Enzymatic Reactions of Methionine Sulfoximine

CONVERSION TO THE CORRESPONDING α-IMINO AND α-KETO ACIDS AND TO α-KETOBUTYRATE AND METHANE SULFINIMIDE*

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L-Methionine sulfoximine is a substrate of L-amino acid oxidase (Crotalus adamanteus), glutamine transaminase, and γ-cystathionase. In the reaction catalyzed by L-amino acid oxidase, methionine sulfoximine is converted to α-imino-γ-methylsulfoximylbutyrate, which undergoes rapid γ elimination yielding methane sulfinimide and 2-imino-3-butenolic acid. Methane sulfinimide is converted to methane sulfonamide, methane sulfonic acid, and methane sulfonic acid; 2-imino-3-butenolic acid is hydrolyzed to vinylglycine, which polymerizes to an insoluble product. When the reaction is carried out in the presence of semicarbazide, the imine formed initially is quantitatively trapped as α-keto-γ-methylsulfoximylbutyrate semicarbazone, from which the free α-keto acid may be obtained. When the reaction is carried out in the presence of a mercaptan (RSH), a γ exchange reaction occurs leading to formation of a new α-keto acid substituted in the γ position by an SR-group; thus, α-keto-γ-(β-hydroxyethanol)butyric acid (8-hydroxyethyl)-2-keto-4-mercaptobutyric acid) was obtained when L-methionine sulfoximine was oxidized in the presence of 2-mercaptoethanol, and enzymatic transamination of this α-keto acid with L-glutamine gave the new amino acid, L-ω-hydroxyethionine. The reaction of L-methionine sulfoximine with γ-cystathionase yields 1 mol each of α-ketobutyrate and methane sulfinimide; the latter is hydrolyzed almost exclusively to methane sulfonic acid. Transamination of L-methionine sulfoximine yields the corresponding α-keto acid (α-keto-γ-methylsulfoximylbutyrate), which is stable. Some of these reactions may occur in vivo, and thus contribute to the toxicity of L-methionine sulfoximine.

MATERIALS AND METHODS

The amino acids, pyridoxal, crystalline beef liver catalase, and purified L-amino acid oxidase (Crotalus adamanteus) were obtained from Sigma. Methane sulfonic acid was obtained from Aldrich. Methane sulfonyl chloride was synthesized from methane sulfonyl chloride and ammonia (33). α-Vinylglycine was a gift of Dr. R. R. Rando (Department of Pharmacology, Harvard Medical School). Glutamine...
The values for the sulfoximine, sulfone, and sulfoxide derivatives are 10, 5, and 0.35 mg in a final volume of 0.2 ml. The mixtures were incubated at 37°C, and aliquots (10 µl) were withdrawn and assayed for ammonia (Curve D) by Conway microdiffusion and Nesslerization, and α-keto acid (Curve 2) by addition of 0.1 ml of 2,4-dinitrophenylhydrazine (0.1% in 2 N HCl) followed by incubation at 37°C for 5 min, and addition of 0.9 ml of 1 N KOH; the absorbance at 480 nm was measured (+, 2,4-dinitrophenylhydrazine = 1.7 x 10³). Curve D, semicarbazone formation in reaction mixtures that also contained 0.1 M semicarbazide.

Transaminase K was purified from rat kidney as described (32). Rat liver γ-cystathionase, purified essentially by the method of Greenberg et al. (34) was kindly donated by W. S. Washietl (Department of Biochemistry, Brandeis University). The isomers of L-methionine sulfoximine (23) were prepared by Dr. W. B. Rowe of this laboratory. Solute pig heart glutamate-aspartate transaminase was obtained from Boehringer Mannheim.

PMR spectra were recorded with a Bruker WH 90 Fourier transform spectrometer. The solvent employed was deuterium oxide (99.7%, Willard Glass Co.), containing 100 mM potassium phosphate buffer (pH 7.2). Chemical shifts (δ) were measured as parts per million (ppm) from the methyl resonance of the internal standard 3-(trimethylsilyl)propane sulfonate (DSS).

