Cytosolic serine hydroxymethyltransferase has been shown previously to exhibit both broad substrate and reaction specificity. In addition to cleaving many different 3-hydroxyamino acids to glycine and an aldehyde, the enzyme also catalyzes with several amino acid substrate analogs decarboxylation, transamination, and racemization reactions. To elucidate the relationship of the structure of the substrate to reaction specificity, the interaction of both amino acid and folate substrates and substrate analogs with the enzyme has been studied by three different methods. These methods include investigating the effects of substrates and substrate analogs on the thermal denaturation properties of the enzyme by differential scanning calorimetry, determining the rate of peptide hydrogen exchange with solvent protons, and measuring the optical activity of the active site pyridoxal phosphate. All three methods suggest that the enzyme exists as an equilibrium between "open" and "closed" forms. Amino acid substrates enter and leave the active site in the open form, but catalysis occurs in the closed form. The data suggest that the amino acid analogs that undergo alternate reactions, such as racemization and transamination, bind only to the open form of the enzyme and that the alternate reactions occur in the open form. Therefore, one role for forming the closed form of the enzyme is to block side reactions and confer reaction specificity.

Serine hydroxymethyltransferase (SHMT) catalyzes the interconversion of serine and glycine with H$_2$PteGlu serving as the one carbon carrier (Schirch, 1982). In addition to the physiological reaction, all forms of this enzyme catalyze the aldol cleavage of a wide variety of 3-hydroxyamino acids. These reactions do not require, in most cases, H$_2$PteGlu as a one carbon carrier of the aldehyde product. Because of the ability of SHMT to use many substrates in the aldol cleavage reaction, it is considered to have broad substrate specificity.

In addition to cleaving 3-hydroxyamino acids, SHMT also catalyzes transamination, decarboxylation, and racemization reactions with several amino acids (Shostak and Schirch, 1988; Palekar et al., 1973). Although some of the reactions have turnover times measured in min, the studies of these slow alternate reactions suggest that reaction specificity for SHMT is determined by the structure of the amino acid. The goal of the present research was to try to understand the relationship of the structure of the amino acid to the properties of reaction specificity in SHMT.

The approach used in this study of reaction specificity was to determine the nature of the interaction of a wide variety of amino acid and folate ligands with the enzyme. Three different methods were used to study the effect of ligand structure on the properties of the enzyme. First, the effect of ligands on the thermal denaturation of the enzyme was investigated using differential scanning calorimetry. Second, the rate of exchange of peptide hydrogens of the enzyme, in the absence and presence of the ligands, with solvent protons was determined. Third, the effect of ligands on the CD spectral properties of the bound pyridoxal-P was studied. We conclude from these studies that several ligands stabilize a closed conformation of the enzyme. The relationship of open and closed conformations to reaction specificity is discussed.

**Experimental Procedures**

**Materials**—All amino acids were obtained from Sigma. All reduced folate compounds were purchased from Fluka (Ronkonkoma, NY). Tritiated water was from Amersham Corp. and had a specific activity of 100 mCi/ml. Cytosolic SHMT was purified from rabbit liver as described previously (Schirch and Peterson, 1980).

**Concentration of Ligand Solutions**—The experiments reported in this study were done by determining the properties of cSHMT in the presence of several amino acid and folate ligands. The concentrations of these ligands in each experiment were as follows: L-serine, 40 mM; glycine, 50 mM; aminomethylphosphonate, 40 mM; D-alanine, 100 mM; L-alanine, 150 mM; L-threonine, 150 mM; L-allo-threonine, 200 mM; H$_2$PteGlu, 500 µM; 5-CH$_2$-H$_4$PteGlu, 200 µM; 5-CHO-H$_2$PteGlu, 200 µM. For each ligand the concentrations are at least 5 times their $K_a$ values.

