Regulation of Glutamine Synthetase from *Bacillus subtilis* by Divalent Cations, Feedback Inhibitors, and L-Glutamine

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SUMMARY

Glutamine synthetase purified to apparent homogeneity from *Bacillus subtilis* is subject to feedback inhibition by multiple end products of glutamine metabolism, as well as by the reaction product L-glutamine. AMP, glutamine, and histidine are potent inhibitors when any of the substrates glutamate, MnATP, or ammonia are present in limiting concentration during assay, but the strong inhibition by glycine and alanine is influenced far less by substrate concentration. In the Mg\(^{2+}\)-dependent biosynthetic assay, AMP and glutamine are potent inhibitors of glutamine synthetase, whereas alanine and glycine do not inhibit this activity at all. Tryptophan, which is not an inhibitor in the Mn\(^{2+}\) biosynthetic assay, exhibits some inhibition in the Mg\(^{2+}\) assay. Synergistic inhibition of the Mn\(^{2+}\)-dependent glutamine synthetase activity is present when AMP and glutamine are studied together; glutamine alone is not inhibitory when present at 5 mM concentration in the saturating assay, but produces 85% inhibition when studied in the presence of 2.5 mM AMP. Histidine and AMP are also synergistic, but glutamine acts independently of histidine. Alanine and glycine reciprocally reduce the effectiveness of one another as inhibitors.

Glutamine appears to play a major role in the over-all regulation of its own synthesis. In *Escherichia coli*, glutamine modulates the glutamine synthetase adenylylation-deadenylylation system, thereby controlling over-all enzyme activity as well as responsiveness to feedback inhibitors. *B. subtilis* appears to lack the mechanisms to adenylylate and deadenylylate preformed glutamine synthetase. The organism does, however, appear to achieve similar regulatory effects by the direct inhibition of glutamine synthetase by glutamine, and by the striking potentiation of AMP inhibition that occurs with low concentrations of glutamine. Therefore, in *B. subtilis* as well as *E. coli*, intracellular glutamine levels play an important role in the fine control of the over-all glutamine synthetase activity, and in the control of cellular anabolic nitrogen metabolism.

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Regulation of complex metabolic pathways in bacteria is accomplished by a multiplicity of mechanisms (1, 2). Among these mechanisms, feedback inhibition of the first enzyme unique to a biosynthetic pathway by the ultimate end products of that pathway has proven to be of great importance. The regulation of glutamine synthetase in *Escherichia coli* has been particularly well studied (1) and feedback inhibition of this enzyme has been shown to correlate well with over-all cellular nitrogen metabolism. In this communication, feedback inhibition of *Bacillus subtilis* glutamine synthetase is shown; the preliminary findings have been reported elsewhere (3).

Inhibition by end products of glutamine metabolism varies with the divergent cation in the biosynthetic assay and with the concentrations of various substrates of the reaction. Synergistic effects are seen with specific feedback inhibitors. The immediate product of the reaction, L-glutamine, may be regarded as central in the expression of catalytic activity; glutamine acts as a direct inhibitor of activity, and also markedly potentiates inhibition by other feedback inhibitors. Whereas glutamine modulates glutamine synthetase activity through a very different mechanism in *E. coli* (1), both *B. subtilis* and *E. coli* appear to share in common the importance of cellular glutamine levels for the expression of enzymatic activity. Similar patterns of inhibition have been demonstrated previously with partially purified glutamine synthetase from *Bacillus licheniformis* (4, 5).

The discovery of glutamate synthase by Meers et al. (6) has demonstrated a direct link between glutamine synthetase and various transaminases, providing an ATP-dependent synthesis of various amino acids from the respective α-keto acids (see “Discussion” for details of these reactions). Alanine and glycine are shown in this communication to be potent inhibitors of glutamine synthetase from *B. subtilis*; such inhibition therefore is likely to be due to simple feedback by end products of glutamine metabolism (6, 12).

