Glutamate γ-Semialdehyde as a Natural Transition State Analogue Inhibitor of *Escherichia coli* Glucosamine-6-phosphate Synthase†

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ABSTRACT: Pyrroline-5-carboxylate, an intermediate in the biosynthesis and degradation of glutamate, proline, and ornithine, acts as a strong reversible inhibitor of glucosamine-6-phosphate synthase, competitive with respect to glutamine. Proton magnetic resonance spectroscopy shows that, under these conditions, pyrroline-5-carboxylate exists in rapid equilibrium with glutamate γ-semialdehyde (0.05%). The observed variation of K_i with pH is consistent with inhibition by this rare species. Glutamate γ-semialdehyde is expected to react reversibly with a cysteine residue at the active site, identified by earlier inactivation studies, to form an analogue of a tetrahedral intermediate in glutamine hydrolysis. The apparent K_i value of glutamate γ-semialdehyde is approximately 3×10^{-8} M.

Glucosamine-6-phosphate synthase (L-glutamine:D-fructose-6-phosphate amidotransferase; GlmS;[1] E.C. 2.6.1.16) catalyzes the first step in hexosamine metabolism, converting fructose-6-phosphate (Fru-6-P) into glucosamine-6-phosphate using glutamine as the ammonia source (Scheme 1) (Gosh *et al.*, 1960; Badet-Denisot *et al.*, 1993). Glucosamine-6-phosphate is eventually transformed into uridine diphospho-*N*-acetylglucosamine, from which other amino sugar-containing molecules are derived. One of these products, *N*-acetylglucosamine, is a major constituent of the peptidoglycan layer of bacterial and fungal cell walls. Accordingly, GlmS offers a potential target for antibacterial and antifungal agents (Andruszkiewicz *et al.*, 1990; Badet-Denisot *et al.*, 1993).

In the sequence of reactions catalyzed by glucosamine-6-phosphate synthase, glutamine is first hydrolyzed to yield glutamate and nascent ammonia, and ammonia is then transferred to fructose-6-phosphate. Finally, Fru-6-P is isomerized from a ketose to an aldose, in a reaction corresponding to a Heyns rearrangement (Kort, 1970; Golinelli-Pimpaneau *et al.*, 1989). GlmS is inactivated by iodoacetamide (Badet *et al.*, 1987), the glutamine analogue 6-diazo-5-oxonorleucine (Gosh *et al.*, 1960; Badet *et al.*, 1987), and N^3-fumaroyl-L-2,3-diaminopropionate derivatives (Kucharczyk *et al.*, 1990). Each of these reagents derivatizes the thiol function of the N-terminal cysteine residue, which is believed to form a γ-glutamyl thioester intermediate during glutamine hydrolysis (Scheme 2A). In accord with this scheme, conversion of the N-terminal cysteine residue to alanine by site-directed mutagenesis results in a total loss of enzymatic activity (Badet-Denisot *et al.*, 1993).

Aldehydes, structurally related to the acyl portion of substrates, act as potent reversible inhibitors of proteases that contain a reactive cysteine (Westerik & Wolfenden, 1972) or serine (Thompson, 1973) residue at the active site. Inhibition of papain involves formation of a thiohemiacetal (Lewis & Wolfenden, 1977; Bendall *et al.*, 1977; Mackenzie *et al.*, 1986) that appears to resemble intermediates in the formation and breakdown of a covalently bound thiol ester intermediate in substrate hydrolysis. If the cysteine residue at the active site of GlmS were to perform a similar function in glutamine cleavage, we reasoned that glutamate γ-semialdehyde might serve as a strong reversible inhibitor (Scheme 2B). Of special interest is the fact that glutamate γ-semialdehyde exists in unfavorable equilibrium with pyrroline-5-carboxylate (P5C) (Schöpf & Oechler, 1936; Schöpf & Steuer, 1947; Vogel & Davis, 1952; Mezl & Knox, 1976), an intermediate in the metabolism of glutamate, proline, and ornithine [for reviews, see Jones (1983, 1985)]. This paper describes the inhibition of *Escherichia coli* glucosamine-6-phosphate synthase by glutamate γ-semialdehyde and the position of its equilibrium of cyclization to form P5C.

