This work is aimed at understanding how protein structure and conformation regulate activity and allosteric communication in the tryptophan synthase $\alpha\beta_2$ complex from *Salmonella typhimurium*. Previous crystallographic and kinetic results suggest that both monovalent cations and a salt bridge between $\alpha$ subunit Asp$^{56}$ and $\beta$ subunit Lys$^{167}$ play allosteric roles. Here we show that mutation of either of these salt bridging residues produced deleterious effects that could be repaired by increased temperature in combination with CsCl or with NaCl plus an $\alpha$ subunit ligand, $\alpha$-glycerol 3-phosphate. Arrhenius plots of the activity data under these conditions were nonlinear. The same conditions yielded temperature-dependent changes in the equilibrium distribution of enzyme-substrate intermediates and in primary kinetic isotope effects. We correlate the results with a model in which the mutant enzymes are converted by increased temperature from a low activity, “open” conformation to a high activity, “closed” conformation under certain conditions. The allosteric ligand and different monovalent cations affected the equilibrium between the open and closed forms. The results suggest that $\alpha$ subunit Asp$^{56}$ and $\beta$ subunit Lys$^{167}$ are not essential for catalysis and for allosteric communication between the $\alpha$ and $\beta$ subunits but that their mutual interaction is important in stabilization of the active, closed form of the $\alpha\beta_2$ complex.

An enzyme must undergo conformational transitions to selectively stabilize each intermediate along a reaction pathway. Catalysis can be modulated by factors that change the enzyme conformation or alter the equilibrium distribution of preexisting conformations. These factors include ligands, solvents, chaotropic agents, temperature, and single site mutations. In the present work, we ask whether factors that stabilize the active conformation of an enzyme can also overcome the deleterious effects of mutations.

We have recently investigated the effects of temperature on the activity of the tryptophan synthase $\alpha\beta_2$ complex (EC 2.5.1.20) in the reaction shown in Scheme I (1). We found that the conditions that yield nonlinear Arrhenius plots of the activity data also yield temperature-dependent changes in the equilibrium distribution of the E-Ser and E-AA intermediates shown in Scheme I and in primary kinetic isotope effects. The results provide evidence for a temperature-induced conversion from a low activity open conformation to a high activity closed conformation (1). Different monovalent cations, which bind to the $\beta$ subunit, and an allosteric ligand (in-$\alpha$-glycerol 3-phosphate (GP)), which binds to the $\alpha$ subunit, affect the equilibrium distribution of the open and closed forms. Here we investigate the effects of increased temperature and of other factors that stabilize the active, closed conformation of tryptophan synthase on the effects of mutations in residues that can form a regulatory salt bridge between the $\alpha$ subunit and the $\beta$ subunit.

Three-dimensional structures of wild-type and mutant forms of the tryptophan synthase $\alpha\beta_2$ complex from *Salmonella typhimurium* have revealed many features including the arrangement of the $\alpha$ and $\beta$ subunits, the location of residues in the interface between the $\alpha$ and $\beta$ subunits, and a monovalent cation binding site in the $\beta$ subunit (2–7). Although Na$^+$, K$^+$, and Cs$^+$ bind to the same site in the $\beta$ subunit, differences in

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1 The abbreviations used are: GP, Dl-$\alpha$-glycerol 3-phosphate; Bis-Tris, 1,3-bis(tris(hydroxymethyl)methylamino); E-Ser, external aldime of $\alpha$-serine; E-AA, external aldime of aminoaacrylate; E-Trp, external aldime of $\gamma$-tryptophan; E-Q, and E-Q$_2$, quinonoid intermediates.

