Monovalent Cation Activation of Tryptophanase*

CLARENCE H. SUELTERS AND ESMOND E. SNELL

From the Department of Biochemistry, University of California, Berkeley, California 94720

The interaction of monovalent cations with holotryptophanase has been examined by spectral and kinetic methods. Using S-orthocitrophenyl-L-cysteine as a substrate, activation by the following monovalent cations was demonstrated: values of $K_a$ (mm, in italics) and $V_{max}$ ($\mu$mol min$^{-1}$ mg$^{-1}$) are given in parentheses; Li$^+$ (54 ± 11.6, 4.3 ± 0.28), Na$^+$ (40 ± 0.06, 18), K$^+$ (1.44 ± 0.06, 41.1 ± 3.5), Tl$^+$ (0.95 ± 0.1, 39 ± 4.4), NH$_4^+$ (0.23 ± 0.01, 57.9 ± 2.6), Rb$^+$ (3.5 ± 0.3, 33.5 ± 1.8), Cs$^+$ (14.6 ± 2.6, 21 ± 2.3). It was demonstrated by circular dichroic spectra that the competitive inhibitor, ethionine, interacts with the holoenzyme in the absence of activating monovalent cations, although it does not undergo labilization of the $\alpha$ proton. On addition of monovalent cation to the holoenzyme-ethionine complex, a marked increase occurs in absorption at 508 nm resulting from labilization of the $\alpha$ proton with formation of the quinoid form of the pyridoxal phosphate moiety of the enzyme-substrate complex at the catalytic center (Morino, Y., and Snell, E. E. (1967) J. Biol. Chem. 242, 2890-2899). The extent of formation of this quinoid intermediate was linearly related to the maximum velocity observed with each cation except NH$_4^+$, which was anomalously active. When measured at 500 nm, the change in absorption is related to $\Delta A = 0.45$ mm$^{-1}$ of tryptophanase for NH$_4^+$ to 0.06 mm$^{-1}$ for Li$^+$. Two moles of thallium (I) were bound per mole of subunit. The data are most consistent with the interaction of monovalent cation at or near the catalytic center in such a way that it either participates directly in the reaction or is required for the critical alignment of one or more functional groups necessary for catalysis.

Monovalent cations such as K$^+$, Na$^+$, or NH$_4^+$ participate in biological processes in a variety of ways. For example, they act as charge carriers in nerve impulses, cofactors in transport, cofactors maintaining osmotic balance, important elements of the environment of halophiles and aquatic organisms, and activators of enzymes. Our interest in these cations is as participants directly in the reaction or is required for the thallium (I) were bound per mole of subunit. The data are consistent with the interaction of monovalent cation at or near the catalytic center in such a way that it either participates directly in the reaction or is required for the critical alignment of one or more functional groups necessary for catalysis.

EXPERIMENTAL PROCEDURES

Materials - KCl, RbCl, CaCl$_2$, Tl(NO$_3$)$_2$, KCl, NaCl, and LiCl were obtained from Ventron, Danvers, Mass., as ultrapure products. Ammonium chloride was Mallinckrodt, analytical reagent. Ethionine and L-threonine derivatives were from Sigma Chemical Co. Pyridoxal phosphate and 4-(2-hydroxyethyl)-piperazinopropanesulfonic acid (Heppes) were obtained from Sigma Chemical Co. Aldrich Chemical Co. was recrystallized from N-propyl alcohol before use. (CH$_3$_NOH was prepared fresh before use by passage of CH$_3$NOH over Dowex 1-OH. Tryptophanase - Tryptophanase from Escherichia coli B/7-A was prepared as described by Watanabe and Snell (6) incorporating the modifications of Suelter et al. (7). Protein was judged to be greater than 90% tryptophanase by polyacrylamide gel electrophoresis. Apoenzyme was activated by incubating it in an activating buffer composed of 0.025 mM CH$_3$_NOH, pH 8.0, 20% glycerol, 1 mM EDTA, 50 mM NH$_4$Cl, 1 mM diethioerythritol, and 1.0 mM pyridoxal phosphate for 1 h at 37°C. Occasionally activation was achieved in the same buffer at 50°C for 15 min as suggested by Högebeg-Raibaud et al. (5). The enzyme had a specific activity of 30 to 35 $\mu$mol min$^{-1}$ mg$^{-1}$ when assayed with 0.6 mM S-orthocitrophenyl-L-cysteine (SOPC) in 50 mM potassium phosphate, pH 8.0, at 25°C (8). Activating monovalent cations were removed from enzyme solutions by passage over a Sephadex G-25 column equilibrated with the desired buffer or by extensive dialysis. Protein concentration was determined spectrophotometrically using $e = 0.795$ mm$^{-1}$ cm$^{-1}$ (4).