MATERIALS AND METHODS

Q-Sepharose Fast Flow and Phenyl Sepharose CL-4B chromatography gels were purchased from Pharmacia, Inc. Ultrogel AcA 34 was purchased from Sepracor Corp.

Δ^1-Pyrroline-5-carboxylate (P5C). P5C was synthesized by oxidation of a mixture of D,L- and D,L-allo-δ-hydroxylysine with periodic acid and purified by ion-exchange chromatography as described by Williams and Frank (1975). The concentration of P5C was determined by the colorimetric method of Mezl and Knox (1976), using the molar extinction coefficient (ε) of the condensation product of P5C with *o*-aminobenzaldehyde ($\epsilon = 2580$ M^{-1} cm^{-1}) reported by these authors.

Δ^1-Pyrroline-2-carboxylate (P2C). P2C was synthesized by treatment of 3,3-dichloro-2-piperidone with barium hydroxide as described by Lewis *et al.* (1993) and Osugi (1958). 3,3-Dichloro-2-piperidone was prepared by treatment of 2-piperidone with PCl_5 and chlorine as described by Wineman *et al.* (1958).

Glutaric Semialdehyde. Glutaric semialdehyde (GSA) was prepared by oxidation of D,L-α-aminoadipic acid with

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[1] Abbreviations: DSS, 3-(trimethylsilyl)propanesulfonic acid; Fru-6-P, fructose-6-phosphate; GlmS, glucosamine-6-phosphate synthase; Gln, glutamine; GSA, glutaric semialdehyde; P2C, Δ^1-pyrroline-2-carboxylate; P5C, Δ^1-pyrroline-5-carboxylate.

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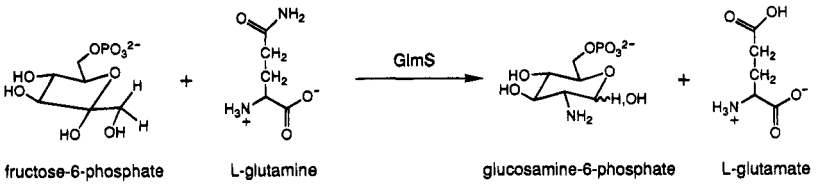
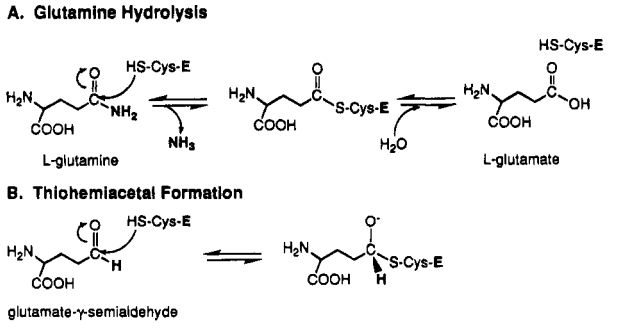
Scheme 1



Scheme 2



chloramine-T, as described by Adams and Chang (1971). Concentrations of the semialdehyde were determined using the colorimetric method of Small and Jones (1990).

Enzyme Purification. *E. coli* 3000 Hfr (ATCC 25257) was grown in 9 L batches to late exponential phase in CGPY medium (Lugtenberg & de Haan, 1971) in a fermenter (MF-114, New Brunswick Scientific Co., Inc.) at 37 °C from a 1% culture inoculum. Cells were collected using a Sorvall RC-3B centrifuge, washed with 100 mM potassium phosphate and 2 mM EDTA buffer, pH 7.5, frozen, and stored at −20 °C until use. A 9 L culture yielded approximately 75 g of cells (wet weight). A crude extract was prepared by disruption with two weights of alumina per cell weight. Glucosamine-6-phosphate synthase was purified from the crude extract essentially as described by Badet *et al.* (1987) and Dutka-Malen *et al.* (1988), except that the organomercurial agarose chromatography and FPLC steps were omitted. Protein concentrations were determined using the method of Bradford (1976). The final purified enzyme preparation had a protein concentration of 3.2 mg/mL and a specific activity of 0.25 unit/mg of protein. One unit was defined as the amount of enzyme that catalyzed the formation of 1 μmol of glucosamine-6-phosphate per minute at saturating substrate concentrations.

