L-Methionine SR-Sulfoximine-resistant Glutamine Synthetase from Mutants of Salmonella typhimurium*

(Received for publication, September 11, 1980, and in revised form, February 4, 1981)

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Two mutants of Salmonella typhimurium resistant to growth inhibition by the glutamine synthetase transition state analog, L-methionine SR-sulfoximine, were isolated and characterized. These mutants are glutamine bradytrophs and cannot use growth rate-limiting nitrogen sources. Although this phenotype resembles that of mutants with lesions in the regulatory gene for glutamine synthetase, glnG, these mutations do not lie in the glnG gene. Purification and characterization of the glutamine synthetase from one of the mutants and a control strain demonstrated that the mutant enzyme is defective in the reverse γ-glutamyltransferase activity but has biosynthetic activity that is resistant to inhibition by L-methionine SR-sulfoximine. The mutant enzyme also has a 4.4-fold higher apparent $K_{\text{m}}$ for glutamate (0.2 mM versus 2.1 mM, respectively) and a 13.8-fold higher $K_{\text{m}}$ for NH$_3$ (6.4 mM versus 0.48 mM) than the enzyme from the control. These data show that the glutamine synthetase protein has been altered by this mutation, designated as glnA982, and suggest that the L-methionine SR-sulfoximine resistance is conferred by a change in the NH$_3$ binding domain of the enzyme.

In Escherichia coli and Salmonella typhimurium, glutamine synthetase activity is modulated by adenylation-deadenylylation modification, feedback inhibition, and by the presence of divalent metal cations (for reviews, see Refs. 1-4). In analyzing the structure and catalytic mechanism of glutamine synthetase, the effects of the proposed transition state analog L-methionine S-sulfoximine have been useful (1, 3). L-Methionine S-sulfoximine inhibits glutamine synthetase in an apparent two-step sequence which initially involves a reversible binding at the active site that is competitive with glutamate (3, 5-9). Subsequently, in the presence of ATP and divalent metals, phosphorylation occurs, causing an irreversible inhibition with L-methionine S-sulfoximine and ADP remaining tightly bound to the enzyme (3, 5-8, 10). Studies with the analog strongly support the existence of γ-glutamylphosphate as a catalytic transition state or intermediate (3). More recently, enzyme-bound L-methionine S-sulfoximine has been shown to affect spectral perturbations at the two metal ion binding sites (11-13) and at the covalent modification site for adenylylation (14, 15) on each subunit.

The quantity of information available on the mechanism of L-methionine S-sulfoximine inhibition prompted studies on the physiological response of enteric bacteria to this analog (16-19). Other S. typhimurium mutants resistant to growth inhibition by L-methionine SR-sulfoximine have reduced glutamate synthase activities and confer resistance by a change independent of an alteration in glutamine synthetase (18, 19). Although no mutants have been identified where the glutamine synthetase is affected, it seemed that the combination of the information on L-methionine S-sulfoximine with an analysis of a resistant enzyme would provide insight into the catalytic mechanism and regulation of this complex protein.

In the present study, the characterization of mutants of S. typhimurium with glutamine synthetase activities resistant to MSO$^2$ is described. In addition to analog resistance, the altered enzyme from one of the mutants is defective in the reverse γ-glutamyltransferase activity and has apparent $K_{\text{m}}$ values for the substrates NH$_3$ and glutamate that are severalfold higher than those of the native enzyme.

**EXPERIMENTAL PROCEDURES**

**Chemicals and Media**—MSO was obtained from the Sigma Chemical Co., St. Louis, MO. All reagents are commercially available. The Luria broth and glucose ammonia medium (which contains 17 mM (NH$_4$)$_2$SO$_4$) were as previously described (17). Alternative nitrogen sources were added to 0.4% glucose minimal salts media at a final concentration of 35 mM. Amino acids and MSO (except where noted) were all added at 0.5 mM. L-Glutamine was supplemented at 2.5 mM. Tetracycline resistance was scored on LB agar plates containing 50 μg of the antibiotic/ml.

