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TRANSPORT AND UTILIZATION OF METHIONINE SULFOXIDE IN THE RABBIT

VADIVEL GANAPATHY and FREDERICK H. LEIBACH *

Department of Cell and Molecular Biology, Medical College of Georgia, Augusta, GA 30912 (U.S.A.)

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Methionine sulfoxide is transported into purified intestinal and renal brush border membrane vesicles from rabbit by an Na^+-dependent mechanism and is accumulated inside the vesicles against the concentration gradient. Both in intestine and kidney, the rate of transport is enhanced with increasing concentrations of Na^+ in the external medium. Increasing the Na^+ gradient reduces the apparent K_t for methionine sulfoxide without causing any change in V_{max}. With an outward K^+ gradient (vesicle > medium), valinomycin stimulates the Na^+-gradient-dependent transport of methionine sulfoxide in the kidney, showing the electrogenicity of the transport process. A number of amino acids inhibit methionine sulfoxide transport in both the intestine and kidney. An enzymatic activity capable of reducing methionine sulfoxide to methionine is present in the intestinal mucosa, renal cortex and liver. The activity is highest in renal cortex and lowest in intestine. The methionine sulfoxide-reducing activity is stimulated by NADH, NADPH, glutathione and dithiothreitol and the potency of the stimulation is in the order: dithiothreitol > NADPH > glutathione > NADH.

Introduction

Methionine sulfoxide and methionine sulfone are the two main derivatives formed by oxidation of methionine. Methionine sulfoxide is readily formed under mild oxidative conditions while the formation of methionine sulfone requires relatively severe oxidative conditions [1]. The presence of methionine sulfoxide in collagen, glomerular basement membrane and lens capsule [2,3] has been demonstrated although the contents are low. Recently, a high content of methionine sulfoxide in the resilium protein of surf clams was reported [4] and 70–90% of methionine in this protein was present in the sulfoxide form.

Apart from the methionine sulfoxide naturally present in many proteins, the possibility that methionine in proteins may be oxidized to sulfoxide during food processing, because of various oxidative treatments, has been pointed out by many investigators [1,5–8]. Such oxidative treatments include the use of hydrogen peroxide to sterilize milk, whey, and milk containers [9–11] and to bleach or detoxify protein concentrates [8–12]. Oxidizing agents such as benzoyl peroxide are used to bleach and improve flour. Heat, traces of metals, and γ-irradiation are also known to catalyze oxidation of food proteins by oxygen. However, not enough attention has been paid to the methionine sulfoxide content of various food proteins probably because of difficulties involved in differential determination of methionine and methionine sulfoxide. Methionine sulfoxide is partly reduced to methionine during acid hydrolysis, a procedure normally employed in the de-

* To whom correspondence should be addressed.

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; PPO, 2,5-diphenyloxazole; POPOP, *p*-bis[2-(5-phenyloxazolyl)]benzene.

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termination of the amino acid composition of proteins.

It is of obvious interest to know the nutritional consequences of the oxidation of free or protein-bound methionine. Although methionine sulfone cannot replace methionine in the diet and may even be toxic [12–15], results concerning methionine sulfoxide are inconsistent. Methionine sulfoxide was nutritionally as effective as methionine when it was added to diets with sulfur amino acids as the limiting factor [16–18], whereas Miller and Samuel [14,15] found the sulfoxide to be less effective. Similarly, poorer utilization of methionine sulfoxide than of methionine has been reported when the sulfoxide replaced methionine in a synthetic amino acid mixture [7]. There is also a discrepancy in the results concerning the utilization of protein-bound methionine sulfoxide. Ellinger and Palmer [19] reported that oxidized casein containing methionine sulfoxide was nutritionally inferior to normal casein. However, another study by Slump and Schreuder [20] showed that methionine sulfoxide both in casein and in fish meal was completely available. Similarly, the digestibility and nutritional availability of oxidized casein in which 98% of the methionine residues were converted to the sulfoxide were not different from those of normal casein in rats [21].

These studies show that a large proportion of the methionine sulfoxide residues in dietary proteins are effectively absorbed and utilized. Since free methionine sulfoxide cannot be utilized as such for protein synthesis [22], it follows that conversion of methionine sulfoxide to methionine occurs in vivo. Methionine sulfoxide reductase capable of reducing methionine sulfoxide to methionine in bacteria, yeast and higher plants has been studied [23–26]. A methionine sulfoxide-reducing system has also been shown to be present in rat tissues [27].

We report here for the first time the presence of an Na^+-dependent concentrative mechanism for the transport of methionine sulfoxide in rabbit intestinal and renal brush border membrane vesicles. We also provide evidence for the presence in rabbit liver and kidney of an enzymatic activity capable of reducing methionine sulfoxide to methionine.

