Formation Constants and Coordination Thermodynamics for Binary and Ternary Complexes of Copper(II), L-Hydroxyproline, and an Amino Acid Enantiomer

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While a rich database is available at or near room temperature (288.15 to 298.15 K) defining the chemical equilibria of solutions containing the copper(II) ion and an amino acid, data describing the temperature dependence of reaction thermodynamics for these systems remain scarce. In addition, data defining enthalpy, entropy, and heat capacity changes for the formation of mixed amino acid chelate complexes are extremely limited, hindering our understanding of the driving forces for complex formation and stabilization. Here, protonation constants and concentration-based equilibrium constants for Cu(II)−amino acid complexes are reported from 288.15 to 348.15 K for leucine, valine, proline, phenylalanine, and hydroxyproline in aqueous solutions containing 0.1 M KNO_3. The logarithmic (ln) values of the protonation and binary stepwise concentration equilibrium constants (K_i) for each amino acid ligand are found to be linearly dependent on the inverse of temperature, indicating negligible change in heat capacity for each of these protonation and complexation reactions. However, ln(K_i) data for ternary complexes formed between Cu(II), L-hydroxyproline, and any one of the other amino acid enantiomers show a nonlinear dependence on inverse temperature, indicating a negative change in heat capacity. Enthalpy and entropy changes for ternary complex formation are therefore temperature-dependent quantities. Our thermodynamic data, when combined with statistical analysis of reaction stoichiometry, reveal that ternary Cu(II)(D′ or L′)(L-hydroxyproline) complexes are consistently hyperstable as compared to their parent bis-binary complexes at all solution temperatures studied.

Introduction

Amino acids are important low molecular weight ligands in humans[1−4] and other biosystems.[5,6] Their involvement in Cu(II) transport and metabolism is well-documented.[7,8] In addition, the reactivity of amino acids and other small organic molecules is often modified when they are coordinated to metal ions. Nature exploits this effect through the construction of metal-ion binding cavities within the active sites of many enzymes (e.g., pyruvate kinase, superoxide dismutase) that serve to accelerate reactions that would otherwise proceed too slowly to be useful in a living system. For example, the extraordinarily high rates of cleavage observed for the enzymatic hydrolysis of carboxylic esters and amides can often be accounted for through the effect of a bound metal ion acting either as a Lewis acid catalyst by coordinating to a carbonyl oxygen atom and thereby polarizing the carbonyl group or as a source of a nucleophile through coordination to a hydroxide ion.[9]

Transition metal ion chelate complexes are also exploited by industry in the large-scale purification of α-amino acids and a wide range of drugs and drug precursors containing an aminocarboxylic acid moiety.[10,11] Chiral ligand exchange chromatography, which utilizes stereoselective binding to an immobilized chiral ligand (selector), is widely used in industry for the separation of racemic mixtures of amino acids and their derivatives.[12,13] Newer technologies for scalable continuous separation of chiral racemates present the selector either directly in solution[14] or on the surfaces of stable micelles.[15,16] These industrial applications are often best carried out at temperatures far from ambient or physiological conditions. Efficient design and optimization of these technologies therefore requires knowledge of chemical equilibria within the system and its dependence on temperature.

In principle, acidic, polar, and basic amino acids can bind metal ions through a mixture of available donor groups that includes α-carboxylate and α-amino groups as well as appropriate groups on the side chain of the α-aminocarboxylic acid: for example, the β-hydroxy group on the side chains of threonine and serine, the β-thiol on cysteine, and the β- and γ-amides on asparagine and glutamine, respectively. While electron donors on the amino acid side chain can be effective ligands, glycine-like bidentate coordination through the α-carboxylate and α-amino groups is often favored thermodynamically among amino acids at neutral and acidic pH.