L-Amino acid oxidase activity was determined by the general procedure of Haefner and Wellner (35, 36) in which the initial product of the reaction (the imine) is trapped as the corresponding semicarbazone. The reaction mixtures contained sodium borate buffer (100 mM; pH 8.0), L-amino acid oxidase, semicarbazide (100 mM), and beef liver catalase (100 units); final volume, 1 ml. The increase in the absorbance at 248 nm due to the semicarbazone (ε = 10³) was continuously recorded at 37°C. The rate of oxidation of L-methionine-(RS)-sulfoximine was 1 µmol/h/mg of enzyme or about 1% of the rates found with L-methionine sulfone and methionine sulfoxide can be obtained in good yields by oxidation with L-amino acid oxidase (see Ref. 37 for general procedure).

Paper chromatography also revealed the formation of methionine sulfoximine preparations are usually contaminated with small amounts of methionine sulfone and methionine sulfoxide which seems to explain the formation of α-keto-γ-methylsulfonylbutyrate. The α-keto analog of methionine sulfoxide and methionine sulfoxide can be obtained by good yields by oxidation with L-amino acid oxidase (see Ref. 27 for general procedure).

Action of L-Amino Acid Oxidase on L-Methionine Sulfoximine—When L-methionine sulfoximine was incubated with L-amino acid oxidase (Fig. 1; Curve I), the formation of ammonia (6.2 µmol after 5 h) was substantially greater on a molar basis than the amount of amino acid present (1 µmol), suggesting that both the α-amino and sulfoximine nitrogen atoms are converted to ammonia. The formation of α-keto acid was equivalent to no more than about 10% of the added amino acid; the α-keto acid product was identified by paper chromatography as α-keto-γ-methylsulfonylbutyrate (see miniprint, p. 1). (L-Methionine sulfoximine preparations are usually contaminated with small amounts of methionine sulfone and methionine sulfoxide which seems to explain the formation of α-keto-γ-methylsulfonylbutyrate. The α-keto analog of methionine sulfoxide and methionine sulfoxide can be obtained by good yields by oxidation with L-amino acid oxidase (see Ref. 37 for general procedure).

That the methyl resonance observed in Fig. 2 (X; Δν = 2.25) represents methionine sulfoxide is indicated by the following: (a) a reaction mixture (5 ml), equivalent to that given in Fig. 1 (after 6 h of oxidation), was streaked on a sheet of Whatman No. 1 paper and chromatographed as described in the mini-

Some of the data are presented as a miniprint supplement immediately following this paper. For the convenience of those who prefer to obtain supplementary material in the form of 5 pages of full size photocopies, these same data are available as JBC Document No. 76M-625. Orders for supplementary material should specify the title, authors, and reference to this paper and the JBC Document Number, and the number of copies desired. Orders should be addressed to The Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014, and must be accompanied by a remittance to the order of the Journal in the amount of $1.90.
An acidic compound was eluted with water from a strip 5 cm wide centered around the RF value of 0.80. The pH of this solution was adjusted to 5 by adding saturated barium hydroxide solution, and a white solid precipitated after addition of 5 volumes of ethanol. Calculated for (CH,02S)2Ba:S, 21.8%; found, 20.0%. (b) It was found that in strong acid (1.0 M HCl) both the (Av = 2.75) peak (methane sulfonic acid) and the (Av = 2.25) peak were shifted downfield by about 0.3 ppm, suggesting that the (Av = 2.25) methyl resonance is adjacent to an ionizable group. (c) The PMR spectra of 100 mM L-cysteine sulfonic acid and 100 mM L-cysteine sulfonic acid (cysteic acid) were obtained in deuterium oxide (pH 7.0), and it was found that the p protons of cysteine sulfonic acid (Av = 3.3) absorb further downfield than those of cysteine sulfonic acid (Av = 2.7) by about 0.6 ppm. This difference is similar to that (0.54 ppm) between Av = 2.79 (methane sulfonic acid) and Av = 2.25. (Because of the deshielding effects of adjacent amino and carboxylate groups the methylene protons of cysteine sulfonic acid and of cysteine sulfonic acid absorb further downfield than the methyl resonances of the corresponding methane sulfur acids. The sulfonate group is deshielding relative to the sulfinate group presumably because of the increase in electronegativity of the sulfur atom at higher oxidation states.) (d) Mass spectral data (see miniprint, Fig. 1) present additional strong evidence that the (Av = 2.25) peak obtained in the PMR spectra of the l-amino acid oxidase reaction and in the γ-cystathionase reaction (see below) is due to the methyl group of methane sulfonic acid. Thus, molecular ions at m/e values of 80, 65, and 63 were obtained in both reactions.