2 The term $\beta_2$ subunit is used for the isolated enzyme in solution; $\beta$ subunit is used for the enzyme in the $\alpha\beta_2$ complex, for a specific residue in the $\beta$ subunit or for the dissociation of the $\alpha$ and $\beta$ subunits.
coordinated give rise to two distinctly different protein conformations (3) (reviewed in Ref. 8, Scheme II). In the presence of Na\(^+\), the carboxylate of \(\beta\)Asp\(^{305}\) forms a salt bridge with the \(\epsilon\)-amino group of \(\beta\)Lys\(^{167}\) (Scheme IIA). In the presence of K\(^+\) or Cs\(^+\), the carboxylate of \(\beta\)Asp\(^{305}\) is in an alternative conformation and the \(\epsilon\)-amino group of \(\beta\)Lys\(^{167}\) moves about 7 Å and makes a salt bridge across the subunit interface with the carboxylate of \(\alpha\)Asp\(^{56}\) (Scheme IIB). Previous studies have shown that the activities of enzymes having mutations at \(\beta\)Lys\(^{167}\) are not essential for catalysis and for allosteric formations (3) (reviewed in Ref. 8, Scheme II). In the presence of \(\text{Na}^+\) or between \(\text{Na}^+\) and \(\text{Na}^+\), the carboxylate of \(\text{Asp}^{305}\) makes a salt bridge across the subunit interface with the carboxylate of \(\text{Asp}^{56}\). Previous studies have shown that the mutagenesis of the residues involved in the salt bridge switch alters the catalytic and allosteric properties of the \(\alpha_2\beta_2\) complex. Increased temperature in the presence of CsCl or of NaCl plus GP reversed the deleterious effects of the mutations. The results indicate that the \(\alpha\) subunit \(\text{Asp}^{56}\) and \(\beta\) subunit \(\text{Lys}^{167}\) are not essential for catalysis and for allosteric communication between the \(\alpha\) and \(\beta\) subunits but that their mutual interaction is important in stabilizing the closed form of the \(\alpha_2\beta_2\) complex.

**MATERIALS AND METHODS**

**Chemicals and Buffers**—L-Serine, \(\text{Na}_2\text{Serine, GP, pyridoxal 5'-phosphate, and Bis-TriA propionate were from Sigma. [\(\alpha\text{-H}\)]-Serine was prepared as reported previously (11). Sodium-free GP was prepared by repeated passage of the solution of the disodium salt over an ion exchange column (DE-52 in the H\(^+\) form, Sigma) (12); the pH of the final eluate was adjusted to 7.8 with Bis-TriA propionate. All experiments were carried out in 50 mM Bis-TriA propionate buffer containing 0.5 mM dithiothreitol. The pH of this buffer was adjusted to 7.8 by the addition of HCl at 25 °C with no compensation for pH variation with temperature (1).**

**Bacterial Strain, Plasmids, and Enzymes**—Wild type and mutant plasmids pEBA-10 and pEBA-4A8 (13) were used to express wild type and \(\text{d}56\text{EA}\) tryptophan synthase \(\alpha_2\beta_2\) complex and the \(\alpha\) subunit from S. typhimurium, respectively, in Escherichia coli CB 149 (14), which lacks the \(\text{trp}\) operon. The wild type and mutant \(\alpha\) and \(\beta\) were purified to homogeneity as described previously (15). The wild type \(\text{asubunit was purified from extracts containing the } \text{asubunit alone (13, 16). The \(\text{d}56\text{EA } \text{asubunit was separated from the apo } \text{asubunit complex. The \(\beta\)K167T } \text{asubunit complex was obtained as described previously (9). The enzymes were dialyzed against monovalent cation-free Bis-TriA propionate buffer, pH 7.8, before use. Protein concentrations were determined from the specific absorbance at 278 nm using } \text{Asubunit } = 6.0 \text{ for the } \text{asubunit complex and } \text{Asubunit } = 4.4 \text{ for the } \text{asubunit (18).}**

**Enzyme Assays and Spectroscopic Methods**—One unit of activity is the formation of 0.1 \(\mu\)mol of product in 20 min at the indicated temperature. The activity in the \(\beta\)-replacement reaction with l-serine and indole was measured by a direct spectrophotometric assay (18) in the presence of excess \(\alpha\) subunit (5 \(\mu\m)\). [\(\text{H}\)]-Serine and [\(\text{H}\)]-l-serine were used in the place of l-serine for investigations of primary kinetic isotope effects. The reaction mixtures were equilibrated for at least 5 min at the desired temperature before the addition of the enzymes. The concentration of l-serine (50 \(\mu\m)\) was used for assays saturating because the same initial rates of the wild type and the mutant \(\alpha_2\beta_2\) complexes were obtained with 25, 50, and 100 \(\mu\m\) l-serine at 5 and 45 °C.

Absorption spectra and assays were measured using a Hewlett-Packard 8452 diode array spectrophotometer thermostated by a Peltier junction temperature controlled cuvette holder, which was calibrated with a thermometer. Buffers containing indicated components were equilibrated at the desired temperature for at least 5 min; spectra were obtained immediately upon the addition of a twentieth volume of the enzyme.