Enzyme Assay. Glucosamine-6-phosphate was determined using a modified Morgan−Elson procedure as described by Gosh and Roseman (1962). Enzyme (10 μL) was added to a solution (1.2 mL) containing fructose-6-phosphate (10 mM), glutamine (15 mM), EDTA (1 mM), and potassium phosphate buffer (0.1 M, pH 7.5), and the mixture was incubated for 30−60 min at 37 °C. The reaction was then terminated by boiling at 100 °C for 4 min. Any protein precipitate was removed by centrifugation, and 0.5 mL of the supernatant was analyzed for glucosamine-6-phosphate (Reissig *et al.*, 1955; Zalkin, 1985). Each determination was compared with a standard curve prepared using authentic glucosamine-6-phosphate. The color yields were found to be linear over the concentration range from 0.01 to 1.0 mM. The validity of the fixed-time assay was established by demonstrating that glucosamine-6-phosphate production varied linearly with time over 60 min at all substrate concentrations. In addition, the color yield was found to vary linearly with increasing enzyme concentrations over the range of 10 to 100 μL of the purified preparation.

Enzyme Inhibition. Assays were performed in potassium phosphate buffer (0.1 M, pH 7.5, containing 10^{-3} M EDTA) at 37 °C, except where noted. Pyrroline-5-carboxylate is stable in acid solution, but unstable in neutral solution in which polymerization is believed to occur (Williams & Frank, 1975; Mezl & Knox, 1976). For that reason, acidic preparations of P5C were neutralized only immediately before use. For inhibition studies with P5C, the enzyme was dialyzed for 24 h against potassium phosphate buffer (0.1 M, pH 7.5, containing 10^{-3} M EDTA) to remove 2,4-dithiothreitol. Reagents present in assays for inhibition by P5C included GlmS (6.7×10^{-3} units/mL), Fru-6-P (10 mM, approaching saturation), Gln (0.125−10.0 mM), and P5C (0−0.2 mM). P5C inhibition of GlmS activity was also examined at lower pH, using potassium phosphate buffer (0.1 M, pH 6.2, containing 10^{-3} M EDTA) at 37 °C and an enzyme concentration of 13×10^{-3} units/mL. Effects of D,L-proline (20 mM), L-2-pyrrolidone-5-carboxylate (20 mM), pyrrole-2-carboxylate (20 mM), and pyrroline-2-carboxylate (10 mM) on GlmS activity were examined at the concentrations indicated. Kinetic data were analyzed by nonlinear regression analysis of Michaelis-Menten plots using the program *EnzymeKinetics* (1990) from Trinity Software. Inhibition constants were determined in triplicate, and average values are reported. The reported errors are standard deviations.

Enzyme Inactivation. Reaction was initiated by addition of enzyme (60 μL) to a solution of glutaric semialdehyde (40 μL, such that final concentrations ranged from 0 to 1.0 mM) in potassium phosphate buffer (0.1 M, pH 7.5, containing 10^{-3} M EDTA). Reaction mixtures were incubated at room temperature (25 °C), and 20 μL aliquots were withdrawn at various time points, diluted 60-fold, and assayed for activity. Inactivation followed first-order kinetics, and the first-order rate constants for inactivation were calculated from plots of activity remaining as a logarithmic function of the time of incubation.