Methods and Materials

Preparation of labeled methionine sulfoxide. L-[*methyl*-^{14}C]Methionine sulfoxide was prepared by mild oxidation of L-[*methyl*-^{14}C]methionine as described in Ref. 27. 250 μCi of L-[*methyl*-^{14}C]-methionine in ethanol/water (7 : 3, v/v) were treated with 1.5 M H_2O_2 in a final volume of 3 ml at 37°C for 2 h. After incubation, the mixture was freeze-dried and the residue was dissolved in 2.5 ml of ethanol/water (7 : 3, v/v). This procedure was found to convert methionine quantitatively to methionine sulfoxide. No attempt was made to separate the two enantiomers resulting from the chirality of the sulfur atom in the sulfoxide.

Unlabeled methionine sulfoxide was prepared from methionine in a similar way.

Preparation of brush border membrane vesicles. Intestinal and renal brush border membrane vesicles were prepared from rabbit as described earlier [28]. Membrane purity was routinely determined by assay of marker enzymes [28,29]. Freshly prepared membrane vesicles were used for transport assays and these vesicles were free of basolateral membranes as assessed by total absence of $(Na^+ + K^+)$-ATPase activity.

Transport assay. Transport of methionine sulfoxide into intestinal and renal brush border membrane vesicles was measured as previously described [28]. When the effects of other amino acids on methionine sulfoxide transport were studied, the pH of the transport buffer was adjusted to 7.5 with either Tris base or HCl. The experiments were always done in duplicate and the variations between the individual values and the mean value was usually less than $\pm$ 10%.

Intravesicular contents were recovered for analysis as described in Ref. 30.

Preparation of tissue homogenates. After the rabbits, weighing 6.0 $\pm$ 1.5 lb, were killed by a lethal dose of nembutal, liver, kidney and the entire small intestine from the pyloric end to the ileocaecal junction were taken out. The intestine was washed with ice-cold KCl (0.154 M) and cut open longitudinally. The mucosa was scraped off and stored on ice until used. A 20% homogenate of the intestinal mucosa, liver or renal cortex was prepared in 0.1 M potassium phosphate buffer (pH 7.4) in a loose-fitting Dounce glass homogenizer

using 15–20 strokes. The homogenate was filtered through four layers of cheese-cloth and the filtrate was employed to determine the methionine sulfoxide-reducing activity.

Assay of methionine sulfoxide-reducing activity. The assay mixture contained 1 μCi labeled methionine sulfoxide (22 nmol), potassium phosphate buffer (10 μmol), pH 7.4, and homogenate (2–3 mg protein) in a final volume of 0.1 ml. The assay mixture also contained NADH (0.1 μmol), NADPH (0.1 μmol) or glutathione (0.5 μmol) when the effects of these reducing agents were studied. Incubations were performed at 37°C for 3 h. Heat-inactivated homogenates (95°C, 5 min) served as proper blanks. The reaction was stopped by heat inactivation of the enzyme and the precipitated protein was removed by centrifugation for 10 min in a table-top clinical centrifuge. The clear supernatants were used to determine the radioactivity associated with methionine.

Separation of methionine from methionine sulfoxide. Methionine and methionine sulfoxide in the supernatants were separated by paper chromatography (Whatman No. 1, 14 h run) using *n*-butanol/glacial acetic acid/water (4 : 1 : 1, v/v). After the development of the chromatogram, the radioactive spots were detected on a Packard radiochromatogram scanner. The portions of paper containing labeled methionine were cut out, suspended in 10 ml of scintillation mixture and counted. The scintillation mixture consisted of toluene-Triton X-100 in a ratio 2 : 1 (v/v) containing 4 g of PPO and 0.1 g of POPOP per l of toluene.

R_f values of methionine, unlabeled methionine sulfoxide (prepared and commercial) and labeled methionine sulfoxide (prepared) were calculated by paper chromatography using three different solvent systems: (a) *n*-butanol/glacial acetic acid/water (4 : 1 : 1, v/v); (b) 2-propanol/water (4 : 1, v/v) and (c) chloroform/methanol/17% NH_4OH (3 : 6 : 1, v/v). R_f is the mobility of the compound relative to the mobility of the solvent front.

Unlabeled compounds on the chromatograms were detected by ninhydrin staining and labeled compounds by radiochromatogram scanner.

Protein was determined by the method of Lowry et al. [31] using crystalline bovine serum albumin as standard.

Materials. Unlabeled amino acids were obtained from California Corporation for Biochemical Research. L-Methionine DL-sulfoxide, NADH, NADPH, glutathione and dithiothreitol were from Sigma Chemical Co. 2-Mercaptoethanol was purchased from Eastman Kodak Co. All other chemicals were of purest analytical grade available.

L-[*methyl*-^{14}C]Methionine (45.7 mCi/mmol) was obtained from New England Nuclear Corp., Boston, MA.