Potentiometry experiments have provided reliable protonation constants and formation constants for many binary metal−ion (amino acid) complexes in aqueous solutions of 0.1 M or 0.15 M (physiological) ionic strength at 293.15 K, 298.15 K, or 310.15 K.[17,18] KNO_3 or $NaClO_4$ is most often employed as the background electrolyte. In certain cases, coordination enthalpies and entropies are reported at either 298.15 K or 310.15 K.[19−22] The decomposition of a formation constant into its enthalpic and entropic contributions is of fundamental importance to understanding the factors that influence the coordination reaction and the stability of the complex. These factors may include solvation, steric, and electronic effects. Although they can be measured more accurately and directly by calorimetry, coordination enthalpies ($\Delta_r H_c$) at a given

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temperature are most often obtained through measurement of formation constants (K_i) at surrounding temperatures and application of the van't Hoff equation:

$$\frac{d(\ln K_i)}{d(1/T)} = -\frac{\Delta_r H_c}{R} \quad (1)$$

This approach provides a good understanding of the thermodynamics of the complexation reaction at the given temperature, but extension to other temperatures is limited by the fact that coordination enthalpies (and entropies) are temperature dependent, and the ΔC_p data required to determine coordination thermodynamics at other temperatures through application of the Kirchoff equation are generally not available.

We report concentration (i.e., concentration-based) protonation constants and formation constants for Cu(II)-(amino acid) complexes from 288.15 to 333.15 K for leucine, valine, proline, phenylalanine, and hydroxyproline in aqueous solutions containing 0.1 M KNO_3. These data are obtained by potentiometry and used to evaluate nonlinearities in the van't Hoff plot and to estimate ΔC_p values for each complex formed. Coordination enthalpies and entropies are then computed as a function of temperature to evaluate the factors that influence the stability of a given complex and the relative dependence of these factors on temperature. Statistical effects in the formation of binary and ternary complexes are also considered and discussed. Particular attention is paid to complexes containing L-hydroxyproline, as this ligand is often used as the immobilized selector in chiral ligand-exchange chromatography.

Materials and Methods

Materials. Aminocarboxylic acid enantiomers, nitric acid (0.0983 M HNO_3 standard), potassium nitrate (KNO_3), and copper nitrate ($Cu(NO_3)_2$) were purchased from Sigma-Aldrich Chemicals Canada Ltd. (Oakville, ON). These reagents have reported purities of greater than 99 % and were used without further purification. For the preparation of all aqueous solutions, water was first double distilled and then treated with a Nanopure II ultrafiltration system (Barnstead; Dubuque, IW). KOH standard (0.1 M) was prepared by diluting KOH Titrisol ampules (Merck) according to the manufacturer's instructions. All reagents and solutions were prepared immediately prior to use in an experiment.

Potentiometric Titrations. All potentiometric titrations were carried out in an electrically insulated Schott Titronic T110 automatic titrator (Schott Instruments, Germany). Premixed samples were introduced into the 3 mL or 10 mL water-jacketed titration vessel and maintained at the desired reaction temperature by an external circulating water bath (Julabo-UC circulator, Germany) and a Teflon-coated magnetically driven stir bar. The titration cell was maintained under nitrogen atmosphere, and aliquots of the base titrant solution (5−20 μL) were introduced into the reaction cell using a Schott TA01 automatic buret. The solution pH was measured continuously with a Metrohm micro-combination pH glass electrode (Brinkmann Instruments Ltd., Mississauga, ON).

The standard electrode potential ($E°$) was determined immediately prior to each titration. A standard stock solution of 10 mM HNO_3 solution was prepared by adding KNO_3 as a background electrolyte to create a degassed 0.1 M solution ($p[H^+] = 2$). The electrode was then calibrated according to the classic procedure of Carpeni et al.[23] by titrating 5.0 mL of this HNO_3 solution with a 0.1 M KOH standard for which the concentration of KOH was verified potentiometrically by titration against a 10 mM potassium hydrogen phthalate (KHP) primary standard ($I = 0.1$ M). The resulting titration data were analyzed using the nonlinear least-squares program CHEMEQ,[24] which regresses $E°$ as well as the initial total proton concentration in the vessel.

