SPECIFIC CATALYTIC CHANGES ASSOCIATED WITH LIMITED SULFHYDRYL MODIFICATION

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SUMMARY

Bacillus subtilis glutamine synthetase readily reacts with iodoacetamide, producing very specific changes in the catalytic parameters characteristic of the native enzyme. The alkylation reaction is influenced both by substrates and feedback inhibitors of the enzyme; whereas ammonia and glutamate are without effect, Mg\(^{2+}\)-ATP enhances the alkylation reaction, and Mn\(^{2+}\)-ATP effectively prevents the catalytic changes that accompany incubation of the enzyme with the alkylating agent. Histidine, tryptophan, and glutamine also potentiate the response of the enzyme to iodoacetamide. With limited alkylation, a marked increase in activity is seen using Mn\(^{2+}\) as divalent cation, a sharp reduction in specific activity is seen in the assay using Mg\(^{2+}\) as divalent cation, and the response of the enzyme to feedback inhibitors is altered. Whereas these changes in B. subtilis catalytic activity are qualitatively similar to changes seen with enzymatic adenylylation of Escherichia coli glutamine synthetase, no role in vivo for sulfhydryl modification of B. subtilis enzyme has been found. When assayed with Mn\(^{2+}\) as divalent cation, alkylated glutamine synthetase has a higher apparent \(K_a\) for glutamate than does native enzyme; the substrate saturation function with the alkylated enzyme is hyperbolic and has lost the inhibition by high concentrations of glutamate, which is characteristic of the native enzyme. When assayed with Mg\(^{2+}\) as divalent cation, the alkylated enzyme shows loss of apparent affinity for glutamate at high glutamate concentrations. Sulfhydryl titration indicates that 1 cysteine residue is readily alkylated per subunit, with two to three additional groups titrated after dissociation of the enzyme. The alkylation reaction is specific for cysteine residues. These results thus provide by chemical modification of the B. subtilis glutamine synthetase a system that is qualitatively analogous to the enzymatic adenylylation of the E. coli glutamine synthetase; the results also exhibit a clear structural distinction between the two enzymes. Under similar conditions of study, the sulfhydryl groups of the B. subtilis enzyme are readily titrated by iodoacetamide, whereas those of E. coli glutamine synthetase are not accessible to the alkylating agent.

During the purification of Bacillus subtilis glutamine synthetase, it was noted that Mg\(^{2+}\)-activated catalytic activity was less stable than Mn\(^{2+}\)-activated activity. It was also noted that the Mg\(^{2+}\)-dependent activity could be largely stabilized by the addition of mercaptans and ethylenediaminetetraacetate to the buffer used for enzyme purification and storage. Further interest in these divalent cation specific effects was prompted by two considerations: (a) in Escherichia coli, loss of Mg\(^{2+}\)-dependent catalytic activity is provoked by enzyme-catalyzed adenylylation of glutamine synthetase, in response to nitrogen availability for cell growth, and this loss is coupled with an increase in Mn\(^{2+}\)-dependent activity; and (b) B. subtilis glutamine synthetase behaves very differently catalytically, depending upon whether Mn\(^{2+}\) or Mg\(^{2+}\) activates the enzyme during assay. That sulfhydryl groups might be of critical importance for catalytic activity of the enzyme was suggested by the protective effects of mercaptans during purification and subsequent storage of the enzyme. This paper presents data to demonstrate that modification of sulfhydryl groups of the enzyme alters catalytic activity in a very selective manner, which may be influenced by either substrate or feedback inhibitors of the enzyme, and provides by chemical modification of the B. subtilis glutamine synthetase a system analogous to enzymatic adenylylation of the E. coli glutamine synthetase.