Equilibrium Dialysis - An ammonium sulfate suspension of apo-tryptophanase (1.2 ml of 52 mg ml$^{-1}$) was centrifuged and decanted. The precipitate was dissolved in a minimum volume (about 0.5 ml) of activating buffer (9.5 mM pyridoxal-P, 9.5 mM diethoerythritol, 0.095 mM (CH$_3$_NOH, pH 8.0), and held at 37°C for 1.5 h. This sample was then dialyzed extensively to remove the activating NH$_4^+$ ion. Since sulhydryl reagents interact with thallium (9), diethioerythritol was replaced as a reducing agent with tributyl phosphine (10). The enzyme solution was first dialyzed against three changes of 100 ml of 0.095 mM (CH$_3$_NOH, pH 8.0), containing 1 mM diethioerythritol, each for a period of 2 to 3 h. It was then dialyzed against three changes (100, 100, and 50 ml) of 0.025 mM (CH$_3$_NOH, pH 8.0, containing 2 mM L-threonine), and 25 $\mu$ of tributyl phosphate per 100 ml. The last 50-ml volume was equilibrated for 10 h. Fifty microliters of the dialyzed enzyme (44.8 mg/ml) was placed on one side of an equilibrium dialysis cell (60-$\mu$l capacity) and dialyzed against 50 $\mu$l of 0.5 mM to 6 mM in the same solvent as enzyme for 4 to 5 h at room temperature, 25-30°C. The samples (10 $\mu$l) were then taken and the total Tl$^+$ concentration was assessed by counting radioactivity. Thallium solutions of 10-10$^6$ cpm $\mu$l$^{-1}$ were employed so that aliquots gave 1000 to 4000 cpm. The amount of Tl$^+$ bound per mol of subunit was determined by difference.

1 The abbreviations used are: Heppes, 4-(2-hydroxyethyl)-1-piperazinopropanesulfonic acid; SOPC, S-orthocitrophenyl-L-cysteine.
Kinetics—Kinetic constants at 25° were determined from initial velocities at SOPC concentrations ranging from 0.02 to 0.6 mM in 0.025 mM (CH₃)₂N/Hepps, pH 8.0, or by analysis of complete reaction progress curves starting at an initial concentration of 0.12 mM SOPC (11). The data were analyzed according to Wilkinson (12).

Spectral Titrations—Dissociation constants for interaction of each cation with tryptophanase in 0.025 mM (CH₃)₂N/Hepps, pH 8.0, containing 10 mM ethionine were determined by following changes in absorption at 500 nm after addition of 1- to 50-μl aliquots of concentrated solution of cation in 0.025 mM (CH₃)₂N/Hepps. After correction of each ΔAₜ, for dilution, the data were plotted as 1/ΔAₜ,versus 1/M⁺. The data were analyzed according to Wilkinson (12).

Visible absorption and circular dichroic spectra were obtained with the Cary 14 and Cary 90 spectrophotometers, respectively.

