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

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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_m for glutamate (0.2 mM *versus* 2.1 mM, respectively) and a 13.8-fold higher K_m for NH_3 (6.4 mM *versus* 0.46 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 adenylylation-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-glutamyl phosphate 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[1] 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_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 $(NH_4)_2SO_4$) 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 KB1 (*int*-1) (21).

Isolation of MSO-resistant Strains—Approximately 5×10^9 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, JB1093 and JB1094, 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

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[1] The abbreviations used are: MSO, L-methionine *SR*-sulfoximine; Tes, *N*-{tris[hydroxymethyl]methyl-2-amino}ethanesulfonic acid; SDS, sodium dodecyl sulfate.

TABLE I

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

Strain	Pertinent genotype	Source/construction
JL610	*cysA1348, hisC527*	J. L. Ingraham
JL907	*gln*$^+$	J. L. Ingraham
TT395	*glnF986*::Tn10	J. Roth
JB667	*gln*-1010	Ref. 20
JB1093	*cysA1348, hisC527, glnA982*	Spontaneous MSO-resistant derivative of JL610
JB1094	*cysA1348, hisC527, glnA983*	Spontaneous MSO-resistant derivative of JL610
JB1107	*gln*$^+$	Transductant of JB667 with KB1 phage from JL610
JB1108	*glnA982*	Transductant of JB667 with KB1 phage from JB1093
JB1110	*glnA983*	Transductant of JB667 with KB1 phage from JB1094

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 hexadexyltrimethylammonium 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. Na_2HPO_4 was used to prepare a standard curve to determine the P_i concentration. One unit of specific activity is the formation of 1 μmol of γ-glutamyl hydroxamate or 1 μmol of P_i/min/mg of protein. Protein was measured by the methods of Lowry *et al.* (25) and Kalb and Bernlohr (26).

Inhibition and Kinetic Analyses—For inhibition and kinetic analyses, glutamine synthetase was purified by Zn^{2+} 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 ATP, NH_2OH, Mg^{2+}, and various amounts of the inhibitor. The reaction was started by the addition of 100 mM Na-glutamate and terminated after 30 min. Apparent K_m determinations were made using the biosynthetic phosphate release assay in the presence of excess concentrations of substrates except for various concentrations of the substrate being tested. Double reciprocal plots from four to eight replicates of the data pairs were derived from the least squares regression line and plotted by an on-line CALCOMP 936 plotter. The apparent K_m values were obtained from the intersection of the regression line on the abscissa.

RESULTS[2]

Isolation of Strains Resistant to Growth Inhibition by MSO—Cells from 24 separate colonies of strain JL610 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 glutamate synthase activities (18, 19), strains JB1093 and JB1094 were purified and characterized further.

Genetic Analysis of Mutations in MSO-resistant Strains—KB1 phage lysates were prepared from the MSO-resistant strains JB1093 and JB1094 and used to transduce the glutamine auxotroph, strain JB667, to prototrophy. Of 387 Gln$^+$ transductants scored for each cross, 98% were also MSO-resistant and unable to grow on nitrogen sources other than $(NH_4)_2SO_4$ (17 mM) or L-glutamine (30 mM). Similar high cotransductional linkages are observed in crosses with JB1093 and JB1094 and other glutamine auxotrophs, and the results show that the mutations are closely linked to the glutamine synthetase structural gene, *glnA*.

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 strains 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, locate 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 $(NH_4)_2SO_4$. In addition to 17 mM $(NH_4)_2SO_4$, 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.

TABLE II

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

Strain	Enzyme activities[a]				
	μ[b]	Glutamate dehydrogenase	Glutamate synthase	Glutamine synthetase[c]	
				Transferase	Biosynthetic
JB1107 (*glnA*$^+$)	0.95	0.366	0.198	0.084	0.012
JB1108 (*glnA982*)	0.77	0.414	0.123	0.011	0.011
JB1110 (*glnA983*)	0.77	0.411	0.107	0.011	0.011

[a] Cultures were grown in 0.4% glucose with 17 mM $(NH_4)_2SO_4$ 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.

