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A SULFOXIDE-REDUCING ENZYME SYSTEM CONSISTING OF ALDEHYDE OXIDASE AND XANTHINE OXIDASE
—— A NEW ELECTRON TRANSFER SYSTEM ——

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Recently, we found that liver aldehyde oxidase functions as sulfoxide reductase in the presence of its electron donor. In addition, the present study provides the first evidence that a combination of liver aldehyde oxidase and milk xanthine oxidase also exhibits sulfoxide reductase activity in the presence of xanthine, an electron donor of xanthine oxidase. Based on these facts, we propose a new electron transfer system consisting of these flavoenzymes.

KEYWORDS ——— sulfoxide reduction; sulfoxide compound; liver aldehyde oxidase; milk xanthine oxidase; sulfoxide-reducing enzyme system; electron transfer system

Previously, we demonstrated that flavoenzymes such as xanthine oxidase and NADPH-cytochrome c reductase supplemented with their electron donors showed sulfoxide reductase activity 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 an electron transfer system in which the soluble factor functions as an electron carrier coupled with the flavoenzymes was responsible for sulfoxide reduction.[1] However, the nature of the soluble factor remained unknown. Here we describe evidence that liver aldehyde oxidase functions as the soluble factor for sulfoxide reduction. In this study, sulindac (cis-5-fluoro-2-methyl-1-[p-(methylsulfinyl)benzylidenyl]indene-3-acetic acid) was used as a substrate and milk xanthine oxidase as a flavoenzyme coupled with the soluble factor.

As shown in Table I, the 30-45% ammonium sulfate precipitate from guinea pig liver cytosol or xanthine oxidase by itself showed no sulfoxide reductase activity in the presence of an electron donor of xanthine oxidase, xanthine. However, when the ammonium sulfate precipitate was combined with xanthine oxidase, a significant reductase activity was observed in the presence of the electron donor. The activity was markedly enhanced by FAD, FMN, riboflavin, methyl viologen or benzyl viologen (data not shown). Thus, the ammonium sulfate precipitate contains a factor(s) essential for sulfoxide reduction.

When the ammonium sulfate precipitate was subjected to DEAE-cellulose column chromatography as described in Experimental, the elution peak of aldehyde oxidase free of xanthine oxidase occurred at the 0.125 M concentration of NaCl. This peak

TABLE I. Milk Xanthine Oxidase-linked Sulfoxide Reductase Activity in the 30-45% Ammonium Sulfate Precipitate from Guinea Pig Liver Cytosol

Enzyme	Electron donor	Sulfoxide reductase activity
		nmol/30 min/mg protein
Ammonium sulfate precipitate (I)	Xanthine	0
Xanthine oxidase (II)	Xanthine	0
I plus II	Xanthine	31
	None	0

Each value represnts mean of four experiments.

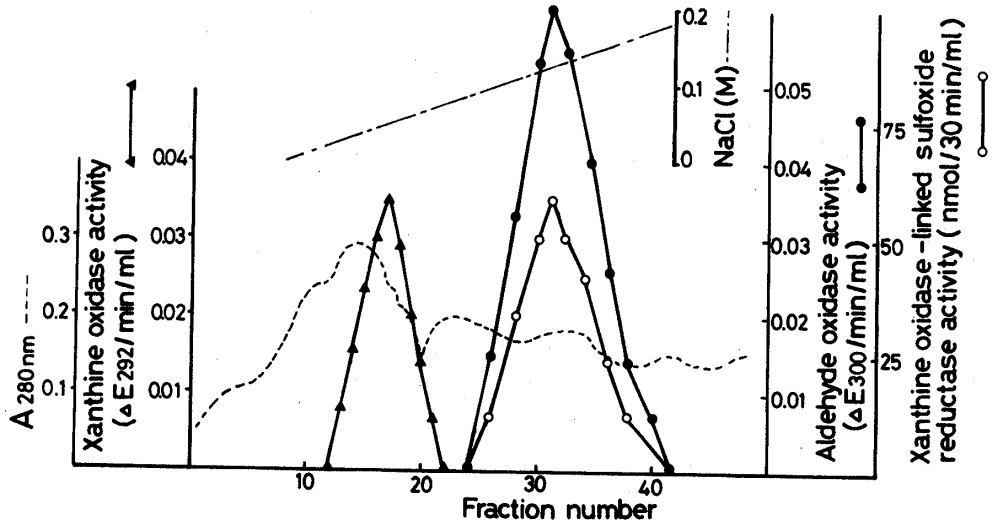

Fig. 1. Chromatography of Milk Xanthine Oxidase-linked Sulfoxide Reductase, Aldehyde Oxidase and Xanthine Oxidase in the 30-45% Ammonium Sulfate Precipitate from Guinea Pig Liver Cytosol on a DEAE-Cellulose Column

position was identical with that of the soluble factor, which was assayed by its capacity to support sulfoxide reduction by xanthine oxidase supplemented with xanthine (Fig. 1).