**Differential Scanning Calorimetry Determinations**—The denaturation of SHMT solutions was performed in an MC-2 differential scanning microcalorimeter from Microcal Inc., Amherst, MA. The instrument used a computerized data acquisition system, and data analysis was performed with software provided by Microcal Inc. SHMT, 3.16 mg/ml, was dialyzed for at least 24 h against 20 volumes of 50 mM potassium BES, pH 7.3. The enzyme and buffer were degassed immediately prior to use. Ligands were added as µL additions of concentrated solutions to the enzyme and buffer. At least triplicate determinations were made for each ligand solution. The scanning rate was 30°C/h. The enthalpy of denaturation ($\Delta H_m$) was determined both by summing the values of deconvoluted curves, determined by the available software, and by cutting out the plotted thermal denaturation curves, weighing them, and comparing the weights with a standard curve generated by the instrument. There was excellent agreement between the two methods for all data reported in this study.

**Peptide Hydrogen Exchange with Solvent Protons**—The amide hydrogens of SHMT were labeled with tritium by adding a 100 mg/
ml solution of enzyme to an equal volume of tritiated water (100 mCi/ml). This solution was incubated at 32 °C for 4 h and at 4 °C for an additional 72 h. Immediately before use, the excess tritiated water was removed by applying 50 μl of the enzyme solution to a Sephadex G-25 column at 0 °C, in a 1-ml plastic syringe, equilibrated with the same pH 7.3 buffer used in the DSC experiments. The enzyme was forced onto the column by applying a light pressure. The enzyme was washed into the column with an additional 100 μl of buffer at 0 °C. The column was then centrifuged at 1,000 rpm for 1 min suspended in a 13 x 100-mm glass tube. The enzyme was eluted in the tube in a volume of 0.36 ml. Less than 0.001% of the tritiated water was in the eluate, but greater than 80% of the enzyme was in the eluate. The total time involved in removing the tritiated water was 4 min, and for all of this period the enzyme was between 0 and 4 °C. The eluted enzyme solution was put on ice and divided into four equal aliquots. To three of these aliquots was added up to 10 μl of a concentrated solution of the ligand to be studied. The four tubes were then placed in a water bath at 30 °C for 30 min. At the end of the reaction all four tubes were placed in ice and 50 μl removed from each tube and placed on a Sephadex G-25 column in a 1-ml plastic syringe. The concentration of enzyme used to obtain spectra was 1-2 mg/ml. For each experiment, concentrated solutions of cSHMT were diluted at least 3-fold into 50 mM potassium BES, pH 7.3. For most experiments, the radioactivity of the stock enzyme solution. From this number the resulting in changes in both metric enthalpy of 66.9 °C (Fig. 1, curve I). The calori-

The number of tritiated hydrogens on SHMT was determined from the cpm/mmol hydrogen in the water was determined. This number was divided into the cpm/mmol enzyme after the 50-min incubation. This gave the number of labeled hydrogens that had not exchanged during the incubation.

*Spectroscopy—UV-Vis spectra were recorded on a Cary 210 spectrophotometer. CD spectra were recorded on a Jasco J-500 spectropolarimeter with a 1-cm path length thermostated cell. The concentration of enzyme used to obtain spectra was 1-2 mg/ml. For each experiment, concentrated solutions of cSHMT were diluted at least 3-fold into 50 mM potassium BES, pH 7.3. For most experiments, spectra were recorded at 30 °C, but for spectra using l-threonine, allothreonine, and d-alanine as ligands, spectra were recorded rapidly at 8 °C.