As shown below, the interaction of γ-cystathionase and L-methionine sulfoximine yields 1 mol each of α-ketobutyrate and elimination product. However, in the PMR spectrum of the products of the l-amino acid oxidase reaction there are no resonances corresponding to the β and γ protons of α-keto-γ-methylenesulfoximinylbutyrate or of the expected elimination product, i.e. vinylglyoxylate (Fig. 3). This initially puzzling finding seems to reflect the high reactivity of vinylglyoxylate, which readily polymerizes to an insoluble precipitate. We attempted to obtain vinylglyoxylate by incubating 40 mM DL-vinylglycine with the enzyme (2.5 mg; final volume, 5 ml) at pH 8 and 37°. After 4 min, the reaction mixture contained 2,4-dinitrophenylhydrazone-forming material equivalent to

Fig. 3. Interaction of l-methionine sulfoximine with l-amino acid oxidase, and proposed nonenzymatic breakdown of the initial oxidation product (α-imino-γ-methylsulfoximinylbutyrate). The imine (I) derived from l-methionine sulfoximine (I) spontaneously undergoes γ elimination, but may be trapped with semicarbazide (in a transamination reaction that eliminates ammonia) to yield the semicarbazone of α-keto-γ-sulfoximinylbutyrate (III). α-Keto-γ-sulfoximinylbutyrate (IV), the α-keto analog of methionine sulfoximine, may be obtained by passage of III through Dowex 50-H+. The γ elimination reaction yields 1 mol each of methane sulfinamide (V) and 2-imino-3-butenoic acid (VI). Methane sulfinamide (V) is unstable and may be oxidized to methane sulfonamide (VII) or hydrolyzed to methane sulfinic acid (VIII), which may be oxidized to the corresponding sulfonic acid (IX). 2-Imino-3-butenic acid (VI) is hydrolyzed to vinylglyoxylate (X) which in turn, via a series of aldol condensations, forms an insoluble polymer (XI). Structure (VI) may be trapped by carrying out the reaction in the presence of a mercaptan. Thus, addition of a thiol anion at the γ position and a proton at the β position of Structure VI yields a saturated α-imino acid, which on hydrolysis and loss of ammonia yields a sulfur containing α-keto acid (XII). α-Keto acid (XII) may also arise via β,γ addition to the unsaturated α-keto acid (X). Enzymatic transamination of (XII) yields a new l-amino acid (XIII).
about 25% of theoretical. By measuring the decrease in α-keto acid (as the 2,4-dinitrophenylhydrazone) with time, the half-life of vinylglyoxylic acid (at 37°C in 100 mM sodium borate buffer, pH 8.0) was estimated to be 1 h. After 24 h no 2,4-dinitrophenylhydrazone-forming material was found, nor were there any peaks in the PMR spectrum of this solution. Vinylglyoxylic acid has evidently not been extensively studied, however, small quantities of the 1-14C-labeled compound have been prepared and found to be very unstable. It is known that pyruvate polymerizes in aqueous solution (41), and vinylglyoxylic acid probably has an even greater tendency to polymerize because its β proton would be expected to be much more labile than the β protons of pyruvate. The unsaturated ketone, methylvinylketone is known to polymerize very readily and is used in the preparation of certain plastic (42). Thus, polymerization of vinylglyoxylic acid, via a series of aldol condensations, would explain the disappearance of the carbonyl function from reaction mixtures containing L-amino acid oxidase and DL-vinylglycine or L-methionine sulfoximine (Fig. 9).