Results

Identification and purity of labeled methionine sulfoxide

The R_f value of labeled methionine sulfoxide prepared from labeled methionine by H_2O_2 treatment was determined and compared with that of commercial methionine sulfoxide in three different solvent systems. As shown in Table I, the R_f values of unlabeled methionine sulfoxide (commercial and prepared) and labeled methionine sulfoxide (prepared) were identical. The purity of labeled methonine sulfoxide was checked by paper chromatography using 2-propanol/water (4 : 1, v/v) as the solvent system. Fig. 1 shows that H_2O_2 treat-

TABLE I

R_f VALUES OF METHIONINE AND METHIONINE SULFOXIDE (PREPARED AND COMMERCIAL) IN DIFFERENT SOLVENT SYSTEMS

R_f values were calculated using paper chromatography (Whatman No. 1, 14 h run) in three different solvent systems: (I) *n*-butanol/glacial acetic acid/water (4 : 1 : 1, v/v), (II) 2-propanol/water (4 : 1, v/v), (III) chloroform/methanol/17% NH_4OH (3 : 6 : 1, v/v). Unlabeled compounds on paper chromatograms were detected using ninhydrin and labeled compounds were detected using Packard Radiochromatogram Scanner.

Compound	R_f V		
Solvent system	I	II	III
Methionine	0.46	0.50	0.66
Methionine sulfoxide	0.16	0.26	0.53
Methionine after H_2O_2 treatment	0.16	0.26	0.53
Labeled methionine after H_2O_2 treatment	0.16	0.26	0.53

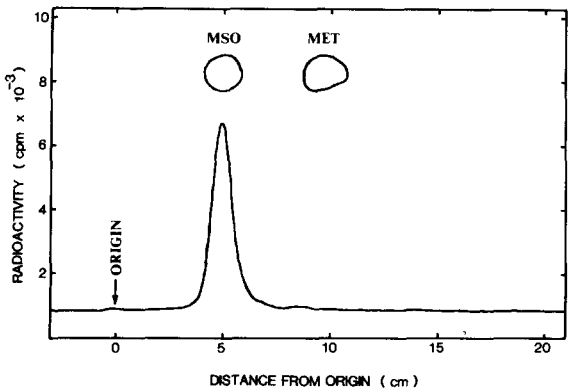

Fig. 1. Radiopurity of methionine sulfoxide (MSO) prepared from methionine (MET). The chromatogram was developed on a Whatman No. 1 paper (14 h run) using 2-propanol/water (4:1, v/v) as the solvent system. Scanning was done with a Packard radiochromatogram scanner.

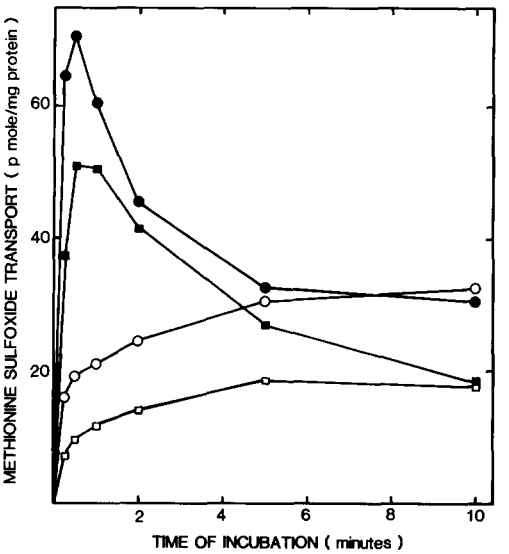

Fig. 2. Time course of methionine sulfoxide (20 μM) transport into intestinal and renal brush burder membrane vesicles in NaCl and KCl media. Intestinal and renal brush border membrane vesicles (0.5–0.8 mg protein) were incubated with 20 μM L-[*methyl*-^{14}C]methionine sulfoxide at 25°C over a period of 10 min in NaCl and KCl media. (●) Renal transport in NaCl medium, (○) renal transport in KCl medium, (■) intestinal transport in NaCl medium, (□) intestinal transport in KCl medium.

ment resulted in quantitative oxidation of methionine to methionine sulfoxide as evidenced by the total absence of labeled methionine. Methionine sulfoxide was the single most predominant labeled compound present and its purity was found to be greater than 98% in all three solvent systems.

Transport of methionine sulfoxide

Transport of methionine sulfoxide (20 μM) into purified intestinal and renal brush border membrane vesicles was studied in the presence and absence of an Na^+ gradient. Fig. 2 shows the time course of methionine sulfoxide transport. Methionine sulfoxide exhibited the 'overshoot' phenomenon in NaCl medium in intestine as well as in kidney. The accumulation of methionine sulfoxide inside the vesicles was maximal at 30 s in both cases. The overshoot phenomenon was absent in the absence of the Na^+ gradient. The amount of methionine sulfoxide transport at the overshoot as well as at equilibrium was greater in kidney than in intestine. The transport at the overshoot was 70.5 ± 4.2 pmol/mg protein in renal brush border vesicles and 50.8 ± 1.1 pmol/mg protein in intestinal brush border vesicles. The equilibrium transport was 31.5 ± 1.7 pmol/mg protein in kidney and 18.0 ± 1.8 pmol/mg protein in intestine. The presence of an Na^+ gradient stimulated methionine sulfoxide transport in both intestine and kidney about 4–5-fold at the overshoot. At the peak of the overshoot, the ratio of intravesicular concentration to the equilibrium value was 2.2 in kidney and 2.8 in intestine.

