Studies on the Inhibition of Glutamine Synthetase by Methionine Sulfone†

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ABSTRACT: Both L- and D-methionine sulfone are effective reversible inhibitors of glutamine synthetase. Incubation of L- or D-[*methyl*-^{14}C]methionine sulfone with glutamine synthetase in the presence of ATP and magnesium ions leads to the formation of a new ^{14}C compound. Formation of the ^{14}C product requires enzyme, ATP, and magnesium ions, and is accompanied by stoichiometric cleavage of ATP to ADP and P_i. The ^{14}C product exhibits the properties of an anion, does not contain phosphate, and is converted to methionine sulfone on treatment with dilute hydrochloric acid or sodium hydroxide. The product is tentatively considered to be a cyclic derivative of methionine sulfone whose formation from methionine sulfone by glutamine synthetase is analogous to the previously demonstrated formation of pyrrolidonecarboxylate from glutamate catalyzed by this enzyme. That γ-glutamyl phosphate is an intermediate in the conversion of glutamate to pyrrolidonecarboxylate is in accord with the ability of glutamine synthetase to catalyze the phosphorylation of methionine sulfoximine to yield methionine sulfoximine phosphate. The findings support the conclusion that methionine sulfone and methionine sulfoximine inhibit glutamine synthetase by serving as analogs of the postulated enzyme-bound tetrahedral intermediate formed by reaction of ammonia with γ-glutamyl phosphate. That the sulfone inhibits reversibly and that the sulfoximine inhibits irreversibly may be explained by greater tendency of the latter compound to be phosphorylated on the enzyme, thus forming the more stable methionine sulfoximine phosphate which is tightly bound to the enzyme.

Earlier studies in this laboratory showed that L-methionine-*S*-sulfoximine inhibits glutamine synthetase irreversibly in a reaction in which the inhibitor is converted, in the presence of ATP and magnesium ions, to L-methionine-*S*-sulfoximine phosphate, which binds tightly to the enzyme (Ronzio and Meister, 1968; Ronzio *et al.*, 1969; Rowe *et al.*, 1969; Manning *et al.*, 1969). On the other hand, it was found that L-methionine sulfone, which is also a very effective inhibitor of glutamine synthetase, inhibits the enzyme in a reversible manner and does not remain attached to the enzyme (Ronzio *et al.*, 1969). The stereochemical basis of inhibition by these methionine derivatives was investigated by a computer analysis of the active site of glutamine synthetase (Gass and Meister, 1970); the calculations performed are in accord with the conclusion that methionine sulfone and methionine sulfoximine are analogs of the tetrahedral intermediate formed (by reaction of ammonia with enzyme-bound γ-glutamyl phosphate) in the course of glutamine synthesis. Thus, it was found that L-methionine *S*-sulfoximine is bound to the enzyme in such a manner that the sulfoximine nitrogen atom lies very close to the site normally occupied by the oxygen atom of glutamate that is phosphorylated. Similarly, one of the sulfone oxygen atoms of L-methionine sulfone and of D-methionine sulfone also lie close to this "oxygen-phosphorylation" site (Gass and Meister, 1970). While L- and D-methionine sulfone (as shown here) are effective inhibitors of glutamine synthetase, neither isomer of methionine sulfone has been found to be phosphorylated under conditions in which methionine sulfoximine is phosphorylated. In the present article data are given which indicate that both isomers of methionine sulfone are converted by glutamine synthetase in the presence of ATP and magnesium ions to a new product, which is probably a cyclic derivative of the sulfone. The occurrence of this reaction is in accordance with previous calculations and considerations relating to the active site of glutamine synthetase, and with the view that the enzyme can catalyze the phosphorylation of the sulfone.

Experimental Section

Materials. L-Methionine, L-methionine sulfoxide, and D-methionine were obtained from Schwarz-Mann. L-Methionine sulfone and D-methionine sulfone were prepared from the corresponding isomers of methionine by the method of Toennies and Kolb (1941). D-Methionine sulfoxide was prepared as described by Toennies and Kolb (1939). AMP, ADP, and ATP were obtained from Sigma. Dicyclohexylcarbodiimide and cyanoethyl phosphate were obtained from Calbiochem. Polyphosphoric acid was obtained from BDH Chemicals, Ltd., and crystalline phosphoric acid was obtained from Fluka AG through Columbia Organic Chemicals.