RESULTS

To evaluate the effect of monovalent cations on an enzyme, it is essential that the effect of each cation on the kinetic parameters, Kₘ and Vₘₓₓ, for that enzyme be examined. Hence the first experiments involved a determination of the Kₘ for SOPC at various concentrations of K⁺. Fig. 1 is a Lineweaver-Burk plot for SOPC at five different concentrations of K⁺ ranging from 0.2 to 30 mM. The inset shows the same data plotted as a function of K⁺ concentrations at each SOPC concentration ranging from 0.02 mM to 0.45 mM levels. There is a nonlinear behavior at low concentrations of both substrate and cation. Similar behavior was observed by Högberg-Rai baud et al. (5) for the activation of tryptophanase by K⁺ at subsaturating concentrations of S-methylcysteine, and by us when tryptophan was used as a substrate (data not given). However, linear Lineweaver-Burk plots were obtained at saturating concentrations of either K⁺ (Curves A, inset, Fig. 1) or SOPC (Curves D and E, Fig. 1). This latter behavior allowed the determination of activation constants (Kₐ) for each cation at 0.6 mM SOPC and the Kₘ and Vₘₓₓ values for SOPC at saturating levels of each cation.

![Fig. 1. Lineweaver-Burk plot for saturation of tryptophanase with SOPC at: A, 0.2 mM K⁺; B, 0.8 mM K⁺; C, 2 mM K⁺; D, 8 mM K⁺; and E, 30 mM K⁺. The inset shows the same data plotted as a function of the K⁺ concentration at: I, 0.025 mM SOPC; 2, 0.15 mM SOPC; 3, 0.075 mM SOPC; and 4, 0.45 mM SOPC. Reactions were conducted in 0.025 mM (CH₃)₂N/Hepps, at pH 8.0, as described under “Experimental Procedures.”](http://www.jbc.org/issue/7/3/1853/supplement-1)

Table I lists the activating constant, Kₐ, for each cation obtained at 0.6 mM SOPC, and also the kinetic constants for SOPC at saturating concentrations of each cation. The concentration of each cation was, at least, 10 times the Kₐ. Several points can be made. First, each cation activates, lithium being the least effective and ammonium the best. Second, the Kₐ for SOPC is not greatly affected by ionic strength. Third, the Kₐ for SOPC is not affected significantly by varying the activating cation, except for Na⁺, whereas the Kₐ for each cation varies from 54 mM for Li⁺ to 0.2 mM for NH₄⁺. The Kₐ for SOPC in the absence of monovalent cation was not determined, since the activity in the absence of monovalent cations is very small and is affected by the ammonia released during the reaction.

**Table I**

| Cation | Kₐ (mM) | Concentration of activating ion | Kₘ (mM) | Vₘₓₓ (μmol min⁻¹ mg⁻¹) |
|--------|--------|--------------------------------|--------|------------------------|
| Li⁺    | 0.08   | 0.068 ± 0.007                  | 4.3 ± 0.28 |
| Na⁺    | 0.2    | 0.245 ± 0.03                   | 18.8 ± 1.0 |
| K⁺     | 0.95   | 0.092 ± 0.03                   | 39 ± 0.4 |
| Ti⁺    | 0.1    | 0.090 ± 0.01                   | 39 ± 0.4 |
| NH₄⁺   | 0.2    | 0.066 ± 0.03                   | 57 ± 2.3 |
| Rb⁺    | 0.2    | 0.065 ± 0.03                   | 57 ± 2.3 |
| Cs⁺    | 0.3    | 0.064 ± 0.04                   | 37 ± 2.4 |

* Standard deviation.

The values given in parentheses were obtained by titrating the ethionineholoenzyme complex with cation, and following the change in absorption at 500 nm. These data were obtained from initial velocities at various substrate concentrations, rather than from analysis of complete reaction progress curves (11).
tive inhibitor ethionine is added in addition to K\(^+\), a reduction in absorption at 420 nm and 337 nm is noted with a marked increase in absorption at 508 nm (Spectrum 3).

The extent of the spectral perturbation at 508 nm in the presence of 10 mM ethionine (\(K_a = 0.52\) mM) was next measured as a function of increasing concentration of monovalent cation. The \(K_a\) for ethionine in the presence of each monovalent cation was not determined, since the \(K_a\) for SOPC was not affected significantly by each cation. These data were plotted as a function of \(1/M^+\) versus \(1/A_{A500\text{nm}}\) and extrapolated to infinite concentration of monovalent cation. A linear plot was observed in each case. The dissociation constants obtained from these data are given in Table I. The \(A_{A500\text{nm}}\) at infinite concentration of \(M^+\) was then plotted as a function of the maximum velocity observed with each cation, using SOPC as the substrate (Fig. 3). For all the cations except ammonia, a nearly direct relationship exists between \(A_{A500\text{nm}}\) and \(V_{max}\). That is, the maximum velocity for a given cation is directly proportional to the extent to which it elicits formation of the 500 nm absorbing species with ethionine.