The equilibrium transport of methionine sulfoxide into intestinal and renal brush border membrane vesicles was inversely proportional to the medium osmolality, showing the accumulation of the compound in an osmotically responsive intravesicular space. The nonspecific binding calculated by extrapolation of the results to infinite osmolality was negligible compared to the transport observed at 300 mosM. Analysis of the intravesicular contents in intestine as well as in kidney showed that methionine sulfoxide was transported into the vesicles without undergoing any metabolic change.

Effect of Na^+ concentration on methionine sulfoxide transport

The effect of increasing concentrations of Na^+ in the incubation medium, added at the initiation

of the incubation, on the transport of methionine sulfoxide was studied in intestinal and renal brush border membrane vesicles (Fig. 3). Mannitol was used to maintain medium isosmolality. In the absence of Na^+, the rate of transport was 24.9 ± 3.1 pmol/30 s per mg protein in the kidney and 13.8 ± 2.4 pmol/30 s per mg protein in the intestine. Increasing concentrations of Na^+ increased the rate of transport. At an external concentration of 100 mM NaCl, the rate of methionine sulfoxide transport was stimulated (3.6 ± 0.2)-fold in the kidney and (4.1 ± 0.1)-fold in the intestine, compared to the rate of transport in the absence of Na^+. Though the extent of stimulation at 100 mM NaCl was the same in both kidney and intestine, the transport in intestine appeared to be saturable with respect to Na^+ while in kidney there was almost a linear relationship between increase in the transport rate and increase in the concentration of Na^+.

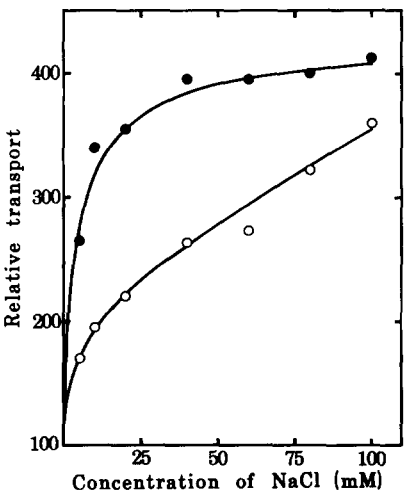

Fig. 3. Effect of Na^+ concentration on the transport of methionine sulfoxide (20 μM) into renal and intestinal brush border membrane vesicles. Membrane vesicles were suspended in 1 mM Hepes-Tris buffer, pH 7.5, containing 300 mM mannitol. The transport buffer contained varying concentrations of NaCl in 1 mM Hepes-Tris buffer, pH 7.5 and labeled methionine sulfoxide. Mannitol was used to maintain isosmolality. The final concentration of NaCl in the incubation medium varied over the range 0–100 mM. The time of incubation was 30 s. (○) Renal brush border membrane vesicles, (●) intestinal brush border membrane vesicles.

Effect of Na^+ on kinetics of methionine sulfoxide transport

Transport of methionine sulfoxide with 30 s incubation was measured over the concentration range 0.2–20 mM. The experiment was done with three different concentrations of NaCl in the incubation medium (0, 5 and 100 mM). Fig. 4 shows the results obtained with intestinal brush border membrane vesicles.

Least-squares-fit analysis of the data showed that the transport of methionine sulfoxide in the intestine in the absence of Na^+ was a saturable process with a K_t of 9.2 mM and a V_{max} of 7.5 nmol/30 s per mg protein. Increasing concentrations of Na^+ in the incubation medium lowered the apparent K_t for transport without significantly affecting V_{max}. The apparent K_t values were 4.8 and 3.0 mM methionine sulfoxide at 5 and 100 mM NaCl respectively, while the V_{max} was unaltered (8.7 ± 1.1 nmol/30 s per mg protein). Similar results were obtained with renal brush border membrane vesicles. In the absence of Na^+, K_t for methionine sulfoxide transport was 8.4 mM and V_{max} was 10.2 nmol/30 s per mg protein. The K_t values at 5 and 100 mM NaCl were 6.4 and 1.9 mM, respectively, with no significant change in V_{max} (9.8 ± 1.6 nmol/30 s per mg protein).