Amino acid purities (99.27 % (w/w) L-hydroxyproline, 99.31 % L/D-leucine, 99.81 % L/D-phenylalanine, 99.42 % L/D-proline, 99.45 % L/D-valine) and protonation constants were determined by titrating solutions of 0.1 M KNO_3 (ca. $p[H^+] = 2$) containing 10 mM of the pure amino acid. Similarly, determination of concentration formation constants for binary complexes was based on a 0.1 M KNO_3 solution containing 10 mM amino acid and 5 mM $Cu(NO_3)_2$; solutions for determination of ternary concentration formation constants contained equimolar (ca. 10 mM) concentrations of each aminocarboxylic acid ligand and $Cu(NO_3)_2$. The concentration of Cu^{2+} in stock solutions of $Cu(NO_3)_2$ was determined by titration with a standard EDTA solution using 1-(2-pyridylazo)-2-naphthol (PAN) indicator dye. A defined volume of copper stock solution diluted in 5.0 mL of deionized water, 200 μL of 0.1 M HNO_3, and 2−3 drops of PAN (3 % solution in methanol) was titrated by dropwise addition of EDTA stock solution to equivalence, which was indicated by a color change from a light purple to light gray. In all titration experiments, a premixed 0.1 M KOH standard solution (Merck) served as the titrant and was sequentially added in aliquots of either 5 or 10 μL to the titration vessel by volumetric pipet. After equilibrium was reached, the electrode potential and the amount of the base added to the system were recorded.

Data Regression. Both standard formation constants (also known as stability constants; denoted by the symbol β) and stepwise stability constants (denoted by the symbol K) are reported in this work. To fix ideas, consider the binary metal (M)−ligand (L) complex ML_n. The reaction

$$M + nL \overset{\beta_{10n0}}{\rightleftharpoons} ML_n \text{ (constant } T \text{ and } P)$$

may be used to define the standard formation constant β for the complex,

$$\beta_{10n0} = \frac{[ML_n]}{[M][L]^n} \quad (2)$$

which is defined in terms of equilibrium concentrations of the reactants. We therefore designate β_{0n01} and all others reported in this work as concentration formation constants to clearly differentiate them from standard (state) formation constants based on the activities of the reactants. The subscript $10n0$ on the β defined in eq 2 indicates the molecules of Cu^{2+} (1), protons (0), amino acid enantiomer (n), and L-hydroxy proline (0), respectively, present in the complex. A negative number in the first subscript register indicates the presence and number of hydroxyl ions in the complex.

The stepwise (concentration) formation constant for the same binary ML_n complex may be defined from the reaction

$$ML_{n-1} + L \overset{K_n}{\rightleftharpoons} ML_n \text{ (constant } T \text{ and } P)$$

as

$$K_n = \frac{[ML_n]}{[ML_{n-1}][L]} \quad (3)$$

Table 1. Amino Acid Concentration-Based Protonation Constants (I_c = 0.1 M KNO_3)[a]

		β at T/K				
system	$\log_{10}$	288.15	298.15	310.15	318.15	333.15
hydroxyproline	β_{0101}	9.7 ± 0.1	9.46 ± 0.01	9.15 ± 0.03	9.1 ± 0.1	8.80 ± 0.01
	β_{0201}	11.5 ± 0.2	11.31 ± 0.02	10.9 ± 0.1	10.83 ±0.02	10.58 ± 0.02
proline	β_{0110}	10.66 ± 0.1	10.50 ± 0.01	10.17 ± 0.01	9.92 ± 0.04	9.7 ± 0.1
	β_{0210}	12.6 ± 0.1	12.46 ± 0.02	12.10 ± 0.02	11.81 ± 0.04	11.7 ± 0.1
valine	β_{0110}	9.74 ± 0.03	9.48 ± 0.01	9.23 ± 0.02	8.98 ± 0.04	8.77 ± 0.02
	β_{0210}	12.03 ± 0.03	11.77 ± 0.01	11.53 ± 0.03	11.28 ± 0.03	11.5 ± 0.1
phenylalanine	β_{0110}	9.32 ± 0.02	9.07 ± 0.02	8.76 ± 0.02	8.62 ± 0.01	8.31 ± 0.01
	β_{0210}	11.52 ± 0.02	11.27 ± 0.04	10.94 ± 0.03	10.8 ± 0.1	10.52 ± 0.03
leucine	β_{0110}	9.81 ± 0.03	9.52 ± 0.02	9.29 ± 0.01	9.11 ± 0.02	8.8 ± 0.1
	β_{0210}	12.2 ± 0.05	11.86 ± 0.02	11.62 ± 0.01	11.44 ± 0.02	11.2 ± 0.1

[a] Errors reported as standard deviation from the mean for 10 to 15 independently regressed data sets.