**RESULTS**

*Effect of Ligands on the Thermal Unfolding of cSHMT—* Increasing the temperature of a solution of cSHMT irreversibly denatures the enzyme. A differential scanning calorimetry (DSC) thermogram of this process shows a single endothermic transition with a T_m of 66.9 °C (Fig. 1, curve 1). The calorimetric enthalpy (AH_m) for this process is 660 kcal/mol. Previous investigators have shown that binding of ligands to proteins often stabilizes a conformation of the protein, resulting in changes in both T_m and enthalpy values (Shriver and Kamath, 1990; Farach and Martinez-Carrion, 1983; Relimpio et al., 1981; Strong et al., 1987). cSHMT has an active site that binds both amino acids and reduced folate derivatives, with serine and glycine being the physiological amino acid substrates and H4PteGlu, and CH2-H4PteGlu, being the physiological folate substrates. As shown in Fig. 1, curve 3, saturation of the enzyme with L-serine results in large increases in both T_m (78 °C) and ΔH_m (1,150 kcal/mol). However, saturation of the enzyme with L-alanine, which differs from serine in not having the 3-hydroxyl group, decreases both T_m (64 °C) and ΔH_m (590 kcal/mol) (Fig. 1, curve 2). These differences in T_m and ΔH_m for the enzyme-serine complex and the enzyme-L-alanine complex suggest that the two ligands bind to different conformational states of the enzyme.

The effect of reduced folate compounds on the thermal denaturation properties of the enzyme is more difficult to determine because of the susceptibility of H4folate to oxidation during the length of time and high temperature used in the DSC experiments. To circumvent this problem we have used both 5-CHO-H4PteGlu and 5-CH3-H4PteGlu as folate ligands. Each of these compounds is more stable than the physiological folate substrates and has been shown to bind to the folate site of cSHMT with the same affinity as H4PteGlu and CH2-H4PteGlu (Schirch and Ropp, 1967). As we will discuss later, these folate analogs are similar in structure to putative intermediates on the reaction pathway. In Fig. 1, curve 4, is shown the thermogram of cSHMT saturated with both glycine and 5-CHO-H4PteGlu. Again, a large increase in T_m (79.5 °C) and ΔH_m (900 kcal/mol) is observed compared with the values determined for the free enzyme (curve 1). Neither glycine nor 5-CHO-H4PteGlu alone results in large changes in T_m and ΔH_m (see Figure 5A). These results again suggest that the enzyme-glycine-5-CHO-H4PteGlu ternary complex has a conformation different from that of the enzyme or the enzyme binary complexes. In both the absence and presence of ligands the DSC curves for denaturation of cSHMT (Fig. 1) are asymmetric. The curves for the enzyme and all enzyme-ligand complexes, with the exception of the cSHMT-serine complex, can be resolved into three sequential two-state steps (Fig. 2B). In order to resolve the denaturation curve for the enzyme-serine complex, four sequential two-state steps are required (Fig. 2A).

**Fig. 1.** DSC thermograms of cSHMT with three ligands. Curve 1, cSHMT; curve 2, cSHMT-L-alanine complex; curve 3, cSHMT-L-serine complex; curve 4, cSHMT-5-CHO-H4PteGlu ternary complex. For each curve the concentration of cSHMT was 3.16 mg/ml at pH 7.3.

**Fig. 2.** Resolution of DSC curves for cSHMT and its serine complex into sequential two-state steps. A, DSC trace of cSHMT-serine complex (—) and its component contributions (--.--). B, DSC trace of cSHMT (—) and its component contributions (--.--).
gands of cSHMT (see Fig. 5, A and B). The results are recorded as the difference in $\Delta H_{\text{cat}} (\Delta (\Delta H) \text{ kcal/mol})$ between the enzyme alone and the enzyme-ligand complex (Fig. 6A). The differences in $T_m$ between the enzyme and enzyme-ligand complexes are recorded as $\Delta T_m$ in Fig. 5B. Thermograms could not be determined for the ligands D-alanine, L-threonine, and allothreonine because these compounds react sufficiently fast to form products during the duration of the DSC experiment. As noted in Fig. 5, A and B, ligands that increase $T_m$ also increase $\Delta H_{\text{cat}}$. Only two binary complexes significantly increased $T_m$ and $\Delta H_{\text{cat}}$: i.e. L-serine and amino-methylphosphonate. Binary complexes of the enzyme with glycine, L-alanine, 5-CH$_3$-H$_4$PteGlu, and 5-CHO-H$_4$PteGlu had little effect on $T_m$ and $\Delta H_{\text{cat}}$ (Fig. 5, A and B). However, all three ternary complexes of enzyme and glycine with either H$_2$PteGlu, 5-CH$_3$-H$_4$PteGlu, or 5-CHO-H$_4$PteGlu increased both $T_m$ and $\Delta H_{\text{cat}}$.