[b] Growth rate constant, in generations/h.

[c] The transferase assay is the reverse γ-glutamyl hydroxamate reaction in the presence of 0.3 mM $MnCl_2$, and the biosynthetic assay is the biosynthetic γ-glutamyl hydroxamate-forming reaction in the presence of 70 mM $MgCl_2$.

[2] 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-1938, cite authors, and include a check or money order for $3.60 per set of photocopies.

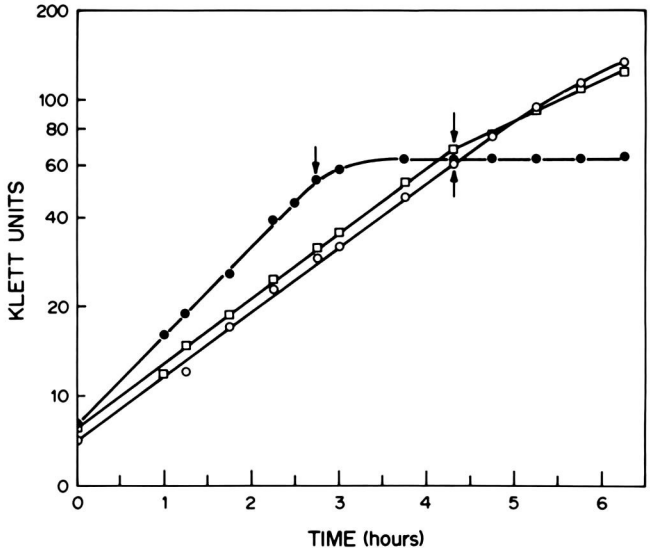

FIG. 1. **Effect of MSO on the growth of the wild type and MSO-resistant mutants.** The analog was added at 0.5 mM at times indicated by *arrows* to cells growing in glucose ammonia medium. ●, strain JB1107 (*gln*$^+$); ○, JB1108 (*glnA982*); □, JB1110 (*glnA983*).

To confirm that the MSO resistance of JB1108 and JB1110 did not affect the glutamate synthase 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 is 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

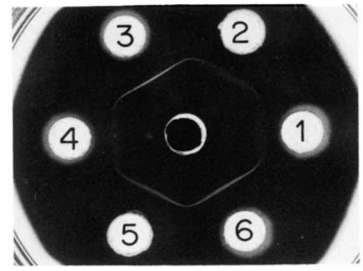

Fig. 2. Immunodiffusion pattern of extracts from wild type and MSO resistant strains. The center well contained 20 μl of anti-glutamine synthetase rabbit antibody, and the outer wells contained 20 μl (230 μg total protein) of crude cell extract from JB1107 (1 and 4), JB1108 (2 and 5), and JB1110 (3 and 6).