From the above definitions, it is evident that

$$\beta_{10n0} = K_1K_2...K_n = \prod_{i=1}^{n} K_i \tag{4}$$

Protonation constants and concentration formation constants were regressed from potentiometric data using the program CHEMEQ previously developed by our group.[24] CHEMEQ minimizes the weighted sum U of the squared residuals between observed (E_i^{obs}) and calculated (E_i^{calc}) electrode potentials:

$$U = \sum_{i=1}^{n} \frac{(E_i^{obs} - E_i^{calc})^2}{\sigma_i^2} = \sum_{i=1}^{n} W_i(E_i^{obs} - E_i^{calc})^2 \tag{5}$$

The weighting factor (W_i) is defined as the reciprocal of the square of the total estimated error (σ_i) due to errors in the electrode potential (σ_E = 0.1 mV), reagent concentrations (e.g., σ_C = 0.3 mM for L-hydroxyproline), and the titrant volume readings (σ_v = 0.002 cm^3):[25]

$$\sigma_i^2 = \sigma_E^2 + \sum_j \left(\frac{\partial E_i^{obs}}{\partial C_j}\right)^2 \sigma_{C_j}^2 + \left(\frac{\partial E_i^{obs}}{\partial V}\right)^2 \sigma_v^2 \tag{6}$$

Since the electrode potential is not an explicit function of the equilibrium formation constants, the implicit differentiation path of Nagypál and Páka[26] was applied in a manner similar to that employed by Gans et al.[27] in his multi-parameter regression program SUPERQUAD, which includes a Gauss−Newton algorithm and the Levenburg−Marquardt technique to improve convergence.

The program CHEMEQ improves upon the implicit differentiation routine of Gans et al.[27] by establishing methods to greatly reduce computational time when certain components are not present in a given complex, to reduce round-off errors due to redundant matrix elements, and to improve parameter regression statistics. A detailed description of these improvements is provided elsewhere.[24] Each reported parameter (protonation constant, concentration formation constant) was regressed from the combined titration data for 10−15 experimental runs, with the standard deviation computed and reported in each case.

Results and Discussion

Protonation Constants. The side chains of all amino acids studied in this work are charge neutral. Each amino acid (A^-) therefore contains two protonation sites characterized by the following stepwise equilibria:

$$H^+ + A^- \rightleftharpoons HA$$

designated by K_1, $\Delta_rH_c(K_1)$, and $\Delta_rS_c(K_1)$

$$HA + H^+ \rightleftharpoons H_2A^+$$

designated by K_2, $\Delta_rH_c(K_2)$, and $\Delta_rS_c(K_2)$

where K_1 refers to protonation of the α-amino group and K_2 refers to that of the α-carboxylate group. Table 1 reports the log value of the standard protonation constants (β_{0110}, β_{0210}) for leucine, valine, proline, phenylalanine, and hydroxyproline as a function of solution temperature. The standard deviation is provided next to each protonation constant. Martell and Smith[28] report protonation constants for all of these amino acids at 298.15 K and I = 0.1 M, and our results are in good agreement with their values at that solution temperature. Comparative data at other temperatures are available for certain amino acids (proline and hydroxyproline[29,30]) and again are in good agreement with our results.

The more familiar stepwise dissociation constants (pK_a values) can be calculated from the measured β values through the relation

$$pK_{a1} = \log K_2 = \log \beta_{0210} - \log \beta_{0110} \tag{7}$$

$$pK_{a2} = \log K_1 = \log \beta_{0110} \tag{8}$$

The Gibbs energy change (Δ_rG_c) for each protonation reaction is related to the respective stepwise protonation constant:

$$\Delta_rG_c(T) = -RT \ln(K_i) = \Delta_rH_c(K_i) - T\Delta_rS_c(K_i) \tag{9}$$

where the molar reaction enthalpy (Δ_rH_c) and entropy (Δ_rS_c) at system temperature (T) are computed from $K_i(T)$ data using eq 1 and eq 9, respectively. Figure 1 plots $K_1(T)$ and $K_2(T)$ data for proline according to the van't Hoff equation (eq 1). A linear relation is observed, indicating a negligible change in heat capacity for each protonation reaction over the temperature range 288.15 to 333.15 K. As a result, Δ_rH_c and Δ_rS_c are constant over this temperature range for both protonation reactions. Linear dependencies of ln K_1 and ln K_2 on inverse temperature were also observed for all other amino acids studied.