L-[*methyl*-^{14}C]Methionine and [γ-^{32}P]ATP were obtained from New England Nuclear. D-[*methyl*-^{14}C]Methionine was obtained from International Chemical and Nuclear Corp. L-[*methyl*-^{14}C]Methionine sulfoxide and D-[*methyl*-^{14}C]-methionine sulfoxide were prepared by the method of Toennies and Kolb (1939); a small amount of unreacted methionine was removed by ion-exchange chromatography as described earlier for the preparation of L-[*methyl*-^{14}C]methionine sulfoximine (Ronzio *et al.*, 1969). L-[*methyl*-^{14}C]Methionine sulfone and D-[*methyl*-^{14}C]methionine sulfone were prepared by the method of Toennies and Kolb (1941); the products obtained, which contained a small amount of methionine sulfoxide and an unidentified compound, were purified by ion exchange chromatography followed in some prepara-

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tions by paper electrophoresis at pH 2.85 carried out as described below.

Glutamine synthetase was isolated from sheep brain by the procedure of Rowe *et al.* (1970).

Methods. Glutamine synthetase activity was determined by the γ-glutamyl hydroxamate procedure (Wellner and Meister, 1966). ADP was determined by coupling with pyruvate kinase and lactate dehydrogenase using the method previously described, except that one-fifth of the volume previously used was employed (Stephani *et al.*, 1972). Paper electrophoresis was performed on Whatman No. 3MM paper in a cooled flat bed electrophoresis apparatus (Savant) at 10° using the following buffer systems: 0.6 N formic acid (pH 2.05)–0.5 N acetic acid (pH 2.6)–ammonium acetate buffers (0.05 N acetate) (pH 2.85, 3.05, and 5.5). In the studies in which radioactive nucleotides were separated, the papers used for electrophoresis were washed prior to use with 5% formic acid containing 2 mM EDTA followed by washing with water. The effective length of the paper strips was 74 cm and the potential gradient was 33–40 V/cm.

^{14}C product formation from labeled methionine sulfone was determined as follows. The enzyme reaction mixture was subjected to gel fitration on Sephadex G-50 and the second peak to emerge from the column (containing low molecular weight compounds) was subjected to paper electrophoresis at pH 2.85 (0.05 N ammonium acetate buffer). Under these conditions, the product of the enzymatic reaction moved 2–3 cm from the origin in the direction of the anode, while methionine sulfone moved 8–9 cm from the origin in the direction of the cathode.

Ascending paper chromatography was carried out with the following systems: solvent I, *tert*-butyl alcohol–methyl ethyl ketone–formic acid–water (40:30:15:15, v/v); solvent II, 80% phenol.

Determinations of radioactivity were performed with a Nuclear-Chicago liquid scintillation counter as previously described (Ronzio *et al.*, 1969). Infrared spectra were obtained with a Perkin-Elmer Model 257 spectrophotometer using KBr pellets containing a sample concentration of 1%.

Results

Inhibition of Glutamine Synthetase by L- *and* D-*Methionine Sulfone.* Previous studies indicated that L-methionine sulfone is an effective inhibitor of glutamine synthetase (Ronzio *et al.*, 1969). The data given in Table I indicate that D-methionine sulfone is also a good inhibitor although it is somewhat less effective than L-methionine sulfone. Inhibition by D-methionine sulfone is readily reversible by dilution into a solution containing an excess of L-glutamate, by gel filtration, or by dialysis as previously reported for inhibition by L-methionine sulfone (Ronzio *et al.*, 1969).

Enzymatic Formation of a ^{14}C *Product from* [^{14}C]*Methionine Sulfone.* When glutamine synthetase was incubated with 5 mM L-[^{14}C]methionine sulfone,[1] evidence for the formation of a labeled product was obtained. Thus, when the reaction mixtures were subjected to gel fitration and aliquots of the peak containing molecules of low molecular weight were subjected to electrophoresis at pH 2.85, a product which moved toward the anode was found. Formation of the product was linearly proportional to the length of incubation for at least 3 hr. The rate of product formation was also linearly proportional to enzyme concentration forming about 5 mol/mol of enzyme per hr, or about 10% of the rate of pyrrolidonecarboxylate formation from L-glutamate (Wellner *et al.*, 1966). The rate of product formation was not affected by addition of ammonia or hydroxylamine, but was markedly decreased when the reaction was carried out in the usual synthetase assay system. The data given in Table II show that the formation of this product requires the presence of both ATP and $MgCl_2$. A product with identical electrophoretic

[1] This concentration of L-methionine sulfone inhibits about 54% under the conditions described in Table I.