METHODS AND MATERIALS

Iodoacetamide was purchased from Amersham-Searle, Des Plaines, Illinois, and was found to be of about 95% purity when assayed by thin layer chromatography in chloroform-methanol-water (30:35:13 (v/v/v)), as described by Shapiro and Stadtman (4). Iodoacetate, iodoacetamide, and p-chloromercuribenzoate were purchased from Calbiochem. p-Chloromercuribenzenesulfonate was obtained from Sigma. The organic mercurials were standardized by titration against glutathione (Nutritional Biochemicals) before use. N-Acetylthioimidazole was obtained from K and K Laboratories, Plainview, New York, and acetic anhydride was obtained from Allied Chemical Company. All chemicals were of the highest grade commercially available. Glutamine synthetase was purified from B. subtilis grown on glucose and limiting ammonia, and the purified preparations were stored at 60 mg per ml at 4° in 50 mM imidazole chloride (pH 7.0) and 1 mM EDTA. The enzyme was judged to be >95% pure by acrylamide gel electrophoresis, sedimentation velocity and equilibrium measurements, and electron microscopy.
The specific activity (Mg\(^{2+}\)-activated system) of the purified preparations was 11 to 13 \(\mu\)g of inorganic phosphate formed per min per mg of protein (25\(^\circ\)). Slight losses in activity were noted with prolonged storage at 4\(^\circ\). Protein was assayed by a modification of the biuret method (5), unless otherwise noted. Assays were performed using either Mn\(^{2+}\) (7.5 mM) or Mg\(^{2+}\) (50 mM) as divalent cation, in a solution containing 50 mM imidazole chloride (pH 7.1), 7.5 mM ATP, 100 mM L-glutamate, 50 mM NH\(_4\)Cl, and sufficient enzyme to produce 0.20 \(\mu\)g of phosphate over the assay period, at 25\(^\circ\), as adapted from the procedure of Boyer, Mills, and Fromm (6). Reactions with native and alkylated glutamine synthetase (see below) were linear with time and enzyme concentration; no lag time was seen with any of these studies. Alkylated glutamine synthetase was prepared by incubation of the purified enzyme at a concentration of 5 mg per ml for 30 min at 25\(^\circ\), in a reaction mixture containing 100 mM iodoacetamide, 100 mM Tris buffer (pH 9.0), and 4 mM MnCl\(_2\). The reaction was stopped by the addition of an equal volume of cold 2-mercaptoethanol (100 mM), and the protein was equilibrated with 50 mM imidazole buffer (pH 7.0), 0.5 mM EDTA, and 0.5 mM 2-mercaptoethanol by passage over a Sephadex G-75 column previously equilibrated with this buffer. Subsequent studies indicated that the Mn\(^{2+}\) present during the alkylation reaction was not required; Mg\(^{2+}\) (4 mM) or EDTA (\(5 \times 10^{-3}\) M) present during alkylation did not alter the catalytic properties described below for the alkylated enzyme. Control preparations, incubated as above, but without iodoacetamide, did not change activity over the course of these experiments. Sulphydral titrations with \(p\)-chloromercuribenzoate were performed with a modification of the technique of Boyer (7). Radioactive determinations were performed in a Nuclear-Chicago liquid scintillation counter, using Bray’s solution, with appropriate corrections for quenching.

**RESULTS**

Reaction of Glutamine Synthetase with Iodoacetamide—Figs. 1 and 2 illustrate the changes in catalytic activity of *B. subtilis* glutamine synthetase that occur after it is incubated with iodoacetamide for various periods of time prior to assay. It is evident that iodoacetamide differentially affects the Mn\(^{2+}\)- and Mg\(^{2+}\)-dependent activities and that these effects are influenced both by the iodoacetamide and the protein concentrations. At 2 mg per ml of protein, incubation with 100 mM iodoacetamide results in loss of both Mn\(^{2+}\)- and Mg\(^{2+}\)-dependent activities and that these effects are influenced both by the iodoacetamide and the protein concentrations. At 2 mg per ml of protein, incubation with 100 mM iodoacetamide results in loss of both Mn\(^{2+}\)- and Mg\(^{2+}\)-dependent activities; however, the loss of Mn\(^{2+}\)-activity is considerably less than that of Mg\(^{2+}\)-activity. Both changes are time-dependent and are not reversed by subsequent incubation with either 2-mercaptoethanol or dithiothreitol. With higher concentrations of protein (5 or 20 mg per ml), incubation with 100 mM iodoacetamide leads to marked stimulation of the Mn\(^{2+}\)-supported activity, in contrast to inhibition of Mg\(^{2+}\)-supported activity. Inhibition of the Mg\(^{2+}\)-activity is an inverse function of the protein concentration (Fig. 1). Incubation of glutamine synthetase with even higher concentrations of iodoacetamide (200 mM) than shown here results in complete loss of activity as measured in both Mn\(^{2+}\) and Mg\(^{2+}\) systems; prolonged incubation of the enzyme with the alkylation agent (50 mM) exhibits loss of the enhanced Mn\(^{2+}\)-activated activity, continued loss of the Mg\(^{2+}\)-activity, and, eventually, complete inactivation of catalytic activity as measured in this system. The optimum conditions for alkylation of the protein to produce the catalytic changes described are thus dependent upon iodoacetamide concentration and protein concentration over the 10-fold range of protein studied. Although the alkylation reaction proceeds equally well with either Mg\(^{2+}\) or EDTA (see “Methods and Materials”) substituted for the 4 mM Mn\(^{2+}\), a role of bound metal in the exposure of protein sulphydral groups is evident; extensive dialysis of the enzyme against
50 mM imidazole (pH 7.0), 0.1 mM mercaptoethanol, and 1 mM EDTA results in a protein which is considerably more sensitive to the alkylation agent than is the native enzyme stored as described (see "Methods and Materials"). The alkylation of glutamine synthetase thus clearly provides a method of differentiating the activities as measured with Mn2+ and Mg2+.

