Methyl Methanethiosulfonate as an Active Site Probe of Serine Hydroxymethyltransferase*

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Using methyl methanethiosulfonate and other sulhydryl group modification reagents we have studied the structure and function of sulhydryl groups in rabbit liver cytosolic serine hydroxymethyltransferase. From a tryptic digest of the enzyme, seven cysteine-containing peptides were isolated and sequenced. These peptides contained a total of 8 cysteine residues. There are no disulfide bonds in this enzyme. Of the eight sulhydryl groups, four react with methyl methanethiosulfonate. Two sulhydryl groups react rapidly with this reagent without altering enzyme catalytic activity. The remaining two sulhydryl groups react more slowly and cause loss of greater than 90% of the catalytic activity of the enzyme. This nearly inactive enzyme contains pyridoxal-P and can form an enzyme-substrate complex. However, the complex dissociates from the active site suggesting that one possible role for a sulhydryl group is to stabilize the enzyme-substrate complex.

The sequence of the cysteine-containing peptide which is responsible for the mechanism-based inactivation of serine hydroxymethyltransferase by p-3-fluoroalanine was determined. This sulhydryl group was shown not to be essential to the enzyme for catalytic activity. Also, the sequence of one of the cysteine peptides shows considerable homology to the active site cysteine peptide from tryptophan synthase.

Serine hydroxymethyltransferase (EC 2.1.2.1) catalyzes the conversion of serine to glycine and 5,10-methylenetetrahydrofolate (1). This reaction is usually considered to be one of the primary sources of one-carbon groups required for a variety of biosynthetic pathways in the cell (1). Both cytosolic and mitochondrial forms of the enzyme have been purified to homogeneity from rabbit liver (2). The role of pyridoxal-P and tetrahydrofolate in the mechanism of the cytosolic enzyme has been extensively studied (3). However, little is known about what amino acid residues form the active site pocket and what role these residues play in the mechanism of this enzyme. We have previously published evidence that there is at least one sulphydryl group at the active site (4). The work reported here extends this previously published data on the number and role of cysteine residues of the rabbit liver cysteine enzyme.

We first observed that the reagent DTNB reacted rapidly with two sulphydryl groups on the enzyme (5). This reaction leads to the release of the pyridoxal-P and complete loss of catalytic activity. We later showed that removal of pyridoxal-P from the active site uncovers a single sulphydryl group which reacts rapidly with iodoacetate. A chymotryptic dodecapeptide containing the carboxymethylcysteine residue was isolated and sequenced (4). One problem with these studies was that both the DTNB and iodoacetate-inactivated enzymes do not bind pyridoxal-P. This makes it impossible to probe the possible function of the sulphydryl group in the binding of the amino acid substrates or the mechanism of the enzyme. We were also unable to determine if the sulphydryl group which reacted with DTNB in the holoenzyme was the same one which reacted with iodoacetate in the apoenzyme.

Our interest in the role of sulphydryl groups was heightened by the report that p-3-fluoroalanine is a mechanism-based inactivator of this enzyme (6). Wang et al. (6) have shown that the inactivation is due to a sulphydryl group reacting with an enzyme-bound aminoacrylate intermediate. This suggests that a sulphydryl group is located at the subsite for binding the amino acid substrate and implicates a mechanistic role for this group.

Our continued interest in the chemistry of sulphydryl groups on serine hydroxymethyltransferase is also due to the success of other investigators in being able to attach reported molecules such as 13C, 15N, and spin labels, on sulphydryl groups at or near the active site of enzymes. These reporter groups have served as useful tools for determining the pK values of adjacent groups at the active site (7-11).

The work reported in this paper determines the number and structure of all the cysteine-containing tryptic peptides of cytosolic serine hydroxymethyltransferase. The primary sulphydryl-modifying reagent we have used is methyl methanethiosulfonate. This reagent reacts rapidly with sulphydryl groups to form methyl disulfides which introduces a relatively large noncharged group at each exposed cysteine residue (11-13). With the use of this and other reagents, we have demonstrated that there are at least two sulphydryl groups at the active site but only one may be critical for catalytic activity. This critical sulphydryl group is not the one involved in the p-3-fluoroalanine inactivation. Our understanding of the chemical reactivity of the sulphydryl groups on this enzyme with methyl methanethiosulfonate will permit additional studies on the active site of this enzyme.