METHODS AND MATERIALS

Materials were of the highest grade commercially available. Nucleotides were obtained from P-L Biochemicals, Milwaukee, Wis., and amino acids from Schwarz-Mann, Orangeburg, N.Y. *B. subtilis* 60029 (supplied through the kindness of Dr. Ernst Freeze), which lacks alanine dehydrogenase and requires indole, was grown on glucose and limiting ammonia. The cells were harvested, stored in liquid nitrogen, and the enzyme subsequently purified as previously described (7). Purified glutamine synthetase (60 mg per ml of protein concentration) was stored at
4°, in 50 mM imidazole chloride (pH 7.0), 1 mM EDTA, and 1 mM 2-mercaptoethanol. The enzyme was judged to be over 95% pure by acrylamide gel electrophoresis, sedimentation velocity and equilibrium studies, and electron microscopy. The specific activity of the purified enzyme was 11 to 13 μg of inorganic phosphate formed per min per mg of protein at 25° in a Mg2+-dependent assay system (7). Since loss of activity is noted with prolonged storage of the enzyme at 4°, and since the response of the enzyme to various feedback inhibitors is also modified, the results described in this communication were obtained within 2 weeks of isolation of the enzyme.

Protein was measured by a modification of the biuret method (8), using crystalline bovine serum albumin as standard. The biosynthetic activity of glutamine synthetase at 25° was measured with either Mn2+ (7.5 mM) or Mg2+ (50 mM) in a final volume of 0.4 ml, containing 50 mM imidazole chloride (pH 7.1), 7.5 mM ATP, 100 mM L-glutamate, 50 mM NH4Cl, and sufficient enzyme to produce ~0.2 μmole of phosphate in 7.5 min. Inorganic phosphate was measured as previously described (r), as adapted from the method of Boyer et al. (9). Approximately 5 μg of enzyme protein were added when Mn2+ was used in the standard biosynthetic assay, and 2.5 μg when Mg2+ was the divalent cation. Activity was measured in a Klett-Summerson colorimeter equipped with a No. 66 filter (1 Klett unit = 1.3 nmols of inorganic phosphate released per assay volume). The data were plotted with the fractional inhibition (i) on the ordinate and the inhibitor concentration (I) on the abscissa. Fractional inhibition is defined as the activity in the presence of the inhibitor, divided by the activity in the control incubated in the absence of the inhibitor. Analysis of the data was also performed using the double reciprocal plot, l/i versus 1/I. If the extrapolation of the plot so obtained crossed the ordinate at 1/i = 1, it was presumed that saturating concentrations of the inhibitor completely abolished the catalytic activity of the enzyme. Inhibition studies were undertaken at saturating levels of the substrates as described above for both Mn2+ and Mg2+ assay systems, and with limiting levels of substrates in separate assays with the Mn2+ assay (2.5 mM glutamate, 1 mM NH4Cl, and 1 mM MnATP, respectively). In each case, the reaction has been shown to be linear with time and protein concentration. The limiting concentrations of the substrates were chosen to provide sufficient concentrations of the respective substrates such that the velocity is approximately half that obtained when the substrate is present in saturating concentrations.

RESULTS

Nonhyperbolic substrate saturation functions were observed with each of the substrates of B. subtilis glutamine synthetase (3). The response of the enzyme to various feedback inhibitors was equally complex. Table I presents the per cent of activity of glutamine synthetase in both Mn2+ and Mg2+ assays (substrates in saturating concentrations) when each of six inhibitors is added separately at 5 mM concentration. Marked differences are noted when the response of the enzyme to the inhibitor is compared in the Mn2+ and Mg2+ assays. Both alanine and glycine are far more effective in the Mn2+ assay, while AMP

![Graph](http://www.jbc.org/)

**Table I**

<table>
<thead>
<tr>
<th>Metal ion</th>
<th>Percentage of control activity in presence of various inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alanine</td>
</tr>
<tr>
<td>Mn2+ (7.5 mM)</td>
<td>25</td>
</tr>
<tr>
<td>Mg2+ (50 mM)</td>
<td>100</td>
</tr>
</tbody>
</table>

a Inhibitor concentration 5 mM, and substrates in saturating concentrations (see text for details).
and glutamine are potent inhibitors when studied with Mg2+. Histidine and tryptophan are very poor inhibitors under the conditions used.

More detailed studies with glycine as an inhibitor in the Mn2+ assay are illustrated in Fig. 1A. The curves of glycine concentration versus fractional inhibition are hyperbolic, as confirmed in the double reciprocal plot (1/i versus 1/I, Fig. 1B). Extrapolation of this plot suggests that complete inhibition of catalytic activity occurs at saturating concentrations of glycine. Glycine is partially competitive with respect to glutamate; in contrast, with limiting MnATP, the inhibition is less marked than when MnATP is present in saturating concentrations, suggesting that binding of MnATP is required for glycine inhibition. These differences in activity with respect to different inhibitors are slight, but are found consistently in consecutive experiments.