For protection studies, either glutamine (0.4 mM final concentration) or fructose-6-phosphate (2 mM final concentration) was incubated with glutaric semialdehyde, followed by addition of the enzyme. Before inactivation studies, the enzyme was dialyzed for 24 h against potassium phosphate buffer (0.1 M, pH 7.5, containing 10^{-3} M EDTA) to remove dithiothreitol.

NMR Spectroscopy. Proton NMR spectra were obtained using a Bruker AMX-500 spectrometer, with suppression of the HOD signal. Pyrroline-5-carboxylate was prepared for the NMR studies as described above, except that D_2O and DCl were used in place of H_2O and HCl. Before NMR spectroscopy, samples of P5C were adjusted to the desired pD using NaOD. pH meter readings were converted to pD values using the equation of Fife and Bruice (1961). Integrated intensities of peaks corresponding to protons of

Table 1: Effect of P5C, Cyclic P5C Analogues, and Glutaric Semialdehyde on the Activity of *E. coli* GlmS

Compound	Effect on GlmS Activity
pyrroline-5-carboxylate	competitive inhibition with respect to Gln*: pH 6.2, K_m 0.40 (±0.02) mM, K_i 8 (±1) μM; pH 7.5, K_m 0.43 (±0.09) mM, K_i 55 (±8) μM
pyrroline-2-carboxylate	$K_i \geq 28$ mM
pyrrole-2-carboxylate	$K_i \geq 56$ mM
L-2-pyrrolidone-5-carboxylate	$K_i \geq 56$ mM
D,L-proline	$K_i \geq 56$ mM
glutaric semialdehyde	irreversible inactivation $k_2 = 0.84(\pm 0.05)$ $M^{-1}s^{-1}$

free glutamate γ-semialdehyde, aldehyde hydrate, and pyrroline-5-carboxylate were measured at various pD values (see Results for spectral details and peak assignments). Chemical shifts (δ) are reported in ppm relative to DSS.

RESULTS

Effects of Pyrroline-5-carboxylate and Analogues. Inhibition of glucosamine-6-phosphate synthase activity by P5C was found to be competitive with respect to glutamine (K_i = 55 (±8) μM). The enzyme showed a higher affinity for P5C when the pH was reduced to 6.2 (Table 1). To obtain an indication of whether P5C is bound to the enzyme in a cyclic form, analogues of P5C were tested for their effect on GlmS activity. The cyclic analogues D,L-proline (20 mM), L-2-pyrrolidone-5-carboxylate (20 mM), pyrrole-2-carboxylate (20 mM), and pyrroline-2-carboxylate (10 mM) did not produce detectable inhibition of activity at the concentrations tested (Table 1).

Glutaric semialdehyde, analogous in structure to linear glutamate γ-semialdehyde,[2] was found to inactivate glucosamine-6-phosphate synthase irreversibly (Figure 1). The slope of a plot of the half-time for inactivation as a function of GSA concentration (Figure 1B) indicates a pseudo-second-order rate constant for inactivation with a value of 0.84 (±0.05) M^{-1} s^{-1}. The half-time for inactivation was found to increase in the presence of either of the two substrates, glutamine or fructose-6-phosphate (Table 2). In spite of the ability of GSA to interact with the active site of GlmS, as indicated by this substrate protection, the inhibitor's reversible affinity for the active site could not be determined,

[2] Although glutamate γ-semialdehyde lacks an amino function, it may cyclize to form a hemiacylal. However, under neutral conditions, the open chain form is expected to be largely favored (Salomaa, 1964; Fife, 1965).