Furthermore, we compared the effect of some chemicals on xanthine oxidase, liver aldehyde oxidase obtained by DEAE-cellulose column chromatography as described above, and the sulfoxide-reducing enzyme system consisting of these two enzyme. As shown in Table II, all of the chemicals tested exhibited little or no effect on xanthine oxidase activity, whereas the activities of both aldehyde oxidase and sulfoxide reductase were similarly susceptible to inhibition by these chemicals.

These facts indicate that guinea pig liver aldehyde oxidase functions not only as sulfoxide reductase in the presence of its electron donor as described previously,[2] but also as a "soluble factor" which can cooperate with xanthine oxidase

TABLE II. Effect of Chemicals on Milk Xanthine Oxidase, Guinea Pig Liver Aldehyde Oxidase and a Sulfoxide-Reducing Enzyme System Consisting of These Two Enzymes

Addition	Concentration	Xanthine oxidase activity	Aldehyde oxidase activity	Sulfoxide reductase activity
	M		% of control	
None (control)	—	100	100	100
Chlorpromazine	2×10^{-4}	106	20	23
Potassium cyanide	2.5×10^{-4}	90	12	17
Sodium arsenite	1×10^{-4}	104	20	5
Quinacrine	1×10^{-4}	92	10	20

Each value represents mean of four experiments.

in sulfoxide reduction.

To confirm this view, aldehyde oxidase from rabbit liver was purified according to the method of Rajagopalan et al.[3] The purified rabbit liver enzyme showed a significant sulfoxide reductase activity in the presence of an electron donor such as acetaldehyde (data not shown). As shown in Table III, furthermore, the enzyme by itself, like guinea pig liver enzyme, showed no sulfoxide reductase activity in the presence of xanthine, but when the enzyme was combined with xanthine oxidase, such activity occurred. Again the activity was stimulated by FAD, FMN, riboflavin, methyl viologen or benzyl viologen (data not shown). This enzyme system can also catalyze the reduction of diphenyl sulfoxide to diphenyl sulfide.

TABLE III. Sulfoxide Reductase Activity of a Combination of Rabbit Liver Aldehyde Oxidase and Milk Xanthine Oxidase

Enzyme	Electron donor	Sulfoxide reductase activity
		nmol/30 min/ mg protein
Aldehyde oxidase (I)	Xanthine	0
Xanthine oxidase (II)	Xanthine	0
I plus II	Xanthine	145
	None	0

Each value represents mean of four experiments.

The present study provides the first evidence that a combination of aldehyde oxidase and xanthine oxidase exhibits a significant reductase activity in the presence of an electron donor of the latter enzyme. As described previously,[2] the former enzyme alone can also exhibit sulfoxide reductase activity in the

presence of its own electron donor (e.g. aldehydes, and N-heterocyclic compounds such as N^1-methylnicotinamide and 2-hydroxypyrimidine). Based on these facts, we propose here a new electron transfer system consisting of these two flavoenzymes as shown in Chart 1. Our preliminary study showed that this enzyme system can utilize not only sulfoxide compounds, but also N-oxide compounds (e.g. cyclobenzaprine N-oxide) as its electron acceptor.

The electron transfer mechanism in this enzyme system is currently under investigation.

Chart 1. A Postulated New Electron Transfer System Consisting of Aldehyde Oxidase and Xanthine Oxidase

EXPERIMENTAL

Sulindac and sulindac sulfide (5-fluoro-2-methyl-1-[p-(methylthio)benzylidenyl]-indene-3-acetic acid) were kindly donated by Merck Sharp and Dohme Research Laboratories. Purified butter milk xanthine oxidase was purchased from Sigma Chemical Co. Aldehyde oxidase from rabbit livers was purified by the method of Rajagopalan et al.[3] The 30-45% ammonium sulfate precipitate from guinea pig liver cytosol was prepared as described previously.[1]

In the assay of sulfoxide reductase activity, a typical incubation mixture consisted of 0.4 μmol of sulindac, 2 μmol of xanthine, 0.5 unit of milk xanthine oxidase and liver aldehyde oxidase preparation in a final volume of 2.5 ml of 0.1 M K,Na-phosphate buffer (pH 7.4). The mixture was incubated anaerobically for 30 min at 37°C using a Thunberg tube, and the reduction product (sulindac sulfide) of sulindac was determined as described previously.[1] When rabbit liver aldehyde oxidase was used as an enzyme source, 3 mg of bovine serum albumin and 30 μg of catalase were added to the above mixture, with incubation for 10 min.

Aldehyde oxidase and xanthine oxidase activities were assayed by the methods of Felsted et al.[4] and Nakamura and Yamasaki,[5] respectively.

DEAE-cellulose column chromatography was performed as follows: A column (1.5x12 cm) of DE-52 was equilibrated with 10 mM phosphate buffer (pH 7.4). After adsorption of the 30-45% ammonium sulfate precipitate (5 ml, 150 mg of protein) on the column, elution was carried out by a linear gradient method of increasing NaCl concentration in the phosphate buffer up to 0.2 M concentration. Fraction (5 ml) was collected at a flow rate of 25 ml/h.

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