**Influence of Substrates and Effectors on Alkylation Reaction**—Fig. 3 presents data revealing the effects of substrates in modifying the response of the enzyme to alkylation by iodoacetamide. The presence of either ammonia or glutamate during the prior incubation with iodoacetamide does not alter the response of the enzyme to alkylation. However, addition of Mn2+-ATP (7.5 mM) to the incubation mixture almost completely prevents the catalytic changes that accompany alkylation of the protein. In contrast, 30 mM Mg2+-7.5 mM ATP appears to facilitate the effects of incubation of the enzyme with iodoacetamide; parallel effects are seen in both the Mn2+ and Mg2+-catalyzed systems.

As noted earlier (8), B. subtilis glutamine synthetase is inhibited by various end products of glutamine metabolism. The ability of various feedback inhibitors to modify the response of the B. subtilis glutamine synthetase to alkylation with iodoacetamide is shown in Table I. When they are present during the incubation with iodoacetamide, alanine, AMP, and glycine are without effect; however, histidine, tryptophan, and glutamine enhance the response of the enzyme to alkylation.

**Effect of Alkylation on Kinetic Parameters**—Alkylation with iodoacetamide has no demonstrable effect on the relationship between reaction velocity and the concentration of the substrates ammonia and Mn2+-ATP. However, as shown in Fig. 4, the substrate saturation function for glutamate (Mn2+ assay) is significantly altered by alkylation. The glutamate saturation curve for the native enzyme exhibits inhibition of activity at high concentrations of glutamate, and an apparent Km of 1 mM is determined from the double reciprocal plot, in good agreement with previous results (9). With the iodoacetamide-modified enzyme, the kinetics are hyperbolic; no inhibition of activity is seen at high substrate concentrations, and the apparent Km is 5 × 10−3 M. Fig. 5 illustrates the substrate saturation functions for glutamate, using Mg2+ as divergent cation; significant differences between the native and alkylated enzymes are shown.

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With the native enzyme, no apparent saturation of the enzyme is seen at 100 mM glutamate, and the double reciprocal plot is concave downward. Study of the alkylated enzyme shows a hyperbolic substrate saturation curve, and the apparent Km for glutamate is ≈33 mM. The enhanced activity of the alkylated enzyme in the Mn2+-activated system can be entirely explained by the loss of inhibition at high glutamate concentrations characteristic of the native enzyme. With Mg2+ as divergent cation, the loss in activity seen with the alkylated enzyme correlates directly with the apparently lowered affinity of the enzyme for glutamate at high glutamate concentrations, rendering 100 mM glutamate markedly subsaturating for this system.

**Response of Alkylated Enzyme to Feedback Inhibitors**—Fig. 6 illustrates the results of studies comparing the activity of native and iodoacetamide-treated enzyme in the presence of feedback inhibitors. Differences in the susceptibilities of native and alkylated glutamine synthetase to inhibition were observed with all inhibitors studied; these differences are most pronounced with AMP, alanine, glycine, and glutamine. When the E. coli glutamine synthetase is enzymatically adenylated, in response to excess ammonia for cellular growth, there is a marked drop in activity as assayed in the Mg2+-activated system, a marked increase in activity as measured in the Mn2+ system, and an increased sensitivity to feedback inhibitors (2). The catalytic changes in the biosynthetic assay illustrated here for the B.
**Sulfhydryl Modification of B. subtilis Glutamine Synthetase**

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**Fig. 4.** Glutamate substrate saturation curves with double reciprocal plots for native and iodoacetamide-treated B. subtilis glutamine synthetase in Mn2+-activated assay. The alkylated protein was prepared as described under "Methods and Materials." Either native or alkylated purified glutamine synthetase (5 μg) was assayed using only Mn2+ as divalent cation and varying glutamate concentrations as shown. Velocity is expressed as Klett units (100 Klett units equal 130 μM Pi released per assay volume).