**Data Analysis**—The activity data were plotted as logarithm of activity (ln Activity) versus the reciprocal of the absolute temperature in K (1/T) and fitted to the Arrhenius equation, Equation 1,

\[
\ln \text{Activity} = \ln Z - \frac{E_a}{RT} \tag{Eq. 1}
\]

where \(E_a\) is the energy of activation, \(R\) is the gas constant, and \(Z\) is the preexponential factor.

The results can be readily transformed into a rate constant, \(k_{cat}\) (19).

According to Eyring’s transition state theory, the temperature dependence of a rate constant is given by Equation 2,

\[
k_{cat} = \frac{h}{k_{B}T} \exp \left(\frac{-\Delta G^0}{RT}\right) \tag{Eq. 2}
\]

\[
\Delta S^0 = \sum_{i=1}^{n} s_i \tag{Eq. 3}
\]

Thus, \(\Delta H^0\) and \(\Delta S^0\) can be determined from the Arrhenius plots.

The equilibrium constant, \(K_{eq}\), for the E-Ser to E-AA conversion can be calculated from the temperature dependence of the absorbance using Equation 4,

\[
K_{eq}(T) = \frac{[E - AA][E - Ser]}{[E - Ser][E - AA]} \tag{Eq. 4}
\]

where \(A_p\) is the absorbance of the solution at absolute temperature \(T\), \(A_{\text{E-AA}}\) and \(A_{\text{E-Ser}}\) are the absorbance of E-AA obtained at higher temperature and of E-Ser obtained at low temperature, respectively.

The temperature dependence of \(K_{eq}\) is given by Equation 5,

\[
\Delta G(T_m) = -RT \ln K_{eq}(T_m) = \Delta H_m - T \Delta S_m = \Delta H_m \left(1 - \frac{T}{T_m}\right) \tag{Eq. 5}
\]

where \(\Delta H_m\) and \(\Delta S_m\) are the changes in enthalpy and entropy of the reaction, respectively, and \(T_m\) is the midpoint, where \(K_{eq}(T) = 1\). Assuming no change in enthalpy or entropy with temperature, we can obtain \(\Delta H_m\) and \(\Delta S_m\) from the linear plot of \(\ln K_{eq}(T_m) versus 1/T\).

**RESULTS**

The \(\text{d}56\text{EA} and \(\beta\)K167T Mutations Alter the Temperature Dependence of the Activity of the \(\alpha_2\beta_2\) Complex—Fig. 1, A–C, shows the effects of temperature on the activities of the wild type \(\alpha_2\beta_2\) complex (1) and of the \(\text{d}56\text{EA} and \(\beta\)K167T \(\alpha_2\beta_2\) complexes, respectively, in the presence of either NaCl or CsCl and in the absence or presence of the allosteric ligand, GP. Arrhenius plots of the data are shown in Fig. 1, D–F. At low

\[\text{3} \ E. \ Woehl, \ O. \ Hur, \ D. \ Ferrari, \ C. \ Bagwell, \ U. \ Banik, \ L.-H. \ Yang, \ E. \ W. \ Miles, \ and \ M. \ F. \ Dunn, \ manuscript \ in \ preparation.\]

\[\text{4} \ \text{The turnover number, } k_{cat}, \ \text{is equal to a factor } t \ \text{times the specific activity for the } \alpha_2\beta_2 \ \text{complex (} f = 0.00594).\]
temperatures (5–35 °C), the activities of the wild type αββ2 complex were much higher in the presence of CsCl than in the presence of NaCl (1). However, at temperatures above 35 °C, the activities were similar in the presence of NaCl or CsCl (Fig. 1A). The Arrhenius plots (Fig. 1D) of the data were nonlinear in the presence of NaCl but were linear in the presence of CsCl, GP plus NaCl, or GP plus CsCl.

The activities of the αD56A and βK167T αββ2 complexes were very low in the presence of NaCl but were dramatically increased in the presence of CsCl. The specific activities of the mutant enzymes were somewhat higher than the activity of the wild type αββ2 complex at 37 °C (Table I) and at high temperatures (Fig. 1) in the presence of CsCl. The addition of GP stimulated the activities of the αD56A or βK167T enzymes in the presence of NaCl but inhibited the activities in the presence of CsCl. Although the activity of either mutant enzyme was lower in the presence of GP plus NaCl than in the presence of GP plus CsCl at low temperatures, the activities under the two conditions became similar at high temperatures (Fig. 1, B, C, E, and F).