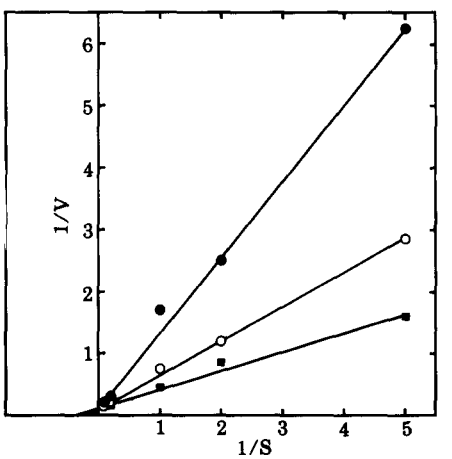

Fig. 4. Effect of Na^+ on the kinetics of methionine sulfoxide transport into intestinal brush border membrane vesicles. The concentration of methionine sulfoxide varied over the range 0.2–20 mM. The experiment was done at 0, 5 and 100 mM NaCl. Mannitol was used to adjust the medium osmolality to 300 mosM. The incubation time was 30 s. (●) In the absence of NaCl, (○) 5 mM NaCl, (■) 100 mM NaCl.

Effect of valinomycin on methionine sulfoxide transport

The effect of valinomycin on Na^+-gradient-dependent methionine sulfoxide transport was studied in intestinal and renal brush border membrane vesicles preloaded with K^+. In these experiments, at the initiation of incubation, there was no Na^+ inside the vesicles while 66 mM NaCl was pesent in the medium. The K^+ concentration inside the vesicles was 6-fold greater than that outside the vesicles. Fig. 5 presents the results obtained with renal brush border membrane vesicles. In the presence of valinomycin (10 μg/mg membrane protein), the transport at the overshoot was stimulated by about 70% over the transport in the absence of the ionophore. This shows that methionine sulfoxide transport in renal brush border membrane vesicles is an electrogenic process.

Fig. 6 shows the effect of valinomycin on methionine sulfoxide transport in intestinal brush

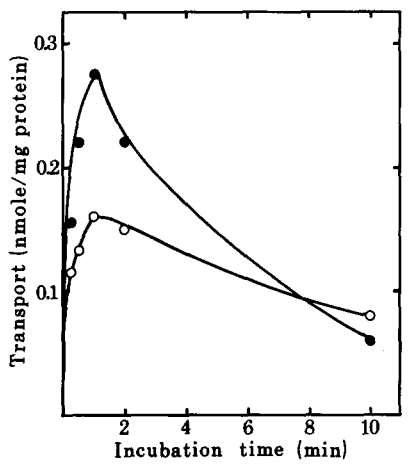

Fig. 5. Effect of valinomycin on Na^+-gradient-dependent methionine sulfoxide transport in renal brush border membrane vesicles. The membrane vesicles were preloaded with 1 mM Hepes-Tris buffer, pH 7.5, containing 150 mM KCl. The transport buffer contained 100 mM NaCl and 100 mM mannitol in 1 mM Hepes-Tris buffer, pH 7.5. The final concentration of labeled methionine sulfoxide in the incubation medium was 20 μM. The incubation was intiated by the addition of 40 μl of membrane suspension to 200 μl of transport buffer. At the beginning of incubation, the intravesicular K^+ concentration was 6-times greater than the external K^+ concentration. Valinomycin concentration, when present, was 10 μg/mg membrane protein. (○) In the absence of valinomycin, (●) in the presence of valinomycin.

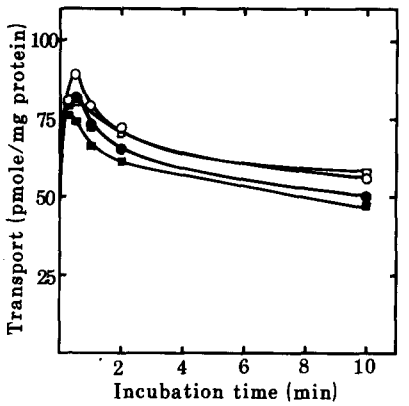

Fig. 6. Effect of valinomycin on Na^+-gradient-dependent methionine sulfoxide transport into intestinal brush border membrane vesicles. The experimental procedure was as described in the legend to Fig. 5. ((○, ●) Chloride salts of Na^+ and K^+, (□, ■) sulfate salts of Na^+ and K^+. Open symbols, absence of valinomycin; closed symbols, presence of valinomycin.

border membrane vesicles. Contrary to the results obtained with kidney, valinomycin showed no effect on the transport of methionine in intestine. This was true even when the sulfate salts of the cations were used instead of chloride salts.

Effects of other amino acids on methionine sulfoxide transport

Table II shows the effects of certain amino acids on the transport of methionine sulfoxide into intestinal and renal brush border vesicles. In both cases, the Na^+-dependent transport of methionine sulfoxide was significantly inhibited by many amino acids suggesting competition. Neutral amino acids like methionine, leucine, gutamine and alanine were more potent as inhibitors than glutamic acid. Lysine, a basic amino acid, also inhibited methionine sulfoxide transport both in intestine and in kidney. Interestingly, proline was a potent inhibitor of methionine sulfoxide transport in intestine, but in kidney it was not.