Effect of Ligands on the Rate of Exchange of Peptide Hydrogens with Solvent Protons—The DSC studies suggest that the binding of several ligands to cSHMT results in stabilization of different conformational states. The effect of these ligands on the conformation of the enzyme was probed further by determining the rate of exchange of amide hydrogens with protons in the solvent. This was accomplished by first lyophilizing the amide hydrogens with tritium by an extended incubation of the tritiated enzyme in tritiated water. The labeled enzyme was then removed rapidly from the tritiated water, and the exchange of the tritiated amide hydrogens with solvent protons was determined as a function of time in the absence and presence of ligands.

Fig. 3 shows the tritium hydrogens remaining on cSHMT, after removal of the labeled solvent, as a function of time. Not shown are the results occurring during the first 15 min of the incubation. There is a rapid loss of 40% of the bound tritium from the enzyme during the first 10 min. After this rapid exchange of hydrogens there is a much slower rate of exchange which lasts for several h. Shown in Fig. 3 is a part of this slower phase of the reaction for the enzyme and the enzyme binary complexes with serine and glycine. The results show that serine, and to a lesser extent glycine, decreases the rate of peptide hydrogen exchange with solvent protons. With a saturating concentration of serine the rate of exchange of about 70-75 hydrogens/molecule of cSHMT (18/subunit) is slower than in the free enzyme (Fig. 3).

The effects of other ligands on the exchange of peptide hydrogens with the solvent were investigated. The labeled enzyme was saturated with different ligands, and the tritium remaining with the enzyme after a 30-min incubation at 30 °C was determined. The difference between the number of hydrogens remaining in the free enzyme and the enzyme-ligand complex is reported in Fig. 5C. The experiment was done in triplicate for each ligand. In addition to serine, the only other binary complex to slow the rate of peptide hydrogen exchange significantly was aminomethylphosphonate. However, ternary complexes of enzyme and glycine with either 5-CH$_3$-H$_4$PteGlu or 5-CHO-H$_4$PteGlu also showed a decreased rate of peptide hydrogen exchange. Binary complexes of the enzyme with glycine, L-alanine, 5-CH$_3$-H$_4$PteGlu, and 5-CHO-H$_4$PteGlu had much smaller effects on the rate of peptide hydrogen exchange (see Fig. 5C). In fact, L-alanine increased the rate of exchange.

Effect of Ligands on the CD Spectra of the Active Site Pyridoxal-P—Pyridoxal-P is bound to the $\epsilon$-amino group of a lysyl residue as an internal aldimine in each of the four subunits of cSHMT (Schirch, 1982). This internal aldimine exhibits maximal absorption at 428 nm. Addition of amino acid ligands converts the internal aldimine to an external aldimine between pyridoxal-P and the amino group of the amino acid ligand. These external aldmines have absorption maxima in the 425-428 nm range and have molar absorptivity coefficients very similar to that of the internal aldimine. When pyridoxal-P is bound to cSHMT, the asymmetry of the active site results in a positive dichroic band at 425 nm for the internal aldimine. Changes in the or of ternary complexes with respect to neighboring residues, at the active site upon forming external aldmines with amino acid ligands may be expected to alter the optical activity of the enzyme-bound pyridoxal-P.