The Arrhenius plots for the mutant enzymes were linear in the presence of NaCl or of GP plus CsCl but highly nonlinear in the presence of CsCl or GP plus NaCl (Fig. 1, E and F). The activation energies (Ea) and activation entropies (ΔS*) calculated from the Arrhenius plots are listed in Table I. The Ea value for either mutant enzyme in the presence of NaCl (141 or 122 kJ/mol) was similar to the Ea value for the wild type enzyme in the presence of NaCl (128 kJ/mol). The Ea value for either mutant enzyme in the presence of CsCl at high temperature (39 kJ/mol) was similar to the Ea for wild type enzyme in the presence of CsCl (34 kJ/mol). The Ea for the higher temperature portion of either mutant enzyme in the presence of NaCl and GP was similar to the Ea in the presence of CsCl and GP and was also similar to the Ea for the wild type enzyme in the presence of GP and NaCl or CsCl. The difference between the Ea and ΔS* values at low temperature and at high temperature calculated from nonlinear Arrhenius plots were also similar for the wild type and mutant enzymes (Table I).

The αD56A and βK167T Mutations Alter the Temperature Dependence of the Absorption Spectra of the αββ2 Complex in the Presence of l-Serine and Other Ligands—The conditions that yielded nonlinear Arrhenius plots (Fig. 1 and Table I) also yielded temperature-dependent absorption spectra for the wild type αββ2 complex (1) and the αD56A and βK167T mutant enzymes (Fig. 2). The spectra obtained from the reactions of the mutant enzymes with l-serine in the presence of CsCl (Fig. 2, A and B) or in the presence of NaCl plus GP (Fig. 2, C and D) were strongly temperature-dependent and were very similar to those reported for the wild type αββ2 complex in the presence of NaCl or GuHCl (1). Decreasing the temperature resulted in decreased absorbance at 340 nm (E-AA) and increased absorbance at 424 nm (E-Ser) (Scheme 1). The presence of isosbestic points in the spectra (solid curves in Fig. 2, A–D) indicates that increasing the temperature shifted the equilibrium distribution of two intermediates from E-Ser to E-AA under these conditions. In contrast, the absorption spectra obtained from the reaction of either the αD56A or the βK167T αββ2 complex with l-serine in the presence of NaCl exhibited a peak at 424 nm (dashed curves in Fig. 2, A and B), which decreased slightly with increasing temperature between 5 and 50 °C (Fig. 2, E and F). Thus, in the presence of NaCl, E-Ser was the predominant intermediate and temperature did not markedly alter the equilibrium distribution of the E-Ser and E-AA intermediates. The reactions of the mutant enzymes with l-serine in the presence of GP plus CsCl yielded spectra that had a peak at 540 nm (dotted curve in Fig. 2, C and D) and were essentially temperature-independent at 4–50 °C (Fig. 2, E and F). The results indicate that the combination of CsCl and GP stabilized the E-AA intermediate of the mutant αββ2 complexes.

Analysis of the absorbance data for the reactions of the mutant enzymes with l-serine in the presence of CsCl or of NaCl plus GP and of the wild type enzyme with l-serine in the presence of NaCl (1) yielded the thermodynamic parameters
were plotted absorbance at 424 nm from these spectra and from analogous experiments was much lower than the \( T_m \) for either mutant enzyme in the presence of CsCl (9 or 11 °C). The absorption spectra of the wild type and mutant enzymes (see “Discussion”). The spectra from E-Ser, which is favored by the open form, to E-AA, 

\[
\alpha \beta \alpha' \beta' \text{complexes in the presence of L-serine. The absorption spectra of the } \alpha D56A \text{ or } \beta K167T \text{ complexes (8 μM) were measured in the presence of 50 mM L-serine and either 0.1 M CsCl (A and B) or NaCl plus 50 mM GP (C and D) (solid lines). The spectra for either mutant enzyme were essentially temperature-independent in the presence of 0.1 M NaCl (dashed lines for data at 25 °C in A and B) or of 0.1 M CsCl + 50 mM GP (dotted lines for data at 25 °C in C and D). The absorbance at 424 nm from these spectra and from analogous experiments were plotted versus °C for the } \alpha D56A \text{ or } \beta K167T \text{ complex.}
\]