**EXPERIMENTAL PROCEDURES**

The following chemicals were purchased from Sigma: L-serine, D-alanine, glycine, Hfolate, pyridoxal-P, NADH, NADP+, alcohol dehydrogenase, iodoacetate, methyl methanethiosulfonate, sodium acid; HPLC, high performance liquid chromatography; Hfolate, tetrahydrofolate.
periodate, and DTNB. [1-14C]Iodoacetate acid, 16 mCi/mmol, was obtained from New England Nuclear. The enzyme formylmethenyltetrahydrofolate synthetase (combined) was purified from rabbit liver as previously described (14). 

Cytosolic serine hydroxymethyltransferase was purified and crystallized by the method of Schirch and Peterson (2). Aporosine hydroxymethyltransferase was prepared by transamination of the pyridoxal-P with L-alanine to form pyridoxamine-P and pyruvate (15). The pyridoxamine-P was removed from the apoenzyme by gel filtration.

Two methods were used to measure serine hydroxymethyltransferase activity in this study. The principal assay utilizes allothreonine as substrate. The product acetaldehyde was continuously measured as the decrease in absorbance at 340 nm upon reduction with NADH and alcohol dehydrogenase (16). This assay does not require H$_2$folate as a cosubstrate. When needed to monitor the effect of sulfhydryl reagents on H$_2$folate function, we utilized L-serine and tetrahydrofolate as substrates. The production of the product 5,10-methylene tetrahydrofolate was continuously monitored at 340 nm upon oxidation with 5,10-methylenetetrahydrofolate with NADP$^+$ and formylmethenyltetrahydrofolate synthetase (14).

Methylmethanethiosulfonate was dissolved in absolute ethanol before each experiment. The concentration of this stock solution was determined by the decrease in absorbance at 260 nm upon reduction with NADPH. Five micromolar concentrations of NADPH were added to a 1 mg/ml solution. The buffer used for all studies was 0.1 M sodium bicarbonate, pH 7.0.

The presence of disulfides in the protein was determined by the procedure described in the previous paragraph. The solutions containing 5 to 10 mg of enzyme were made 5.5 M in guanidine hydrochloride and adjusted to pH 2.85 with phosphoric acid, in the reservoir. Fractions of 0.1 mM pyridoxal-P to the assay restores the reaction to near linearity with time. The pyridoxal-P in these partially purified peptides contained carboxymethylcysteine residues (0.5-1.0 nmol) were hydrolyzed in sealed, evacuated tubes with constant boiling HCl for 24 h at 110 °C. Amino acid analyses were then determined using a Durrum M6F amino acid analyzer.

The remaining two peptides were rechromatographed on the same HPLC system and by taking smaller fractions these peptides were also obtained free of contaminants.

Aliquots of the purified peptides containing carboxymethylcysteine residues (0.5-1.0 nmol) were hydrolyzed in sealed, evacuated tubes with constant boiling HCl for 24 h at 110 °C. Amino acid analyses were then determined using a Durrum M6F amino acid analyzer. The sequences of the peptides were determined by the manual Edman degradation method of Tarr (30). Usually 2 nmol of peptide were used. The released phenylthiohydantoin amino acids were identified by HPLC on a Beckman phenylthiodantoin amino acid column or by amino acid analysis after hydrolysis with HCl. The presence of phenylthiodantoin Cys(Cm) was verified by counting an aliquot of those fractions which gave alanine after HCl hydrolysis.