**Bacterial Strains and Phage**—All strains used are derivatives of S. typhimurium LT-2 and are listed in Table I. Transductions for strain construction were performed as described (19, 20) using the phage KBI (int-1) (21).

**Isolation of MSO-Resistant Strains**—Approximately 5 × 10$^8$ cells of strain JL610 were plated onto glucose ammonia agar supplemented with L-cysteine, L-histidine, and 1 mM MSO. After 48-72 h at 37 °C, isolated colonies were scored for their inability to grow on minimal media containing either L-arginine, L-proline, or L-glutamate as sole sources of nitrogen. Two independent, resistant strains, JB1083 and JB1084, were isolated, purified under nonselective conditions, and further characterized.

**Ouchterlony Immunodiffusion**—Plates 5 cm in diameter were prepared containing 0.8% agarose in 0.1 M Na-phosphate buffer, pH 7.5, and 1 mM Na-azide. To wells 1.5 cm apart was added 20 μl of extract or antibody prepared against purified glutamine synthetase. Plates were incubated at 30 °C for 36 h.

**Enzyme Assays**—Glutamate dehydrogenase and glutamate synthase activities were measured by following the oxidation of NADPH as described (10). Glutamine synthetase activity was measured either by the reverse γ-glutamyltransferase reaction, the biosynthetic γ-glutamyl hydroxamate-forming activity, or by the biosynthetic phosphate release assay. The reverse γ-glutamyltransferase activity was measured...
**Table I**

**List of S. typhimurium LT-2 strains used in this study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Pertinent genotype</th>
<th>Source/construction</th>
</tr>
</thead>
<tbody>
<tr>
<td>JLB60</td>
<td>cryA1348, hisC527</td>
<td>J. L. Ingraham</td>
</tr>
<tr>
<td>JLB60</td>
<td>glt+</td>
<td>Ref. 20</td>
</tr>
<tr>
<td>TT395</td>
<td>gltN989::Tn10</td>
<td>J. L. Ingraham</td>
</tr>
<tr>
<td>JLB67</td>
<td>glt-1010</td>
<td>Ref. 20</td>
</tr>
<tr>
<td>JB1093</td>
<td>cryA1348, hisC527,</td>
<td>Spontaneous MSO-resistant derivative of JLB60</td>
</tr>
<tr>
<td></td>
<td>gltA982</td>
<td></td>
</tr>
<tr>
<td>JB1094</td>
<td>cryA1348, hisC527,</td>
<td>Spontaneous MSO-resistant derivative of JLB60</td>
</tr>
<tr>
<td></td>
<td>gltA983</td>
<td></td>
</tr>
<tr>
<td>JB1107</td>
<td>glt+</td>
<td>Transductant of JLB667 with KB1 phage from JLB60</td>
</tr>
<tr>
<td>JB1108</td>
<td>gltA982</td>
<td>Transductant of JLB667 with KB1 phage from JLB107</td>
</tr>
<tr>
<td>JB1110</td>
<td>gltA983</td>
<td>Transductant of JLB667 with KB1 phage from JLB110</td>
</tr>
</tbody>
</table>

Assayed by the procedure of Stadtman et al. (22), with the modification that the buffer was 50 mM TES, pH 7.55, at 25 °C. The biosynthetic γ-glutamyl hydroxamate-forming activity was measured by the procedure of Bender et al. (23) with the modifications that the buffer was 50 mM TES, pH 7.55, at 25 °C and water was substituted for hexadecyltrimethylammonium bromide since extracts or purified enzyme was used in place of whole cells. The phosphate release activity (24) was measured in 50 mM TES buffer, pH 7.0, at 25 °C using an amount of enzyme producing 0.25 μmol or less total phosphate in 15 min. Na2HPO4 was used to prepare a standard curve to determine the P concentration. One unit of specific activity is the formation of 1 μmol of γ-glutamyl hydroxamate or 1 μmol of P/mn/mg of protein.