Fig. 4B shows the absorption spectra of cSHMT (curve 1) and its binary complexes with L-alanine (curve 2) and glycine (curve 3). The spectrum of the enzyme saturated with serine is very similar to that of the enzyme alone and was not included in Fig. 4B. The addition of glycine results in the appearance of three absorption bands at 343, 425, and 495 nm. Each of these absorption bands corresponds to a different structure of the cofactor at the active site. The three complexes are in rapid equilibrium, with the complex absorbing at 425 nm being the external aldimine. In Fig. 4A are shown the CD spectra of the same solutions used to record the absorption spectra in Fig. 4B. For each CD spectrum, the optical activity was normalized to mdeg/absorbance unit for either the external or internal aldimine at 423 nm. For the enzyme alone this value was 83 mdeg/A$_{nm}$. The optical activity of the external aldmines formed from glycine and L-alanine
was very close to the value of the enzyme, being 79 and 77 mdeg/A<sub>425</sub>, respectively. However, the value for the enzyme-serine complex was 37 mdeg/A<sub>425</sub>. This shows that in the enzyme-serine complex, the orientation of pyridoxal-P with respect to the neighboring groups is less asymmetric.

The effects of a number of other ligands on the CD spectra of the external and internal aldimines of pyridoxal-P were determined by the same procedure used to obtain the data recorded in Fig. 4, A and B. The results are shown in Fig. 5D as the difference in optical activity in mdeg/A<sub>425</sub> between enzyme and the enzyme-ligand complex. Of the ligands used in both the DSC and peptide hydrogen exchange experiments, only serine and aminomethylphosphonate resulted in major decreases in optical activity of the coenzyme in the external aldimine. Binary complexes with 5-CH<sub>3</sub>-H<sub>4</sub>PteGlu and 5-CHO-H<sub>4</sub>PteGlu also did not change the optical properties of the external aldimine. We could not investigate the ternary complexes of the enzyme with glycine and either 5-CH<sub>3</sub>-H<sub>4</sub>PteGlu or 5-CHO-H<sub>4</sub>PteGlu because of the large absorption band that forms at 500 nm in these ternary complexes (Schirch and Ropp, 1967). This large absorption at 500 nm makes an accurate determination of the absorption of the external aldimine at 425 nm impossible.

The determination of the optical activity of the external aldimines formed from d-alanine, L-threonine, and allo-threonine was done at 8 °C. These spectra were recorded with a large excess of each ligand. The absorption spectra were recorded within 1 min after adding the ligand to the enzyme, and the CD spectra were recorded within 4 min. By doing the reaction at low temperature, less than 1% of the threonine, allo-threonine, and d-alanine was converted to product during the measurement of the CD spectra. The addition of serine to the enzyme gave the same results at 8 °C as observed at 25 °C. The results show that d-alanine did not cause a change in the optical activity of the enzyme-bound cofactor, but both allo-threonine and L-threonine decreased the optical activity to an extent similar to that observed with serine. These results are recorded as ligands J, K, and L in Fig. 5D.

**Discussion**

Our goal in this research was to gain some insight into why amino acid substrate analogs of serine and glycine undergo side reactions, such as decarboxylation, transamination, and racemization, with SHMT. Side reactions are not observed with either the physiological substrates or other substrate analogs such as l-threonine and allo-threonine. To study this problem we have looked at three different properties of cSHMT in the presence of various ligands. DSC determines the stability of the enzyme and the heat required to go from the native state to the denatured state. If one assumes that ligands do not bind to the denatured state, then the differences in <i>Tm</i> and DH<sub>cat</sub> values between the free enzyme and the enzyme-ligand complexes are the result of differences in energy between the native enzyme and its complexes. With the enzyme-ligand complex, the enthalpy of binding will appear as a part of DH<sub>cat</sub>. However, the enthalpy of binding is usually much smaller than the enthalpy of denaturation. This method has been used with a variety of proteins to observe conformational changes induced by ligand binding (Relimpio et al., 1981; Farach and Martinez-Carrion, 1983; Strong et al., 1987; Shriver and Kamath, 1990). One of these is the pyridoxal-P containing enzyme aspartate aminotransferase (Relimpio et al., 1981).