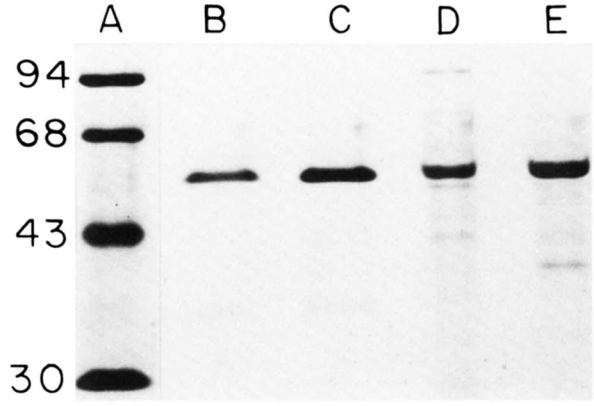

Fig. 3. SDS-polyacrylamide gel of glutamine synthetase purified by Zn^{2+} precipitation and Affi-Gel Blue (Bio-Rad Laboratories, Richmond, CA) affinity chromatography. Zinc purified enzyme preparations (0.92 mg protein from JL907 and 1.22 mg protein from JB1108) were applied to 2 ml Affi-Gel Blue columns, washed with 50 ml TES buffer (50 mM, pH 7.2, 2.5 mM $MgCl_2$), then eluted with 5 mM ATP in the same buffer. Fractions with the highest activity were pooled, lyophillized, then reconstituted in 200 μl buffer. After boiling in SDS and β-mercaptoethanol, 50 μl samples were applied to the gel. Lane A, proteins used as MW standards: phosphorylase b (94 K), bovine serum albumin (68 K), ovalbumin (43 K), and carbonic anhydrase (30 K). Lane B, glutamine synthetase from JL907 purified by Zn^{2+} precipitation and Affi-Gel Blue chromatography. Lane C, glutamine synthetase from JB1108 purified by Zn^{2+} precipitation and Affi-Gel Blue chromatography. Lane D, glutamine synthetase from JL907 after Zn^{2+} precipitation only. Lane E, glutamine synthetase from JB1108 after Zn^{2+} precipitation only.

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 Mn^{2+} or Mg^{2+} 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 Zn^{2+} 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 Zn^{2+}-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 Zn^{2+} precipitation. Incubations were carried out with glutamine synthetase in the presence of inhibitor, ATP, NH_2OH, and Mg^{2+} 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 ($IC_{0.5}$ = 3.8 μM),[3] whereas the

[3] $IC_{0.5}$ = concentration of MSO with half-maximal biosynthetic γ-glutamyl hydroxamate-forming activity of glutamine synthetase remaining.

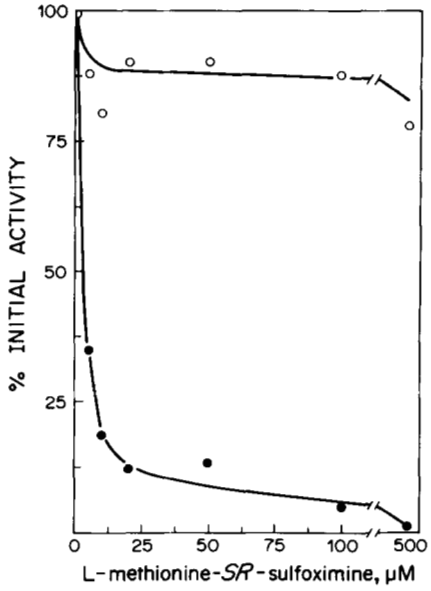

FIG. 4. **Sensitivity of purified glutamine synthetase from strains JB1107 and JB1108 to inactivation by MSO.** Glutamine synthetase purified by Zn^{2+} precipitation from JB1107 (●) or JB1108 (○) was incubated for 5 min in the presence of varying concentrations of the inhibitor as described under "Experimental Procedures."