That vinylglyoxylic acid is produced by the action of α-amino acid oxidase on L-methionine sulfoximine and on DL-vinylglycine is further supported by the observation that when the reaction described in Fig. 1 was carried out in the presence of an excess of methyl mercaptan, a good yield (96%) of α-keto-γ-methylmercaptobutyrate (5-(methyl)-2-keto-4-mercaptobutyrate) was obtained after 6 h. The α-keto acid produced in this reaction exhibited an Rf value identical with that of authentic α-keto-γ-methylmercaptobutyrate (see Miniprint, p. 1), and to the adduct produced when DL-vinylglycine was oxidized by L-amino acid oxidase in the presence of excess methyl mercaptan. The methyl mercaptan employed in these studies was generated in situ by shaking dimethyl disulfide with 200 mM dithiothreitol in 100 mM potassium pyrophosphate buffer (pH 8.3); the aqueous phase (50 μl) was added to a reaction mixture (50 μl) containing buffer, L-amino acid oxidase, and catalase (final concentrations as given in Fig. 1). The generality of this mercaptan addition reaction is supported by the observation that 2-mercaptoethanol also forms a vinylglyoxylic acid adduct. Thus, when the reaction was carried out in the presence of 2-mercaptoethanol a high yield of the corresponding α-keto acid (α-keto-γ-(β-hydroxyethyl)butyrate; S-(hydroxyethyl)-2-keto-4-mercaptobutyrate) was obtained. The preparations of this α-keto acid, the corresponding 2,4-dinitrophenylhydrazone, and the corresponding α-amino acid (L-α-hydroxyhomocysteine) are described in the Miniprint, p. 2 or 3. α-Keto-γ-(β-hydroxyethyl)butyrate can be detected on paper chromatography as a new orange α-phenylethylamine-positive compound (Rf 0.81) (see Miniprint, p. 1). A compound with the same properties was detected when DL-vinylglycine was oxidized with L-amino acid oxidase in the presence of 200 mM 2-mercaptoethanol. It seems probable that the mercaptan adds to carbon atom 4 of 2-imino-3-butenolic acid, followed by hydrolysis to the corresponding sulfur-containing α-keto acid. However, mercaptans can add to the free α-keto acid. Thus, when vinylglyoxylic acid was incubated with a 2 fold molar excess of 2-mercaptoethanol, there was no loss of carbonyl groups over a period of 5 h (37°C, 100 mM borate buffer, pH 8.4) and α-keto-γ-(β-hydroxyethyl)butyrate was detected by paper chromatography. In contrast, when vinylglyoxylic acid was incubated in the absence of 2-mercaptoethanol, rapid loss of α-keto acid was observed, presumably due to polymerization. In these studies vinylglyoxylic acid was generated by employing an amount of L-amino acid oxidase, such that oxidation of DL-vinylglycine was complete in 10 min; after incubating for an additional 10 min (to ensure complete hydrolysis of the imine (35)), the mercaptan was added.

The reaction of α-amino acid oxidase with L-methionine-(RS)-sulfoximine and associated reactions are summarized in Fig. 3. The ratio of methane sulfonic acid (VIII) to methane sulfonic acid (IX) depends on the method of isolation. Methane sulfonic acid is stable at pH 7.0 but acid facilitates its oxidation to the sulfonic acid. Thus, when the reaction mixture is passed through Dowex 50 H+ followed by rapid neutralization and lyophilization, methane sulfonic acid predominates rather than methane sulfonic acid. Methane sulfonimide, the initial elimination product, is unstable and may be oxidized to the stable sulfonamide or hydrolyzed to the relatively stable sulfonic acid. The amount of sulfonamide produced relative to the other sulfur compounds seems to depend on the oxidizing power of the medium. No sulfonamide was detected in the γ-cystathionase reaction. Under the conditions employed, CH₃SOONH⁺ appears to be a much better leaving group than are −OH, −SH, and −SCH₃. Thus, homoserine, homocysteine, and methionine are oxidized by L-amino acid oxidase to yield essentially equivalent amounts of the corresponding α-keto acids.

