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RABBIT LIVER ENZYMES RESPONSIBLE FOR REDUCTION OF NITROPOLYCYCLIC AROMATIC HYDROCARBONS

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The present paper is the first description of mammalian nitroreductases acting upon nitropolycyclic aromatic hydrocarbons. Rabbit liver microsomal and cytosolic fractions have ability to reduce 1-nitropyrene or 2-nitrofluorene to the corresponding amine under anaerobic conditions. The nitroreductase activity in the former fraction is mainly due to a cytochrome P-450 system and that in the latter fraction is due to aldehyde oxidase.

KEYWORDS ——nitropolycyclic aromatic hydrocarbon; 1-nitropyrene; 2-nitrofluorene; nitroreduction; rabbit liver; microsome; cytosol; nitroreductase activity; cytochrome P-450; aldehyde oxidase

Recent studies showed that nitropolycyclic aromatic hydrocarbons (nitro-PAHs) are present in airborne particulates[1-3] and diesel engine emission,[4] and also in xerographic toners[5] and copies.[6] Nitrobenzo[a]pyrenes, nitropyrenes, nitroanthracenes, nitrochrysenes, nitrofluoranthenes, nitroacenaphthenes, nitroperylenes and nitrofluorenes are, all of them, among the most potent mutagens according to the Salmonella typhimurium assay,[6-8] and some of them have proved to be carcinogenic.[9-11] Therefore, these nitro compounds are noteworthy as a new type of evironmental pollutants. Generally, the metabolic nitroreduction appears to be essential for the occurrence of biological activities of nitro compounds. Indeed, the mutagenicity of nitro-PAHs in S. typhimurium depends upon nitroreduction of these compounds by nitroreductases of the tester strains.[7,12] However, little is known about the nature of mammalian and bacterial enzymes responsible for reduction of nitro-PAHs. The present communication is the first report of such mammalian nitroreductases. In the study, rabbit liver enzymes were examined using carcinogenic 1-nitropyrene and 2-nitrofluorene as substrates.

As shown in Table I, reduction of 1-nitropyrene and 2-nitrofluorene to the corresponding amines was catalyzed by rabbit liver microsomes supplemented with NADPH or NADH. In this case, NADPH was much more effective than NADH as an electron donor. The NADPH-linked nitroreductase activity was inhibited by carbon monoxide or SKF 525-A, indicating the involvement of cytochrome P-450 in the reduction of these nitro-PAHs by rabbit liver microsomes. This microsomal activity was almost completely abolished under aerobic conditions (data not shown).

On the other hand, rabbit liver cytosol by itself, unlike the liver microsomes described above, exhibited some nitroreductase effect on the nitro-PAHs. The

TABLE I. Reduction of 1-Nitropyrene and 2-Nitrofluorene by Rabbit Liver Microsomes

Addition	Amino compound formed (nmol/30 min/g liver)	
	1-Nitropyrene	2-Nitrofluorene
None	0	0
NADPH	148	126
NADH	21	30
NADPH-CO*	0	0
NADPH-SKF 525-A (10^{-3}M)	92	79

Values represent means of four experiments.

*The assay was performed under an atmosphere of carbon monoxide.

TABLE II. Reduction of 1-Nitropyrene and 2-Nitrofluorene by Rabbit Liver Cytosol

Addition	Amino compound formed (nmol/30 min/g liver)	
	1-Nitropyrene	2-Nitrofluorene
None	119	82
NADPH	122	90
NADH	158	124
Acetaldehyde	823	306
Benzaldehyde	924	359
N^1-Methylnicotinamide	1372	467
2-Hydroxypyrimidine	950	403
Methotrexate	881	318
Xanthine	110	80

Values represent means of four experiments.

activity was markedly enhanced by addition of an electron donor of aldehyde oxidase such as acetaldehyde, benzaldehyde, N^1-methylnicotinamide, 2-hydroxypyrimidine or methotrexate. However, little or no stimulatory effect was observed with NADPH or NADH, or xanthine which is an electron donor of xanthine oxidase (Table II). These facts strongly suggest that aldehyde oxidase is a principal enzyme responsible for the reduction of the nitro-PAHs by rabbit liver cytosol. Accordingly, the activities of both nitroreductase and aldehyde oxidase of the liver cytosol were similarly inhibited by menadione, chlorpromazine, sodium arsenite and potassium cyanide, respectively (Table III). Finally, we demonstrated that purified rabbit liver aldehyde oxidase in the presence of its electron donors exhibits a significant nitroreductase effect on 1-nitropyrene (Table IV).

