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INVOLVEMENT OF LIVER ALDEHYDE OXIDASE IN THE REDUCTION OF NICOTINAMIDE N-OXIDE

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SUMMARY. The present paper describes that mammalian liver aldehyde oxidase is involved in the reduction of nicotinamide N-oxide to nicotinamide. Rabbit liver aldehyde oxidase supplemented with its electron donor exhibited a significant nicotinamide N-oxide reductase activity under anaerobic conditions. Liver cytosols from rabbits, hogs, guinea pigs, hamsters, rats and mice, all of them, similarly exhibited the N-oxide reductase activity in the presence of an electron donor of aldehyde oxidase, but not xanthine oxidase. The cytosolic N-oxide reductase activity was almost completely inhibited by menadione, an inhibitor of aldehyde oxidase.

Nicotinamide N-oxide, which occasionally produced a marked stimulation of the squalene oxidocyclase activity (1), was found as an in vivo metabolite of nicotinamide in rats (1) and mice (2,3). Generally, N-oxide compounds undergo the biological reduction to the corresponding heterocyclic or tertiary amino compounds. However, only a little is known about the enzymes involved. As regards nicotinamide N-oxide, Chaykin and Bloch (1) demonstrated first that hog liver homogenate catalyzes its reduction to nicotinamide. An enzyme involved in the N-oxide reduction was isolated from hog liver and identified as xanthine oxidase (4,5).

On the other hand, recently, we found that liver aldehyde oxidase can catalyze the reduction of sulfoxides (6,7), nitrosamines (8,9), hydroxamic acids (10,11), azo dyes (12) and nitropolycyclic aromatic hydrocarbons (13). The present communication provides evidence that aldehyde oxidase functions as a major

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liver enzyme responsible for the reduction of nicotinamide N-oxide to nicotinamide.

MATERIALS AND METHODS

Enzymes. Liver cytosol was prepared as follows: The liver was homogenized in four volumes of 1.15 % KCl, the homogenate was centrifuged for 20 min at 9,000 x g, and cytosol was separated from microsomes by centrifugation for 60 min at 105,000 x g. Rabbit liver aldehyde oxidase was purified according to the method of Rajagopalan et al. (14). Bovine liver catalase was purchased from Sigma Chemical Co.

Assay of N-oxide reductase activity. The incubation mixture consisted of 0.5 μmol of nicotinamide N-oxide, 2 μmol of an electron donor, 0.005 unit of aldehyde oxidase, 12 μg of catalase and 1.25 mg of bovine serum albumin in a final volume of 1 ml of 0.1 M phosphate buffer (pH 7.4). When liver cytosol was used in place of aldehyde oxidase, both catalase and bovine serum albumin were omitted from the incubation mixture. The incubation was performed using a Thunberg tube under anaerobic conditions. The side arm contained an electron donor and the body contained all other components. The tube was gassed for 3 min with deoxygenated nitrogen, evacuated with an aspirator for 10 min and again gassed with the nitrogen. The reaction was started by mixing the compounds of the side arm and the body together, continued for 5 min at 37° and stopped by adding 0.1 ml of 5 N NaOH. A control tube, lacking nicotinamide N-oxide, was run with each reaction tube to correct for formation of nicotinamide other than that from nicotinamide N-oxide. The mixture, after adding 30 μg of benzamide as an internal standard and 0.1 g of NaCl, was extracted twice with 5 ml each of ethyl acetate and the combined extract was evaporated to dryness in vacuo. The residue was dissolved in 0.1 ml of methanol and then subjected to high pressure liquid chromatography (hplc). Hplc was performed in a Gilson 1B high pressure liquid chromatograph equipped with a M & S Variactor 311 ultraviolet absorption detector. The instrument was fitted with a 15 cm x 4.6 mm (I.D.) M & S Pack C18 column. The mobile phase was methanol-water (1:9). The chromatograph was operated at a flow rate of 1.1 ml/min at ambient temperature and at a wavelength of 254 nm. Nicotinamide (elution time, 6.3 min) formed was determined from its peak area.