A second method determined the exchange of peptide hydrogens with solvent protons. This technique is more specific than the DSC experiment since one is only measuring the accessibility ofamide hydrogens to solvent. The rate of exchange of amide hydrogens of a protein has been suggested to reflect the compactness and “breathing” motion of the protein (Englander, 1975; Pfister et al., 1978). This technique has also been used with aspartate aminotransferase (Pfister et al., 1978). The third method, determining CD spectra of the active site pyridoxal-P, is much more specific than the other two methods since it is measuring only the orientation of pyridoxal-P at the active site.

We have investigated the effect of binding of six different ligands to SHMT by all three methods described in the previous paragraphs. The results are in agreement with the idea that serine and aminomethylphosphonate cause a structural change in the enzyme when they bind at the active site. No evidence was found that glycine, L-alanine, 5-CH<sub>3</sub>-H<sub>4</sub>PteGlu, and 5-CHO-H<sub>4</sub>PteGlu cause major structural changes when they bind at the active site. It appears that each of the three methods provides similar information on structural changes induced by binding of serine and aminomethylphosphonate to SHMT. We conclude from the studies on these six ligands that any one of the three methods can
give information concerning conformational changes that occur during ligand binding. Thus, we conclude that the results of DSC thermodynamic studies are consistent with a conformational change taking place during the formation of ternary complexes with glycine and either H$_4$PteGlu, 5-CH$_3$H$_4$PteGlu, or 5-CHO-H$_4$PteGlu (Fig. 5, A, B, and C). Likewise, results of CD experiments are consistent with a conformational change taking place during formation of binary complexes with either L-threonine or allothreonine. However, results of CD studies suggest that saturation of the enzyme with D-alanine does not cause a conformational change (Fig. 5D).

The sequence of reactions shown in Scheme 1 is consistent with the results recorded in Fig. 5. In this scheme the enzyme active site exists as an equilibrium between open and closed forms (structures I and VI). In the absence of any ligands the equilibrium lies in favor of the open form. The addition of serine, which is converted first to the external aldimeine of pyridoxal-P, results in a shift in the equilibrium to favor the closed form (structures II and VII). In the closed form the enzyme exhibits a higher $T_m$, a larger $\Delta H_{cal}$ for denaturation, and decreased optical activity of the pyridoxal-P. The increased $\Delta H_{cal}$ and decreased rate of peptide hydrogen exchange are consistent with the closed form of the enzyme being a more compact structure.

The results, summarized in Fig. 5, suggest that binding of folate analogs as binary complexes does not shift the equilibrium toward the closed form. However, the addition of H$_4$PteGlu to the enzyme serine complex, as shown by structure VIII in Scheme 1, most likely maintains the ternary complex in the closed form. This is suggested from the results of studies with the ternary complexes of SHMT with glycine and both 5-CH$_3$H$_4$PteGlu and 5-CHO-H$_4$PteGlu. These two analogs of H$_4$PteGlu bind as tightly at the active site as the substrate folates and are structurally related to the proposed H$_4$PteGlu 5-iminium cation intermediate in the reaction mechanism (Kallen and Jencks, 1966).

The next step in the reaction is the transfer of the hydroxymethyl group of serine to form CH$_2$-H$_4$PteGlu, followed by release of the one-carbon carrier, leaving the enzyme-glycine binary complex (structure X). The results shown in Fig. 5 suggest that this binary complex the equilibrium lies toward the open form (structures V and X).

The data recorded in Fig. 5 and the sequence of reactions shown in Scheme 1 suggest that amino acid substrates enter and leave the open form of the active site but that the folate substrates can enter either the open or closed form. These results are consistent with our previous conclusions from kinetic studies that serine and H$_4$PteGlu add by a sequential random mechanism (Schirch et al., 1977). If H$_4$PteGlu could add only to the open or closed form then addition of serine and H$_4$PteGlu would have been sequentially ordered. We conclude that the structural changes that take place upon serine binding to SHMT do not alter the structure of the folate binding site significantly.