From these results, we conclude that a microsomal cytochrome P-450 system and cytosolic aldehyde oxidase are mainly responsible for reduction of nitro-PAHs in rabbit liver.

TABLE III. Effect of Some Chemicals on Nitroreductase and Aldehyde Oxidase Activities of Rabbit Liver Cytosol

Addition	Concentration	Amino compound formed*		Aldehyde oxidase activity
		1-Nitropyrene	2-Nitrofluorene	
	M	% of control		
None (control)	—	100	100	100
Menadione	5×10^{-4}	2	11	0
Chlorpromazine	5×10^{-4}	2	6	0
Sodium arsenite	1×10^{-4}	4	15	11
Potassium cyanide	2.5×10^{-3}	5	18	18

Values represent means of four experiments.
*The assay was performed in the presence of N^1-methylnicotinamide.

TABLE IV. Reduction of 1-Nitropyrene by Rabbit Liver Aldehyde Oxidase

Addition	1-Aminopyrene formed (nmol/30 min/mg protein)
None	0
Acetaldehyde	1119
N^1-Methylnicotinamide	2149
2-Hydroxypyrimidine	1264
NADPH	2
NADH	3
Xanthine	2

Values represent means of four experiments.

EXPERIMENTAL

2-Nitrofluorene and 1-aminopyrene were purchased from Tokyo Kasei Kogyo Co.,Ltd. and 2-aminofluorene from Nakarai Chemicals,Ltd. 1-Nitropyrene was synthesized by the method of Ristagno and Shine.[13]

Male albino rabbits (2.0-2.5 kg) were killed and their livers were removed. The tissue was homogenized in 4 volumes of 1.15% KCl, the homogenate was centrifuged for 20 min at 9,000 x g, and the supernatant fraction was centrifuged for 60 min at 105,000 x g. The microsomal fraction was washed by resuspension in the KCl solution and resedimentation for 60 min at 105,000 x g. Rabbit liver aldehyde oxidase was prepared by the method of Rajagopalan *et al.*[14]

The incubation mixture for liver microsomes or cytosol consisted of 0.2 μmol of 1-nitropyrene or 2-nitrofluorene, 4 μmol of an electron donor and liver preparation equivalent to 0.1-0.5 g of liver in a final volume of 2.5 ml of 0.1 M phosphate

buffer (pH 7.4). For purified rabbit liver aldehyde oxidase, the incubation mixture consisted of 0.2 μmol of the substrate, 4 μmol of an electron donor, 0.025 μmol of FAD, 1 mg of the enzyme, 7.5 mg of bovine serum albumin and 72 μg of catalase in a final volume of 2.5 ml of the phosphate buffer. Prior to incubation, a Thunberg tube was gassed for 5 min with deoxygenated nitrogen, evacuated with an aspirator for 5 min and again gassed with nitrogen. In some experiments, carbon monoxide was used in place of nitrogen. The reaction was started by mixing the solution of the side arm and the body together, and continued for 30 min at 37°C. With 1-nitropyrene, the reaction mixture was adjusted to pH 10 with NaOH solution and then extracted twice with 5 ml each of heptane. With 2-nitrofluorene, the mixture was extracted twice with 5 ml each of ethyl acetate. The extracts were subjected to GLC to determine the reduction products of these nitro-PAHs. The gas chromatographic analysis was carried out using a Hitachi 163 gas chromatograph equipped with a flame ionization detector and a 2 m x 3 mm i.d. glass column packed with 3% Dexsil 400 GC on Chromosorb W. Operating conditions: Injection port and detector temperature 310°C for 1-aminopyrene, and 260°C for 2-aminofluorene; column temperature 260°C for 1-aminopyrene, and 210°C for 2-aminofluorene; internal standard cyproheptadine for 1-aminopyrene, and phenothiazine for 2-aminofluorene. Retention time of the reduction products was: 1-Aminopyrene, 14.0 min (1-nitropyrene, 16.5 min), and 2-aminofluorene, 8 min (2-nitrofluorene, 14.4 min). For identification of these reduction products, GC-mass spectra were recorded with a Shimadzu 7000S mass spectrometer. Assay of aldehyde oxidase was carried out according to the method of Felsted et al.[15]

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