Inactivation of serine hydroxymethyltransferase by 2,3-fluorol alanine was performed as follows. To several milligrams of enzyme in 0.1 mM sodium 2-[bis(2-hydroxyethyl)amino]ethanesulfonic acid buffer, pH 7.0, were added 5 mg of 2,3-fluorol alanine. A 10-μl aliquot was removed, and catalytic activity determined in the allothreonine assay containing 10 μM pyridoxal-P. The enzyme-d-3-fluorol alanine solution showed no loss of activity during a 10-min incubation at 30 °C. After 10 min, 20 μl of a 6 mM solution of tetrahydrofolate were added, and the enzymic activity determined at several intervals. The enzyme lost all activity during the next 20 min. The H$_2$folate stock solution was then prepared by dissolving 25 mg of tetrahydrofolate in 2.5 ml of 200 mM potassium phosphate, 50 mM ascorbate, pH 7.5. This solution showed the presence of some thiol compound as determined by reaction with DTNB. To remove this thiol from the stock tetrahydrofolate solution, DTNB was added to a final concentration of 1 mM.

RESULTS

Inhibition by Methyl Methanethiosulfonate—When a 10-fold excess of methylmethanethiosulfonate is added to serine hydroxymethyltransferase the enzyme loses activity over a 1-h period as shown in Fig. 1. Increasing concentrations of the reagent increase the rate at which activity is lost although the loss of activity is never described by a simple first order reaction. The rate of inhibition by methylmethanethiosulfonate is decreased in the presence of the amino acid substrates serine and glycine (Fig. 1). However, removal of pyridoxal-P increases the rate of inactivation (Fig. 1). The assay used to measure enzyme activity in this study was allothreonine which does not require tetrahydrofolate as a cosubstrate. When the study was repeated with L-serine and H$_2$folate as substrates, essentially identical curves with those recorded in Fig. 1 were obtained. The data in Fig. 1 suggest that critical sulfhydryl groups at the amino acid and pyridoxal-P binding sites are reacting with methyl methanethiosulfonate. There do not appear to be critical sulfhydryl groups at the H$_2$folate binding site which react uniquely with methyl methanethiosulfonate.

Methylmethanethiosulfonate not only inactivates serine hydroxymethyltransferase but it also produces an enzyme which gives nonlinear kinetics with time (Fig. 2). Inactivation studies were performed by removing 10-μl aliquots of an enzyme-methylmethanethiosulfonate solution and adding it to an allothreonine assay solution. The formation of the product acetaldehyde was monitored by measuring the decrease in absorbance at 340 nm due to the alcohol dehydrogenase-catalyzed oxidation of NADH. Normally this assay is linear with time until the NADH is depleted. As shown in Fig. 2, for aliquots of enzyme from a methyl methanethiosulfonate inactivation study, the rate of product formation decreases with time. This nonlinearity appears to be the result of the loss of pyridoxal-P from the enzyme during the assay. Addition of 0.1 mM pyridoxal-P to the assay restores the reaction to near linearity with time. The pyridoxal-P in these partially

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Fig. 1. Loss of catalytic activity of serine hydroxymethyltransferase in the presence of methyl methanethiosulfonate. x—x, reaction contained 0.2 mM serine hydroxymethyltransferase and 10 mM methyl methanethiosulfonate. At several time intervals, aliquots were removed and catalytic activity determined in an assay with allothreonine as substrate. □—□ and △—△, the study was repeated except 20 mM glycine (□) or L-serine (△) was added to the reaction solution. ○—○, the study as in x—x was repeated with the apoenzyme.

Fig. 2. Loss of linearity of serine hydroxymethyltransferase assay during inactivation by methyl methanethiosulfonate. The reaction conditions were the same as recorded for the holoenzyme in Fig. 1. The decrease in absorbance at 340 nm is due to the alcohol dehydrogenase-catalyzed reduction of the product acetaldehyde by NADH. The numbers on the curves are the minutes the enzyme had been preincubated with methyl methanethiosulfonate.

inactivated enzyme studies is lost from the active site only in the presence of the amino acid substrates. This suggests that modifying sulfhydryl groups with methyl methanethiosulfonate results in an enzyme in which the intermediates involving pyridoxal-P-substrate complexes are dissociating from the active site.