Enzymatic reduction of methionine sulfoxide

The results of several experiments with liver, kidney and mucosal homogenates showed that all three tissues possessed an enzymatic activity capable of reducing methionine sulfoxide to methionine. Kidney cortex had the highest methionine sulfo-

TABLE II

EFFECTS OF AMINO ACIDS ON TRANSPORT OF METHIONINE SULFOXIDE INTO INTESTINAL AND RENAL BRUSH BORDER MEMBRANE VESICLES

Intestinal and renal brush border membrane vesicles were incubated with L-[*methyl*-^{14}C]methionine sulfoxide (20 μM) for 30 s at 25°C in NaCl and KCl media. The concentration of unlabeled amino acids in the incubation meidum was 1 mM. The Na^+-dependent transport of methionine sulfoxide was calculated by subtracting the transport observed in KCl medium from that in NaCl medium. The control value for the Na^+-dependent transport was 46.0 ± 2.5 pmol/mg protein in intestine and 64.6 ± 3.1 pmol/mg protein in kidney and these values were taken as 100. The results are expressed as the mean ± S.D. from three experiments.

Addition	Relative transport	
	Intestine	Kidney
None	100 ± 6	100 ± 5
Methionine sulfoxide	78 ± 4	51 ± 4
Methionine	42 ± 4	32 ± 1
Glutamine	64 ± 3	53 ± 11
Alanine	69 ± 1	38 ± 1
Leucine	46 ± 5	31 ± 2
Lysine	64 ± 4	55 ± 7
Proline	53 ± 2	101 ± 6
Glutamic acid	79 ± 1	109 ± 1

xide-reducing activity. Fig. 7 shows that a significant amount of methionine was formed from methionine sulfoxide with kidney cortical homogenate without addition of any reducing compound. Heat-treated homogenate did not have any methionine sulfoxide-reducing activity. Addition of 1 mM NADH or HADPH significantly stimulated the reduction of methionine sulfoxide, and NADPH seemed more active than NADH in enhancing the reduction. Similar results were obtained with liver and mucosal homogenates, although the reducing activities per mg protein varied significantly from one another.

As shown in Table III, all three tissues had significant methionine sulfoxide-reducing activity. Without the addition of any reducing agent, kidney cortex was 3-times more active than liver, whereas intestine was the least active. Addition of NADH stimulated methionine formation 2-fold over the control value in all the tissues while NADPH caused 4–5-fold stimulation. Glutathione at higher concentrations served as an effective reducing agent.

Fig. 8 shows the efficiency of 2-mercaptoethanol, dithiothreitol, glutathione and NADPH at varying concentrations in serving as the reducing

TABLE III

FORMATION OF METHIONINE FROM METHIONINE SULFOXIDE WITH MUCOSAL, KIDNEY CORTICAL AND LIVER HOMOGENATES

L-[*methyl*-^{14}C]Methionine sulfoxide (0.22 mM) was incubated with different homogenates in potassium phosphate buffer (0.1 M), pH 7.4 (final volume, 0.1 ml), for 3 h at 37°C in the absence and presence of NADH (1 mM), NADPH (1 mM) or glutathione (5 mM). Protein concentration in the reaction mixture was in the range 2–3 mg. Heat-inactivated homogenates (95°C, 5 min) were treated in a similar way to serve as blanks. The reaction was stopped by heat treatment and the precipitated protein was removed by centrifugation. Methionine and unreacted methionine sulfoxide in the supernatant were separated by paper chromatography. Methionine was estimated by counting radioactivity associated with methionine. The value of the -fold stimulation with various reducing agents over the control value is given in parentheses. The results are expressed as the mean ± S.D. from three experiments.

Tissue	nmol methionine formed/mg protein per 3 h			
	Control	NADH	NADPH	Glutathione
Intestine	0.12 ± 0.04	0.25 ± 0.13 (2.0 ± 0.4)	0.61 ± 0.02 (5.3 ± 1.6)	0.56 ± 0.08 (3.7 ± 0.6)
Kidney	1.49 ± 0.28	2.61 ± 0.07 (1.8 ± 0.4)	6.60 ± 1.27 (4.5 ± 0.1)	4.81 ± 1.11 (3.7 ± 0.8)
Liver	0.48 ± 0.18	1.05 ± 0.30 (2.3 ± 0.2)	2.03 ± 0.18 (4.5 ± 1.3)	1.63 ± 0.37 (2.7 ± 0.5)

