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INVOLVEMENT OF LIVER ALDEHYDE OXIDASE IN SULFOXIDE REDUCTION

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The present study provides the first evidence that guinea pig liver aldehyde oxidase, which is in the cytosolic fraction, in the presence of its electron donors such as aldehydes or N-heterocyclic compounds, functions as a sulfoxide reductase towards sulindac and other sulfoxide compounds.

KEYWORDS —— sulfoxide compound; sulfoxide reduction; sulfoxide reductase; aldehyde oxidase; guinea pig liver; sulfides

Enzymatic sulfoxide reduction is responsible for activation, inactivation and alteration of action of sulfoxide compounds in animal bodies; e.g., the pharmacological activities of sulindac (*cis*-5-fluoro-2-methyl-1-[*p*-(methylsulfinyl)benzylidenyl]indene-3-acetic acid), one of nonsteroidal anti-inflammatory agents, are attributable to the reduction product sulindac sulfide (5-fluoro-2-methyl-1-[*p*-(methylthio)benzylidenyl]indene-3-acetic acid).[1] However, little has been known about the nature of mammalian sulfoxide reductase. Recently, Anders et al. suggested the involvement of thioredoxin in the reduction of sulindac by cytosolic enzyme(s) of rat liver[2] and kidney.[3] And we demonstrated that some flavoenzymes such as NADPH-cytochrome c reductase and xanthine oxidase supplemented with their electron donors exhibited sulfoxide reductase activity in the presence of the soluble factor in guinea pig liver cytosol.[4,5] The present study provides the first evidence that guinea pig liver aldehyde oxidase is involved in sulfoxide reduction.

First, the ability of guinea pig liver 9,000 x *g* supernatant to catalyze the reduction of sulindac was assessed in the presence of NADPH, NADH or acetaldehyde. The liver preparation was found to exhibit a significant sulfoxide reductase activity in the presence of acetaldehyde under anaerobic conditions. The acetaldehyde-linked activity occurred in the cytosolic fraction, but not in the microsomal fraction (Table I).

Furthermore, when the liver cytosol was subjected to ammonium sulfate fractionation as described in EXPERIMENTAL, the acetaldehyde-linked activity was associated with a protein which was precipitated between 30 and 45% ammonium sulfate saturation. The ability of the precipitate to reduce sulindac was again examined in the presence of aldehydes, N-heterocyclic compounds or reduced pyridine nucleotides under anaerobic conditions. The precipitate showed sulfoxide reductase activity in the presence of aldehydes or N^1-methylnicotinamide or 2-hydroxypyrimidine in varying degree, but not in the presence of NADPH or NADH (Table II). These aldehydes and

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TABLE I. Intracellular Localization of Sulfoxide Reductase Activity in Guinea Pig Liver

Fraction	Electron donor	Sulfoxide reductase activity* Anaerobic	Aerobic
		μmol/30 min/g liver	
9,000xg Supernatant	None	0.20	0.04
	NADPH	0.41	0.04
	NADH	0.20	0.03
	Acetaldehyde	3.58	0.16
Microsomes	None	0	0
	Acetaldehyde	0.02	0
Cytosol	None	0.26	0.05
	Acetaldehyde	3.65	0.27

Each value represents mean of four experiments.
*The assay was carried out using sulindac as described in EXPERIMENTAL.

TABLE II. Electron Donor Requirements for Sulfoxide Reductase Activity of the 30-45% Ammonium Sulfate Precipitate from Guinea Pig Liver Cytosol

Electron donor	Sulfoxide reductase activity*
	nmol/30 min/mg protein
None	0
Acetaldehyde	114
Propionaldehyde	136
Butyraldehyde	97
Benzaldehyde	97
N^1-Methylnicotinamide	100
2-Hydroxypyrimidine	152
NADPH	10
NADH	6

Each value represents mean of four experiments.
*The assay was carried out using sulindac as described in EXPERIMENTAL.

N-heterocyclic compounds are known as electron donors of aldehyde oxidase which is one of liver cytosolic enzymes. Boiling the ammonium sulfate precipitate prior to incubation completely abolished its ability to reduce sulindac. These results suggest that aldehyde oxidase present in the cytosolic fraction of guinea pig liver functions as sulfoxide reductase.

Next, the comparative ability of some chemicals to inhibit the aldehyde oxidase and sulfoxide reductase activities of the 30-45% ammonium sulfate precipitate was examined. As shown in Table III, both activities were similarly susceptible to inhibition by all of these chemicals except allopurinol, which is a specific

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TABLE III. Effect of Various Chemicals on Sulfoxide Reductase and Aldehyde Oxidase Activities of the 30-45% Ammonium Sulfate Precipitate from Guinea Pig Liver Cytosol

Addition	Concentration	Sulfoxide reductase activity*	Aldehyde oxidase activity
	M	% of control	
None (control)		100	100
Menadione	5×10^{-5}	2	0
Chlorpromazine	2×10^{-4}	22	19
Amidol	2×10^{-4}	3	0
Potassium cyanide	2.5×10^{-4}	6	9
Sodium arsenite	1×10^{-4}	5	18
p-Chloromercuri-benzoic acid	1×10^{-4}	3	9
Quinacrine	1×10^{-4}	41	37
Dicumarol	1×10^{-5}	66	67
Allopurinol	2×10^{-4}	93	100

Each value represents mean of four experiments.

