

Absorption of methionine and methionine sulphoxide in rat intestine and the effect of glutathione

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1. The absorption of methionine and methionine sulphoxide was compared in inverted rings from three sections of rat intestine.
2. In all sections, absorption of unoxidized methionine was 20-40% higher than that of methionine at low physiological substrate concentrations. At higher substrate levels, the difference was not so pronounced.
3. Inhibition studies indicated that absorption of methionine and of methionine sulphoxide took place by different absorption systems.
4. Glutathione increased the absorption of methionine and, to a greater extent, the absorption of methionine sulphoxide.

Because methionine is the first limiting amino acid in many foods and feeds there has been some concern as to the biological availability of the oxidized forms of methionine. There is general agreement that methionine sulphone is unavailable as a source of methionine, but there has been some controversy concerning the biological availability of methionine sulphoxide (Anderson *et al.* 1976; Gjoen & Njaa, 1977; Cuq *et al.* 1978; Njaa & Aksnes, 1982). This controversy has been partly resolved by the observation that the free sulphoxide was utilized better when cystine was present in the diet, than without it (Gjoen & Njaa, 1977). However, most of the reported studies in which free methionine and methionine sulphoxide were compared showed better utilization of the former, but the difference was small when the amino acids were protein bound (Gjoen & Njaa, 1977; Cuq *et al.* 1978). These observations may be explained by different rates of absorption in the alimentary tract. The fact that high levels of sulphoxide were found in blood plasma of rats given diets containing methionine sulphoxide (Gjoen & Njaa, 1977) shows that absorption takes place. Little is known, however, about the efficiency of this absorption under physiological conditions.

The present paper reports a study of the absorption of methionine and methionine sulphoxide *in vitro* using inverted intestinal rings from three sections of the tract. Also included are studies on the effect of reduced glutathione on the absorption pattern.

MATERIALS AND METHODS

Reduced glutathione (GSH) and glycyl-L-methionine were obtained from Sigma (Poole, Dorset), L-cysteine and L-methionine sulphoxide from Koch-Light (Haverhill, Suffolk), L-methionine from Merck (Poole, Dorset) and L-[methyl-³H]methionine (12 Ci/mmol) from New England Nuclear (Southampton). The radioactive methionine gradually oxidized to sulphoxide and portions for 1 week's use were therefore purified by chromatography on a Dowex 50 × 4 column (50 × 5 mm) using 50 mM-sodium citrate buffer, pH 3.6, for equilibration and elution. Of the original labelled methionine solution 40 µl was diluted to 400 µl with 50 mM-citrate buffer, pH 2.5, and applied to the column. The fractions containing methionine were used in the experiments. L-[Methyl-³H]methionine sulphoxide was prepared from the purified labelled methionine by oxidation with hydrogen peroxide (2 × stoichiometric amounts) at pH 2-3.

The absorption studies were performed on small intestines from adult male rats (250–550 g). After an overnight fast they were anaesthetized by intraperitoneal injection of Mebumal (pentobarbital sodium) and the small intestine, between 100 mm posterior to the pylorus and 100 mm anterior to the caecum, was dissected. It was divided into three parts of approximately equal length; sections A, B and C referring to the anterior, middle and posterior parts respectively. The appropriate section was inverted and cut into short cylinders with a polyedged knife. The length of the cylinders (later referred to as intestinal rings) was 5.2 mm. Separate rings were suspended on hooks made from injection needles.

Gas (oxygen-carbon dioxide (95:5, v/v)) was bubbled through the needles. The rings were preincubated for 5 min in 3 ml modified Ringer solution (130 mM-NaCl, 10 mM-KCl, 10 mM-NaHCO₃, 1.2 mM-K₂HPO₄, 0.2 mM-KH₂PO₄, 1.2 mM-CaCl₂, 1.2 mM-MgCl₂, pH 7.2) containing 16.7 mM-glucose. The rings hanging on the hooks were then transferred to test tubes containing the specified amount of Ringer solution and the substance to be studied. After the incubation period the rings were washed in 2 ml 0.3 M-mannitol for 2–3 s and then extracted overnight in 0.1 M-nitric acid, or in 50 mM-sodium citrate buffer, pH 2.5, if the extracts were to be chromatographed. Both extraction media were equally effective. All incubations were done at 37°. Absorption was measured by the decrease in radioactivity in the incubation medium or by the radioactivity extracted during the overnight extraction. No further radioactivity was extracted when the extraction time was prolonged. The wet weight of the intestinal rings varied from 50 to 67 mg. The differences were due mainly to varying amounts of adipose and connective tissue adhering to the intestine. The absorption rate was therefore expressed as $\mu\text{mol}/\text{min}$ per ring.