**Fig. 5.** Glutamate substrate saturation curves with double reciprocal plots for native and iodoacetamide-treated B. subtilis glutamine synthetase in the Mg2+ assay. The alkylated protein was prepared as described under "Methods and Materials." Native and alkylated purified glutamine synthetase (3.5 μg and 7.0 μg, respectively) were assayed using only Mg2+ as divalent cation and varying glutamate concentrations as shown. Velocity is expressed as Klett units (100 Klett units equal 130 μM Pi released per assay volume).

*B. subtilis* glutamine synthetase as a consequence of sulfhydryl modification mimics, therefore, the changes seen when the *E. coli* enzyme is enzymatically adenylylated.

**Effect of Other Sulfhydryl Reagents—**In view of the major changes in catalytic properties of the *B. subtilis* glutamine synthetase resulting from chemical modification of the enzyme with iodoacetamide, other compounds that modify sulfhydryl residues in proteins were tested for their effect on the catalytic properties of the enzyme. Fig. 7 illustrates the activity in both Mn2+- and Mg2+-activated reactions after incubation with p-chloromercuriphenylsulfonate (10^-6 M) for the times shown. The enzyme is sensitive to the mercurial reagent and responds in a different manner than to alkylation by iodoacetamide. Thus, a minimal increase in activity is seen in the Mn2+ assay, whereas inactivation in the Mg2+-catalyzed system is essentially complete in 15 min. Other compounds that may react with sulfhydryl groups were tested with *B. subtilis* glutamine synthetase; the results of these studies are shown in Table II. Alkylation with iodoacetate is qualitatively similar to alkylation with iodoacetamide, although the enzyme is less sensitive to iodoacetate at the concentration studied (100 μM). Little or no change is seen in the Mn2+-activated system after the enzyme is reacted with either N-acetylimidazole or acetic anhydride, but marked inhibition is exhibited in the Mn2+-activated system; these results are, therefore, qualitatively similar to those seen after the enzyme is reacted with p-chloromercuriphenylsulfonate. The response of the enzyme modified with these reagents to AMP inhibition was also measured in the Mn2+-activated system. In all cases, chemical modification results in increased sensitivity to AMP, even though little or no change in the Mn2+-catalyzed activity in the absence of the inhibitor can be shown, as revealed in Table II.

**Sucrose Gradient Analysis of Native and Alkylated Enzyme—**Sucrose gradient analysis (data not shown) of the native and iodoacetamide-treated enzyme shows no differences in the sedimentation properties of the native (S20,W = 19.3 S) (1) and alkylated enzyme. If the gradient is prepared with CMS (10^-6 M) or iodoacetamide (60 μM), the enzyme dissociates over the course of the sedimentation run into lower molecular weight material, and complete loss of catalytic activity results. These experiments indicate that gross dissociation of enzyme does not result from limited alkylation; however, prolonged exposure of the enzyme to either CMS or iodoacetamide disrupts the quaternary structure of the enzyme with dissociation of the protein into lower molecular weight species than the native enzyme.

**Number of Reactive Sulfhydryl Groups—**Table III presents...
**Table II**

**Effect of chemical modifiers on catalytic properties of B. subtilis glutamine synthetase**

B. subtilis glutamine synthetase was incubated with the compounds listed below and then assayed in the standard biosynthetic assay system, using either Mn\(^{2+}\) or Mg\(^{2+}\) as divalent cation. Results are expressed as percentage of activity relative to a control incubated in the absence of a chemical modifier. The activities were also tested in the presence of 20 mM AMP and are expressed as percentage of activity relative to the activity in the absence of inhibitor.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Activity</th>
<th>Activity with 20 mM AMP-Mn(^{2+}) assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mn(^{2+}) assay</td>
<td>Mg(^{2+}) assay</td>
</tr>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Iodoacetamide(^a)</td>
<td>220</td>
<td>25</td>
</tr>
<tr>
<td>Iodoacetate(^b)</td>
<td>108</td>
<td>36</td>
</tr>
<tr>
<td>Acetic anhydride(^c)</td>
<td>104</td>
<td>34</td>
</tr>
<tr>
<td>N-Acetylimidazole(^d)</td>
<td>101</td>
<td>16</td>
</tr>
<tr>
<td>CMS(^e)</td>
<td>115</td>
<td>8</td>
</tr>
</tbody>
</table>