Preparation of the α-Keto Acid Analog of Methionine Sulfoximine (α-Keto-γ-methylsulfoximinylbutyrate; 2-Oxo-4-(S-methylsulfoximido)butanoate) using L-Amino Acid Oxidase—The initial product formed in the oxidation of L-methionine sulfoximine by L-amino acid oxidase is the corresponding imine (II, Fig. 3). As first shown by Hafner and Wellner (35, 36) such imines may be quantitatively trapped as the corresponding semicarbazones, and data given in Fig. 1 (Curve 3) indicate that such a trapping procedure is applicable to the imine derived from methionine sulfoximine. We have found that the α-keto acid analog of methionine sulfoximine (Fig. 3, IV) can be regenerated from the corresponding semicarbazone; the α-keto acid was prepared as follows. A reaction mixture containing 4 mmol of semicarbazide, 1 g of dried crude snake venom (37), 181 mg of L-methionine-(RS)-sulfoximine, and 1,000 units of catalase (final volume, 20 ml; pH, 8.0) was incubated with aeration at 37°C for 47 h. The protein was removed by filtration through an Amicon UM 10 membrane, and the protein-free solution was applied to the top of a Dowex 50 H+ column (1 × 5 cm). The column was eluted with 200 ml of water and the eluate was evaporated to 5 ml. This solution which contained traces of the semicarbazone, was reapplied to a similar Dowex 50 H+ column and eluted with 200 ml of water. The effluent was evaporated to 5 ml decolorized with charcoal, and the pH was adjusted to 4.5 by adding saturated barium hydroxide. The solution was evaporated to 1 ml and the barium salt of the α-keto acid was precipitated by adding 5 volumes of ice-cold ethanol. The barium salt was reprecipitated twice
The keto acid gave a single α-phenylenediamine-positive spot on paper chromatography (see miniprint, p. 1); the $R_f$ value (0.70) was identical with that found as a product of the glutamine transaminase K catalyzed reaction between L-methionine sulfoximine and phenylpyruvate.

**Interaction of L-Methionine Sulfoximine with Glutamine Transaminase K—L-Methionine** and a number of methionine analogs, including L-methionine-(RS)-sulfoximine are substrates of glutamine transaminase K and L (31, 32). Transamination between L-methionine sulfoximine and phenylpyruvate was followed by observing the disappearance of the enol·borate complex of phenylpyruvate (43) (Fig. 4A, Curve 2). When glutamine was the amino donor, the disappearance of 2,4-dinitrophenylhydrazone-forming material (Fig. 4B, Curve 3), followed that of the disappearance of the enol·borate complex (Fig. 4A, Curve 3), since the α-keto analog of glutamine exists predominantly in a cyclic form and does not readily form a hydrazone (44). However, when L-methionine sulfoximine was the amino donor, after complete disappearance of phenylpyruvate there was only a small decrease in the absorbance at 430 nm due to 2,4-dinitrophenylhydrazone-forming material (Fig. 4B, Curve 3), reflecting the presence of the 2,4-dinitrophenylhydrazone of α keto γ methylsulfoximyliminobutyrate, which has a lower extinction coefficient at 430 nm than that of phenylpyruvate. The data (Fig. 4B, Curve 2) indicate that the α-keto acid analog of methionine sulfoximine is stable and disappears relatively slowly. When the reaction mixture (Fig. 4B, Curve 2) was incubated for 1 h, and then passed through a Dowex 50-H⁺ column (followed by neutralization and lyophilization), α-keto-γ-methylsulfoximyliminobutyrate was detected by paper chromatography (see miniprint, p. 1). However, when the reaction mixture was incubated for 48 h, no such product was detected, and the PMR spectrum revealed the presence of methane sulfonamide (3%), methane sulfonic acid (20%), and methane sulfonic acid (77%).

L-Methionine-S-sulfoximine and L-methionine-(R)-sulfoximine are both substrates for glutamine transaminase. Thus, the rates of disappearance of phenylpyruvate were in the ratio of 1:1:1.3 for L-methionine-(RS)-sulfoximine, L-methionine-(R)-sulfoximine, and L-methionine-(S-sulfoximine, respectively, under the conditions given in Fig. 4.