TABLE I: Effect of Various Methionine Derivatives on Glutamine Synthetase Activity.[a]

Compound Added	Concn (mM)	% Inhibition
L-Methionine sulfone	10	78
D-Methionine sulfone	10	60
L-Methionine-*S*-sulfoximine	2	65

[a] The reaction mixtures contained enzyme (11.5 nM), sodium L-glutamate (0.05 M), NH_2OH (0.10 M), NaATP (0.01 M), $MgCl_2$ (0.02 M), imidazole-HCl buffer (pH 7.2; 0.05 M), 2-mercaptoethanol (0.025 M), and methionine derivative as indicated; final volume 1.0 ml. After incubation at 37° for 15 min, the formation of γ-glutamyl hydroxamate was measured and compared with a control in which no methionine derivative was added.

TABLE II: Requirements for Formation of Product from Methionine Sulfone.[a]

Compd Omitted[b]	Product Formation (nmol per hr per nmol of Enzyme)
None	4.8
$MgCl_2$	0
ATP	0.2
None (Mg^{2+}, 10 mM)	4.2
None (Mg^{2+}, 20 mM)	2.3

[a] The reaction mixtures contained imidazole hydrochloride buffer (pH 7.2; 25 mM), 2-mercaptoethanol (1 mM), sodium ATP (10 mM), and magnesium chloride (5 mM or as indicated), L-[^{14}C]methionine sulfone (10 mM; 5.0×10^5 cpm/μmol), and enzyme (10.4 nmol) in a final volume of 2.0 ml. After incubation for 3 hr at 37°, the mixtures were applied to Sephadex G-50 columns (0.5 × 15 cm) equilibrated with ammonium bicarbonate buffer (pH 7.8; 25 mM); elution was carried out at 4° with the same buffer. Aliquots of the fraction containing low molecular weight compounds were subjected to electrophoresis at pH 2.85 to separate the product from methionine sulfone; see Methods; the amount of product was determined by liquid scintillation counting. [b] No product was found when the enzyme was omitted.

TABLE III: Stoichiometry of Product Formation.[a]

Expt	Reaction Components	nmol Formed: Product	ADP	P_i
1	Methionine sulfone + [γ-^{32}P]ATP		370	410
2	[^{14}C]Methionine sulfone + ATP	370	430	

[a] The reaction mixtures contained imidazole hydrochloride buffer (pH 7.2; 43 mM), 2-mercaptoethanol (2.5 mM), sodium ATP (5 mM; in expt 1, [γ-^{32}P], 7.4 $\times$ 10^5 cpm/μmol), magnesium chloride (10 mM), L-methionine sulfone (10 mM; in expt 2, ^{14}C, 4.45 $\times$ 10^5 cpm/μmol), and enzyme (6.4 nmol) in a final volume of 1.0 ml. The mixtures were incubated at 37° for 3 hr. The formation of ADP was determined on an aliquot (0.2 ml) as described under Methods. The remainder of the reaction mixtures was added to the top of Sephadex G-50 columns (0.5 $\times$ 15 cm) equilibrated with ammonium bicarbonate buffer (pH 7.8, 25 mM) and the columns were eluted with the same buffer at 4°. Fractions of 0.7 ml were collected and assayed for protein and radioactivity. In expt 1 [^{32}P]P_i was separated by paper electrophoresis (pH 3.05) and determined by scintillation counting. In expt 2, the fractions containing ^{14}C were combined, adjusted to pH 2 by addition of hydrochloric acid, and then applied to a column of Dowex 50 H^- (1 $\times$ 8 cm) equilibrated with 0.01 N hydrochloric acid, to remove most of the methionine sulfone. The column was treated with 50 ml of 0.01 M hydrochloric acid and the effluent was lyophilized. The residue was dissolved in 0.5 ml of water and an aliquot was subjected to paper electrophoresis at pH 2.85 to separate the product from the small amount of remaining methionine sulfone; the radioactivity of the product was determined by scintillation counting. In a control experiment with [γ-^{32}P]ATP (no methionine sulfone), the amounts of ADP and P_i found were 6 and 12%, respectively, of the values obtained in expt 1.

properties was formed from D-[*methyl*-^{14}C]methionine sulfone at about half the rate found with L-methionine sulfone. Neither L- nor D-methionine sulfoxide is an effective inhibitor of glutamine synthetase (Ronzio *et al.*, 1969). In the present studies no evidence was obtained for the formation of a new product using the procedures described above in experiments with the L and D isomers of [^{14}C]methionine sulfoxide.