Expt 1. The time-course of methionine and methionine sulphoxide absorption

The absorptions of methionine and methionine sulphoxide in intestinal section A were determined after 1, 2, 3, 5, 10, 15 and 20 min by the decrease in radioactivity in the incubation medium. Three replicate analyses were performed with two intestinal rings in 2 ml incubation medium containing 1 mM of the substrate to be studied.

Expt 2. Absorption of methionine and methionine sulphoxide in sections of the small intestine at different substrate concentrations

Absorption was determined as the extractable amounts of radioactivity in the three sections of intestine using substrate concentrations of 0.10, 0.25, 0.50, 1.0 and 5.0 mM and an incubation period of 2 min. From seven to eleven replicate analyses were performed at each concentration level with one intestinal ring in 1 ml incubation mixture.

Expt 3. Inhibition studies and the effect of GSH

Studies of the inhibitory effects of methionine, methionine sulphoxide and glycyl-L-methionine on the absorption of methionine and methionine sulphoxide were carried out with rings from section A at 1 mM concentrations. One intestinal ring in 1 ml incubation mixture was used and seven replicate analyses were performed. As the extracted fractions were to be chromatographed for amino acid identification, the incubations were run for 5 min and the absorbed amounts were measured by the decrease in radioactivity in the incubation medium and by the extracted radioactivity after absorption. All studies were also carried out in the presence of 2 mM-GSH in the incubation mixtures.

RESULTS

The absorption of methionine and methionine sulphoxide determined over 20 min in Expt 1 are shown in Fig. 1. The mean (with SE) amounts of radioactive methionine and methionine sulphoxide extracted from the intestinal rings at the end of the incubation period accounted

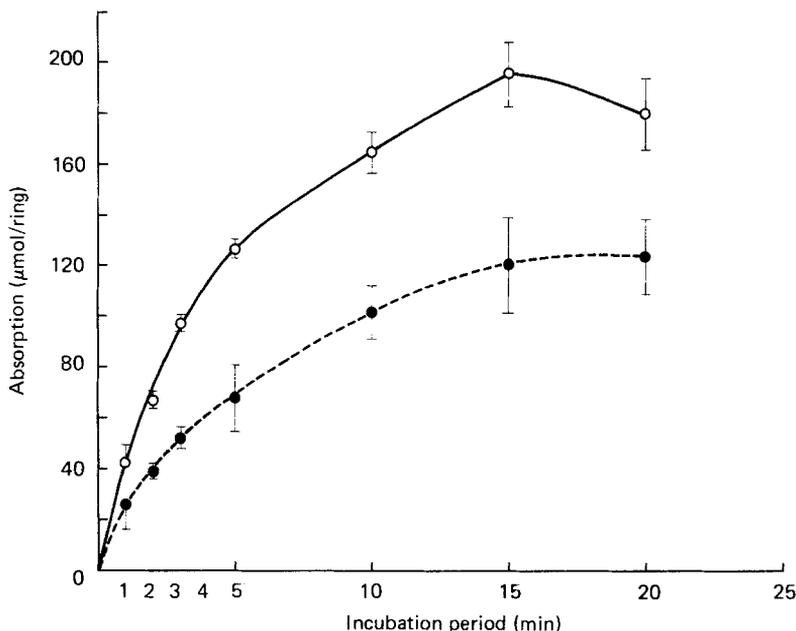


Fig. 1. Absorption of methionine (○) and methionine sulphoxide (●) v. time in intestinal section A (see p. 584 at 1 mM initial substrate concentration. Three replicate analyses were performed with two intestinal rings in 2 ml incubation medium. Points are mean values with their standard errors represented by vertical bars.

for 96.0 (8.3) and 95.4 (13.0) % of the amounts measured by difference. The absorption of methionine was higher than that of methionine sulphoxide by approximately 40%. The amounts absorbed were highest after 15–20 min in which case 20% of methionine was absorbed. After this time period, leakage from the intestinal rings to the environment exceeded the absorption. In a separate experiment the leakage was measured after 5 min absorption by transferring the intestinal rings to a gassed medium without radioactive amino acids. After 10 min approximately 40% of the total extractable radioactivity was found in the medium. Therefore, absorption experiments at various substrate concentrations were studied with a 2 min incubation period. As only small amounts were absorbed after this short incubation time (2–6% of the initial amounts) the absorbed amounts were measured by the extracted radioactivities. In these experiments absorption of methionine was better than that of methionine sulphoxide at low substrate concentrations. At higher concentrations the difference was not so pronounced and in the posterior part of the intestine methionine sulphoxide was absorbed better than methionine (Table 1).