- Purified glutamine synthetase was incubated at 2 mg per ml in 100 mM Tris (pH 9.0), 100 mM iodoacetamide, and 4 mM MnCl\(_2\) at 25\(^\circ\) for 15 min before assay.
- Purified glutamine synthetase was incubated at 1 mg per ml in 100 mM Tris (pH 9.0), 100 mM iodoacetate, and 4 mM MnCl\(_2\) at 25\(^\circ\) for 60 min before assay.
- Purified glutamine synthetase was incubated at 1 mg per ml with 100 mM dimethylglutarate buffer (pH 7.6) and 14 mM acetic anhydride at 4\(^\circ\) for 15 min before assay.
- Purified glutamine synthetase was incubated at 1 mg per ml in 100 mM Tris buffer (pH 8.0) with 100 mM N-acetylimidazole for 15 min at 25\(^\circ\) before assay.
- Purified glutamine synthetase was incubated at 2 mg per ml for 15 min at 4\(^\circ\) with 10\(^{-4}\) M CMS in 100 mM Tris buffer (pH 8.0). Assay was performed as above.

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1. Reaction of the protein with p-chloromercuribenzoate in 1\% sodium dodecyl sulfate, conditions fully dissociating the protein (1), leads to the titration of 3.3 sulfhydryl residues, whereas reaction of the dissociated enzyme with \(^{14}\)C-iodoacetamide leads to alkylation of 3.8 sulfhydryl groups/50,000 g of protein. Therefore, it is probable that between 3 and 4 cysteine residues of the dissociated subunits react with the sulfhydryl reagents. When the enzyme is alkylated under conditions that produce maximal changes in the Mn\(^{2+}\) and Mg\(^{2+}\) activity, treatment of the native (undissociated) enzyme with \(^{14}\)C-iodoacetamide leads to alkylation of 1.1 sulfhydryl groups/50,000 molecular weight. Moreover, when the enzyme is similarly alkylated, using unlabeled iodoacetamide (see "Methods and Materials"), and is subsequently dissociated with 1\% sodium dodecyl sulfate, the dissociated enzyme contains 0.9 fewer titratable groups than were found with dissociated enzyme titrated without prior treatment with iodoacetamide. It is concluded that the catalytic changes described herein (Figs. 1 and 2) are produced by the alkylation of a single sulfhydryl group per subunit and that two to three other sulfhydryl groups are relatively inaccessible to the reagent and are titrated only upon dissociation of the protein. These results thus illustrate another important difference between the glutamine synthetases from E. coli and B. subtilis, in that with the E. coli enzyme, under conditions that readily modify B.
Sulphydryl groups of B. subtilis glutamine synthetase

<table>
<thead>
<tr>
<th>Method of determination</th>
<th>Sulphydryl groups per 40,000 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acid analysis*</td>
<td>3.6</td>
</tr>
<tr>
<td>Reaction with 14C-iodoacetamide</td>
<td>3.8</td>
</tr>
<tr>
<td>Native enzyme*</td>
<td></td>
</tr>
<tr>
<td>Disaggregated enzyme (1% SDS)*</td>
<td>3.2</td>
</tr>
<tr>
<td>Reaction with CMB⁴</td>
<td></td>
</tr>
<tr>
<td>Disaggregated enzyme (1% SDS)</td>
<td>3.3</td>
</tr>
<tr>
<td>Treated enzyme,‡ disaggregated (1% SDS)</td>
<td>2.4</td>
</tr>
</tbody>
</table>

* The amino acid analysis was performed as described previously (1). The value given is uncorrected for loss during hydrolysis.

† Purified glutamine synthetase at a concentration of 2 mg per ml was incubated with 14C-iodoacetamide at a concentration of 50 mM in 100 mM Tris buffer (pH 9.0) and 4 mM MnCl₂. The reaction, at 25° for 30 min, was stopped with 1 x HCl in 50% acetone (8). The precipitate was washed 3 times and lyophilized. The lyophilized protein was then dissolved in 0.1% NaOH for scintillation counting. The reaction conditions were selected to determine sulphydryl groups alkylated, giving stimulation of Mn⁺⁺ activity to ~230% of nonalkylated control and inhibition of Mg⁺⁺ activity to ~20% of nonalkylated control.

‡ Purified glutamine synthetase at a concentration of 2 mg per ml was incubated with 50 mM 14C-iodoacetamide, 100 mM Tris (pH 6.0), 4 mM MnCl₂, and 1% SDS, after incubation of the enzyme at 3° for 30 min in 1% SDS. The incubation at 25° for 2 hours was stopped as described in footnote b and the 14C-iodoacetamide incorporated determined as described in footnote b.