The next question of interest is whether or not the substrate binds in the absence of cations. Fig. 4A shows that the absorption spectrum of holoenzyme in the absence of monovalent cation, plus or minus the competitive inhibitor, ethionine, are nearly identical. On the other hand, circular dichroic spectra of holotryptophanase, plus or minus the competitive inhibitor ethionine, in the absence of activating monovalent cations (Fig. 4B), are very different and are consistent with a complex between holoenzyme and ethionine. The difference between spectra 1 and 2 in the 360 to 440 nm range cannot be due to contamination with a monovalent cation such as ammonia, since the change in circular dichroism at 420 nm is much larger than that at 508 nm, where the maximum change occurs following interaction of monovalent cation (Spectrum 3). The formation of a complex between ethionine and holoenzyme in the absence of monovalent cations is consistent with the observation that saturating SOPC allows Michaelis-Menten kinetics for the activation with \(K^+\) (Fig. 1).

Finally, stoichiometry of binding of \(M^+\) to tryptophanase was examined in an effort to evaluate the specificity of its interaction with monovalent cations. The data of Fig. 5 indicate the binding of 2 mol of \(M^+\) to holoenzyme with \(K_a\) equivalent to that measured by kinetics or spectral titration.

**DISCUSSION**

The sigmoid kinetics for activation of tryptophanase by \(K^+\) at subsaturating concentrations of substrate, first reported by Högberg-Raibaud et al. (5), were confirmed in this study. Fig. 1 indicates nonlinear Michaelis-Menten kinetics for the activation by \(K^+\) at low SOPC concentrations and for the reaction with SOPC at low \(K^+\) concentrations. However, at saturating concentrations of either \(K^+\) or SOPC, we observed linear Michaelis-Menten kinetics. A similar behavior was noted with tryptophan as the substrate (data not given).

Several models may explain these data. Högberg-Raibaud et al. (5) suggested for one model that a minimum of 3 sites per subunit of enzyme, consistent with an observed Hill slope of 2.7, must be complexed with monovalent cation to achieve the activated enzyme form. No interactions between protomers would be necessary. Alternatively, they suggested cooperative binding of potassium. We suggest a third possibility, namely, that the \(K_a\) for the pyridoxal phosphate-apoenzyme complex is sufficiently large in the absence of monovalent cations and

---

**Figures and Tables**

- **Fig. 2**: Absorption spectra of holotryptophanase in 0.025 M (CH\(_2\))\(_2\)N/Hepps, pH 8.0, at room temperature. Holotryptophanase was prepared as described under "Experimental Procedures," and spectra were recorded at 1 mg/ml. Spectrum 1, holotryptophanase; Spectrum 2, holotryptophanase plus 0.1 M KCl; Spectrum 3, holotryptophanase plus 0.1 M KCl and 10 mM ethionine.

- **Fig. 3**: A plot of \(A_{A500\text{nm}}\) per mg of tryptophanase in 0.025 M (CH\(_2\))\(_2\)N/Hepps, pH 8.0, and 10 mM ethionine at infinite concentration of each cation, versus the maximum velocity with SOPC at saturating concentrations of each cation (see text).