(\( \Delta H_{eq} \) and \( \Delta S_{eq} \)) and \( T_m \) values for the conversion of E-Ser to E-AA (Table II). The \( \Delta H_{eq} \) and \( \Delta S_{eq} \) values were similar for the wild type and mutant enzymes (see “Discussion”). The \( T_m \) value for either mutant enzyme in the presence of CsCl (9 or 11 °C) was much lower than the \( T_m \) in the presence of NaCl and GP (18 or 21 °C). This result indicates that a higher temperature was needed to shift the equilibrium distribution of intermediates from E-Ser, which is favored by the open form, to E-AA, which is favored by the closed form, in the presence of GP and NaCl than in the presence of CsCl. Therefore CsCl, which binds at a site in the \( \beta \) subunit, was more effective in stabilizing the closed form than GP, which binds at the active site of the \( \alpha \) subunit.

The \( \alpha D56A \) and \( \beta K167T \) Mutations Alter the Temperature Dependence of Isotope Effects—The conditions that yielded nonlinear Arrhenius plots (Fig. 1 and Table I) and temperature-dependent absorption spectra in the presence of L-serine (Fig. 2 and Table II) also yielded temperature-dependent changes in the kinetic isotope effects for the reaction of L-serine and indole for the wild type \( \alpha \beta \alpha' \beta' \) complex (1) and the \( \alpha D56A \) and \( \beta K167T \) \( \alpha \beta \alpha' \beta' \) complex (Fig. 3). The primary kinetic isotope effects were small (−1.3) and essentially temperature-independent for the wild type \( \alpha \beta \alpha' \beta' \) complex in the presence of CsCl (1) and for the mutant enzymes in the presence of CsCl plus GP (Fig. 3). The kinetic isotope effect for either mutant enzyme in the presence of NaCl was large (−5) and changed only slightly with temperature. In contrast, the kinetic isotope effects in the presence of CsCl or NaCl plus GP were temperature-dependent and decreased with increasing temperature, as observed with the wild type enzyme in the presence of NaCl or GuHCl (1).

**DISCUSSION**

Previous investigations of the tryptophan synthase \( \alpha \beta \) complex have shown that both the binding of a monovalent cation to a site in the \( \beta \) subunit and a salt bridge between \( \beta Lys^{167} \) and \( \alpha Asp^{56} \) in the contact region between the \( \alpha \) and \( \beta \) subunits (Scheme II B) are important for the transmission of allosteric signals between the \( \alpha \) and \( \beta \) subunits (8–10, 20). Our present results provide additional evidence that mutation of either \( \beta Lys^{167} \) or \( \alpha Asp^{56} \) affects allosteric communication and catalysis, most likely by altering the conformational states of the \( \alpha \) and \( \beta \) subunits. Our most important finding is that

### TABLE I

**Effects of monovalent cations on the apparent activation energies and the activation entropies of the reaction rates of the tryptophan synthase \( \alpha \beta \) complex**

Conditions for activity measurements and data analysis by Arrhenius plots are given in Fig. 2 and in the text. Activity is specific in units/mg at 37 °C. Data for the wild type enzyme are from Ref. 1. WT, wild type.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Effectors</th>
<th>Arrhenius plot</th>
<th>Activity at 37 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>( E_a ) (kJ/mol)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Low T</td>
</tr>
<tr>
<td>WT</td>
<td>Na⁺</td>
<td>Nonlinear</td>
<td>1310</td>
</tr>
<tr>
<td>WT</td>
<td>Cs⁺</td>
<td>Linear</td>
<td>1400</td>
</tr>
<tr>
<td>WT</td>
<td>Na⁺ + GP</td>
<td>Linear</td>
<td>310</td>
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<tr>
<td>WT</td>
<td>Cs⁺ + GP</td>
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<td>200</td>
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<tr>
<td>K167T</td>
<td>Cs⁺ + GP</td>
<td>Linear</td>
<td>760</td>
</tr>
</tbody>
</table>