**Enzyme activities**—For inhibition and kinetic analyses, glutamine synthetase was purified by Zn2+ precipitation (27) and examined by SDS-polyacrylamide disc gel electrophoresis (28).

Sensitivity of purified glutamine synthetase to MSO was examined using the biosynthetic γ-glutamyl hydroxamate-forming activity. Enzyme was incubated for 5 min at 37 °C in the presence of 0.3 mM MnCl2, and the biosynthetic assay was started by the addition of 100 μM Na-glutamate and stopped by the addition of 100 μM Na2HP04. The reaction was incubated for 5 min at 37 °C in the presence of 0.3 mM MnCl2, and the biosynthetic assay was started by the addition of 100 μM Na-glutamate and stopped by the addition of 100 μM Na2HP04.

**Inhibition and Kinetic Analyses**—For inhibition and kinetic analyses, glutamine synthetase was purified by Zn2+ precipitation (27) and examined by SDS-polyacrylamide disc gel electrophoresis (28).

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**RESULTS**

**Isolation of Strains Resistant to Growth Inhibition by MSO**—Cells from 24 separate colonies of strain JLB610 were plated on a medium containing MSO to select for independent and nonmutagenized mutants. Since strains that are unable to use limiting nitrogen sources were desired, MSO-resistant colonies were scored for their inability to grow with L-arginine, L-proline, or L-glutamate as the only source of ammonia. Three of 173 MSO-resistant colonies had the altered phenotype, of which two strains, JB1093 and JB1094, had only 13% of the glutamine synthetase γ-glutamyltransferase activity of the control. Because these MSO-resistant strains differed from previously described mutants that have lost glutamine synthetase activity (18, 19), strains JB1093 and JB1094 were purified and characterized further.

**Genetic Analysis of Mutations in MSO-resistant Strains**

Portions of this paper (including Figs. 2, 3, 5, and 6) are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 80M-1958, cite authors, and include a check or money order for $5.00 per set of photocopies.

**TABLE II**

**Activities of nitrogen metabolism enzymes in wild type and L-methionine SR sulfoximine-resistant mutants**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Glutamate dehydrogenase</th>
<th>Glutamate synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td>JB1107 (glnA +)</td>
<td>0.95</td>
<td>0.366</td>
</tr>
<tr>
<td>JB1108 (glnA982)</td>
<td>0.77</td>
<td>0.414</td>
</tr>
<tr>
<td>JB1110 (glnA983)</td>
<td>0.77</td>
<td>0.411</td>
</tr>
</tbody>
</table>

* Cultures were grown in 0.4% glucose with 17 mM (NH4)2SO4 as the nitrogen source and harvested at 100 ± 5 Klett units. Crude extracts were prepared and enzymes assayed as described under "Experimental Procedures." Specific activity is micromoles per min per mg of protein.

* Growth rate constant, in generations/h.

The transferase assay is the reverse γ-glutamyl hydroxamate reaction in the presence of 0.3 mM MnCl2, and the biosynthetic assay is the biosynthetic γ-glutamyl hydroxamate-forming reaction in the presence of 70 mM MgCl2.

Mutants unable to grow with L-arginine or L-glutamate as nitrogen sources have been identified as having mutations in the glnA-linked gene glnG (29, 30). The glnG mutations have the property of suppressing glutamine auxotrophy caused by mutations in the genetically unlinked glnF gene. To determine whether the mutations in strain JB1093 and JB1094 lie in the glnG gene, KB1 phage was used to transduce the glnF986::Tn10 mutation into these strains selecting for the tetracycline resistance encoded by the Tn10 transposon. These derivatives became glutamine auxotrophs, showing that the mutations conferring MSO resistance in JB1093 and JB1094 do not suppress glnF986::Tn10 and are not in the glnG gene. These data, together with the enzymatic alterations shown below, locates the mutations in the glnA gene, and they are designated glnA982 and glnA983.