Number of Groups Modified by Methyl Methanethiosulfonate—In this experiment less than stoichiometric amounts of methyl methanethiosulfonate were added to 1 ml of a 9 mg/ml solution of enzyme. After incubation for 15 min at 23 °C a 100-μl aliquot of the reaction mixture was passed through a small Sephadex G-75 column to remove any unreacted modifying reagent. The enzyme was analyzed for both catalytic activity and the total number of free sulfhydryl groups. The procedure was repeated by the addition of more methyl methanethiosulfonate to the stock enzyme solution until the enzyme had lost all of its activity. With no modifying reagent, the enzyme was found to have 7.9 to 8.0 sulfhydryl groups/subunit. Fig. 3 shows that as one titrates the enzyme with methyl methanethiosulfonate, a total of four sulfhydryl groups are blocked in the holoenzyme. The data in Fig. 3 also show that the first two sulfhydryl groups that are blocked do not cause a loss of activity in the allothreonine assay. However, modifying the third and fourth sulfhydryl groups leads to a loss of 94% of the catalytic activity. The addition of more methyl methanethiosulfonate did not lead to any additional loss of free sulfhydryl groups.

This study was repeated using serine and H4folate as substrates to measure catalytic activity. As shown in Fig. 3, the same results were found as when allothreonine was used as substrate. This further confirms that the four sulfhydryl groups which are blocked by methyl methanesulfonate probably do not play an important role in H4folate binding or function.

Titration of sulfhydryl groups by methyl methanethiosulfonate was also performed on apoenzyme hydroxymethyltransferase. In the absence of pyridoxal-P, a total of five sulfhydryl groups are blocked with a complete loss of catalytic activity. The catalytically important sulfhydryl groups appear to react rapidly with methyl methanesulfonate since even the lowest concentration of reagent results in a loss of activity. This is consistent with the kinetics of inhibition for the apoenzyme recorded in Fig. 1.

When the modified and inactive enzyme is incubated with
Methyl methanethiosulfonate is an enzyme inactivator of rabbit liver serine hydroxymethyltransferase. It appears critical for catalytic activity, as blocked sulfhydryl groups from the active site are not inhibited by DTNB (Fig. 5). Inactivation by methyl methanethiosulfonate until 2.2 mM results in 80 to 90% loss of catalytic activity. We define this as the one catalytically important sulfhydryl group.

To determine the number and structure of the tryptic peptides containing cysteine residues, we labeled the enzyme in 5.5 M guanidine HCl with [1-14C]iodoacetate. After removal of the guanidine HCl, the carboxymethylated enzyme was digested with trypsin. About 200 µg of the trypsin digest were eluted from an HPLC C-18 reverse phase column with a phosphoric acid-acetonitrile gradient. The peptide elution profile monitored by absorbance at 215 nm is shown in Fig. 6B. Fractions, containing 1 ml of eluate, were collected and analyzed for radioactivity. Seven radioactive peaks containing carboxymethylcysteine were found as shown in Fig. 6A.
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We also attempted to determine which 4 of the cysteine residues are not blocked by methyl methanethiosulfonate in the holoenzyme. This was done by denaturing the enzyme with four blocked sulfhydryl groups in guanidine HCl and reacting it with [1-14C]iodoacetate. Tryptic peptides were isolated as before and the elution profile on the HPLC System determined. Only peptides 1 and 7 (Table I) had greatly reduced radioactivity. The fact that only two peptides are missing in the enzyme with four blocked sulfhydryl groups suggests that during the denaturation of the enzyme with guanidine HCl, some disulfide interchange took place.