Fig. 2A shows that alanine is a potent inhibitor of *B. subtilis* glutamine synthetase also. Alanine is partially competitive with respect to glutamate as well, but is a more effective inhibitor when ammonia is present in saturating, rather than limiting concentrations. It is likely that ammonia binding is required for alanine to be an effective inhibitor. Alanine concentration is a hyperbolic function of the fractional inhibition under all conditions studied, and as shown in the double reciprocal plot (Fig. 2B), complete inhibition of catalytic activity is shown by extrapolation to infinite alanine concentration. Neither alanine nor glycine inhibits activity when Mg2+ is used, in striking contrast to the results with Mn2+ (data not shown).

The amide nitrogen of glutamine is directly incorporated into the amino acids tryptophan and histidine. Fig. 3 illustrates slight activation of *B. subtilis* glutamine synthetase by low concentrations of histidine in all assay conditions; higher concentrations of the inhibitor were required for significant inhibition of the activity. Histidine is partially competitive with respect to glutamate, MnATP, and ammonia, and the curves of inhibitor versus fractional inhibition are nonhyperbolic under all assay conditions studied. Tryptophan (data not shown) does not inhibit *B. subtilis* glutamine synthetase in any of the four Mn2+-dependent assay systems investigated. In Fig. 4, both histidine and tryptophan are shown to be inhibitors of glutamine synthetase activity when Mg2+ is substituted for Mn2+.

The reaction product, L-glutamine, inhibits the activity of *B. subtilis* glutamine synthetase, and is much more effective as an inhibitor when the concentration of any of the three sub-
Histidine

A- A Tryptophan

FIG. 4. Effects of increasing concentrations of histidine and tryptophan on the catalytic activity of Bacillus subtilis glutamine synthetase in the Mg\textsuperscript{2+} biosynthetic assay, studied at saturating concentrations of all substrates (see “Methods and Materials”).

substrates is present in limiting concentrations during assay in the Mn\textsuperscript{2+} system (Fig. 5A). The double reciprocal plot of these data shows that the curves in Fig. 5A are nonhyperbolic. Extrapolation of the curves in the double reciprocal plot to infinite inhibitor concentration suggests complete inhibition of enzyme activity under all conditions of study (data not shown).

AMP is a weak inhibitor of glutamine synthetase in the Mn\textsuperscript{2+}-saturating assay as illustrated in Fig. 5B. As with glutamine, AMP is a far more powerful inhibitor of activity when any of the substrates are present in limiting concentration during the Mn\textsuperscript{2+} biosynthetic assay. The double reciprocal plot of the data illustrated in Fig. 5B for the assays using limiting MnATP and glutamate clearly shows that the relationship of AMP concentration and partial inhibition of activity is not hyperbolic. Additionally, extrapolation to infinite inhibitor concentrations shows that AMP may completely inhibit the catalytic activity of the enzyme (data not shown).

Fig. 6 illustrates the effects of increasing AMP and glutamine concentrations on the activity studied in the Mg\textsuperscript{2+} biosynthetic reaction with saturating concentrations of all substrates. Both AMP and glutamine are very potent inhibitors of activity in the Mg\textsuperscript{2+} system, in contrast to the minimal inhibition seen with Mn\textsuperscript{2+}.

Inhibition of B. subtilis glutamine synthetase by specific inhibitors is modified if a second inhibitor is added to the Mn\textsuperscript{2+} biosynthetic assay with saturating concentrations of substrates. In Fig. 7, alanine at 2.5 mM is shown to reduce AMP inhibition significantly in the Mn\textsuperscript{2+} reaction, whereas histidine potentiates the AMP effect. Less than 10\% inhibition occurs when 10 mM AMP is added to the Mn\textsuperscript{2+} biosynthetic assay; when studied with 2.5 mM histidine present, AMP at 10 mM results in nearly 60\% inhibition. Tryptophan, at 10 mM, has no apparent effect on the inhibition of the reaction by AMP. As shown in Fig. 8, the effect of glutamine in potentiating AMP inhibition is even more striking than that shown for histidine in Fig. 7. At 2 mM concentration, AMP results in nearly complete inhibition of catalytic activity in the presence of 2.5 mM glutamine, and nearly 50\% inhibition with 1 mM glutamine. Thus glutamine, the product of the biosynthetic reaction, is a very powerful direct inhibitor of the enzyme activity in Mg\textsuperscript{2+}, and strongly potentiates AMP inhibition with Mn\textsuperscript{2+}. Glutamine thus may play
Fig. 6. Effects of increasing concentrations of glutamine and AMP on the catalytic activity of *Bacillus subtilis* glutamine synthetase in the Mg$^{2+}$ biosynthetic assay, studied at saturating concentrations of all substrates (see "Methods and Materials").