**Interaction of L-Methionine Sulfoximine with γ-Cystathionase** γ-Cystathionase catalyzes conversion of L-cystathionine to α-ketobutyrate, cysteine, and ammonia, and also certain other α elimination reactions (45). We have found that γ-cystathionase can also catalyze relatively slow γ elimination reactions involving L-methionine sulfoximine, L-methionine sulfoxide, and L-methionine sulfone to yield α-ketobutyrate; methionine is not a substrate. The interaction of γ-cystathionase with L-methionine sulfoximine was studied by examination of the PMR spectra of the isolated reaction products (see miniprint, Fig. 2). Resonance signals due to the β protons of α-ketobutyrate (quartet, $\Delta \tau = 2.72$), the γ protons of α-ketobutyrate (triplet, $\Delta \tau = 1.65$) and the methyl group of methane sulfonic acid (singlet, $\Delta \tau = 2.25$) were detected. The integrals of these peaks were, within experimental error, in the ratio 2/3/3, respectively. The data thus indicate that the molar ratio of α-ketobutyrate to elimination product (methylene sulfonic acid) is approximately 1/1. The amount of methane sulfonic acid was less than 3% of the sulfonic acid; methanol sulfonamide could not be detected. A scheme for the interaction of γ-cystathionase with L-methionine sulfoximine is given in Fig. 5.

**DISCUSSION**

These studies, which have elucidated several reactions of methionine sulfoximine, seem to explain in part the reported in vivo conversion of methionine sulfoximine to other sulfur-containing compounds (30). It seems probable (on the basis of chromatographic data) that two of the 35S-containing compounds found previously after injection of mice with L-[35S]methionine sulfoximine are methane sulfonic acid and methane sulfonic acid; this indicates that methionine sulfoximine is metabolized in vivo by pathway(s) similar to those found here. Although such metabolites may contribute to the toxicity of methionine sulfoximine, it is notable that both isomers of L-methionine sulfoximine participate in the reactions summarized in Figs. 3 and 5, while only L-methionine-(S)-sulfoximine (and not the corresponding (R) isomer) produces convulsions (23) and inhibits glutamine and γ-glutamylcysteine synthetase (92-99). Thus, it seems probable that inhibition of one or both of the synthetases is closely associated with the production of convulsions. Whereas L-methionine-(S)-sulfoximine inactivates the synthetases presumably by mimicking the tetrahedral intermediates or transition states formed in these reactions, methionine sulfoximine may inhibit pyridoxal phosphate enzymes by other mechanisms. Thus, γ elimination might lead to a vinlyglycine ketimine intermediate which could be attacked by Michael addition of a basic group at the active site of the enzyme. Rando (46) has suggested a similar mechanism for inactivation of glutamate-aspartate transaminase by vinylglycine. Preliminary studies in this laboratory indicate that L-methionine sulfoximine slowly and
The finding that methionine sulfoximine interacts with several transaminases and γ-cystathionase suggests that it might be worthwhile to also examine other vitamin B₆-containing enzymes.⁴

The γ elimination reactions observed here involve elimination of methane sulfinimide associated with collapse of hexavalent sulfur to the tetravalent state. While reactions involving such a change in the oxidation state of sulfur are well known, we are aware of only one other reaction in which a sulfinimide is produced from a sulfoximine (48); in this case the elimination reaction is brought about by a cyclization reaction that releases a product containing a three-membered ring. The formation of α-keto-γ-(β-hydroxyethyl)butyrate that occurs when L-methionine sulfoximine is oxidized by L-amino acid oxidase in the presence of 2-mercaptoethanol involves a nonenzyatic γ exchange reaction. This reaction is analogous to one catalyzed by L-cystathionine γ-synthase (L-Ο-succinylhomoserine + L-cysteine → L-γ-cystathionine + succinate) (40), and to a recently described reaction catalyzed by methionine; (L-methionine + 2-mercaptoethanol → L-Ο-hydroxyethylthione + methylmercaptan) (50). By varying the mercaptan employed in the γ displacement reaction a number of α-keto acids substituted in the γ position by an ER group are potentially available; these can be readily converted to the corresponding methionine analogs by transamination, reductive amination, or controlled reduction of the 2,4-dinitrophenylhydrazone derivative (51).