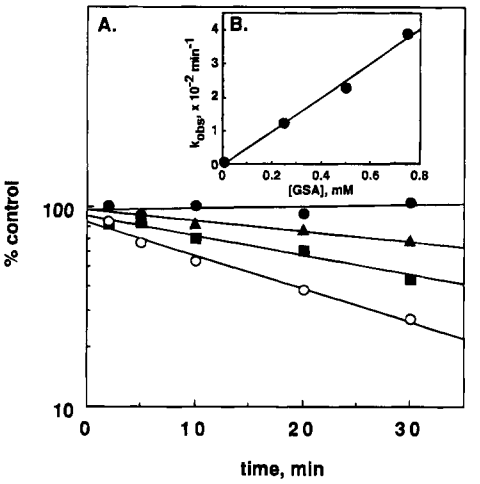


FIGURE 1: *A.* Time-dependent inactivation of GlmS by glutaric semialdehyde. Conditions: 100 mM potassium phosphate buffer, pH 7.5, 1.0 mM EDTA at 25 °C. Concentrations of GSA are 0 mM (●), 0.25 mM (▲), 0.50 mM (■), and 0.75 mM (○). The concentration of GlmS is 0.24 units/mL. B. Apparent first-order rate constants of inactivation plotted as a function of GSA concentration. The linear regression slope is 0.84 (±0.05) M^{-1} s^{-1}.

Table 2: Glucosamine-6-phosphate Synthase Inactivation by Glutaric Semialdehyde: Protection by Substrates at Concentrations Equal to Their K_m Values[a]

protecting agent	concentration, mM	half-time, min
Fru-6-P	2.0	54
Gln	0.4	21
none	0	7

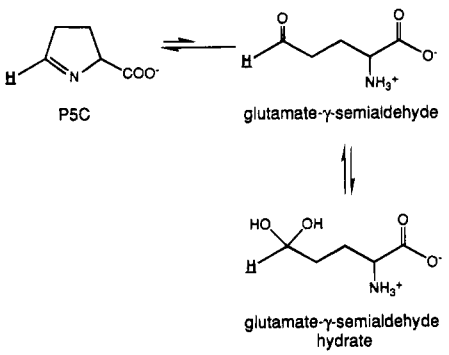
[a] Assay conditions are described in the Materials and Methods.

because the rate of inactivation continued to increase with increasing inhibitor concentrations, even at GSA concentrations 15-fold higher than the apparent K_i value for P5C.

The Position of Equilibrium between Glutamate γ-Semialdehyde and Pyrroline-5-carboxylate. Proton NMR spectra of P5C at different pD values indicate the presence of several rapidly equilibrating species. At low pD values, the spectrum is complex in the high-field region between 2 and 5 ppm. As the pD is raised above 6.2, the spectrum becomes simplified in this region. Three well-separated signals are observed at approximately 5.8, 9.1, and 9.8 ppm. Irradiation at a frequency corresponding to any one of these three signals causes suppression of the other two signals with no effect on the rest of the spectrum. This indicates that the three signals observed between 5.5 and 10 ppm originate from the same proton in several species that are in rapid equilibrium. This proton was identified as the proton attached to C-5 in the open chain semialdehyde, which becomes C-1 in the cyclic imine (shown underlined in Scheme 3). Downfield peaks are observed for the free aldehyde at ≈9.8 ppm, the *gem*-diol at ≈5.8 ppm, and the imine at ≈ 9.1 ppm. The relative concentrations of free aldehyde, aldehyde hydrate, and cyclic imine that were present at equilibrium at various pD values were determined by integration of the intensities of signals corresponding to each of these species.

Figure 2A shows the relative concentrations of aldehyde hydrate and imine at various pD values. We report these concentrations as ratios because it is likely that at different pD values other species are also present at low concentrations (cf. Lewis *et al.* (1993)]. Of the three major forms of glutamate γ-semialdehyde, the free aldehyde could be