No mutual inhibition was observed between methionine and methionine sulphoxide, and glycyl-L-methionine did not inhibit the absorption of either (Table 2). The extracted radioactivities were 60–80% of that measured by difference. There was, however, significant correlation between the two measurements of absorption ($r +0.97$). When GSH was present in the incubation mixture, the absorption of methionine was increased by 40% as measured by decrease in radioactivity in the incubation medium. Absorption of methionine sulphoxide increased even more and approached that of methionine. Measured by the extracted radioactivity, however, neither amino acid showed increased absorption, and the extracted amounts in these cases accounted for only 25–60% of the absorbed amount as measured by difference. Moreover, there was no significant correlation between the

Table 1. Absorption of methionine (MET) and methionine sulphoxide (MS) ($\mu\text{mol}/\text{min}$ per intestinal ring) in different sections of small intestine and at different substrate concentrations*
(Mean values with their standard errors)

Section†...	A				B				C			
	MET		MS		MET		MS		MET		MS	
	Mean	SE										
Substrate concentration (mM)												
0.10	4.98	0.26	3.81	0.26	4.86	0.38	3.20	0.22	5.60	0.10	3.59	0.14
0.25	15.2	0.8	8.8	0.5	10.6	1.0	8.3	0.5	11.7	0.8	10.2	0.6
0.50	26.6	1.9	18.7	1.7	21.5	3.2	16.2	1.8	24.8	1.5	18.2	0.6
1.0	47.8	1.6	36.4	1.1	38.5	4.5	33.9	2.8	40.8	2.7	41.9	1.8
5.0	189	14	166	5.7	122	9.8	161	9.5	153	8.3	203	3.4

* From seven to eleven replicate analyses were performed at each concentration level with one intestinal ring on 1 ml incubation medium.

† Sections A, B and C refer to the anterior, middle and posterior parts respectively of the dissected small intestine (100 mm posterior to the pylorus and 100 mm anterior to the caecum) divided into three parts of approximately equal length.

Table 2. Absorption of methionine (MET) and methionine sulphoxide (MS) in intestinal section A (see p. 584) and the effect of reduced glutathione (GSH), methionine, methionine sulphoxide and glycyl-L-methionine*
(Mean values with their standard errors)

Inhibitor	Absorption ($\mu\text{mol}/\text{min}$ per ring)															
	MET			MS			MET+GSH			MS+GSH						
	Difference†	Extract†	Mean	Difference	Extract	Mean	Difference	Extract	Mean	Difference	Extract	Mean				
None	102	10	81	14	86	8	52	6	142	11	79	9	137	13	57	6
MS	93	14	73	15	—	—	—	—	121	17	77	8	—	—	—	—
MET	—	—	—	—	68	9	40	5	—	—	—	—	135	11	36	5
Glycyl-L-methionine	86	9	60	4	71	11	39	6	129	12	76	6	147	9	40	5

* Seven replicate analyses were performed with one intestinal ring in 1 ml incubation medium.

† Decrease in radioactivity in incubation medium.

‡ Extracted amount from intestinal ring after absorption.

measurements of absorption ($r = 0.50$). The same effect as for GSH was observed for cysteine. The extracted radioactivity in the experiments reported in Table 2 were chromatographed on an ion-exchange column. Only the radioactive amino acid added to the incubation medium was eluted. It has not been possible to identify the non-extractable radioactive compound.

DISCUSSION

The method used in the present work is not well suited for calculation of kinetic constants for absorption. It is, however, a much-used method and is suitable for comparative studies. Because of its simplicity it was used in this work to compare the absorptions of methionine and methionine sulphoxide *in vitro*.