- **Fig. 4**: A, Absorption spectra of holotryptophanase (2.5 mg/ml) in 0.025 M (CH\(_2\))\(_2\)N/Hepps at pH 8.0 and room temperature. Spectrum 1, holotryptophanase; Spectrum 2, holotryptophanase plus 10 mM ethionine. B, circular dichroic spectra of holotryptophanase (3.2 mg/ml) in 0.025 M (CH\(_2\))\(_2\)N/Hepps at pH 8.0 and room temperature. Spectrum 1, holotryptophanase; Spectrum 2, holotryptophanase plus 10 mM ethionine; Spectrum 3, holotryptophanase plus 0.1 mM KCl and 10 mM ethionine.
substrate that the holoenzyme partially dissociates in the assay mix at low concentrations of enzyme, substrate, and metal ion activator. Presumably, K+ or substrate brings about an increased affinity of enzyme for pyridoxal-P, which results in an increase in active enzyme at higher substrate or K+ concentrations, resulting in the nonlinear Michaelis-Menten kinetics. The binding studies with Tl+ indicate 2 mol of Tl+ bound per protomer, and if this is true also for K+, the first model suggested by Högberg-Raibaud et al. (5), as an explanation of nonlinear K+ saturation at subsaturating substrate, could not be true. Sufficient data are not available to distinguish between the other alternatives. However, addition of pyridoxal-P to an assay at low K+ and SOPC concentrations eliminates much of the curvature of the Lineweaver-Burk plot. This observation favors dissociation of pyridoxal-P from the holoenzyme in the assay mix as an explanation of the sigmoid kinetics.

The data of Table I indicate that Na+ elicits nearly 30% of the activity observed with NH4+. Further, a reaction with Na+ at 1 M, 0.17 M S-methylcysteine (KNa = 10 mM) (15) 0.025 M (CH3)3N/HOppe, pH 8.0, 25°C, gave a rate equal to 16.5% of that observed with 0.05 M NH4+. These observations are in direct contrast to earlier reports with other substrates, in which Na+ was shown to elicit less than 5% of the activity observed with NH4+ (3-5, 16). The earlier results are consistent, however, with our findings (cf. Table I) that much greater concentrations of cation, substrate, or both are required to demonstrate activity of Na+. and that the Vmax observed with this cation is much lower than that observed with K+. For example, in the experiment reported by Högberg-Raibaud et al. (5), S-methylcysteine (KNa = 10 max (15)) and Na+ were employed at concentrations of only 70 and <50%, respectively, of those required for enzyme saturation. Under these conditions, the enzyme would show much lower activity than the maximum expected assuming Michaelis-Menten kinetics. Thus, failure to note activity with Na+ results primarily from the fact that insufficient Na+ and substrate were added to the assay mixture.

The circular dichroic data of Fig. 4B provide convincing evidence that the competitive inhibitor, ethionine, interacts, with the holoenzyme in the absence of monovalent cation, but does so in such a way that the 508 nm absorbing species is not formed. The structure of the former adduct is not known; one possibility (since, as shown in Fig. 4A, it has the same absorption spectrum as enzyme alone) is that it has Structure II (Fig. 6). The addition of cations shifts the reaction from II to III, a or b (Fig. 6), which for ethionine absorbs maximally at 508 nm (Fig. 2).

As indicated in Fig. 3, the maximum absorption at 508 nm, and thus the extent of formation of the quinoid intermediate III elicited by each monovalent cation in the presence of the inhibitor, ethionine, is directly proportional to the Vmax observed with each cation and SOPC except for NH4. The activity with ammonia is much greater than expected, perhaps due to the stereochemical nature of the interaction with its ligands. Since the extent of formation of the quinoid intermediate reflects the labilization of the amino acid α proton (4), the data are consistent with the view that labilization of the α proton is the rate-limiting step in the reaction with SOPC. This is in contrast to the data obtained for a variety of other substrates of tryptophanase, including l-tryptophan, serine, S-ethyl-L-cysteine, and S-methyl-L-cysteine (4). When reactions with these substrates are allowed to proceed to partial completion in tritiated water, the unreacted substrate contained tritium. This is consistent with the argument that the rate-limiting step in the reaction occurs after the quinoid intermediate III. Fig. 6. Morino and Snell (4) supported this argument by showing the existence of appreciable intermediates near 500 nm during the steady state reaction with several substrates, such as tryptophan, S-methyl-, and S-ethyl-L-cysteine. However, the Vmax for SOPC is substantially higher than that for any of the previously tested substrates, and this higher rate could well be associated with a change in the rate-limiting step. In accordance with this, experiments similar to those reported previously (4), but with SOPC as substrate in H2O containing 0.05 M NH4+, 0.1 M K+, or 1 M Na+, showed no incorporation of tritium into the unreacted SOPC. In addition, no 500 nm absorbing species could be detected by a scanning stopped flow (17) spectrophotometric study of the reaction with SOPC in 0.1 M KCl; appreciable 500 nm absorption was detected when tryptophan, S-ethyl-, and S-benzyl-L-cysteine were used as substrates in the tryptophanase reaction. Thus, the rate-limiting step with SOPC occurs before elimination of the β substituent; labilization of the α proton as the rate-limiting step is consistent with these observations.