RESULTS

As shown in Table I, rabbit liver aldehyde oxidase exhibited a significant nicotinamide N-oxide reductase activity in the presence of its electron donor such as 2-hydroxypyrimidine, N^1-methylnicotinamide, benzaldehyde or butyraldehyde. In this case, 2-hydroxypyrimidine was most effective. Rabbit liver cytosol, which contains aldehyde oxidase together with other flavoenzyme such as xanthine oxidase and DT-diaphorase, also exhibited the N-oxide reductase activity in the presence of the electron donors

Table I. Reduction of nicotinamide N-oxide by rabbit liver aldehyde oxidase and cytosol

Addition	Nicotinamide formed	
	Aldehyde oxidase (μmol/5 min/mg protein)	Cytosol (μmol/5 min/g liver)
None	0	0.1
2-Hydroxypyrimidine	51.5	116.4
N^1-Methylnicotinamide	9.1	12.6
Benzaldehyde	6.4	13.3
Butyraldehyde	6.8	12.1
Xanthine	0	0.2
NADH	0	0.2
NADPH	0	0.2
2-Hydroxypyrimidine plus menadione	0	0
2-Hydroxypyrimidine plus p-benzoquinone	0	0

Each value represents mean of four experiments.

of aldehyde oxidase. The 2-hydroxypyrimidine-linked activity of the cytosol as well as aldehyde oxidase was markedly inhibited by menadione and p-benzoquinone which are known as strong inhibitors of aldehyde oxidase. On the other hand, no significant N-oxide reductase activity above the control was found when xanthine, NADH or NADPH, which is an electron donor of xanthine oxidase or DT-diaphorase, was added. Furthermore, the ability of rabbit liver microsomes to reduce nicotinamide N-oxide was examine under anaerobic conditions. However, the liver preparation exhibited no N-oxide reductase activity even in the presence of NADPH or NADH (data not shown). Thus, the present study demonstrated that aldehyde oxidase functions as a major nicotinamide N-oxide reductase in the rabbit liver.

As shown in Table II, the liver cytosols from several mammalian species besides rabbits also exhibited, in varying degrees,

Table II. Nicotinamide N-oxide reductase activity of liver cytosols from several mammalian species

Addition	Nicotinamide formed (μmol/5 min/g liver)				
	Hog	Guinea pig	Hamster	Rat	Mouse
None	0.3	0.2	0.2	0.1	0.1
2-Hydroxypyrimidine	67.9	64.7	208.4	5.1	14.8
Xanthine	0.3	0.2	0.2	0.1	0.1
2-Hydroxypyrimidine plus menadione	1.4	0.8	0.6	0	0

Each value represents mean of four experiments.

a significant nicotinamide N-oxide reductase activity in the presence of 2-hydroxypyrimidine, but not xanthine. In either case, the 2-hydroxypyrimidine-linked activity was again markedly inhibited by menadione. From these results, we concluded that liver aldehyde oxidase plays a prominent role in the reduction of nicotinamide N-oxide not only in rabbits, but also in other mammalian species.

DISCUSSION

Xanthine oxidase supplemented with its electron donor such as xanthine or NADH has been shown to be capable of reducing nicotinamide N-oxide to nicotinamide under anaerobic conditions (5). The enzyme can also reduce the N-oxide group of several Purine N-oxide derivatives (15). Therefore, a preliminary attempt was made to roughly compare nicotinamide N-oxide reductase activity of xanthine oxidase with that of aldehyde oxidase. When nicotinamide N-oxide (0.5 μmoles) was anaerobically incubated at 37° with milk xanthine oxidase (1 unit) and xanthine (2 μmoles) in 0.1 M phosphate buffer, pH 7.4, the amount of nicotinamide formed was only 3.6 nanomoles per 5 minutes. The value is too much lower, compared with 62 micromoles of nicotinamide formed per 5 minutes per unit of rabbit liver aldehyde oxidase when nicotin-

amide N-oxide was incubated with the enzyme and 2-hydroxypyrimidine as described in the experimental section. This seems to be a reason why all of mammalian liver cytosols tested exhibited no significant nicotinamide N-oxide reductase activity over the control in the presence of xanthine.

Another preliminary study showed that aldehyde oxidase activity of liver cytosols from six mammalian species tested, which was assayed by using 2-hydroxypyrimidine as an electron donor and ferricyanide as an electron acceptor, was almost parallel to their 2-hydroxypyrimidine-linked nicotinamide N-oxide reductase activity. This fact supports the idea that the 2-hydroxypyrimidine-linked N-oxide reductase activity in these liver cytosols is due to aldehyde oxidase. In addition, the study showed that rabbit liver aldehyde oxidase in the presence of 2-hydroxypyrimidine can also catalyze the reduction of N-oxide compounds such as nicotinic acid N-oxide, quinoline N-oxide, 2-bromopyridine N-oxide and β-picoline N-oxide.

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