The physiological concentration of amino acids in the intestine after a normal meal range from 70 to 700 μM (Lerner, 1973). At these concentrations, methionine sulphoxide is less-well absorbed than methionine. The absorption rate of methionine in rat intestine is in the same range as that reported by others using similar methods; from Fig. 1 and Table 2 the absorption rate of methionine at 1 mM initial concentration was estimated to be 2.0–2.5 $\mu\text{mol}/5$ min per g wet weight intestine, compared with 3 $\mu\text{mol}/5$ min per g wet weight intestine as reported by Cheng *et al.* (1971) at 1.25 mM substrate concentration.

Under the conditions described, the absorption of methionine seems to take place by active transport as 20% of the radioactive methionine was found in the tissue, which accounted for only approximately 5% of the total volume. The transport from incubation medium to intestinal rings was more effective for unoxidized than for oxidized methionine, except at the highest substrate level where methionine sulphoxide was transported more effectively in sections B and C. This indicates a lower affinity and a higher maximal velocity for transport of methionine sulphoxide in these sections. The results are in accordance with those of Higuchi *et al.* (1982) who recently reported similar rates of absorption at the 5 mM substrate level.

The fact that methionine absorption was not inhibited by methionine sulphoxide, and the absorption of methionine sulphoxide was not inhibited by methionine, indicates that the absorption process for the two amino acids is different. Different absorption mechanisms are reported for acid, basic and neutral amino acids (Munck, 1981). As methionine sulphoxide has a lower isoelectric point than methionine, the absorption mechanism may change by oxidation of methionine. Absorption of both amino acids is also different from that of peptides as glycyl-L-methionine did not inhibit the absorptions. Different systems for absorption have also been reported for unoxidized and oxidized methionine in bacterial transport (Ayling, 1981), but Higuchi *et al.* (1982) concluded that they have a common transport system in rat small intestine.

The increased absorption of the two amino acids in the presence of GSH indicates that this peptide is important in the absorption of methionine and methionine sulphoxide in rat intestine. GSH is present in the intestine in relatively high concentrations and is said to be involved in the absorption of amino acids in various tissues (Meister, 1982). That methionine is absorbed by a system involving GSH was indicated by Griffith *et al.* (1978) who found that the level of GSH in the kidney decreased at elevated methionine concentrations; however, the importance of GSH in the absorption of methionine is disputed (Bidot-Lopez & Schinbeckler, 1981; Kalra *et al.* 1981). Our results give more support to the assumption that methionine and, to a larger extent, methionine sulphoxide are absorbed by a system involving GSH and that this system is only one of the systems transporting methionine in the intestine. Methionine sulphoxide is reported to inhibit γ -glutamylcysteine synthetase (EC 6.3.2.2) which is involved in the GSH-absorption system (Richman *et al.* 1973). This inhibition may therefore explain, to some extent, the

slower absorption of oxidized than of unoxidized methionine. The effect of cysteine may be caused by increased concentration of GSH as the synthesis of this tripeptide is significantly influenced by the intracellular concentration of cysteine (Meister & Tate, 1976).

The amounts of absorbed amino acid obtained by measuring the concentrations in the incubation mixtures were different from those obtained by extraction of the intestinal rings (Expts 1 and 3). This may be due to methodological limitations: the shorter the incubation periods, the smaller the amounts absorbed and the greater the errors in the differences. The important observation is, however, that with the same incubation period, the presence of GSH seemed to increase the differences while leaving the extracted amounts unchanged. Thus GSH may have trapped some of the absorbed amino acid to a non-extractable form. One possible form is γ -glutamyl-amino acid which is postulated to be formed during amino acid absorption in the presence of GSH (Meister, 1982).

Unpublished analyses (A. Aksnes) of faeces from rats given methionine sulphoxide alone or in combination with cystine show complete absorption of methionine sulphoxide in the small intestine. This indicates that the absorption rate observed for methionine sulphoxide probably provides for near quantitative uptake of this amino acid in rat intestine. This is not surprising as methionine is effectively absorbed in the small intestine, and a 40% lower absorption, as observed for methionine sulphoxide, is comparable with the absorption rate of several other amino acids (Munck, 1981). Therefore, the difference in utilization of methionine and methionine sulphoxide and the increased utilization of the latter in the presence of cysteine (Gjøen & Njaa, 1977) are probably not caused by the observed difference in absorption and the effect of GSH on this process.

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