Stoichiometry of Product Formation. The experiments described in Table III give data on the stoichiometry of the reaction catalyzed by glutamine synthetase in which a new product is formed from methionine sulfone. Identical experiments were performed using labeled methionine sulfone in one reaction and labeled ATP in a parallel reaction carried out under the same conditions. As indicated in Table III, close to 1 mol of ADP and 1 mol of inorganic phosphate were formed for each mole of product formed from L-methionine sulfone.

Properties of the Enzymatically Formed Product. Several properties of the product formed by the action of the enzyme on methionine sulfone were examined using a sample of about 300 nmol of the ^{14}C product. The low molecular weight peak obtained from gel filtration on Sephadex G-50 of the enzymatic reaction mixture was passed through a small column (1 $\times$ 6 cm) of Dowex 50 (H^+) to remove the bulk of the unreacted methionine sulfone. The material that was not retained by the column was lyophilized and the residue obtained was dissolved in 1 ml of water and then subjected to paper electrophoresis at pH 2.85. The area of the paper 1–6 cm anionic to the origin was eluted with water and the eluate was lyophilized. The product exhibited the following properties. (a) It was not bound by Dowex 50 (H^+) at pH 7.0. (b) It migrated as an anion at pH values of 2.85, 3.05, and 5.5, and remained at the origin at pH 2.05. (c) It exhibited an R_f value of 0.90 in both solvent I and solvent II; the respective R_f values for methionine sulfone were 0.41 and 0.65. (d) On treatment with 1 N HCl (1 hr, 100°), about 90% of the radioactivity was converted to a substance which exhibited paper chromatographic and electrophoretic properties indistinguishable from those of methionine sulfone. (e) Treatment of the product with 1 N sodium hydroxide (1 hr, 100°) gave a 70% yield of [^{14}C]methionine sulfone.

In an experiment in which both L-[^{14}C]methionine sulfone and [γ-^{32}P]ATP were used, the product was isolated by gel filtration of the reaction mixture followed by Dowex 50 (H^-) chromatography and paper electrophoresis at pH 2.85 as described above; the radioactive area of the paper 2–5 cm anionic to the origin contained the ^{14}C product and also a small amount of ^{32}P. The latter was shown by reelectrophoresis at pH 2.85 in the presence of carrier ATP, ADP, and AMP to be [^{32}P]AMP,[2] whose mobility under these conditions was 6–7 cm in the direction of the anode. After the second electrophoresis, the radioactive area of the paper 2–5 cm anionic to the origin contained only ^{14}C and no detectable ^{32}P. In another experiment, the peak obtained on gel filtration containing the low molecular weight compounds was passed through a column of activated charcoal (Darco G-60) prior to chromatography on Dowex 50 (H^-) and paper electrophoresis. In this experiment also, the product was found to contain ^{14}C and no ^{32}P.

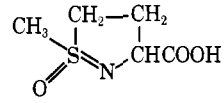
The findings show that the product exhibits the properties of an anion and that it is converted to methionine sulfone on treatment with acid or alkali. The formation of the product (which does not contain phosphate) requires enzyme, ATP, and Mg^{2+}, and is associated with equimolar cleavage of ATP to ADP and P_i. The conditions required for the formation of the product are thus identical with those required for the catalytic formation of pyrrolidonecarboxylate from glutamate by the enzyme. Furthermore, the properties of the product as stated above are analogous to those of pyrrolidonecarboxylate. It thus appears probable that the product formed from methionine sulfone is a cyclic compound analogous in structure to pyrrolidonecarboxylate. In an attempt to establish this definitely we tried in a number of ways to prepare such a cyclic compound from methionine sulfone by chemical methods, so that comparative studies could be carried out. Reactions were carried out in which [^{14}C]methionine sulfone was treated under a variety of conditions similar to those which are effective in the cyclization of glutamate (Orlowski and Meister, 1971a,b). In none of these did we detect formation of a compound with the paper electrophoretic properties of the enzymatically formed product. Under the conditions employed, conversion of 1% of the methionine sulfone to the product could have been detected. The following approaches