Table 2 reports regressed Δ_rH_c and Δ_rS_c values for each amino acid studied. Protonation thermodynamics measured by calorimetry have previously been reported at 298.15 K, and our results are in reasonably good agreement with those earlier studies. However, our Δ_rS_c values are slightly higher (on average ca. 4.2 to 8.4 J/mol·K) than previously

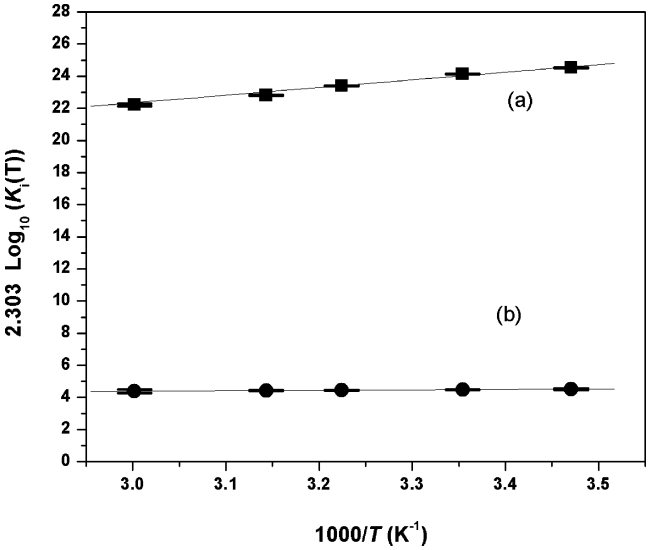

Figure 1. van't Hoff plots for stepwise protonation of proline: (a) K_1, (b) K_2.

Table 2. Enthalpy and Entropy Changes for Amino Acid Protonation over the Temperature Range T = 298.15 K to 333.15 K (I_c = 0.1 M KNO_3)

analyte	equilibrium	$\Delta_r S_c$ (J·mol^{-1}·K^{-1})	$\Delta_r H_c$ (kJ·mol^{-1})
leucine	[HL]/[H][L]	49.8 ± 0.8	−39.7 ± 0.4
	[H_2L]/[HL][H]	47.3 ±1.3	0.8 ± 0.4
valine	[HL]/[H][L]	46.5 ± 0.8	−40.2 ± 0.4
	[H_2L]/[HL][H]	54.4 ± 0.8	3.4 ± 0.4
proline	[HL]/[H][L]	57.4 ± 1.6	−43.1 ± 0.8
	[H_2L]/[HL][H]	29.3 ± 0.8	−2.5 ± 0.4
phenylalanine	[HL]/[H][L]	35.2 ± 0.8	−41.5 ± 0.4
	[H_2L]/[HL][H]	42.7 ± 0.4	0.4 ± 0.4
hydroxyproline	[HL]/[H][L]	52.7 ± 1.7	−38.5 ± 0.8
	[H_2L]/[HL][H]	22.2 ± 0.8	−3.8 ± 0.4

measured by calorimetry, possibly due to the intrinsically higher level of error associated with indirectly determining reaction enthalpies from potentiometry data. As our results indicate that $\Delta_r H_c$ and $\Delta_r S_c$ remain constant from 288.15 to 333.15 K, the value of K_1 or K_2 at any temperature over this range can be computed from the data at 298.15 K provided in Tables 1 and 2 using the relation

$$\ln K_i(T) = \ln K_i(T^\circ) + \Delta_r H_c^\circ(K_i)\frac{(T - T^\circ)}{2.303RTT^\circ} \quad (10)$$

where T° is the reference temperature (298.15 K) and $\Delta_r H_c^\circ$ is the enthalpy change for the protonation reaction at that temperature. For each amino acid studied, protonation of the α-amino group is exothermic, with a $\Delta_r H_c^\circ(K_1)$ near −40 kJ mol^{-1}. In contrast, protonation of the α-carboxylate group is found to be nearly athermal for each amino acid. Both results are in good agreement with previous results obtained from calorimetric method.[31,32] Due to the observed exothermic α-amino protonation reaction, we find that the concentrations of protonated states of the amino acids will decrease with increasing temperature.