Scheme 3



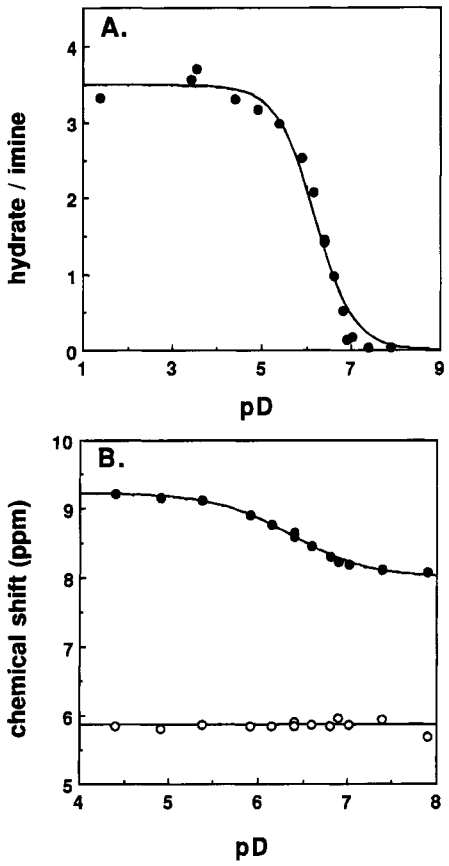


FIGURE 2: A. Ratio of glutamic γ-semialdehyde (hydrate) to pyrroline-5-carboxylate (imine) as a function of pD. Points are experimental hydrate to imine ratios (H/I) while the curve is from a fit of the equation $(H/I)^{obs} = [(H/I)^{max}(1 + 10^{pD-pK_{ao}})]/[(1 + 10^{pD-pK_{ac}})]$, where pK_{ao} and pK_{ac} are the pK_a values for the conjugate acids of glutamate γ-semialdehyde hydrate and P5C, respectively. The hydrate/imine ratio shows a sharp transition corresponding to a pK_{ac} value of 6.20 (±0.05). The value for pK_{ao} is ≥9.5. B. Chemical shift (ppm) of the imine proton signal (●) and the hydrate proton signal (O) as a function of pD. Points are experimental values, while the curve for the imine proton signal is from a fit of the equation $\delta^{obs} = [\delta^{max} + (\delta^{min})(10^{pD-pK_a})]/[(10^{pD-pK_a}) + 1]$. The pK_a value was determined to be 6.67 (±0.08). The curve for the hydrate proton signal is the linear regression least-squares line.

detected only at low pD values where the open chain forms predominate. At pD 4.9, the free aldehyde to hydrate ratio is about 1:73, increasing to 1:22 at pD 1.4. At pD 6.2, there is a sharp transition in the ratio of hydrate to imine, so that the latter species becomes strongly predominant under more alkaline conditions. The chemical shift of the imine proton was found to vary strongly with changing pD (Figure 2B). This variation was used to evaluate the pK_a value of the imine as 6.67 (±0.08). The chemical shift of the hydrate proton, however, showed no significant variation with pD. These observations are consistent with the peak assignments.

DISCUSSION

Glucosamine-6-phosphate synthase catalyzes the hydrolysis of glutamine to glutamate and ammonia, and ammonia is then transferred to fructose-6-phosphate to produce glucosamine-6-phosphate. Aldehydes, related in structure to the acyl portion of substrates, have been found to serve as potent reversible inhibitors of several hydrolases (Wolfenden, 1978, and references cited therein). Inhibition by aldehydes appears to be due to their ability to form thiohemiacetals that resemble tetrahedral intermediates in the formation and breakdown of acyl-enzyme intermediates. Similarly, the N-terminal cysteine residue of GlmS is believed to participate in the formation of a γ-glutamyl thioester intermediate during glutamine hydrolysis (Badet-Denisot *et al.*, 1993). Accordingly, we reasoned that the glutamine analogue glutamate γ-semialdehyde, a metabolite in the biosynthesis and catabolism of the amino acids glutamate, proline, and ornithine, might react at the glutamine binding site to form a thiohemiacetal and reversibly inhibit glucosamine-6-phosphate synthase activity (Scheme 2B).

Glutamate γ-semialdehyde exists in equilibrium (Scheme 3) with its intramolecular cyclization product pyrroline-5-carboxylate. In neutral aqueous solutions, the equilibrium is believed to favor the cyclized form strongly (Schöpf & Oechler, 1936; Schöpf & Steuer, 1947; Vogel & Davis, 1952; Mezl & Knox, 1976), but the position of this equilibrium does not appear to have been reported.