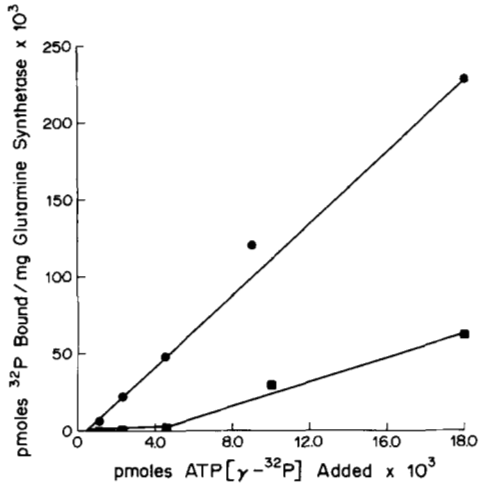

Fig. 5. Binding of glutamine synthetase-MSO-PO_4 complex to nitrocellulose filters using glutamine synthetase from wild type, JL907 (●), and mutant, JB1108 (○), strains. Enzyme (10 μg) was preincubated for 5 min at 37°C in 200 μl of 78 mM TES, pH 7.15, 47 mM $MgCl_2$ and 330 μM MSO. γ-^{32}P ATP (5 μCi/pmol) was added as indicated and the mixture incubated for an additional 30 min. Nitrocellulose filters, 0.45 μm (Schleicher and Schuell, Keene, NH), were boiled for 5 min in H_2O and washed with the preincubation solution. After incubation, 200 μl of each sample was applied to the filters under low vacuum (2 ml/min) then washed with 2 ml of the preincubation solution. Filters were dried for 30 min at 100°C then counted for 5 min in 8 ml Aquasol-2 (New England Nuclear, Boston, MA) using a Beckman LS-230 Liquid Scintillation Counter. Each sample was done in duplicate. A background of 9.5×10^{-5} pmoles γ-^{32}P ATP bound to bovine serum albumin was subtracted from each point.

glutamine synthetase from strain JB1108 retained 83% of the initial activity at concentrations 100-fold higher than the $IC_{0.5}$ of the control enzyme. To determine whether the presence of NH_2OH in the preincubation mixture could enhance the resistance of the mutant enzyme by binding the NH_3 site, these experiments were repeated by incubating the enzymes for 5 min with $MgCl_2$, ATP, and 0.5 mM MSO. The reactions were started by the addition of NH_2OH and glutamate. These experiments demonstrated that the mutant enzyme retained 81% activity, whereas the wild type enzyme retained only 5% activity, showing that the resistance is independent of NH_2OH in the preincubation mixture. Thus, the glutamine synthetase from JB1108 is altered such that MSO either no longer binds to the active site or, once bound, is not susceptible to phosphorylation.

To determine whether the formation of the glutamine synthetase-MSO-PO_4 complex occurred to the same extent with the mutant enzyme as for the wild type, Zn^{2+}-purified preparations were incubated with MSO and [γ-^{32}P]ATP and then bound to nitrocellulose filters. From the data shown in Fig. 5, the mutant enzyme retained ^{32}P in the presumed complex form at levels at least 4.5-fold lower than that observed for the wild type enzyme. Since some phosphorylation was seen to occur with the mutant enzyme at the higher ATP concentrations, these data are consistent with the slight inhibition of