[2] The preparation of γ-^{32}P used in these studies was found to contain trace amounts of label in the α and β phosphoryl groups.

were employed: methionine sulfone was heated at 180° in a sealed tube under nitrogen for 2–20 hr. The sulfone was also heated at 110° in a sealed tube with 2 N HCl, 1 N NaOH, sodium acetate buffers (pH 4–9), and potassium phosphate buffers (pH 4.5–9) for 7–10 days. A number of phosphorylation procedures were tried since we postulated that methionine sulfone phosphate would cyclize in a manner analogous to the cyclization of γ-glutamyl phosphate. Methionine sulfone methyl ester was treated with dicyclohexylcarbodiimide and cyanoethyl phosphate (or KH_2PO_4) as described by Tener (1961). Under these conditions, good yields of methionine sulfoximine phosphate have been obtained from methionine sulfoximine (Rowe *et al.*, 1969). Phosphorylation was also unsuccessfully attempted with phosphorus oxychloride (Neuhaus and Korkes, 1958), phosphoric acid–phosphorus pentoxide (Peterson *et al.*, 1953) and diphenyl phosphochloridate (Lardy and Fischer, 1952). Methionine sulfone (unlabeled) was also treated with polyphosphoric acid (Cherbuliez and Rabinowitch, 1956a,b) and with crystalline phosphoric acid (Hanna and Mendicino, 1970) at 100–105° with stirring for 3 days; although the starting material disappeared, a product with the properties of the enzymatically formed product was not obtained, nor was one with the expected properties of methionine sulfone phosphate achieved. We also attempted without success to prepare a cyclic compound from D-[^{14}C]methionine sulfone by the action of D-glutamic acid cyclase, an enzyme that converts D-glutamate to D-pyrrolidonecarboxylate (Meister *et al.*, 1963). Phosphorylation of methionine sulfoxide was unsuccessfully attempted using dicyclohexylcarbodiimide and cyanoethyl phosphate (or KH_2OP_4) (Tener, 1961), and the phosphoric acid–phosphorus pentoxide method (Peterson *et al.*, 1953).

Discussion

The data indicate that both L-methionine sulfone and D-methionine sulfone are effective inhibitors of glutamine synthetase. Computer studies of the active site of glutamine synthetase indicate that L- and D-methionine sulfone act as analogs of the transiently formed tetrahedral intermediate produced by reaction of ammonia with enzyme bound γ-glutamyl phosphate (Gass and Meister, 1970). L-Methionine-*S*-sulfoximine inhibits the enzyme in an essentially similar manner, but inhibition by this compound is not reversible because the sulfoximine nitrogen atom is phosphorylated on the enzyme to yield L-methionine-*S*-sulfoximine phosphate, which binds tightly to the enzyme. The isomers of methionine sulfone can bind to the enzyme in virtually the same manner as L-methionine-*S*-sulfoximine, but evidently the phosphorylated sulfone is unstable and does not remain tightly bound to the enzyme. That methionine sulfone interacts with ATP on the enzyme is indicated by the findings that methionine sulfone is converted to a product whose formation requires both ATP and magnesium ions, and that there is stoichiometry between formation of product and formation of ADP and inorganic phosphate. While the structure of this product has not yet been definitely established, the data indicate that it is not a phosphorylated derivative of methionine sulfone analogous to methionine sulfoximine phosphate. It is converted on treatment with dilute acid or alkali to methionine sulfone. The product does not bind to Dowex 50 (H^+) and it behaves as an anion on paper electrophoresis. The findings, which suggest that the compound does not have a free α-amino group, are consistent with a structure analogous to pyrrolidonecarboxylate. We therefore tentatively assign the following structure to the product formed from methionine sulfone by glutamine synthetase



Proof of structure must await synthesis by nonenzymatic methods.[3] Our inability to prepare a phosphorylated derivative of methionine sulfone is consistent with the expected electronegativity of the sulfone oxygen atoms. If such a derivative is formed on the enzyme (and then cyclizes rapidly), it would seem that the binding of methionine sulfone to the enzyme either (a) alters the electronegativity of one of the sulfone oxygen atoms so as to facilitate its phosphorylation, or (b) that such binding is associated with an orientation of the sulfone on the enzyme in which phosphorylation by ATP is favored. The latter possibility would be in accord with the computer studies of the interaction of methionine sulfone with the enzyme (Gass and Meister, 1970). The findings suggest that methionine sulfone phosphate is less stable than methionine sulfoximine phosphate; this is in accord with our previous conclusion that the phosphate moiety of methionine sulfoximine phosphate is attached to the sulfoximine nitrogen atom rather than to its oxygen atom (Rowe *et al.*, 1969).