We found that pyrroline-5-carboxylate inhibits glucosamine-6-phosphate synthase activity competitively with respect to glutamine ($K_i = 5.5 \times 10^{-5}$ M at pH 7.5). To determine whether inhibition might be due to cyclic P5C or to the rarer open chain semialdehyde, we examined the effect of analogues of P5C and glutamate γ-semialdehyde on enzyme activity (Table 1). Both L-2-pyrrolidone-5-carboxylate and pyrrole-2-carboxylate mimic P5C in having an sp^2-hybridized carbon atom adjacent to the ring nitrogen, but we observed no significant inhibition by the cyclic analogues D,L-proline, L-2-pyrrolidone-5-carboxylate, pyrrole-2-carboxylate, or pyrroline-2-carboxylate. In contrast, glutaric semialdehyde, an analogue of linear glutamate γ-semialdehyde, irreversibly inactivated glucosamine-6-phosphate synthase. In addition, the presence of either of the substrates fructose-6-phosphate or glutamine decreased the rate of enzyme inactivation by glutaric semialdehyde (Table 2), indicating that GSA probably interacts with the enzyme at or near both the glutamine and fructose-6-phosphate, binding sites.[3] The failure of cyclic analogues of P5C to inhibit GlmS activity and also the ability of GSA to interact with GlmS appear to be

[3] It seems plausible that the structural similarity of glutaric semialdehyde to glutamine allows it to bind at the enzyme's glutamine binding site. Irreversible reaction between the aldehyde function and an amino function in the Fru-6-P binding site leading to imine formation may be responsible for the observed inactivation. Such imine formation seems plausible, since pyridoxal has been shown to inactivate glucosamine-6-phosphate synthase by reacting with a lysine residue in the fructose-6-phosphate binding site (Golinelli-Pimpaneau & Badet, 1991). Why the aldehyde function of glutamate γ-semialdehyde does not react irreversibly with the enzyme is unclear.

consistent with the view that the glutamic semialdehyde, rather than P5C, is responsible for inhibition. Further, if glutamate γ-semialdehyde were responsible for the observed inhibition of GlmS activity, then inhibition should be more pronounced at lower pH values where equilibrium favors cyclization less strongly. Lowering the pH from 7.5 to 6.2 does indeed enhance the enzyme's apparent affinity for the inhibitor as evidenced by a 7-fold reduction in the K_i value (Table 1).

If the open chain form, rather than cyclic P5C, is responsible for the inhibition observed, then the apparent dissociation constant of P5C underrepresents the enzyme's real affinity for the form of the inhibitor that is actually bound, and it would be desirable to know the abundance of this minor species in solution. It has been suggested that equilibrium favors the cyclized product (Schöpf & Oechler, 1936; Schöpf & Steuer, 1947), but the position of this equilibrium was not determined. Lewis *et al.* (1993) found that pyrroline-2-carboxylate exhibited a complex and pH-dependent proton NMR spectrum and reported pK_a values of 1.8 and 6.2, respectively, for the carboxylic acid group and pyrrolinium moiety. These authors observed only the cyclic form of this compound at pH 6.5 and above, at which the concentration of noncyclic forms becomes too low to observe.

Previous attempts to determine the position of equilibrium of ring opening appear to have been impeded by the tendency of P5C to undergo irreversible and concentration-dependent polymerization (Mezl & Knox, 1976). Following the preparative method of Mezl and Knox (1976), we were able to prepare solutions of pyrroline-5-carboxylate in D_2O that were sufficiently stable for characterization by NMR, and integration of the downfield signals allowed us to estimate the relative amounts of glutamate γ-semialdehyde hydrate and cyclic imine present at various pD values. At pD 7.4, the cyclic imine was found to be approximately 26 times more abundant than the hydrate. As the pD value decreases, there is a sharp transition in the hydrate to imine ratio at pD 6.2, the hydrate becoming predominant at lower pD values (Figure 2A). This transition presumably corresponds to the ionization of the pyrrolinium moiety of the pyrroline-5-carboxylate. In accord with this interpretation [see also Lewis *et al.* (1993)], the chemical shift of the imine proton signal is pD-dependent, with an apparent pK_a value of 6.67 ($\pm$0.08).

