr), deoxycholate, and others, and that the activation of AFB₁ into DNA-binding form (s) and the hydroxylation to AFM₁ were selectively catalyzed by cytochrome P-450 I-a (maximal CO-differential spectrum at 450.0 nm and low-spin form) and cytochrome P-450 II-a (448.0 nm and high-spin form), respectively.

The hepatic microsomes derived from rats transformed emodin (EM; 1,3,8-trihydroxy-6-methyl-anthraquinone) into at least 10 anthraquinoid metabolites, and among these metabolites 2-hydroxy-EM (1,2,3,8-tetrahydroxy-6-methyl-anthraquinone)

is a direct-acting mutagen in Ames assay^{3,4)}.

In order to characterize the role of cytochrome P-450 isozymes in metabolic transformation of mycotoxins, we described the biotransformation of ochratoxin A (OA) and EM in the reconstituted cytochrome P-450 system.

Materials and Methods

Mycotoxins: OA and EM were isolated from *Aspergillus ochraceus* and rhubarb, respectively. Standard 4(R)- and 4(S)-4-hydroxy-OAs were kindly gifted by Dr. Størmer (University of Oslo).

Enzymes and assay: Microsomes were prepared from the livers of rats¹⁾, and cytochrome P-450 s were fractionated from the livers of PCB (Kanechlore 400)-induced rats²⁾. In the microsomal system, potassium phosphate buffer (pH 7.2) 100 μ moles, MgCl₂ 2.5 μ moles, NADP 2.5 μ moles, glucose-6-phosphate (G-6-P) 5 μ moles, G-6-P dehydrogenase 1 unit, microsomal protein 2-3 mg, and OA 0.125 or EM 37 μ moles, in total volume of 1.0 ml, were incubated at 37°C for 20-60 min. In the

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reconstituted cytochrome P-450 system, cytochrome P-450 0.1 nmole, fpr 0.75 unit, dilauroyl L-3 phosphatidyl choline 15 μ g, sodium deoxycholate 50 μ g, EDTA 0.05 μ mole, MgCl_2 7.5 μ moles, NADP 0.25 μ mole, G-6-P 2.5 μ moles, G-6-P dehydrogenase 0.5 unit, potassium phosphate buffer (pH 7.4) 25 μ moles, and OA 39 or EM 18 μ moles, in total volume of 0.5 ml, were incubated at 37°C for 60 min.

Analysis of metabolites: As for the metabolism of OA, the reaction was terminated by an addition of 0.5 ml of 2 N HCl solution, and the metabolites were extracted with methanol-chloroform mixture (2:1) and followed by TLC analysis. Silicagel plates were developed in chloroform-ethyl acetate-formic acid (10:3:1, v/v). After an additional development with the same solvent, the metabolites were determined by fluorodensitometry (CS-910 dual-wavelength chromatoscanner, Shimadzu; excitation at 345 nm and emission at 450 nm). The reaction mixture of EM metabolism was acidified with HCl, followed by extraction with ethyl acetate extract, and the ethyl acetate extract was analyzed by TLC. The silicagel plate, pretreated with 1 N oxalic acid, was developed in chloroform-cyclohexane-dioxane (10:1:1), and the metabolites were quantitated at 450 nm.

Results and Discussion

TLC analysis of the microsomal metabolites of OA revealed, besides of OA remained (Rf. 0.52), 4(S)-4-hydroxy-OA (Rf. 0.37), 4(R)-4-hydroxy-OA (Rf. 0.31), and a polar metabolite (Rf. 0.05). These two hydroxylated metabolites were co-migrated with the standards under different solvent systems such as chloroform-ether-acetic acid (17:5:1) and toluene-ethyl acetate-formic acid (6:3:1).

Fig. 1 showed the hydroxylating activity of hepatic microsomes prepared from the control and induced rats. In the control microsomes, the formation of 4(R)-4-hydroxy-OA was about 4 times higher than 4(S)-4-hydroxy-OA, and it was enhanced in PCB- and 3 MC-induced rats. In the

Fig. 1. Microsomal hydroxylation of ochratoxin A

Fig. 2. pH Dependency of the hydroxylation reaction of ochratoxin A in the hepatic microsomes derived from 3 MC-induced rats

3 MC-microsomes, the maximal production of 4(R)-4-hydroxy-OA was observed at pH 6.0 (Fig. 2). Since the maximal production of AFM₁ and 2-hydroxy-EM was observed at pH 8.0⁶⁾ and pH

Fig. 3. Metabolic transformation of ochratoxin A in the reconstituted P-450 system

7.0³⁾, respectively, the optimal pH for hydroxylation reaction is presumed to be dependent on the substrates used.

In the experiments with the reconstituted cytochrome P-450 system, formation of 4(R)-4-hydroxy-OA was the highest with cytochrome P-450 II-a, and followed by II-b and II-c, as shown in Fig. 3. The type I isoymes also catalyzed this reaction but their activity was far less than the type II. Formation of 4(S)-4-hydroxy-OA was observed in the various cytochrome P-450 isoymes, and the highest activity was demonstrated with P-450 I-c, and followed by I-d. The present data showed that cytochrome P-450 isoymes catalyzed the stereospecific hydroxylation of OA, and cytochrome P-450 II-a plays an principal role for the formation of 4(R)-4-hydroxy-OA, a major metabolite in the hepatic microsomes of rats.

As summarized in Table 1, the cytochrome P-450 I isoymes (a-d) produced no detectable amount of 2-hydroxy-EM by TLC analysis. On the other hand, cytochrome P-450 II-a produced 2-hydroxy-EM (35 nmol/nmol P-450/20 min), and other II-type isoymes produced this direct-acting mutagen but their activity was far less than II-a.

Table 1. Formation of 2-hydroxy-emodin in the reconstituted cytochrome P-450 system

Cytochrome P-450 isoymes	Maximal CO-difference spectra (nm)	2-Hydroxy-EM formed (nmol/nmol P-450/20 min)
I- a	450.0	ND*
I- b	452.0	ND
I- c	450.0	ND
I- d	450.0	ND
II- a	448.0	35.0
II- b	449.5	7.9
II- c	447.0	7.9
II- d	447.5	3.5

* ND: not detected.

Thus, the cytochrome P-450 II-a is the major isoyme in biotransformation of EM into a direct-acting mutagen, 2-hydroxy-EM.

Summing up the preceding^{1,4)} and present data, the cytochrome P-450 II-a, characterized by maximal CO-differential spectrum at 448.0 nm and a high-spin form, has a high activity in the hydroxylation of AFB₁, OA, and EM into AFM₁, 4(R)-4-hydroxy-OA, and 2-hydroxy-EM, respectively, and the reconstituted cytochrome P-450 system is useful for analysis of the biotransformation reaction of mycotoxins and other environmental toxins.

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