For further physiological and biochemical characterization, transductants of JB667 inheriting the glnA982 and glnA983 mutations (designated as JB1108 and JB1110) were used. An isogenic Gln⁺ transductant, JB1107, was used as a control strain.

**Growth Properties and Levels of Ammonia Assimilatory Enzymes in MSO-resistant Strains**—Growth of MSO-resistant strains was examined on a number of nitrogen compounds, including L-glutamate, L-glutamine, L-asparagine, L-serine, L-alanine, cytidine, N-acetylglucosamine, and 1 mM (NH4)2SO4. In addition to 17 mM (NH4)2SO4, only L-glutamine is capable of supporting growth of these strains. Even with excess ammonia, the mutants grow slower than the control (0.77 versus 0.95 generation/h) (Table II). This slow growth is reversed by the addition of 2.5 mM glutamine to the medium. Thus, strains JB1108 and JB1110 are glutamine bradytrophs and are unable to utilize growth rate-limiting nitrogen sources. The resistance of the mutants to MSO was demonstrated by the addition of 0.5 mM MSO to a growing culture (Fig. 1). In the wild type culture, growth ceases, whereas the analog has little effect on the growth of the mutant strains.

**RESULTS**

Isolation of Strains Resistant to Growth Inhibition by MSO—Cells from 24 separate colonies of strain JLB610 were plated on a medium containing MSO to select for independent and nonmutagenized mutants. Since strains that are unable to use limiting nitrogen sources were desired, MSO-resistant colonies were scored for their inability to grow with L-arginine, L-proline, or L-glutamate as the only source of ammonia. Three of 173 MSO-resistant colonies had the altered phenotype, of which two strains, JB1093 and JB1094, had only 13% of the glutamine synthetase γ-glutamyltransferase activity of the control. Because these MSO-resistant strains differed from previously described mutants that have lost glutamine synthetase activity (18, 19), strains JB1093 and JB1094 were purified and characterized further.

**Genetic Analysis of Mutations in MSO-resistant Strains**—
Methionine Sulfoximine-resistant Glutamine Synthetase

To confirm that the MSO resistance of JB1108 and JB1110 did not affect the glutamate synthetase activity, as had been found for other MSO-resistant mutants (18, 19), the primary ammonia assimilatory enzymes were assayed. Only slight differences were observed in the activities of glutamate dehydrogenase and glutamate synthase (Table II). However, a marked reduction of glutamine synthetase activity, as measured by the reverse γ-glutamyltransferase reaction, was seen in the MSO-resistant strains. These values, 13% of normal, are extremely low considering the only slightly reduced growth rate of the mutants in glucose ammonia medium. This suggested that although the reverse γ-glutamyltransferase activity is low, significant biosynthetic activity could be present in the growing cells. Therefore, the biosynthetic γ-glutamyl hydroxamate-forming activity of glutamine synthetase was measured with glutamate, ATP, and hydroxylamine as substrates. This assay is less sensitive than the reverse γ-glutamyltransferase, but the results show that the mutants have biosynthetic glutamine synthetase activity comparable to the control (Table II).

Antibody Reaction with Glutamine Synthetase—The amount of glutamine synthetase protein in these strains was further examined immunologically. Immunoprecipitation and immunodiffusion were used to determine the amount and antigenic nature of the glutamine synthetase produced in the MSO-resistant strains JB1108 and JB1110. Extracts of glucose ammonia-grown cells were prepared and incubated with various amounts of specific antiserum. Protein assays of the precipitate indicated that the same amount of cross-reacting material is produced in JB1108 and JB1110 as in JB1107 (data not shown). In addition, immunodiffusion plates showed no differences in antigenic properties (since no spurs were observed) and precipitin lines of comparable intensity (Fig. 2). These data support the conclusion that strains JB1108 and JB1110 have biosynthetically active glutamine synthetase proteins present in amounts comparable to the control but that the reverse γ-glutamyltransferase activities are considerably reduced for the mutants.