The extent of the 508 nm perturbation, and therefore, the

C. H. Sueter and E. E. Snell, unpublished observations.

D. S. June and C. H. Sueter, unpublished observations.
extent of formation of the quinoid intermediate resulting from lability of the α proton, is dependent on the monovalent cation. This observation raises two questions. First, what property of the cation is responsible for the effect, and second, does the cation participate directly in the reaction at the catalytic center?

Based on crystallographic structures of monovalent cation complexes such as nonactin (18) and enniatin B (19), it is expected that the monovalent cation would be complexed without water of hydration. Therefore, the interaction of a cation with an enzyme must be considered in terms of the following scheme:

\[ E(H_2O)_n + M^+ (H_2O)_m \rightarrow E - M(H_2O)_{n+x} + (x + y - z)H_2O \]

Assuming that the hydration energy of the enzyme cation binding site is negligible, the affinity for a cation is primarily controlled by the difference in free energy of hydration of the cation and the free energy of interaction of the naked cation with its site on the enzyme surface (20). For example, the free energy of hydration ranges from approximately 96 kcal/mol for Li⁺ to 47 kcal/mol for Cs⁺. The free energy of interaction of monovalent cation with nonactin, when referred to a vacuum, is essentially the same magnitude (20). The differences between these values gives the net free energy of interaction (ranging from 1 to 4 kcal/mol) defining the order of affinities of nonactin with M⁺ in aqueous solution, K⁺ > Rb⁺ > Cs⁺ > Na⁺ > Li⁺, which is identical to that observed with tryptophanase disregarding NH₄⁺ and Tl⁺. The binding constants for NH₄⁺ and Tl⁺ with nonactin are anomalously high (20). Based on these arguments, it is expected that the affinities of the naked cation for the enzyme are approximately the same as those with nonactin, and that their sequence follows the same order, namely, Li⁺ > Na⁺ > K⁺ > Rb⁺ > Cs⁺. Therefore, once bound, the actual attractive force between the cation and the interacting groups on an enzyme surface bears little resemblance to the order of maximum velocities. Furthermore, this attractive force is much larger than that indicated by the dissociation constant of M⁺ with enzyme and may provide important clues regarding the role of monovalent cations in catalysis. A similar argument was made by Jencks (21) for other systems.

Another interesting feature of these cations is their size. Cations with ionic radii smaller than 1.3 Å, or larger than 1.5 Å, are poor activators. This relationship between the size of a cation, including its volume and surface area, and the maximum velocity are presented in Fig. 7. The plot is given as a function of optimum size assuming that the size of NH₄⁺ is optimum, but that the rate with NH₄⁺ is anomalous. The parameters are presented in terms of a percentage of the optimum. When the parameter was larger than the optimum, the excess over 100 was subtracted from 100. The data suggest that the forces of attraction between the naked cation and the enzyme, which, in fact, differ little, bring about the alignment of the peptide backbone into an optimum configuration. An optimum configuration requires an optimum size or volume for the monovalent cation, so that the proper alignment of reacting groups needed for efficient catalysis can be achieved. The anomalous behavior of NH₄⁺ suggests that the directionality of the attractive force may be important in the critical alignment of the interacting groups.