Concentration Formation Constants for Binary Complexes. All of the amino acids investigated in this study exhibit glycine-like coordination chemistry with the Cu^{2+} ion, but differences in the electron-withdrawing strengths of the side chains alter binary Cu^{2+}(amino acid) complex stabilities relative to those observed in the Cu^{2+}-(glycine) complex. Standard binary formation constants for Cu^{2+}−amino acid (A) complexes are defined in this study by the following reaction equilibria:

$$Cu^{2+} + A^- \overset{\beta_{1010}}{\rightleftharpoons} CuA^+$$

$$Cu^{2+} + 2A^- \overset{\beta_{1020}}{\rightleftharpoons} CuA_2$$

except in the case of L-hydroxyproline (hereafter referred to as L-HyPro), where the formation constants for the equivalent binary complexes are β_{1001} and β_{1002}, respectively. Standard formation constants at 298.15 K for all binary Cu^{2+} $^-$ amino acid complexes are reported in Table 3 along with their standard deviations. Sóvágó et al.[33] previously measured formation constants for these binary complexes at 298.15 K but did not study their temperature dependence. The results published here at 298.15 K are in good agreement with their data.

Given their nonpolar and neutral side chains, the amino acids included in this study are not expected to associate strongly with either the inner or the outer coordination sphere of Cu(II). Not surprisingly then, the stabilities of the two binary homo-chiral complexes (Cu^{2+}(L)$_2$ and Cu^{2+}-(D)$_2$, where L and D represent the Fischer-based chirality of the amino acid) and the binary hetero-chiral complex (Cu^{2+}(L)(D)) were found to be equal to within our experimental error. For example, the formation constant for the hetero-chiral Cu^{2+}(L)(D) bis-binary complex formed at 298.15 K with D-phenylalanine (L-Phe-Cu-D-Phe) was determined in this study to be $\log_{10}\beta_{1020}$= 14.48 ± 0.03, and that for D-valine (L-Val-Cu-D-Val), was $\log_{10}\beta_{1020}$= 14.8 ± 0.1. Comparison with the corresponding data for homo-chiral bis-binary complex shown in Table 3 reveals no statistically relevant difference in stability. However, this is not the case with all binary metal−ion (amino acid) complexes.[34] For example, calorimetry studies by Sharrock and Raymond[35] show that metal coordination of serine is stereoselective due to participation of the hydroxy group in the complexation of Cu(II) through hydrogen bonding to an axially coordinated water molecule.

Stepwise formation constants were also calculated and plotted versus inverse temperature to estimate the en-

Table 3. Binary Amino Acid/Copper Concentration Formation Constants (I_c = 0.1 M KNO_3)

		β at T/K				
system	$\log_{10}$	288.15	298.15	310.15	318.15	333.15
hydroxyproline	β_{1001}	8.63 ± 0.01	8.48 ± 0.03	8.22 ± 0.01	8.19 ± 0.01	8.06 ± 0.01
	β_{1002}	15.92 ± 0.02	15.6 ± 0.1	15.10 ± 0.01	15.04 ± 0.01	14.75 ± 0.02
proline	β_{1010}	8.87 ± 0.01	8.73 ± 0.01	8.61 ± 0.02	8.50 ± 0.01	8.33 ± 0.01
	β_{1020}	16.37 ± 0.01	16.08 ± 0.03	15.8 ± 0.1	15.62 ± 0.03	15.3 ± 0.3
valine	β_{1010}	8.1 ± 0.1	8.05 ± 0.01	7.94 ± 0.01	7.89 ± 0.03	7.35 ± 0.02
	β_{1020}	14.90 ± 0.02	14.79 ± 0.02	14.56 ± 0.01	14.47 ± 0.01	13.43 ± 0.05
phenylalanine	β_{1010}	7.86 ± 0.01	7.71 ± 0.01	7.6 ± 0.1	7.56 ± 0.03	7.5 ± 0.1
	β_{1020}	14.76 ± 0.01	14.42 ± 0.02	14.1 ± 0.1	14.04 ± 0.02	13.80 ± 0.09
leucine	β_{1010}	8.12 ± 0.02	8.1 ± 0.1	8.0 ± 0.1	7.92 ± 0.02	7.85 ± 0.02
	β_{1020}	14.9 ± 0.1	14.71 ± 0.01	14.55 ± 0.02	14.48 ± 0.03	14.34 ± 0.03