*The assay was carried out using sulindac as described in EXPERIMENTAL.

inhibitor of xanthine oxidase.

Furthermore, the ammonium sulfate precipitate was chromatographed on DEAE-cellulose (DE-52) column. After adsorption of the protein, elution was carried out with 10 mM phosphate buffer (pH 7.4), followed by a gradient of increasing NaCl concentration up to 0.2 M concentration. As a result, the elution peak of aldehyde oxidase free of xanthine oxidase activity was observed at the 0.125 M concentration of NaCl. This peak position was identical with that of sulindac reductase activity. These facts led us to conclude that guinea pig liver aldehyde oxidase in the presence of its electron donor can catalyze the reduction of sulindac.

Our preliminary study showed that the aldehyde oxidase with acetaldehyde as an electron donor can also catalyze the reduction of a variety of sulfoxide compounds: dimethyl sulfoxide, diphenyl sulfoxide, dibenzyl sulfoxide, dichlorodiphenyl sulfoxide, phenothiazine sulfoxide, sulfinpyrazone, biotin sulfoxide methyl ester and methionine sulfoxide.

Previously, we demonstrated that flavoenzymes such as xanthine oxidase and NADPH-cytochrome c reductase supplemented with their electron donors showed sulfoxide reductase activity towards diphenyl sulfoxide in the presence of the 30-45% ammonium sulfate precipitate from guinea pig liver cytosol, which was temporarily designated as "soluble factor". Based on this, we suggested that a new electron transfer system in which the soluble factor functions as an electron carrier coupled with the flavoenzymes described above was responsible for the sulfoxide reduction.[5)] However, the nature of the soluble factor remained unknown. Our preliminary study showed that when the 30-45% ammonium sulfate precipitate was subjected to DEAE-cellulose column chromatography as described above, the activity of the soluble factor, which was assayed by its capacity to support the reduction of sulindac by

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milk xanthine oxidase supplemented with xanthine, was eluted at the same position as aldehyde oxidase. This fact strongly suggested that guinea pig liver aldehyde oxidase functions not only as sulfoxide reductase in the presence of its electron donor, but also as the soluble factor which can cooperate with other flavoenzymes for sulfoxide reduction.

The purification of aldehyde oxidase from guinea pig liver is in progress in our laboratory.

EXPERIMENTAL

Male guinea pigs (350-400 g) were killed by decapitation 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*. The 105,000 x *g* supernatant (cytosol) was subjected to ammonium sulfate fractionation, and proteins which were precipitated between 0 and 30%, 30 and 45%, and 45 and 60% ammonium sulfate saturation were collected.

In the assay of sulfoxide reductase activity, a typical incubation mixture consisted of 0.4 μmol of sulindac, 2 μmol of an electron donor and 0.5 ml of liver preparation (equivalent to 0.1-0.5 g of liver) in a final volume of 2.5 ml of 0.1 M K,Na-phosphate buffer (pH 7.4). Anaerobic incubation was performed using a Thunberg tube. The tube was gassed for 3 min with deoxygenated nitrogen, evacuated with an aspirator for 5 min and again gassed with 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. In aerobic experiments, the incubation was performed in an open vessel. After incubation, the reduction product (sulindac sulfide) of sulindac was determined according to the method described in a previous paper.[4) Assay of aldehyde oxidase activity was carried out according to the method of Felsted et al.[6)]; the increase in optical density at 300 nm which accompanies the oxidation of N^1-methylnicotinamide to the 2- and 4-pyridones was determined.

REFERENCES

1) D.E. Duggan, K.F. Hooke, E.A. Risley, T.Y. Shen and C.G. Van Arman, J.Pharmacol. Exp.Ther., 201, 8 (1977).
2) M.W.Anders, J.H. Ratnayake, P.E. Hanna and J.A. Fuchs, Biochem.Biophys.Res. Commun., 97, 846 (1980).
3) M.W. Anders, J.H. Ratnayake, P.E. Hanna and J.A. Fuchs, Drug Metab.Dispos., 9, 307 (1981).
4) S. Kitamura, K. Tatsumi and H. Yoshimura, J.Pharm.Dyn., 3, 290 (1980).
5) S. Kitamura, K. Tatsumi, Y. Hirata and H. Yoshimura, J.Pharm.Dyn., 4, 528 (1981).
6) R.L. Felsted, A.E.-Y. Chu and S. Chaykin, J.Biol.Chem., 248, 2580 (1973).

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