Enzymatic Properties of Purified Glutamine Synthetases—Initial experiments with extracts from strains JB1107, JB1108, and JB1110 showed that the glutamine synthetase activities from both the mutants were more resistant to MSO than the control enzyme (data not shown). This resistance was demonstrated with both γ-glutamyltransferase and the biosynthetic γ-glutamyl hydroxamate-forming assays and is thus independent of whether Mn²⁺ or Mg²⁺ is present or whether glutamine or glutamate is the substrate. To demonstrate that this increased resistance was not due to another component in the extracts and to examine other properties of the altered protein, the glutamine synthetase from one mutant was purified. The glutamine synthetase was purified by Zn²⁺ precipitation (27) from glucose ammonia-grown cells of a glnA' strain, JL907, and the MSO-resistant strain JB1108. An apparent equivalent amount of glutamine synthetase protein was purified from 65 g of wet weight cells of each strain as determined by total protein and SDS disc gel electrophoresis. No differences in the mobilities of the glutamine synthetase subunits could be detected on 12% SDS-polyacrylamide gels with the Zn²⁺-precipitated preparations or with enzyme purified to apparent homogeneity by affinity chromatography on Affi-Gel blue (Fig. 3).

Inhibition of the biosynthetic γ-glutamyl hydroxamate-forming activity of glutamine synthetase by MSO was examined using the enzyme prepared by Zn²⁺ precipitation (27) from glucose ammonia-grown cells of a glnA' strain, JL907, and the MSO-resistant strain JB1108. An apparent equivalent amount of glutamine synthetase protein was purified from 65 g of wet weight cells of each strain as determined by total protein and SDS disc gel electrophoresis. No differences in the mobilities of the glutamine synthetase subunits could be detected on 12% SDS-polyacrylamide gels with the Zn²⁺-precipitated preparations or with enzyme purified to apparent homogeneity by affinity chromatography on Affi-Gel blue (Fig. 3).

Inhibition of the biosynthetic γ-glutamyl hydroxamate-forming activity of glutamine synthetase by MSO was examined using the enzyme prepared by Zn²⁺ precipitation. Incubations were carried out with glutamine synthetase in the presence of inhibitor, ATP, NH₂OH, and Mg²⁺ such that phosphorylation and irreversible inhibition (3, 5, 8, 10) could occur. Fig. 4 shows the rapid inactivation of wild type enzyme at low inhibitor concentrations (IC₅₀ = 3.8 µM), whereas the

Fig. 1. Effect of MSO on the growth of the wild type and MSO-resistant mutants. The analog was added at 0.5 mM at times indicated by arrows to cells growing in glucose ammonia medium. ●, strain JB1107 (gln'); ○, JB1108 (glnA982); □, JB1110 (glnA983).

Fig. 2. Immunodiffusion pattern of extracts from wild type and MSO resistant strains. The outer well contained 20 µl of anti-glutamine synthetase rabbit antibody, and the outer wells contained 20 µl of total proteins of crude cell extract from JB1107 (1 and 3), JB1108 (2 and 5), and JB1110 (3 and 4).

Fig. 3. Polyacrylamide gel of glutamine synthetase purified by Zn²⁺ precipitation and affinity blue (Bio-Rad Laboratories, Richmond, CA) affinity chromatography. Lane A, glutamine synthetase purified by Zn²⁺ precipitation and Affi-Gel Blue chromatography. Lane B, glutamine synthetase purified by Zn²⁺ precipitation and Affi-Gel Blue chromatography. Lane C, glutamine synthetase from JB1108 purified by Zn²⁺ precipitation and Affi-Gel Blue chromatography. Lane D, glutamine synthetase from JB1108 after Zn²⁺ precipitation only. Lane E, glutamine synthetase from JB1108 after Zn²⁺ precipitation only.
Methionine Sulfoximine-resistant Glutamine Synthetase