Fig. 7. Inhibition of glutamine synthetase activity by AMP in the presence and absence of other inhibitors. The standard Mn$^{2+}$ biosynthetic assay system (all substrates at saturating concentrations) was used, and inhibitors were added at the final concentrations indicated: AMP alone, O---O; AMP + 2.5 mM alanine, •--•; AMP + 2.5 mM histidine, △-△; and AMP + 10 mM tryptophan, □--□. Control values for the assay in the presence of 2.5 mM histidine and 10 mM tryptophan did not differ significantly from control values in the absence of histidine. Glutamine and AMP therefore potentiate each other, but histidine has no apparent effect.

In other studies (data not shown), it has been shown that histidine is without effect on glutamine inhibition. Also, alanine and glycine are less effective inhibitors when one is studied in the presence of the other.

**DISCUSSION**

In bacteria, glutamine synthetase functions both to assimilate ammonia and to synthesize glutamine itself. The discovery of glutamate synthase by Meers *et al.* (6) has greatly clarified the role of glutamine synthetase in ammonia assimilation. The following sequence of reactions is one route by which ammonia is incorporated into metabolites (6, 12).

\[
\text{Glutamate} + \text{ATP} + \text{NH}_4 \rightleftharpoons \text{glutamine} + \text{ADP} + \text{P}_i \quad (1)
\]

\[
\text{Glutamine} + \alpha\text{-ketoglutarate} + \text{TPNH} \rightleftharpoons \text{glutamate} + 2\text{glutamate biosynthetic} + \text{TPN} \quad (2)
\]

\[
\text{Glutamate} + \text{RCOCOOH} \rightleftharpoons \text{transaminase} \quad (3)
\]

\[
\text{RCNH}_2\text{COOH} + \alpha\text{-ketoglutarate}
\]

The effect of AMP on glutamine inhibition is shown in Fig. 9. AMP at low concentrations very strongly potentiates inhibition by glutamine. Glutamine and AMP therefore potentiate inhibition by each other in the inhibition of glutamine synthetase.

The inhibition of the enzyme by histidine with AMP or glutamine present is shown in Fig. 10. AMP is very effective in potentiating histidine as an inhibitor, but glutamine has no apparent effect.
Glutamine Alone
+0.25 mM AMP
+1.0 mM AMP
+2.5 mM AMP

GLUTAMINE, MILLIMOLAR

FIG. 9. Inhibition of glutamine synthetase activity by glutamine in the presence and absence of AMP. The standard Mn⁴⁺ biosynthetic assay system (all substrates at saturating concentrations) was used, and AMP was added at the final concentrations indicated: glutamine alone, O——O; glutamine + 0.25 mM AMP, ■——■; glutamine + 1.0 mM AMP, △——△; and glutamine + 2.5 mM AMP, □——□. Control values in the presence of AMP did not differ significantly from control values in the absence of AMP.

HISTIDINE, MILLIMOLAR

FIG. 10. Inhibition of glutamine synthetase activity by histidine in the presence and absence of AMP and glutamine. The standard Mn⁴⁺ biosynthetic assay system (all substrates at saturating concentrations) was used, and AMP and glutamine were added at the concentrations indicated: histidine alone, O——O; histidine + 2.5 mM AMP, ■——■; and histidine + 2.5 mM glutamine, △——△. Control values in the presence of inhibitors did not differ significantly from the value in the absence of inhibitor.

The sum of these three reactions is shown below.

\[
\text{RCO}_2\text{COOH} + \text{ATP} + \text{TPNH} + \text{NH}_3 \rightarrow \text{RCNH}_2\text{COOH} + \text{ADP} + \text{P} + \text{TPN} \quad (4)
\]

This series of reactions appears to be the only route for glutamate production in *Bacilli* since these organisms lack glutamate dehydrogenase (10). Enteric bacteria possess both glutamate synthase and a TPNH-dependent glutamate dehydrogenase for the synthesis of glutamate (6, 11) by the reaction

\[
\alpha\text{-Ketoglutarate} + \text{NH}_3 + \text{TPNH} \rightarrow \text{glutamate dehydrogenase} \rightarrow \text{glutamate} + \text{TPN} \quad (5)
\]

Mutants of *Bacillus megaterium* lacking glutamate synthase thus are glutamate auxotrophs (10), in contrast to *E. coli* which must lack both glutamate synthase and glutamate dehydrogenase to manifest glutamate auxotrophy (11).