### TABLE II

**Enthalpy and entropy changes for the equilibrium between E-Ser and E-AA**

Values of \( \Delta H_{eq} \), \( \Delta S_{eq} \), and \( T_m \) were obtained by analysis of the spect罗斯copic data for the wild type \( \alpha \beta \alpha' \beta' \) complex (1) and for the mutant enzymes (Fig. 3). WT, wild type.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Effectors</th>
<th>( \Delta H_{eq} ) (kJ/mol)</th>
<th>( \Delta S_{eq} ) (J/mol K)</th>
<th>( T_m ) °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>Na⁺</td>
<td>92</td>
<td>327</td>
<td>8.4</td>
</tr>
<tr>
<td>αD56A</td>
<td>Na⁺ + GP</td>
<td>96</td>
<td>328</td>
<td>18</td>
</tr>
<tr>
<td>αD56A</td>
<td>Cs⁺</td>
<td>98</td>
<td>347</td>
<td>9</td>
</tr>
<tr>
<td>K167T</td>
<td>Na⁺ + GP</td>
<td>85</td>
<td>289</td>
<td>21</td>
</tr>
<tr>
<td>K167T</td>
<td>Cs⁺</td>
<td>87</td>
<td>304</td>
<td>11</td>
</tr>
</tbody>
</table>
Regulatory Role of a Salt Bridge in Tryptophan Synthase

increased temperature combined with an α subunit ligand (GP) or a certain cation (Cs\(^+\) or NH\(_4\))\(^+\) reversed the deleterious effects of either mutation on activity. We propose that these activating conditions stabilize the active, closed conformation of the mutant enzymes in the presence of NaCl or GuHCl (1).

The occurrence of nonlinear Arrhenius plots under certain conditions (Fig. 2 and Table I) provides evidence for temperature-dependent conformational transitions in the wild type enzyme, as reported previously (1), and in the αD56A and βK167T αββ\(_2\) complexes. A key finding is that there is an exact correlation between the effectors that yield nonlinear Arrhenius plots and the effectors that yield temperature-dependent changes in the equilibrium distribution of enzyme-substrate intermediates and in primary kinetic isotope effects for the wild type (1) and mutant αββ\(_2\) complexes (Fig. 3 and Table III). Another important observation is that the differences between the values of \(E_a\) and \(\Delta S^0\) at low and high temperatures derived from nonlinear Arrhenius plots (Table I) were closely similar to the values of \(\Delta H^0\) and \(\Delta S_{eq}\) for the conversion of E-Ser to E-AA obtained under analogous conditions (Table II) for both the wild type and mutant enzymes. These results support the proposal, made previously for the wild type enzyme (1), that the nonlinear Arrhenius plots result from conformational changes in the mutant enzymes. The absolute values of these thermodynamic constants were closely similar for both the wild type and the mutant enzymes, indicating that the same inferred conformational transition occurred with the wild type and mutant αββ\(_2\) complexes. Stabilization of the higher activity, closed conformation of the mutant enzymes at both high and low temperatures required the combination of two stabilizing effectors (Cs\(^+\) and GP), whereas stabilization of the wild type enzyme required only one stabilizing effector (either Cs\(^+\) or GP) (Table I). This result provides evidence that the mutations shifted the equilibrium toward the more open conformation.

The close parallel between the effects of mutation of either \(β\)Lys\(^{167}\) or αAsp\(^{90}\) on the temperature dependence data argues that interaction between these two residues is important for stabilization of the active, closed conformation of the β subunit. An analogous argument has been made on the basis of the similar effects of these two mutations on the reaction and substrate specificities (10).

We have previously discussed the thermodynamic parameters for the cases that yield nonlinear Arrhenius plots in relation to the simple reaction mechanism shown in Equation 6 (1).

\[
\begin{align*}
E + \text{Ser} &\rightleftharpoons E - \text{Ser} \rightleftharpoons E - AA \rightleftharpoons E + \text{Trp} \\
& E_{cat} = E_{eq} \times k_{cat} \times k_{eq}
\end{align*}
\]

We showed (1) that at low temperatures, in the presence of Na\(^+\), the enzymatic rate constant \(k_{cat} = E_{eq} \times k_{cat} \times k_{eq}\), where \(K_{eq}\) is the apparent steady-state equilibrium constant between E-Ser (open) and E-AA (closed) = \(K_{eq} \times k_{cat} \times k_{eq}\) in Equation 6, and \(k_{eq}\) is the rate constant of the rate-limiting step (E-Q \(\rightarrow\) E-Trp). If these mutations have no effect on substrate binding or on interactions at the β site, the 90-fold decreases in the specific activities of the mutant proteins, detected under these conditions, can be ascribed to similar decreases in \(K_{eq}\). At 20 °C, disruption of the intersubunit salt bridge between \(β\)Lys\(^{167}\) and αAsp\(^{90}\) could destabilize the closed form of the protein by \(-10.9\) kJ/mol (i.e., \(RTln(90)\)).