Two open chain forms of P5C might be considered to be responsible for inhibition. First, glutamate γ-semialdehyde might be bound as the corresponding *gem*-diol. If that species were responsible for the observed inhibition, then its K_i value would be (5.5×10^{-5} M)/26 = 2×10^{-6} M. This mode of binding of inhibitory aldehydes has been observed in the case of leucine aminopeptidase (Andersson *et al.*, 1985) and carboxypeptidase A (Christianson & Lipscomb, 1985), metalloenzymes that appear to bring about substrate hydrolysis by direct water attack. In view of the prominent role of an SH group in the activity of glucosamine-6-phosphate synthase (see introduction), it appears more probable that glutamate γ-semialdehyde is bound directly, forming a hemiacetal that resembles an activated intermediate in glutamine hydrolysis by a double displacement mechanism. This seems likely because of the apparent resemblance of this enzyme to papain, for which inhibition by thiohemiacetal formation has been established (Lewis & Wolfenden, 1977; Bendall *et al.*, 1977; Mackenzie *et al.*, 1986). The ratio of the *gem*-diol to the free aldehyde (1:73, observed at pD 4.9) can be used to estimate the K_i value of glutamate γ-semialdehyde as 2.9×10^{-8} M.

The action of glutamate γ-semialdehyde may be contrasted with that of several naturally occurring compounds that have been reported to inhibit glucosamine-6-phosphate synthase irreversibly, by alkylating the enzyme. Bacilysin (Walker & Abraham, 1970a,b) and chlorotetain (Rapp *et al.*, 1988), produced by *Bacillus subtilis*, contain an N-terminal L-alanine and a β-(4′-oxocyclohexyl)-substituted alanine. In the case of bacilysin, inhibitory activity was shown to be due to the C-terminal β-(2′,3′-epoxy-4′-oxocyclohexyl)-containing amino acid, anticapsin (Chmara, 1985). Reversible binding of anticapsin to the enzyme, prior to the inactivation step, has been reported to occur with a dissociation constant with a value of 0.13 μM (Chmara, 1985). For *E. coli* glucosamine-6-phosphate synthase, anticapsin is the most effective inactivating agent known (Badet *et al.*, 1988). Compound A19009, from a *Micromonospora* species (Molloy *et al.*, 1972), and compound Sch 37137, from *Streptomyces collinus* (Cooper *et al.*, 1988), are dipeptides that contain the exotic amino acid N^3-fumaramoyl-L-2,3-diaminopropionic acid or its epoxide. Synthetic inhibitors that resemble these naturally occurring antibiotics (Chmara, 1985; Chmara *et al.*, 1985; Badet *et al.*, 1988; Auvin *et al.*, 1991; Milewski *et al.*, 1992; Andruszkiewicz *et al.*, 1994, and references cited therein) also inactivate GlmS irreversibly.

The fact that glutamate γ-semialdehyde is in rapid equilibrium with P5C, an intermediate in the formation and degradation of glutamate, proline, and ornithine, is of special interest. Of the mammalian enzymes that might generate P5C, P5C reductase and possibly P5C dehydrogenase occur in the cytosol (Jones, 1983, 1985), where mammalian glucosamine-6-phosphate synthase[4] is also located (Winterburn & Phelps, 1971). The possibility that activity in these pathways might influence hexosamine biosynthesis remains to be explored.

[4] Glucosamine-6-phosphate synthase isolated from rat liver and other mammalian sources is susceptible to feedback regulation by the end product of the hexosamine pathway, UDP-*N*-acetylglucoasamine, whereas the *E. coli* enzyme is not (Winterburn & Phelps, 1971).

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