A COMPARISON OF METHIONINE-S-OXIDE REDUCTASE ACTIVITIES IN LIVER AND KIDNEY BETWEEN CATTLE AND SWINE

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Introduction

Methionine sulfoxide (MSO) is now recognized to be available as a methionine (Met) source for mammals. Recently MSO has been isolated from the incubation medium of rumen ciliate protozoa and identified chemically (Onodera & Takei, 1986). This compound has also been detected in rumen fluid (Salsbury et al., 1971) and in the metabolite of Met by rumen protozoa (Onodera and Migita, 1985). The blood of both steer and goats has been revealed to contain an appreciable amount of MSO which was uaually not present in human blood (Cook et al., 1965).

These data were suggestive enough to arrive at a hypothesis that ruminant animals might have higher methionine-S-oxide reductase (EC 1.8.4.5) (MSO reductase) activity than did simple-stomached animals. The present paper reports a comparative study of MSO reductase activities in liver and kidney between cattle and swine as representatives of ruminants and simple-stomached animals, respectively.

Materials and Methods

Liver and kidney were obtained from cattle and swine slaughtered in a meat plant in Miyazaki city, Japan. Liver and kidney of cattle and swine were minced with scissors (about 0.5 cm) and 1.6g of the tissue preparations were suspended in 10 ml of the homogenization phosphate buffer solution (pH was adjusted with 0.1 M KH_2PO_4 and 0.1 M K_2HPO_4, when necessary). The suspensions were homogenized in a cooled homogenizer (below 4°C) (Homo blendor, Sakuma Seisakusho, Ltd.). Homogenates were then centrifuged at 27,000xg for 30 min or 100,000xg for 60 min, and the suspernatant fluid was usually used as crude enzyme solution, though the homogenate itself and the precipitates resuspended in the same volume of buffer were also used, when necessary.

Incubation mixture consisted of 1 ml of enzyme solution and 1 ml of substrate solution (0.02 to 2.0 mM L-methionine sulfoxide (Sigma) in 0.1 M phosphate buffer solution mentioned above) which also contained a reducing agent such as NADH, NADPH, dithiothreitol or glutathione (final concentration: 1 mM), when the effects of these agents were studied. Incubation was carried out at the optimum temperature or at the temperature of the tissues (39°C) for 3 h. After incubation, the reaction mixture was mixed with 1 ml of 10% (w/v) sulfosalicylic acid (cold) to stop the reaction and left in a refrigerator for more than 6 h to deproteinize. The mixture was, then, centrifuged (27,000xg, 30 min, at 4°C) and the clear supernatant fluid was used for analysis of Met.

Met was analyzed by high speed amino acid analyzer (MLC-203, ATTO, Japan) and MSO reductase activity was expressed as a difference of Met concentrations increased in the reaction mixture with and without substrate per protein or nitrogen in the crude enzyme solution or per g of tissue. Protein and nitrogen were analyzed by the method of Lowry et al. (1952) and microkjeldahl method, respectively.

Results and Discussion

At first, MSO reductase activity was compared among homogenate, supernatant fluid and precipitate. As a result, the activity was found chiefly in homogenates and supernatant fluid in all samples. According to this finding, the supernatant fluid was used hereafter for the assay of the enzyme activity.

Then the characteristics of the enzyme in both tissues of both animals were examined to determine the conditions necessary for the comparison of the enzyme activity between both animals and the results were summarized in table 1. The results suggested that optimum pH and temperature and the affinity for the substrate were different between not only animals but also tissues and the

TABLE 1. CHARACTERISTICS OF METHIONINE-S-OXIDE REDUCTASE IN LIVER AND KIDNEY OF CATTLE AND SWINE

Items	Cattle		Swine	
	Liver	Kidney	Liver	Kidney
Optimum pH	6.0	6.7	7.0	7.0
Optimum temperature (°C)	33	39	37	37
Reducing agent[a] (1 mM)	DTT>NADH= NADPH>GSH> Con	DTT>NADPH> NADH=GSH= Con	DTT>NADH= NADPH>GSH> Con	DTT>NADPH> NADH=Con> GSH
Substrate conc.(mM)[b] (for V_{max})	0.15	0.60	0.25	0.25

[a]DTT, dithiothreitol; GSH, glutathione; Con, control (no agent).

[b]Methionine sulfoxide concentration for getting maximum velocity (activity).

requirement of reducing agent was also different between tissues of both animals, where NADH for liver and NADPH for kidney. Based on these results, the conditions for assessing the ability of the enzyme under physiological states of both animals were determined as follows: pH, optimum pH; temperature, 39°C (temperature of tissues); reducing agent, 1 mM NADH for liver and 1 mM NADPH for kidney; substrate concentration, 0.8 mM for cattle liver, 0.25 mM for swine liver, 0.6 mM for cattle kidney and 1.0 mM for swine kidney. Under these conditions, the enzyme activities of liver and kidney of cattle and swine were determined using more than 10 heads of animals (table 2). The results obtained with both tissues of both animals varied from animal to animal so that individuals had to be divided at least into three groups in both tissues of cattle and into two groups in both tissues of swine. With liver, the activity per unit weight (g) of tissue of high and low group of cattle were about threefold higher than that of high and low group of swine, respectively. With kidney, the activity of high group of cattle was 2.4 times as high as the high group of swine, but the activity of low group of cattle was about a third of that of low group of swine.

In order to compare the standard abilities to reduce MSO to give methionine between cattle

TABLE 2. METHIONINE-S-OXIDE REDUCTASE ACTIVITIES IN LIVER AND KIDNEY OF CATTLE AND SWINE

Groups based on enzyme acitivity	Cattle		Swine	
	Liver	Kidney	Liver	Kidney
High[a]	723 (2)	697±75 (4)	232 (2)	296±28 (8)
Medium[a]	437±52 (7)	303 (2)	—	—
Low[a]	178±34 (8)	53±28 (6)	63±23 (8)	165 (2)
Total activity of[b] liver and kidney	1,100		193	

[a]Unit: nmol of methionine/hr/g of tissue ± SE. Figures in ( ) are heads of animals (frequency).

[b]Total enzyme activity of liver and kidney calculated from the figures of the MOS reductase activities of both tissues of the highest frequency of groups of both animals, provided that liver of cattle and swine were 6,000 and 2,000 g, respectively, and kidney of cattle and swine were 650 and 225 g, respectively and that there were enough substrate.

and swine, the amount of MSO possibly produced by both liver and kidney in both animals was calculated using the figures of the MSO reductase activities of both tissues of the highest frequency (table 2) of groups of both animals, provided that liver of cattle and swine were 6,000 and 2,000 g, respectively, and kidney of cattle and swine were 650 and 225 g, respectively and that there were enough substrate. As a result, the abilities were shown as 1,100 and 193 μg of methionine/hr/head for cattle and swine, respectively (table 2), indicated that the standard abilities of cattle was 5.7 times as high as that of swine.

Wide variations of the activities in both tissues of cattle and swine shown in the present experiments led us to presume that MSO reductase might be an inducible enzyme. If it is true, the higher the supply of MSO, the higher the activity may be. Relatively higher activities in cattle than swine may reflect the higher production of MSO in the rumen and hence the concentration of MSO in the blood (Cook et al., 1965). The hypothesis we arrived at for designing the present experiment that ruminants might have higher MSO reductase activity than swine, therefore, seemed to be not necessarily mistaken.

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(**Key Words:** Methionine-S-oxide Reducatase, Ruminants, Swine)

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