However, it is possible that each mutation also affects the conformation of the subunit in which it resides. Mutations of other β subunit residues that do not form salt bridges with α subunit residues have indeed been found to alter the substrate and reaction specificity (21–23). Our finding that the activity of the wild type β\(_2\) subunit (1) was 6-fold higher than that of the βK167T β\(_2\) subunit (data not shown) in the presence of NaCl and –4-fold higher in the presence of CsCl at both high and low temperatures indicates that the conformation of the β\(_2\) subunit is indeed altered by the βK167T mutation. The present and earlier (9) findings that the deleterious effects of this mutation are repaired by association with the α subunit under some conditions show that the salt bridge is not essential for this intersubunit repair. Thus, activity measurements do not clearly distinguish between the effects of the βK167T mutation on the conformation of the β subunit and on the effects on the salt bridge with αD56A in the αββ\(_2\) complex.

The small decreases in absorbance at 424 nm shown by the mutant complexes with L-serine at 40 °C in the presence of NaCl (Fig. 2, E and F) suggest that less than 5% of the enzymes

---

\(^5\)The temperature dependence of the activity of the βK167T mutant αββ\(_2\) complex in the presence of NH\(_4\)Cl was closely similar to that in the presence of CsCl (data not shown).

**Table III**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Effectors</th>
<th>Arrhenius plot</th>
<th>Spectral changes</th>
<th>KIE changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>Na(^+)</td>
<td>Nonlinear</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>WT</td>
<td>Cs(^+)</td>
<td>Linear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>Na(^+)+GP</td>
<td>Linear</td>
<td></td>
<td></td>
</tr>
<tr>
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**Fig. 3.** Temperature dependence of the kinetic isotope effects for the activities of the αD56A (A) or βK167T (B) αββ\(_2\) complex in the reaction with L-serine and indole. The primary kinetic isotope effect, which is the ratio of the activity with \(α\)-H\(_{Lys}\)-serine to the activity with \(α\)-H\(_{Phe}\)-serine, was plotted versus temperature. The wild type αββ\(_2\) complex exhibits similar temperature-dependent kinetic isotope effects in the presence of NaCl or GuHCl (1).
are in the E-AA (closed) form. Under the same conditions, most of the wild type enzyme (>99%) is in this form (1). Such marked changes in the distribution of enzyme intermediates indicate a large reduction in $K_{eq}$ (>2000-fold) for the mutant proteins, arising from destabilization of their closed form by at least 16.7 kJ/mol (i.e., $RT\ln 2000$).

We next ask what information our results give on the importance of the $\beta$Lys$^{167}$-$\alpha$Asp$^{56}$ salt bridge for allosteric communication? Important features of allosteric communication in the tryptophan synthase $\alpha_2\beta_2$ complex include mutual activation of the $\alpha$ and $\beta$ subunits upon association and ligand-induced alterations of the equilibrium distribution of enzyme-substrate intermediates and of activity. The $\beta$Lys$^{167}$-$\alpha$Asp$^{56}$ salt bridge is not essential for activation of the $\beta$ subunit, as discussed above. Neither is the salt bridge essential for ligand-induced alterations in the distribution of intermediates, as shown by the effects of GP on the absorption spectra (Fig. 2). However, mutation of the salt bridge residues alters dramatically the effects of GP on the activity at the $\beta$ site (Table I). The addition of GP increased the activity of the mutant enzymes in the presence of Cs$^+$ in Scheme I). The smaller inhibition of the high activity form of the wild type enzyme by Cs$^+$ is in this form (1). Such marked reduction in $K_{eq}$ (2000-fold) for the mutant proteins, arising from destabilization of their closed form by at least 16.7 kJ/mol (i.e., $RT\ln 2000$).

Regulatory Role of a Salt Bridge in Tryptophan Synthase

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