§ Described under "Methods and Materials."

¶ "Treated enzyme" refers to alkylated glutamine synthetase.

The enzyme was treated with unlabeled iodoacetamide under conditions described in footnote b. The enzyme was treated as described in footnote b and the 14C-iodoacetamide incorporated determined as described in footnote b.

**The specificity of reaction with iodoacetamide—To prove that treatment with iodoacetamide results in modification of cysteine residues, 14C-iodoacetamide-treated enzyme was subjected to amino acid analysis and radioactive counting of the eluted peaks. The enzyme was subjected to two conditions of 14C-iodoacetamide treatment: (a) limited alkylation, resulting in maximum stimulation of the Mn⁺⁺ activity and depression of the activity of the Mg⁺⁺ system, and (b) alkylation of the dissociated enzyme (1% sodium dodecyl sulfate), as described in Table III. In the first instance, more than 99% of the radioactivity incorporated is found as alkylated cysteine, and in the second instance, more than 95%. In both cases, the remaining activity is scattered in several peaks.

The specificity of the reactions with p-chloromercuriphenylsulfonyl, iodoacetate, N-acetylimidazole, and acetic anhydride has not, however, been directly tested.

**Discussion**

Glutamine synthetase isolated from B. subtilis has a molecular weight of 600,000 and is composed of 12 subunits with molecular weights of 50,000 (1). The data presented in this paper suggest that there are between 3 and 4 sulphydryl residues per subunit, one of which titrates readily with iodoacetamide to give characteristic changes in the catalytic properties of the enzyme. Although the E. coli glutamine synthetase is structurally very similar to B. subtilis glutamine synthetase with regard to both molecular weight and subunit organization, native E. coli glutamine synthetase does not react with iodoacetamide unless the conformation of the enzyme is altered (2, 4). Sulphydrylreactivity thus shows a clear difference between the two enzymes.

The catalytic changes caused by limited alkylation of B. subtilis glutamine synthetase (i.e., an increase in the Mn⁺⁺ activity, a decrease in the Mg⁺⁺ activity, and altered reactivity to feedback inhibitors) are qualitatively very similar to changes in E. coli glutamine synthetase as it is enzymatically deaminylated in response to excess ammonia available to the cell for growth (2). Whereas sulphydryl modification offers to B. subtilis an attractive active mechanism for regulation of glutamine synthetase, no direct evidence has been obtained that regulation in vivo is achieved by this mechanism.

The increased Mn⁺⁺-catalyzed activity and apparent decreased Mg⁺⁺ activity seen with limited alkylation of the native enzyme provide a convenient means to separate these two activities. The Mn-ATP and Mg-ATP functions are also separated by the opposite effects each has on the time course of alkylation by iodoacetamide. The change in the substrate saturation functions for glutamate satisfactorily accounts for the increase in activity associated with the Mn⁺⁺-activated reaction, as well as for the decrease seen in the Mg⁺⁺-activated reaction. These changes are characteristic with separate glutamate sites on the enzyme: one, an active site with different Kₘ values for glutamate, depending upon whether Mn⁺⁺ or Mg⁺⁺ is the activating cation, and the second, a regulatory site, which at high glutamate concentrations is inhibitory in the system catalyzed by Mn⁺⁺ and lowers the apparent Kₘ in the Mg⁺⁺ system. Separate sites may also exist for Mn⁺⁺ and Mg⁺⁺, and the differing effects seen with these divalent cations may be related to different metal interactions at a single active site. The Mn⁺⁺ and Mg⁺⁺ activities are not additive. A conformational change accompanying the reaction of iodoacetamide with the protein sulphydryl groups could also account for most if not all of the changes noted. In addition, the binding of three inhibitors, tryptophan, histidine, and glutamine, can be distinguished by the enhancement each provides to the alkylation reaction with iodoacetamide. These experiments thus show that both substrates for the B. subtilis glutamine synthetase, as well as effectors of the catalytic activity, influence sulphydryl reactivity toward the alkylating reagent iodoacetamide.

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formed the amino acid analyses and concomitant radioactivity measurements of the ¹³C-iodoacetamide-treated enzyme. Messrs. Henry Lutterlough and Maurice Miles were responsible for large scale bacterial growths.

REFERENCES

Bacillus subtilis Glutamine Synthetase: SPECIFIC CATALYTIC CHANGES ASSOCIATED WITH LIMITED SULFHYDRYL MODIFICATION
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