Fig. 4. Sensitivity of purified glutamine synthetase from strains JB1107 and JB1108 to inactivation by MSO. Glutamine synthetase purified by Zn⁺⁺ precipitation from JB1107 (□) or JB1108 (○) was incubated for 5 min in the presence of varying concentrations of the inhibitor as described under "Experimental Procedures."
Methionine Sulfoximine-resistant Glutamine Synthetase

TABLE III

<table>
<thead>
<tr>
<th>Property</th>
<th>Source of glutamine synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type (glnA)</td>
</tr>
<tr>
<td>Subunit molecular weight (d)³</td>
<td>55,000</td>
</tr>
<tr>
<td>Enzymatic reaction⁴</td>
<td>Reverse γ-glutamyltransferase</td>
</tr>
<tr>
<td></td>
<td>Biosynthetic γ-glutamyl hydroxamate activity</td>
</tr>
<tr>
<td>Biosynthetic (P, release)</td>
<td>Mg²⁺ activation</td>
</tr>
<tr>
<td></td>
<td>Mn²⁺ activation</td>
</tr>
<tr>
<td>Apparent Kₐ values (mM)</td>
<td>Glutamate</td>
</tr>
<tr>
<td></td>
<td>Ammonia</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
</tr>
<tr>
<td>IC₅₀ L-Methionine SR-sulfoximine</td>
<td>3.8 ± Resistant</td>
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</table>

<table>
<thead>
<tr>
<th>Property</th>
<th>Source of glutamine synthetase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type (glnA)</td>
</tr>
<tr>
<td>Molecular weight was determined by SDS-12% polyacrylamide gel electrophoresis of Zn²⁺-precipitated enzyme.</td>
<td></td>
</tr>
<tr>
<td>Units are micromoles per min per mg of protein. The various activities of glutamine synthetase require the presence of divalent metals and here included 0.3 mM Mn⁺⁺ for the reverse γ-glutamyltransferase, 70 mM Mg⁺⁺ for the bio synthetic γ-glutamyl hydroxamate-forming activity, and either 100 mM Mg⁺⁺ or 5 mM Mn⁺⁺ for the bio synthetic P; release assay.</td>
<td></td>
</tr>
</tbody>
</table>

Both growth (Fig. 1) and γ-glutamyl hydroxamate-forming activity (Fig. 4) also observed with the mutant strain and the glnA982 enzyme.

A mutation that causes an enzyme to be resistant to a proposed transition state analog might also alter the binding properties for the substrates. Therefore, the biosynthetic phosphate release assay was used to determine the apparent Kₐ values for glutamate, NH₃, and ATP for glutamine synthetase from these strains. Glutamine synthetase preparations, purified by Zn²⁺ precipitation, were incubated in the presence of various concentrations of a single substrate with all other substrates in excess. Least squares regression lines were derived from double reciprocal plots of the varying substrate concentration and average reaction velocities.

A significant increase in the apparent Kₐ for NH₃ by the MSO-resistant glutamine synthetase was observed relative to the native enzyme. The observed apparent Kₐ for NH₃ by the mutant (6.4 mM) was 13.8-fold higher than the 0.46 mM found for the wild type glutamine synthetase (Fig. 6 and Table III). Additionally, there was a 4.4-fold increase in the Kₛ for glutamate, but no significant difference in the Kₛ for ATP by the MSO-resistant enzyme. These altered kinetic parameters, particularly for NH₃, indicate that the acquired MSO resistance of the glutamine synthetase from JB1108 results in altered substrate interactions with the enzyme active site.