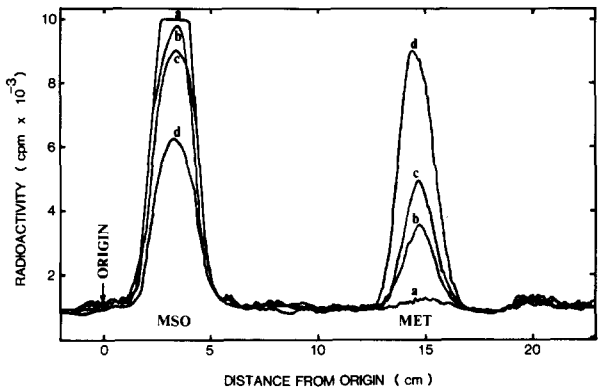

Fig. 7. Formation of methionine from methionine sulfoxide with renal cortical homogenates. 1 μCi of L-[*methyl*-^{14}C]-methionine sulfoxide was incubated with renal cortical homogenates (2 mg protein) in 0.1 M potassium phosphate buffer (pH 7.4) at 37°C for 3 h. The reaction was stopped by heat inactivation (95°C, 5 min) and the precipitated proteins removed by centrifugation. The homogenate subjected to heat treatment prior to incubation was treated in a similar way to serve as the blank. An aliquot of the clear supernatant was used to separate methionine from methionine sulfoxide by paper chromatography (Whatman No. 1, 14 h run). The radioactive peaks were identified with a Packard radiochromatogram scanner. (a) Blank with the heat-inactivated homogenate, (b) control in the absence of reducing agent, (c) 1 mM NADH, (d) 1 mM NADPH. All the four scannings were superimposed.

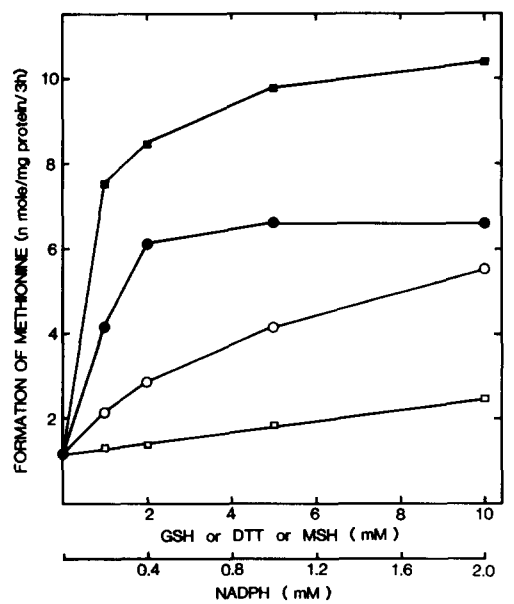

Fig. 8. Effects of reducing agents on the methionine sulfoxide-reducing activity of renal cortical homogenates. Renal cortical homogenate (1.5–2 mg protein) was incubated with 1 μCi of L-[*methyl*-^{14}C]methionine sulfoxide in 0.1 M potassium phosphate buffer (pH 7.4) at 37°C for 3 h in the presence of varying concentration of NADPH, glutathione, 2-mercaptoethanol (MSH) and dithiothreitol (DTT). Methionine was separated from methionine sulfoxide as described in the legend to Fig. 3. The amount of methionine formed was quantitated by measuring the radioactivity associated with methionine on the radiochromatogram. (■), dithiothreitol, (●) NADPH, (○) glutathione, (□) 2-mercaptoethanol.

agent in the reduction of methionine sulfoxide to methionine by kidney cortical homogenate. Of the four compounds tested, dithiothreitol was most potent in promoting the formation of methionine. Although NADPH was significantly active at lower concentrations, increasing its concentration did not cause any additional stimulation. Glutathione was fairly active at higher concentrations whereas 2-mercaptoethanol showed negligible activity.

Discussion

A number of studies have shown that methionine sulfoxide is present in many proteins, and methionine residues in food proteins undergo oxidation to form methionine sulfoxide during food processing. Free as well as protein-bound methionine sulfoxide is nutritionally available in animals and can replace methionine in the diet. These results clearly suggest that methionine sulfoxide can be absorbed as effectively as methionine in the intestine and converted to methionine in vivo to be utilized in protein syntesis and in other metabolic reactions. However, there are no data available on the mechanism of methionine sulfoxide absorption. Our studies demonstrate that intestinal brush border membrane vesicles from rabbit can transport methionine sulfoxide by an Na^+-dependent mechanism and that it is an active process occurring against the concentration gradient. Renal brush border membrane vesicles also possess a similar mechanism for the effective reabsorption of methionine sulfoxide from the glomerular filtrate.

The equilibrium transport of methionine sulfoxide was greater in renal brush border membrane vesicles than in intestinal brush border vesicles even though the initial concentration in the medium was 20 μM in both cases. It has been shown [32] that the average size of the brush border membrane vesicles from renal cortex

(0.2–0.7 μm) is greater than that of the vesicles from small intestine (0.2–0.4 μm). Therefore, even though the equilibrium transport expressed as pmol/mg protein was greater in kidney than in intestine, the intravesicular concentration at equilibium will be nearly equal in both cases.