The interconversion of reaction intermediates occurs in the closed state. Similar mechanistic arguments were made for aspartate aminotransferase from evidence accumulated from the effect of ligands on amide hydrogen-solvent exchange and DSC experiments (Relimpio et al., 1981; Pfister et al., 1978). Since these earlier studies with aspartate aminotransferase, x-ray diffraction studies have confirmed the open and closed forms of the enzyme (Arnone et al., 1985; Jansonius et al., 1985).

The data, presented in Fig. 5, also suggest possible structural elements in the ligands required to shift the equilibrium from the open to the closed form. One requirement is for an amino acid substrate. We have shown previously that the carboxyl group of the amino acid is required for forming a stable external aldimeine (Schirch and Diller, 1971). Although we did not investigate amines without a carboxyl group as ligands in this study, we suggest that the carboxyl group of the amino acid plays a key role in the process of forming the closed form of the enzyme. A second requirement is to have a group in the one-carbon site. This can be either an alcohol group, as found on serine, threonine, or allothreonine, or a carbon group on the 5-position of H$_4$PteGlu. However, a methyl group attached to the amino acid, as found in both L- and D-alanine, does not result in stabilization of the closed form. The only exceptions to these structural requirements are those exhibited by the glycine analog, aminomethylphosphonate. This compound has no group in the one-carbon site. However, it has a double negative charge at the carboxyl binding site. This double negative charge appears to shift the equilibrium of the enzyme to the closed form.

Slow side reactions that occur in pyridoxal-P enzymes have been proposed to occur because of the reactivity of the aldimes formed with the amino acids (Snell, 1985; Miles et al., 1986). The nonenzymatic reactions of pyridoxal amino acid aldimes are well documented (Metzler, 1957; Davis and Metzler, 1972; Martell, 1989). Most of these nonenzymatic reactions are slower than the side reactions observed with enzymes, but because the substrate analogs are held at an active site it is believed that groups on the enzyme account for the increased rates of side reactions. What is unusual about the side reactions for several pyridoxal-P enzymes is that they occur only with substrate analogs and not with the substrates themselves. With SHMT, the most extensively studied side reactions are those that occur with L- and D-alanine (Shostak and Schirch, 1988; Schirch and Jenkins, 1964). The results in these studies show that these amino acids form external aldimes that remain in the open form. This suggests that side reactions occur only in the open form and that the closed form functions to block these side reactions. Therefore, the role of the conformational change, occurring upon binding of serine, is not only to enhance the catalytic activity by aligning catalytic groups at the active site but to also decrease the rate of unwanted side reactions.

L-Threonine is a very poor substrate having a $k_{cat}$ value that is about 5% of the $k_{cat}$ value for serine. However, L-threonine does not undergo side reactions, such as transamination or racemization, at a rate observed with D- and L-alanine.$^2$ The results, shown in Fig. 5D, suggest that L-threonine shifts the equilibrium to the closed form. This explains the lack of side reactions, but it does not explain the low value of $k_{cat}$. One possible explanation for the low $k_{cat}$ value is that the rate-determining step for this substrate is forming the closed state.

The asymmetric thermal denaturation curves for cSHMT, as recorded in Fig. 2, suggest that the subunits do not denature cooperatively. However, the multiple two-state thermal transitions may also reflect the fact that the enzyme is a population of subforms with different negative charges. These subforms have been shown to be the result, at least in part, of the deamidation of Asn5 near the amino terminus of the enzyme (Artigues et al., 1990). Two thirds of this residue is deamidated, resulting in a protein with equal amounts of asparagine, aspartic acid, and isoaspartic acid residues at position 5. These subforms may denature at slightly different temperatures giving rise to the asymmetric thermograms.

REFERENCES