Many α-keto acids can be prepared from the corresponding L-amino acids by oxidation with L-amino acid oxidase (37, 52), but the enzymatic oxidation of L-methionine sulfoximine is unusual in that the intermediate imino acid undergoes rapid γ elimination rather than hydrolysis. Inasmuch as α-imino acids have half-lives of the order of a few seconds (35) the γ elimination reaction involving α-imino-γ-methylsulfoximinylbutyrate must be extremely rapid. Since the corresponding α-keto acid has a half-life of several days under comparable conditions, the rate enhancement of the α-imino acid versus the α-keto acid for this γ elimination reaction must be at least 10⁵. This difference may probably be explained by the increase in the reactivity of a C—H bond adjacent to an amino group as compared to one adjacent to a carbonyl group. Such reactivity seems to explain the large rate enhancement of amine-catalyzed aldol condensations and reverse aldol reactions, and may be important in many enzymatic reactions, e.g. those catalyzed by aldolase and δ-aminolevulinic synthase (53). It has been suggested that a carbamation at the α position of an imino compound may be greatly favored relative to a carbamation in the α position of the corresponding carbonyl compound. Thus, the elaboration of acetone is catalyzed by amines at rates which are proportional to the concentration of the protonated amine; such catalysis may be as much as 10⁴ times faster than that which occurs with carboxylic acids with pKₐ values comparable to amines (53, 54). Similar rate enhancements have been noted for the N-methylamine-catalyzed hydrolysis...
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references

possible for the imino analog of homoserine, the driving force for elimination would be much lower than that for elimination of a sulfinimide. However, water is eliminated from homoserine in the cystathionine-catalyzed γ elimination reaction. Therefore, although protonation of the ω-hydroxyl group is highly unfavorable, strategic positioning of active site bases seems to provide an efficient proton transfer system. Evidence that such basic groups are present at the active site of γ-cystathionase has come from inactivation studies with propargylglycine (60). The active site of γ-cystathionase seems to be quite flexible; thus, there is evidence that the active site has two regions, one of which contains pyridoxal phosphate and the base(s) necessary for a, and b proton abstraction. The other site binds the γ substituent and has a base that interacts with the hydroxyl group of homoserine; this site also interacts with the hydroxyl groups of competitive inhibitors, such as l-serine and l-2-amino-5-hydroxyvaleric acid (W. S. Washstien, A. J. L. Cooper, and R. H. Abeles, unpublished data).

incorporation into 2-D-isobutyraldehyde (55, 56). Such rate enhancements (53-56) are similar to that observed here for the γ elimination reaction of α-imino-γ-sulfinimidine butyrate as compared to that of the corresponding α-keto acid. There is good evidence that the product released from the L-amino acid oxidase reaction is the imine (35, 57). Flavin and Slaughter (57) were able to trap an enamine species with N-ethylmaleimide in the γ-cystathionase and threonine deaminase reactions, but not in the L-amino acid oxidase-catalyzed reaction with L-alanine or DL-α-aminobutyrate. Evidently only in the oxidation reaction with L-phenylalanine is there evidence that the imine released from an active site can equilibrate with an enamine before hydrolysis (58, 59). We have considered the possibility that the reaction of L-amino acid oxidase with methionine sulfoximine may be a special case in which a relatively long lived enamine species orients prior to hydrolysis of the imine. However, we were unable to trap such a species with N-ethylmaleimide under conditions similar to those used by Flavin and Slaughter (53).

We suggest that the nonenzymatic γ elimination reaction of α-imino-γ-sulfinimidine butyrate occurs by a mechanism analogous to that proposed by Jencks (60) for the γ-cystathionase reaction (Fig. 5, Structure II → Structure III). The elimination involves loss of a proton a to the pronated imino group, resulting in a carbanion-enamine intermediate which rapidly breaks down to 2-imino-3-butenoc acid and methane sulfinimide. The carbanion-enamine species is evidently short lived, both in the nonenzymatic γ elimination reaction and in the γ-cystathionase-catalyzed reaction. In the latter the enamine species produced late in the enzymatic reaction sequence can be trapped with N-ethylmaleimide (67). The pKa of a ketimine is at least 3 orders of magnitude lower than of the corresponding amino compound. Therefore, the pKa of an α-imino acid is in the range 5 to 6 (35, 61); at pH at which the L-amino acid oxidase reaction is carried out a small but significant amount of the imine will be protonated. The pKa of dibutylsulfloximine (water; 25°) has been reported to be 3.30 ± 0.05 (62). While the exact value of the pKa of the sulfinimine group of methionine sulfoximine has not yet been determined, it is probably not greatly different from this value. Therefore, the methionine sulfinimine nitrogen atom will be only very slightly protonated at the pH of the L-amino acid oxidase reaction. It is conceivable that the imino acid has a half-life sufficiently long so that the electron flow is toward a fully protonated sulfinimine nitrogen. Another possibility is a concerted reaction, either a direct intramolecular transfer of the electron pair from the sulfinimine double bond to a suitable acceptor, or more likely, an intramolecular reaction involving a seven-membered ring system, i.e., a proton is transferred from the α-nitrogen of the carbanion-enamine transition state intermediate to the nitrogen of the leaving sulfinimide. Examples of reactions involving rings with greater than six members are well known, e.g., the oxidation of cis- or trans-allylic alcohols to α-β-unsaturated aldehydes by selenium dioxide appears to involve an eight-membered transition state (63). The latter reaction also resembles the γ elimination reaction of α-imino γ-sulfinimidine butyrate in that the end product is an α-β-unsaturated carbonyl compound.8