The pleiotropic alterations in the catalytic properties of MSO-resistant glutamine synthetase are summarized in Table III. The ratio of Mg²⁺ to Mn²⁺-stimulated biosynthetic activities of wild type and mutant enzymes was identical, indicating the same divalent cation response and states of adenylation for both preparations. In contrast, the enzyme was essentially unable to react with glutamine in the reverse γ-glutamyltransferase reaction in the presence of either Mn⁺⁺ or Mg⁺⁺ and had significant alterations in substrate kinetic parameters, particularly the Kₛ for NH₃. These data thus identify a unique glutamine synthetase active site mutation that alters the catalytic properties of the enzyme and the growth properties of the cell.

Two independent, spontaneously occurring mutant strains of S. typhimurium were isolated that are resistant to growth inhibition by L-methionine SR-sulfoximine and are unable to grow with organic nitrogen compounds or low concentrations of ammonia as sole nitrogen sources. Interestingly, these growth properties, excluding the MSO resistance and the inability to use limiting ammonia, are the same as those found for Reg⁻ strains (29, 30) with mutations in the glnG gene. However, the mutations described here, unlike the glnG lesions, do not suppress the glutamine auxotrophy caused by glnF mutations and result in the production of normal amounts of glutamine synthetase proteins that have altered catalytic properties (Table III). Therefore, despite certain phenotypic similarities, these mutants have mutations that are distinct from those in the glnG gene. These results demonstrate that the combination of close genetic linkage with glnA and the Reg⁻ phenotype are not sufficient criteria for designating a mutation as being in the glnG gene since mutations within the glnA gene can produce similar properties.

The inability of the MSO-resistant strains to grow with limiting nitrogen sources may be explained if the higher apparent Kₛ values for NH₃ prevent the mutant enzymes from functioning when the NH₃ concentration is low.

Earlier studies (1, 31) suggest that the sulfoximine moiety of the analog interacts with the active site such that the S-methyl group occupies the ammonia binding site. Two predominant alterations in the glutamine synthetase from strain JB1108 are (i) an inability to catalyze the glutamine-dependent reverse γ-glutamyltransferase reaction and (ii) a significant increase in the apparent Kₛ for NH₃ and, to a lesser extent, the Kₛ for glutamate. Thus, if resistance to MSO inactivation were due to altered interactions of the S-methyl group with the ammonia binding domain, then altered interaction with the amide of glutamine and NH₃ itself would be predicted. This is in fact observed (Table III). Irrespective of the specific mode of MSO resistance, the catalytic changes caused by the glnA982 mutation are accommodated by an altered NH₃ binding domain.

The biosynthetic γ-glutamyl hydroxamate-forming activity of glutamine synthetase from the strain with the glnA982 lesion is clearly insensitive to irreversible inactivation when incubated in the presence of MSO, ATP, and Mg⁺⁺ (Fig. 4). It is not known whether this resistance to inactivation is due to reduced inhibitor binding or reduced phosphorylation.

It is clear (Fig. 5) that formation of the glutamine synthetase-MSO complex is significantly lower with the mutant enzyme and that complex formation is detected only at higher ATP concentrations. The detection of less MSO-32p₀ complexes for the mutant enzyme is consistent with either a reduction in MSO binding, a reduction in phosphorylation, or possibly with the release of the MSO-32p₀ from the mutant enzyme. These results establish another difference for the mutant enzyme and are consistent with the alterations observed in catalytic activity.

Since the study of glutamine synthetase has frequently involved the use of MSO, valuable use could be made of the mutant enzyme presently described. The inhibitor-resistant enzyme should be useful in probing the catalytic mechanism of the enzyme, particularly in studies relating MSO inhibition with biophysical parameters (11–15), isotope exchange reactions (32), and the results obtained through the use of other proposed transition state analogs (33). Because glutamine synthetase is both structurally and catalytically a complex protein, further analysis of the enzyme from the mutant strains will provide fundamental information important to understanding this enzyme reaction.
Acknowledgments—We thank Drs. K. M. Herrmann, G. B. Kohlhaw, F. C. Wedler, and H. Zalkin for helpful discussions during the preparation of the manuscript.

REFERENCES