Inhibition of Serine Hydroxymethyltransferase by Periodate—Stoichiometric concentrations of periodate have been shown to be a mild procedure for oxidizing vicinal sulfhydryl groups in enzymes (22). We tested the effect of a 4-fold excess of sodium periodate on the apoenzyme and holoenzyme. In each experiment the enzyme lost all activity in less than 1 min of incubation. Denaturation of the oxidized enzyme in sodium dodecyl sulfate and reaction with DTNB showed the presence of only four sulfhydryl groups. This suggests that periodate has formed two disulfide bonds. Addition of substrates or phosphate did not protect the enzyme from reaction with periodate. Addition of methyl methanethiosulfonate or DTNB to the periodate-oxidized enzyme did not result in a further decrease in the number of DTNB-reactive sulfhydryl groups. This suggests that the four sulfhydryl groups which react with periodate are the same four which react with methyl methanethiosulfonate.

The periodate-oxidized apoenzyme can be reactivated to about 90% activity on incubation for 30 min at 25 °C with 10 mM dithiothreitol. The spectrum of the periodate-oxidized holoenzyme shows an absorption maximum at 429 nm and is essentially identical with the spectrum of the enzyme inhibited with methyl methanethiosulfonate as recorded in Fig. 4.

DISCUSSION

Sulphhydryl groups have been implicated as being at or near the active site of the following pyridoxal-P enzyme. Glutamate decarboxylase (23), isoleucine dehydrogenase (24), lysine dehydrogenase (25), ornithine decarboxylase (26), cysteine sulfinate decarboxylase (27), tryptophan synthase (28), aspartate aminotransferase (29), L-alanine aminotransferase (30), and L-amino acid transaminase (31). Since all pyridoxal-P enzymes probably have similar intermediates it seems likely that a sulfhydryl group at the active site may have a similar function in these enzymes. However, the function of a sulfhydryl group in the mechanism of any pyridoxal-P enzyme has yet to be elucidated. One approach to determining if sulfhydryl groups are playing similar roles in pyridoxal-P enzymes is to isolate cysteine-containing peptides from the various enzymes and look for similarities in amino acid sequence. This approach has been done with the active site pyridoxal-P peptide from a wide variety of B6-enzymes. As we pointed out earlier the pyridoxyl peptide from rabbit liver cytosolic serine hydroxymethyltransferase shows a great deal of homology to the sequences of all bacterial decarboxylases and tryptophan synthase. Unfortunately, the only enzyme of this group to have the active site cysteine peptide sequenced is tryptophan synthase. The amino acid sequence around the active site cysteine in this enzyme is Ala-Leu-Thr-Lys-Cys-Gln-Asn...Lys (33). In peptide 6 from serine hydroxymethyltransferase we find the sequence Ala-Leu-Gly-Ser-Cys-Leu-Asa... where four of the seven amino acids are in the same location. This sequence is also present in mitochondrial serine hydroxymethyltransferase. Although peptide 6 (Table I), is not one

In order to purify the cysteine-containing peptides, the trypsin hydrolysate was first fractionated on a phosphocellulose column. Five fractions containing radioactivity from this column were placed on an HPLC C18 reverse phase column for isolation. The purified peptides corresponding to the reactive peptides in the HPLC elution profile as shown in Fig. 6A.

Table I

Sequence of cysteine-containing tryptic peptides of cytosolic serine hydroxymethyltransferase

The number of the peptide corresponds to the position of the radioactive peptides in the HPLC elution profile as shown in Fig. 6A.

| Peptide | Sequence |
|---------|----------|
| 1       | Gly-Cys(Cm)-Arg |
| 2       | Asn-Thr-Cys(Cm)-Pro-Gly-Asp-Lys |
| 3       | Gln-Val-Val-Ala-Asn-Cys(Cm)-Arg |
| 4       | Val-Leu-Glu-Ala-Cyst(Cm)-Ser-Ile-Ala-Cyst(Cm)-Asp-Lys |
| 5       | Leu-Ile-Ile-Ala-Gly-Thr-Ser-Cys(Cm)-Tyr |
| 6       | Ala-Val-Leu-Glu-Ala-Lys-Glu-Ser-Cys(Cm)-Leu-Asa-Asn |
| 7       | Tyr-Tyr-Gly-Thr-Glu-Cys(Cm)-Ile-Asp-Glu-Leu-Trp,Asx,Glu,Leu,His,Lys |

In Fig. 6, elution profile of tryptic peptides of cytosol serine hydroxymethyltransferase from a C18 reverse phase column on high performance liquid chromatography. A, profile of radioactive enzyme which had been reacted with [1-14C]iodoacetic acid before digestion with trypsin. B, profile of peptides monitored at 215 nm of tryptic digest.