8 We have obtained similar results with dimethysulfloximine, whose pKa value was found by titration to be 2.8 ± 0.2 (H2O; 25°).

* That the imine derived from homoserine does not undergo a γ elimination reaction may possibly be related to the very low pKa value (≈ 2.64) for protonation of the ω-hydroxy group. Although a concerted reaction, similar to that discussed above, is also theoretically
### Enzymatic Reactions of Methionine Sulfoximine

#### Supplement

To: Enzymatic Reactions of Methionine Sulfoximine, Conjugation to the Corresponding α-βeto-
and α-βeto-γ-carboxylic Acid, and to α-keto-γ-carboxylic Acid, and to α-keto-hydroxymethyl Acid.

by Arthur J. L. Cooper, Ralph A. Silver, and Allan Holohan, Departments of Neurology and
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Paper Chromatography of Methionine Sulfoximine and their Metabolites

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<th>Compound</th>
<th>Rf x 100</th>
<th>Method of Detection</th>
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</thead>
<tbody>
<tr>
<td>L-Methionine</td>
<td>71</td>
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<td>L-Methionine sulfoxide</td>
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<tr>
<td>L-Methionine sulfone</td>
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<td>L-D-Hydroxyphosphate</td>
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<td>L-Dehydrophase</td>
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<td>α-Keto-γ-hydroxymethylate</td>
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<td>α-Keto-γ-methylbutyrate</td>
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<td>α-Keto-γ-methylvalerate</td>
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<td>α-Keto-γ-propylolate</td>
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<td>Sulfonate acid</td>
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<td>Sulfuric acid</td>
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</tr>
<tr>
<td>Sulfate acid</td>
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<td>N2,N8</td>
</tr>
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#### Notes

Paper chromatography was carried out on Whatman No. 1 paper by the ascending method.

The reaction was studied by the method of paper chromatography. The reaction was run under standard conditions.

The reaction mixture contained 0.25 μmol of each of α-βeto-
and α-βeto-γ-carboxylic acid, 200 μmol of α-keto-
hydroxymethyl acid, and 100 μmol of L-D-phenylalanine.

The reaction was followed by paper chromatography.

#### Figure 1

- **Part a**: The reaction was carried out as described in micrographs.
- **Part b**: The reaction was carried out as described in micrographs.
- **Part c**: The reaction was carried out as described in micrographs.

#### Figure 2

- **Part a**: The reaction was carried out as described in micrographs.
- **Part b**: The reaction was carried out as described in micrographs.
- **Part c**: The reaction was carried out as described in micrographs.

#### Notes

- The reaction mixture contained 0.25 μmol of each of α-βeto-
and α-βeto-γ-carboxylic acid, 200 μmol of α-keto-
hydroxymethyl acid, and 100 μmol of L-D-phenylalanine.
- The reaction was followed by paper chromatography.
- The reaction was carried out as described in micrographs.
Enzymatic reactions of methionine sulfoximine. Conversion to the corresponding alpha-imino and alpha-keto acids and to alpha-ketobutyrate and methane sulfinimide.

A J Cooper, R A Stephani and A Meister