The transport of methionine sulfoxide in intestine and kidney was stimulated by increasing concentrations of Na^+ in the incubation medium. The Na^+-independent transport process was also saturable in both kidney and intestine. The effect of Na^+ on transport was to increase the affinity of methionine sulfoxide for the carrier, because the presence of Na^+ substantially lowered the apparent K_t for transport without affecting the V_{max}. The role of the electrochemical membrane potential in methionine sulfoxide transport across renal and intestinal brush border membranes was studied by experimentally inducing a membrane potential using valinomycin. In these experiments, there was an outward K^+ gradient (vesicle > outside) at the initiation of incubation, and the presence of valinomycin caused a concentration-dependent efflux of K^+ generating an electrochemical potential. This electrochemical potential stimulated methionine sulfoxide transport in renal brush border membrane vesicles by about 70%, indicating that the transport process in kidney is electrogenic. However, under similar conditions, valinomycin failed to produce any effect on methionine sulfoxide transport across intestinal brush border membranes. Even through this does not prove the absence of electrogenic transport of methionine sulfoxide in the intestine, the results show that the transport processes in intestinal and renal brush border membrane vesicles do differ in this regard.

In kidney, as well as in intestine, transport of methionine sulfoxide was effectively inhibited by many amino acids including methionine. Thus, the oxidation of the sulfur atom in methionine does not seem to affect its affinity for the carrier protein. Recently, it has been reported that methionine sulfoxide is transported by the high-affinity system available for methionine and glutamine in bacteria [33]. The present paper is the first report of the presence of an active transport system for methionine sulfoxide in mammalian intestine and kidney. Proline and glutamic acid caused significant inhibition of methionine sulfoxide transport in the intestine whereas they did not have any effect in the kidney. This is not surprising in the light of recent studies which have shown notable differences between amino acid-transport processes in the intestine and kidney [34].

The presence of an enzyme system to reduce methionine sulfoxide to methionine in bacteria, yeast and higher plants is well known [23–26]. The methionine sulfoxide-reducting activity in yeast has been shown to be a multicomponent system involving thioredoxin, thioredoxin reductase and the enzyme directly responsible for the reduction of methionine sulfoxide [35]. The reduction of methionine sulfoxide in bacteria is also very likely a multicomponent system and studies on the purification of methionine sulfoxide reductase showed that dithiothreitol could replace thioredoxin which simplified the assay considerably [24].

The presence of a methionine sulfoxide-reducting system has been demonstrated in rat kidney and liver [27]. Our results show that rabbit liver and kidney possess significant methionine sulfoxide-reducing activity. The specific activity of the system in the presence of either NADH or NADPH is much higher in the rabbit tissues than the reported values in the rat tissues [27]. Another important difference between these two studies is the efficiency of NADH or NADPH in serving as the reducing agent in the conversion of methionine sulfoxide to methionine. NADH has been reported to be more effective than NADPH in the rat. However, our present study shows that NADPH is at least 2–3-times more efficient than NADH in the rabbit. It is interesting to note here that the methionine sulfoxide-reducing system in bacteria and yeast utilizes NADPH rather than NADH [23,25]. Even though the present results do not provide evidence for the multicomponent nature of the system in rabbit, the fact that dithiothreitol was the most potent reducing agent in the conversion of methionine sulfoxide to methionine suggests that the enzyme system in the rabbit is possibly similar to that in bacteria and yeast.

What is puzzling is the function of this enzyme system. High levels of methionine sulfoxide in animal cells would not normally be expected. However, the enzyme system would definitely be responsible for the reduction of methionine sulfoxide arising from dietary proteins. Moreover, the

oxidation of methionine to methionine sulfoxide in tissue proteins, especially those with a long half-life, would occur in vivo because of the oxidizing agents such as peroxides, hydroxyl radical and superoxides which are constantly being formed. There are reports that there is a relatively large amount of methionine sulfoxide in the cataractous lens protein [36,37]. Thus, the enzyme system would also be responsible for the reduction of methionine sulfoxide arising from the intracellular breakdown of tissue proteins containing methionine sulfoxide.

The purified methionine sulfoxide reductase from *Escherichia coli* is specific for free methionine sulfoxide and does not reduce protein-bound methionine sulfoxide [24]. Very recently, an enzyme catalyzing the reduction of protein-bound methionine sulfoxide has been partially purified from *E. coli* [38] and it appears to be different from methionine sulfoxide reductase [24]. Extracts of rat tissue can also catalyze the reduction of methionine sulfoxide residues in protein [38]. The enzyme activity which reduces the protein-bound methionine sulfoxide may have an important physiological function because many proteins lose their biological activity when their methionine residues are oxidized to the sulfoxide. The ability of various tissues to prevent the accumulation of inactive proteins due to oxidation of methionine may be important for normal function. However, it is not known at present whether rabbit tissues contain an enzymatic activity capable of reducing methionine sulfoxide residues in protein and, if present, whether it is different from the enzymatic activity reported in this paper.

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