In peptide 6 from serine hydroxymethyltransferase shows a great deal of homology to the pyridoxal-P enzyme. Glutamate decarboxylase (23), isoleucine dehydrogenase (24), lysine dehydrogenase (25), ornithine decarboxylase (26), cysteine sulfinate decarboxylase (27), tryptophan synthase (28), aspartate aminotransferase (29), L-alanine aminotransferase (30), and L-amino acid transaminase (31). Since all pyridoxal-P enzymes probably have similar intermediates it seems likely that a sulfhydryl group at the active site may have a similar function in these enzymes. However, the function of a sulfhydryl group in the mechanism of any pyridoxal-P enzyme has yet to be elucidated. One approach to determining if sulfhydryl groups are playing similar roles in pyridoxal-P enzymes is to isolate cysteine-containing peptides from the various enzymes and look for similarities in amino acid sequence. This approach has been done with the active site pyridoxal-P peptide from a wide variety of B6-enzymes. As we pointed out earlier the pyridoxyl peptide from rabbit liver cytosolic serine hydroxymethyltransferase shows a great deal of homology to the sequences of all bacterial decarboxylases and tryptophan synthase (32). Unfortunately, the only enzyme of this group to have the active site cysteine peptide sequenced is tryptophan synthase. The amino acid sequence around the active site cysteine in this enzyme is Ala-Leu-Thr-Lys-Cys-Gln-Asn...Lys (33). In peptide 6 from serine hydroxymethyltransferase we find the sequence Ala-Leu-Gly-Ser-Cys-Leu-Asa... where four of the seven amino acids are in the same location. This sequence is also present in mitochondrial serine hydroxymethyltransferase. Although peptide 6 (Table I), is not one

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| 4       | Val-Leu-Glu-Ala-Cyst(Cm)-Ser-Ile-Ala-Cyst(Cm)-Asp-Lys |
| 5       | Leu-Ile-Ile-Ala-Gly-Thr-Ser-Cys(Cm)-Tyr |
| 6       | Ala-Val-Leu-Glu-Ala-Lys-Glu-Ser-Cys(Cm)-Leu-Asa-Asn |
| 7       | Tyr-Tyr-Gly-Thr-Glu-Cys(Cm)-Ile-Asp-Glu-Leu-Trp,Asx,Glu,Leu,His,Lys |

In order to purify the cysteine-containing peptides, the trypsin hydrolysate was first fractionated on a phosphocellulose column. Five fractions containing radioactivity from this column were placed on an HPLC C18 reverse phase column for isolation. The purified peptides corresponding to the radioactive peaks in Fig. 6A were sequenced by the Edman degradation procedure (20). The sequences of the peptides are recorded in Table I. Peptide 4 contains 2 cysteine residues which accounts for all eight sulfhydryl groups observed on titration with DTNB. Peptide 5 is the active site peptide previously sequenced by us. The cysteine in this peptide is the one which reacts with iodoacetate in the apoenzyme (4). This purified active site tryptic peptide does not contain Lys or Arg. This is probably a chymotryptic peptide which was formed due to the presence of a Lys or Arg-Pro bond near the COOH-terminal end of this peptide.

We next determined which peptide contained the cysteine which reacts with D-3-fluoralanine. This was achieved by denaturing the enzyme which had been inactivated with fluoralanine and reacting all of the exposed sulfhydryl groups with [1-14C]iodoacetate. The HPLC elution profile of the tryptic digest of this enzyme was missing peptide 3.