In the course of further studies on this problem, especially on the synthesis of methionine sulfimine by treatment of methionine with sodium azide in the presence of sulfuric acid (Riemschneider and Kluge, 1953), and on the previously reported (Rowe *et al.*, 1969) reduction of methionine sulfoximine phosphate and methionine sulfoximine to the corresponding sulfimines, we have found that the methionine compounds formed are actually sulfate salts of either methionine sulfoximine or of methionine sulfoximine phosphate rather than the corresponding sulfimines as we previously concluded. The compounds formed by treatment of methionine sulfoximine and methionine sulfoximine phosphate with dithionite (or under some conditions with Na_2SO_4) appear to be stable sulfate salts which are readily crystallizable and which chromatograph with characteristic partition coefficients in several paper chromatographic systems. These salts can, however, be dissociated by ion-exchange chromatography to yield the original sulfoximines. Evidence presented by Furukawa *et al.* (1972) and by Appel and Büchner (1962) as well as our own evidence indicates that alkyl sulfimine derivatives are relatively unstable compounds which can be readily oxidized to sulfoximines.

We have therefore reexamined the properties of methionine sulfoximine phosphate, especially data on the infrared spectrum of this and related compounds. Study of the infrared spectra of several derivatives of methionine and dimethyl sulfide indicates that the characteristic absorption bands of the sulfoximine moiety are the N–H stretching frequency at 3200–3350 cm^{-1} and the bands at 1190–1230 and 1010–

[3] A similar cyclic compound ("dehydromethionine") was proposed as an intermediate in the reaction of methionine with I_2, which has been used for the quantitative iodometric determination of methionine (Lavine, 1943; Bakay and Toennies, 1951). A method of preparation and isolation of this compound was briefly described by Lavine (1945). We carried out the reaction described by Lavine with [*methyl*-^{14}C]-methionine; paper chromatography and paper electrophoresis of the reaction products revealed that all of the radioactivity moved with methionine sulfoxide and methionine. The crystalline material obtained from the reaction mixture was about 95% methionine sulfoxide and the remainder was methionine.

1030 cm^{-1} which are attributed to the S=O and S=N stretching vibrations, respectively (Short and Thompson, 1950); Schmidbaur and Kammel, 1969). In the infrared spectrum of methionine sulfoximine phosphate the 3200-cm^{-1} band (N–H stretching) is absent. The S=N band at 1020 cm^{-1} is shifted to 1050 cm^{-1} and the S=O band at 1200 cm^{-1} is shifted to 1220 cm^{-1}, but both bands are within the expected range for double bonds, thus excluding structures such as $^{2-}HO_3POS{\equiv}N(-CH_3(-CH_2CH_2CHNH_2COOH)$. Corbridge and Lowe (1954) described a characteristic N–P stretching frequency in amide phosphates at 691–697 cm^{-1}; a similar band at 719 cm^{-1} is found with creatine phosphate and we observed a band at 736 cm^{-1} with a preparation of dimethyl sulfoximine phosphate (which contained some dimethyl sulfoximine polyphosphate). This region of the spectrum of methionine sulfoximine phosphate contains medium strength bands at 665 and 732 cm^{-1}; since the former band is also present in methionine sulfoximine, the band at 732 cm^{-1} may be attributed to N–P absorption. These infrared characteristics are therefore in accord with the N-phosphorylated structure of methionine sulfoximine phosphate proposed previously (Rowe *et al.*, 1969).

The present studies, which support the conclusion that both methionine sulfone and methionine sulfoximine inhibit glutamine synthetase by serving as analogs of the proposed enzyme-bound tetrahedral intermediate, suggest the possibility that sulfone and sulfoximine derivatives may also be useful as inhibitors of other enzymatic reactions, especially those in which carboxyl or carbonyl groups undergo transformations involving tetrahedral intermediates of this type. Direct extension of the present and previous work on the inhibition of glutamine synthetase by methionine sulfoximine and methionine sulfone has recently been possible in studies on γ-glutamylcysteine synthetase, an enzyme which is also inhibited by these methionine derivatives (Orlowski and Meister, 1971a,b; Richman *et al.*, manuscript in preparation).

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