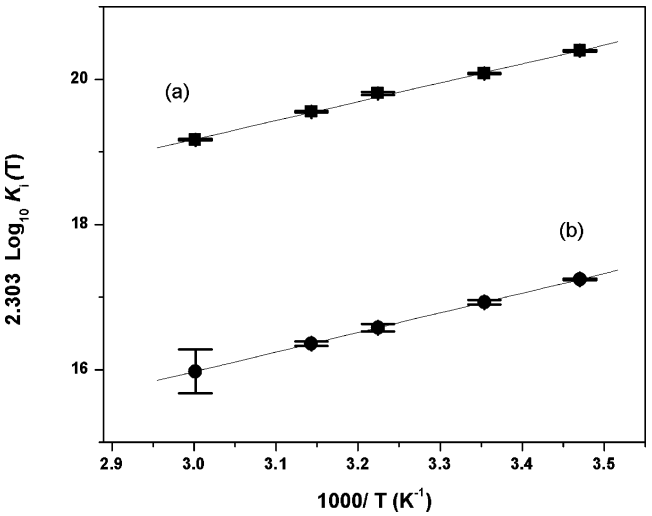

Figure 2. van't Hoff plots for stepwise formation of mono- and bis-binary Cu^{2+} complexes containing proline: (a) K_1, (b) K_2.

Table 4. Enthalpy and Entropy Changes for Mono- and Bis-Binary Complex Formation over the Temperature Range T = 298.15 K to 333.15 K (I_c = 0.1 M KNO_3)

analyte	equilibrium	$\Delta_r S_c$ ($J \cdot mol^{-1} \cdot K^{-1}$)	$\Delta_r H_c$ ($kJ \cdot mol^{-1}$)
leucine	[CuL]/[Cu][L]	116.8 ± 0.8	−11.3 ± 0.4
	$[CuL_2]/[CuL][L]$	92.5 ± 1.3	−10.5 ± 0.4
valine	[CuL]/[Cu][L]	59.0 ± 0.4	−28.5 ± 0.4
	$[CuL_2]/[CuL][L]$	41.9 ± 0.8	−25.9 ± 0.4
proline	[CuL]/[Cu][L]	94.6 ± 0.4	−21.8± 0.4
	$[CuL_2]/[CuL][L]$	65.3 ± 08	−22.6± 0.4
phenylalanine	[CuL]/[Cu][L]	22.9 ± 0.3	−21.7 ± 0.4
	$[CuL_2]/[CuL][L]$	95.9 ±1.7	−22.6 ± 0.8
hydroxyproline	[CuL]/[Cu][L]	69.9 ± 0.4	−27.2± 0.4
	$[CuL_2]/[CuL][L]$	39.8 ± 0.8	−28.9 ± 0.4

thalpy and entropy change for formation of each binary complex and to assess the magnitude of any heat capacity change that accompanies the reaction. As with protonation reactions, the van't Hoff plots were linear for each binary Cu^{2+}−amino acid complex studied, indicating the absence of a measurable change in heat capacity (e.g., Figure 2). The thermodynamic data reported in Table 4 therefore can be assumed valid from 288.15 K to 333.15 K. From these data it is evident for each amino acid that formation of the mono- and bis-binary complexes is both enthalpically and entropically favored. As $\Delta_r H_c(K_1)$ and $\Delta_r H_c(K_2)$ are similar for each amino acid (e.g., $\Delta_r H_c(K_1) = -21.8$ kJ/mol and $\Delta_r H_c(K_2) = -22.6$ kJ/mol for proline), differences in the stabilities of the mono- and bis-binary complexes are primarily due to differences in formation entropies. The relatively low values of $\Delta_r H_c(K_1)$ and $\Delta_r H_c(K_2)$ for leucine are surprising, and although great care was taken during our experiments, an error in potentiometric data acquisition for that system cannot be ruled out.