Does the cation exert its effect by interaction directly with the reactants at the catalytic site, or at some site distant from the catalytic site? Data of Fig. 2 show that the cation binds in the absence of substrate, and those of Fig. 4 show that the substrate binds in the absence of cation. The addition of the cation permits formation of the quinoid intermediate with the concomitant labilization of the α proton and catalysis (Structure II to III, Fig. 6). Since proteins are flexible molecules, it would seem unlikely that the relatively small differential effect of each cation could be mediated if it were bound at some site on the protein surface distant from the active site. The most likely possibilities are that the cation is bound at the catalytic site and participates directly in the reaction, or alternatively is bound near the catalytic center to bring about the critical alignment of one or more functional groups. The fact that most pyridoxal-P-dependent enzymes that catalyze α, β elimination reactions require monovalent cations for maximum activity (1, 13, 22), whereas those catalyzing transamination reactions do not, also supports the view that these cations are involved directly or indirectly in the catalytic process.

A stoichiometry of 2 Tl⁺ per subunit is also consistent with a specific role for the cation, rather than a nonspecific conformational effect. The hyperbolic saturation curve for monovalent cation activation when excess substrate is present is consistent with participation of 1 cation per catalytic center. Whether or not the second mole has mechanistic significance will have to await additional data; for example, x-ray crystallographic evidence.

Acknowledgments—We acknowledge the prior, unpublished work of T. Watanabe, who established the activating effects of Tl⁺ (13), and also the gracious assistance of Dr. Beverly Guirard and Ms. Jean Wang during many phases of this research.

REFERENCES
1. Sueltzer, C. H. (1970) Science 168, 789-795
2. Sueltzer, C. H. (1974) in Metal Ions in Biological Systems, (Sigel, H., ed) Vol. 3, pp. 291-361, Marcel Dekker, Inc., New York
3. Happold, F. C., and Struyvenberg, A. (1954) Biochem. J. 58, 379-386
4. Morino, Y., and Snell, E. E. (1961) J. Biol. Chem. 242, 2800-2809
5. Högberg-Raibaud, A., Raibaud, O., and Goldberg, M. E. (1973) J. Biol. Chem. 249, 3352-3358
6. Watanabe, T., and Snell, E. E. (1972) Proc. Natl. Acad. Sci., U. S. A. 69, 1086-1090
7. Sueltzer, C. H., Wang, J., and Snell, E. E. (1977) Anal. Biochem. 76, 221-232
8. Sueltzer, C. H., Wang, J., and Snell, E. E. (1976) FEBS Lett. 66, 236-239
9. Harmony, J. A. K., Shaffer, P. J., and Himes, R. H. (1974) J. Biol. Chem. 249, 394-401
10. Friedman, M. (1973) in *The Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides and Proteins* p. 206, Pergamon Press, Oxford
11. Yun, S. L., and Suelter, C. H. (1977) *Biochim. Biophys. Acta* 480, 1–13
12. Wilkinson, G. N. (1961) *Biochem. J.* 80, 324–332
13. Snell, E. E. (1975) *Adv. Enzymol. Relat. Areas Mol. Biol.* 42, 287–333
14. Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* 51, 660
15. Newton, W. A., Morino, Y., and Snell, E. E. (1965) *J. Biol. Chem.* 240, 1211–1218
16. Newton, W. A., and Snell, E. E. (1964) *Proc. Natl. Acad. Sci. U. S. A.* 51, 382–389
17. Suelter, C. H., Coolen, R. B., Papadakis, N., and Dye, J. L. (1975) *Anal. Biochem.* 69, 130–135
18. Kilbour, B. T., Dunitz, J. D., Proda, L. A. R., and Simon, W. (1967) *J. Mol. Biol.* 30, 559–563
19. Dobler, M., Dunitz, J. D., and Krajewski, J. (1980) *J. Mol. Biol.* 142, 603–606
20. Krasne, S., and Eisenman, G. (1973) in *Membranes – A Series of Advances* (Eisenman, G., ed) Vol. 2, p. 277, Marcel Dekker, New York
21. Jencks, W. P. (1975) *Adv. Enzymol. Relat. Areas Mol. Biol.* 43, 219–410
22. Davis, L., and Metzler, D. E. (1972) in *The Enzymes* (Boyer, P. D., ed) Vol. 7, pp. 33–74, Academic Press, New York
Monovalent cation activation of tryptophanase.
C H Suelter and E E Snell

J. Biol. Chem. 1977, 252:1852-1857.