The potent inhibition of glutamine synthetase from *B. subtilis* by alanine and glycine appears to be simply end product inhibition of the first enzyme in a biosynthetic pathway (12). The regulation of nitrogen assimilation by alanine feedback inhibition of glutamine synthetase is of special interest, since *Bacilli* contain the enzyme 3-alanine dehydrogenase (13).

\[
\text{l}-\text{Alanine} + \text{NAD} \rightarrow \text{alanine dehydrogenase} \rightarrow \text{pyruvate} + \text{NADH} + \text{NH}_3 \quad (6)
\]

The enzyme appears to function catabolically, is induced by d-alanine, and is not required for ammonia assimilation (13). l-Alanine dehydrogenase thus should itself be under rigorous control to prevent the coupling of Reactions 4 and 5, which would result in a "futile cycle" of amino acid synthesis and degradation leading to the depletion of cellular ATP stores.

In addition to regulation by alanine and glycine, glutamine synthetase from *B. subtilis* is also inhibited by its product, glutamine, and several biosynthetic end products: AMP, CTP, thymoproph, and histidine (3), all of which contain nitrogen derived from the amide group of glutamine. Inhibition by biosynthetic end products is a common property of glutamine synthetase from *E. coli* (14, 14), rat liver (16, 17), as well as other sources.

The inhibition by l-glutamine of glutamine synthetase is common to the enzyme from *Bacilli* and the enzyme derived from rat liver and rat kidney (18). This product inhibition by l-glutamine provides a greatly simplified mechanism to regulate over-all glutamine synthesis when compared to the mechanisms in *E. coli* (19, 20), which involve a complex cascade of enzymes catalyzing the adenylylation and deadenylation of preformed glutamine synthetase. The two types of regulation appear to achieve somewhat similar results (Fig. 11). Inhibition of *B. subtilis* glutamine synthetase by L-glutamine is marked in the Mg⁴⁺-supported reaction, but is minimal when the reaction depends on Mn⁴⁺. In *E. coli*, L-glutamine stimulates adenylylation of glutamine synthetase which results in an inhibition of Mg⁴⁺-dependent activity, but in an enhancement of Mn⁴⁺-dependent activity (19). Adenylation of glutamine synthetase in *Bacilli* has not been observed (7, 21). In mammals, direct inhibition of glutamine synthetase by L-glutamine is also observed (17, 18); glutamine added to media of cells in culture results in a profound decrease in glutamine synthetase activity (22, 23). The molecular basis of this change is not known. These results emphasize the very central role that glutamine plays in controlling its own synthesis.
The inhibition of glutamine synthetase from B. subtilis by L-glutamine also renders the enzyme more susceptible to inhibition by AMP when the reaction is supported by Mn²⁺. This synergistic inhibition by glutamine and AMP is similar to that observed with glutamine synthetase from B. licheniformis (5). An analogous phenomenon is observed in E. coli, in which glutamine-stimulated adenyllylation also increases the sensitivity of glutamine synthetase to AMP inhibition as demonstrated in Fig. 11 (24).

The synergistic inhibition of glutamine synthetase from B. subtilis by AMP with histidine or L-glutamine suggests that AMP also may have a special function in the regulation of glutamine synthetase (Fig. 11). In addition to its role as a biosynthetic end product, AMP may also serve as an indicator of cellular energy metabolism in the regulation of glutamine synthetase. This suggestion is strengthened by the observation of reciprocal regulation of glutamine synthetase and glutaminase B observed in E. coli. In this organism, AMP inhibits glutamine synthetase and slightly activates glutaminase B, and ATP is a substrate for glutamine synthetase but is an inhibitor of glutaminase B (25).