2 L. Schirch, unpublished results.
of the peptides known to be at the active site of the enzyme, we cannot eliminate it as being at that location. We feel that the methodology reported in this paper can be used on other pyridoxal-P enzymes in order to compare sequences around active site cysteine residues.

One of our current interests is to identify the sequences of peptides which define the active site of serine hydroxymethyltransferase. We had isolated and sequenced two peptides previously and in this paper we have added a third sequence. The inactivation studies with D-3-fluorooxalone show that the cysteine in peptide 3 (Table I) lies near the ε-carbon of the amino acid substrate-binding site. This sulfhydryl group reacts rapidly with methyl methanethiosulfonate and DTNB (Fig. 3) but only results in inactivation with DTNB modification. There is at least one more sulfhydryl group at the active site which reacts with methyl methanethiosulfonate. The reaction of this group results in an inactive enzyme and is protected by the addition of substrates (Fig. 1). This sulfhydryl group may be the same one we have shown previously to react with iodoacetate in the apoenzyme and is identified as peptide 5 (Table I) in the HPLC profile (Fig. 6) (22). Even though this sulfhydryl group when blocked gives an inactive enzyme, we cannot assign a functional role in the mechanism of the enzyme. Blocking this group appears to change the environment of the pyridoxal-P as evidenced by the spectral shift from 428 to 415 nm (Fig. 4). This change in environment may mean the pyridoxal-P is no longer in line with critical residues required for catalysis. The critical sulfhydryl group may play a role in stabilizing the enzyme-substrate complex, however, since when it is blocked the pyridoxal-P-substrate complex readily dissociates from the active site (Fig. 2).

There are several experiments which suggest that there may be even more than the two sulfhydryl groups in peptides 3 and 5 (Table I) at the active site. Methyl methanethiosulfonate reacts with four sulfhydryl groups in the holoenzyme but removal of pyridoxal-P uncovers another reactive sulfhydryl group. At this time we can only rule out cysteines in peptides 1 and 7 (Table I) as being buried and not accessible to methyl methanethiosulfonate.

Knowing the location and reactivity of sulfhydryl groups on an enzyme can provide useful tools for mechanistic studies. In this paper we use methyl methanethiosulfonate as a sulfhydryl group-modifying reagent to probe the structure and function of cysteine residues in cytosolic serine hydroxymethyltransferase. The advantage of this reagent is that it modifies sulfhydryl groups as methyl disulfides which should have a minimum steric and charge effect on the structure of the enzyme. The reagent also appears to be specific for sulfhydryl groups. It does react with lysine and histidine, but the reaction is slow and not reversed by thiol compounds (34). The rapid and reversible inactivation of serine hydroxymethyltransferase strongly suggests that in this enzyme we are looking only at sulfhydryl group modifications. In future studies, we hope to be able to use C-labeled methyl methanethiosulfonate as a method of introducing an NMR probe into specific locations in this enzyme.

In addition to furthering our understanding of the number and function of sulfhydryl groups in this enzyme, this study has suggested two additional areas of research. The first is the use of modifying reagents to probe for other active site residues. In the past, we have attempted to use such reagents as phenylglyoxal, ethoxyformic anhydride, and cyanate to test for arginyl, histidyl, and lysyl groups at the active site. In each case, we observed both inhibition and loss of at least one sulfhydryl group. We could not, however, correlate inhibition with only the loss of the sulfhydryl group. It became apparent that we could not probe for the other active site residues unless we could reversibly block the active site sulfhydryl groups during the modification reaction. The experiments in this paper show that both methyl methanethiosulfonate and periodate can serve this function.

A second area of future study is the effect of blocking the sulfhydryl groups as methyl disulfides on the stability of the enzyme. Reacting the enzyme with methyl methanethiosulfonate decreases the stability of the enzyme. With four blocked sulfhydryl groups, the denaturation temperature is decreased by 15 °C as determined by a differential scanning calorimetry study. This suggests that the sulfhydryl groups may be important in maintaining the proper tertiary structure of cytosolic serine hydroxymethyltransferase.

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