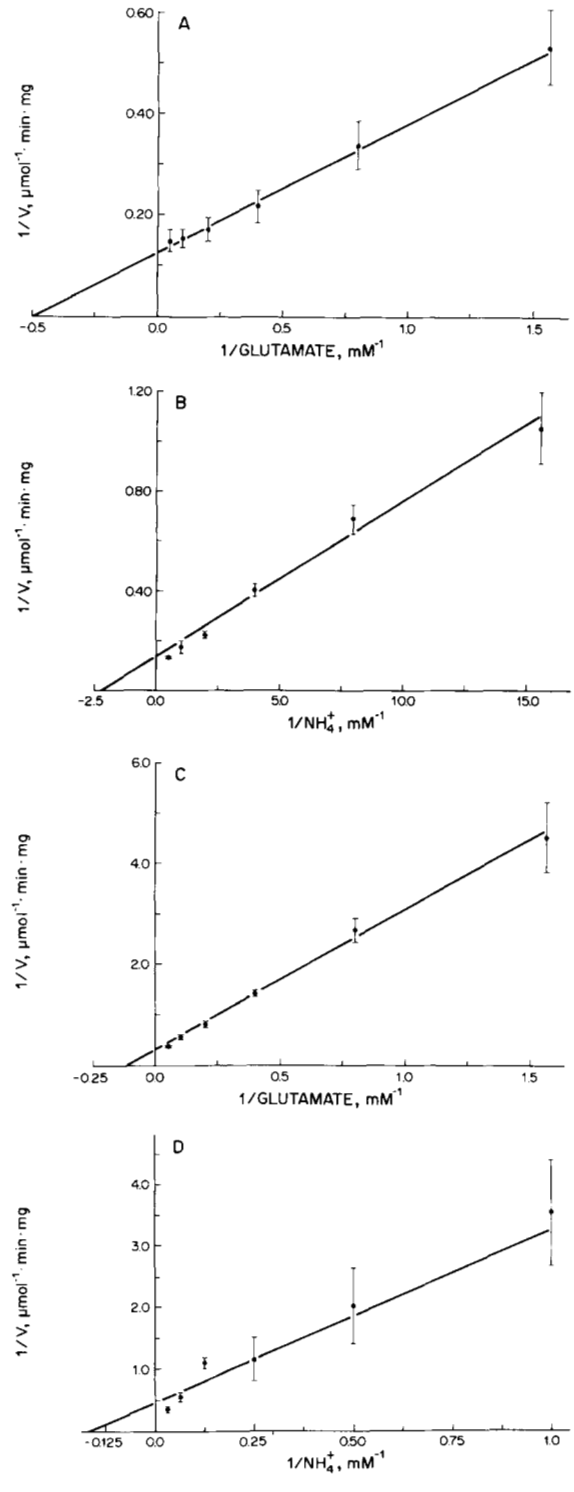

Fig. 6. Double reciprocal plots of average velocities of glutamine synthetase biosynthetic activity as a function of varying substrate concentration. The regression line, the means (○) and ± S.D. (error bars) are indicated for glutamine synthetase purified by Zn^{2+} precipitation from the wild type strain, JL907 (A and B), and the MSO-resistant strain, JB1108 (C and D). Activity was measured by the P_i release assay described in "Experimental Procedures" in the text.

TABLE III

Properties of glutamine synthetase from wild type and L-methionine SR-sulfoximine-resistant S. typhimurium strains

Property	Source of glutamine synthetase	
	Wild type (*glnA*$^+$)	MSO-resistant (*glnA982*)
Subunit molecular weight (d)[a]	~55,000	~55,000
Enzymatic reaction[b]		
Reverse γ-glutamyltransferase	7.85	0.45
Biosynthetic γ-glutamyl hydroxamate activity	1.02	0.53
Biosynthetic (P_i release)		
Mg^{2+} activation	6.33	3.16
Mn^{2+} activation	1.03	0.51
Apparent K_m values (mM)		
Glutamate	2.1	9.2
Ammonia	0.46	6.4
ATP	2.9	4.6
$IC_{0.5}$ L-Methionine *SR*-sulfoximine (μM)	3.8	Resistant

[a] Molecular weight was determined by SDS-12% polyacrylamide gel electrophoresis of Zn^{2+}-precipitated enzyme.

[b] 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^{2+} for the reverse γ-glutamyltransferase, 70 mM Mg^{2+} for the biosynthetic γ-glutamyl hydroxamate-forming activity, and either 100 mM Mg^{2+} or 5 mM Mn^{2+} for the biosynthetic P_i release assay.

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 K_m values for glutamate, NH_3, and ATP for glutamine synthetase from these strains. Glutamine synthetase preparations, purified by Zn^{2+} 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 K_m for NH_3 by the MSO -resistant glutamine synthetase was observed relative to the native enzyme. The observed apparent K_m for NH_3 by the mutant enzyme (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 K_m for glutamate, but no significant difference in the K_m for ATP by the MSO-resistant enzyme. These altered kinetic parameters, particularly for NH_3, 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 Mg^{2+} to Mn^{2+}-stimulated biosynthetic activities of wild type and mutant enzymes was identical, indicating the same divalent cation response and states of adenylylation 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^{2+} or Mg^{2+} and had significant alterations in substrate kinetic parameters, particularly the K_m for NH_3. 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.

DISCUSSION

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 K_m values for NH_3 prevent the mutant enzymes from functioning when the NH_3 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 K_m for NH_3 and, to a lesser extent, the K_m 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 NH_3 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 NH_3 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^{2+} (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-$^{32}PO_4$ complex 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-$^{32}PO_4$ 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 paremeters (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.

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