The level of inhibitor which is required to reduce catalytic activity significantly in vitro may exceed the physiological levels that inhibitors achieve in vivo (e.g. glycine or histidine, Mn⁺⁺ assay). In instances when the inhibitor is partially competitive with respect to one or more substrates, physiological levels of inhibitor are more likely to reduce activity substantially. Because of the relatively high apparent $K_a$ for glutamate (5 mM, Mn⁺⁺ assay), competitive inhibitors with respect to this substrate would be favored as important in vivo mediators of activity. Also, the reciprocal potentiation that glutamine and AMP produce with respect to each other as inhibitors of glutamine synthetase reduce to physiological range the levels of these intermediates required for inhibition. Both AMP and glutamine are strong inhibitors at low levels when Mg⁺⁺ is substituted for Mn⁺⁺. The presence of distinct binding sites on glutamine synthetase-O-AMP refers to adenyllylated E. coli glutamine synthetase. The interconversion of adenyllylated and nonadenyllylated forms of E. coli glutamine synthetase is catalyzed by the E. coli glutamine synthetase adenyllylating and deadenyllylating enzymes, respectively.

As demonstrated in this paper, inhibition of glutamine synthetase by metabolites is dramatically altered by the divalent cations Mg⁺⁺ and Mn⁺⁺. B. subtilis growing logarithmically in the presence of excess Mg⁺⁺ (1 mM) and 0.001 mM Mn⁺⁺ has a total intracellular concentration of 20 mM Mg⁺⁺ and 0.2 mM Mn⁺⁺. Thus, during vegetative growth the total free and bound magnesium is approximately 100 times higher than the total Mn⁺⁺. With the onset of sporulation, since glutamine, but not glutamate is thought to be a repressor of sporulation (28). In addition to modulating the inhibition by feedback inhibitors, Mg⁺⁺ and Mn⁺⁺ also control the activity of glutamine synthetase by forming a complex with the enzyme directly, and by ATP chelation (3, 7). It should be noted that the affinities of various ligands for these two divergent cations can differ considerably (29).

The precise mechanisms regulating the complex response of B. subtilis glutamine synthetase to Mn⁺⁺ and Mg⁺⁺, L-glutamate, and L-glutamine are not known. Glutamine inhibition in the Mn⁺⁺ reaction does not follow hyperbolic kinetics, and glutamine is minimally effective as an inhibitor even at very high concentrations. In contrast, partially alkylated glutamine synthetase is 70% inhibited by 20 mM glutamine (3, 30). The native enzyme in the Mn⁺⁺ reaction is very sensitive to glutamine inhibition when AMP is added. More than 55% reduction of catalytic activity is produced by the addition of 5 mM glutamine to an assay already containing 2.5 mM AMP, whereas in the absence of AMP, 5 mM glutamine is not inhibitory at all. When Mg⁺⁺ replaces Mn⁺⁺, glutamine is a very potent inhibitor of B. subtilis glutamine synthetase. These results are summarized in Table II, and may be explained by the presence of a separate regulatory site for L-glutamine. The data allow no firm basis for distinguishing this possibility however from the possibility that L-glutamine mediates a protein conformational charge altering the active site of B. subtilis glutamine synthetase.

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**Table II**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Native enzyme</th>
<th>Alkylated enzyme (31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn⁺⁺ Inhibition by L-glutamine (5 mM)</td>
<td>None</td>
<td>%</td>
</tr>
<tr>
<td>Inhibition by L-glutamine (20 mM)</td>
<td>2</td>
<td>70</td>
</tr>
<tr>
<td>Inhibition by L-glutamine (5 mM) in presence of 2.5 mM AMP</td>
<td>85</td>
<td>Not studied</td>
</tr>
<tr>
<td>Mg⁺⁺ Inhibition by L-glutamine (5 mM)</td>
<td>98</td>
<td>Not studied</td>
</tr>
</tbody>
</table>

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B. subtilis glutamine synthetase for glycine, alanine, histidine, tryptophan, AMP, and glutamine is suggested by these studies. Further investigation is required to establish this point and precisely define the physiological role each inhibitor plays in the nitrogen metabolism of B. subtilis.
Indeed, if allosteric inhibition of glutamine synthetase by the reaction product L-glutamine does occur, it is not without precedent. Rat brain hexokinase is allosterically inhibited by its product, glucose 6-phosphate (31), while phosphofructokinase from sheep heart is allosterically inhibited by its substrate, ATP (32). Glutaminase B from E. coli appears to be allosterically activated by its product L-glutamate (25), and the inhibition of uridine diphosphoglucose pyrophosphorylase by its product, uridine diphosphoglucose, may be allosteric (33, 34). Many chemical reactions which are freely reversible thermodynamically, but unidirectional enzymatically, probably also involve allosteric inhibition of the enzyme by its product (35).

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