The stepwise thermodynamic data in Table 4 indicate that formation of the mono-binary complex is favored over the bis-binary complex. We interpreted this observation and the role of entropy in the two binary complexation reactions by considering an appropriate energy cycle for converting the bis-binary complex into two mono-binary complexes:

$$Cu^{2+} + CuL_2 \overset{K_B}{\rightleftharpoons} 2CuL^+$$

where K_B, the equilibrium constant for the bis-complex conversion reaction, is given by the ratio K_1/K_2 in which K_1 and K_2 are the concentration formation constants for the stepwise complexation reactions:

$$Cu^{2+} + L^- \overset{K_1}{\rightleftharpoons} CuL^+$$

$$L^- + CuL^+ \overset{K_2}{\rightleftharpoons} CuL_2$$

Thermodynamic changes for the conversion of the bis-binary complex to the mono-binary complex are therefore given by

$$\Delta_r G_B = \Delta_r G_c(K_1) - \Delta_r G_c(K_2) = -RT \ln K_B \quad (11)$$

$$\Delta_r H_B = \Delta_r H_c(K_1) - \Delta_r H_c(K_2) \quad (12)$$

$$\Delta_r S_B = \frac{\Delta_r H_B - \Delta_r G_B}{T} \quad (13)$$

This analysis confirms that the mono-binary complex is energetically favored ($\Delta_r G_B < 0$) due to a large and favorable $\Delta_r S_B$ (Table 5). The origin of the higher entropy of the mono-binary complex can be understood, at least in part, by considering statistical effects.[36] Bjerrum[37] was the first to study the factors that influence consecutive complex formation by dividing the energetics of the bis- to mono-binary conversion reaction into contributions made by "statistical effects" and "residual ligand effects" (i.e., non-ideal effects). This division of energetic contributions recognizes that when the coordination sites of the central metal ion are completely equivalent and remain so during the formation of successive complexes, the ratio of the stepwise formation constants can be determined solely from reaction statistics. The Gibbs energy change for the conversion reaction is then given by

$$\Delta_r G_B = \Delta G_{stat} + \Delta G_r = -RT \ln K_{stat} + \Delta G_r \quad (14)$$

where K_{stat} represents the enhancement in the stability constant of the mono-binary complex due to statistical (ideal) effects, and ΔG_r is the contribution to the Gibbs energy change due to residual (nonideal) ligand effects. Statistically, the tendency of complex CuL_n to split off a ligand is proportional to the number of ways in which the ligand may be removed. Similarly, the tendency to add a ligand to form CuL_n is proportional to the number of ways in which the ligand may be inserted into the available Cu^{2+}

Table 5. Thermodynamic Properties for the Conversion Reaction $Cu^{2+} + CuL_2 \overset{K_B}{\rightleftharpoons} 2CuL^+$ at T° = 298.15 K (I_c = 0.1 M KNO_3)

amino acid	$\log_{10} K_B$	$\Delta_r G_B^\circ$ ($kJ \cdot mol^{-1}$)	$\Delta_r H_B^\circ$ ($kJ \cdot mol^{-1}$)	$\Delta_r S_B^\circ$ ($J \cdot mol^{-1} \cdot K^{-1}$)	ΔG_r° ($kJ \cdot mol^{-1}$)	ΔS_r° ($J \cdot mol^{-1} \cdot K^{-1}$)
valine	1.30	−7.41	−0.6	23.0	−2.23	5.53
leucine	1.21	−6.91	−2.4	15.1	−1.76	−2.25
proline	1.38	−7.87	0.9	29.3	−2.71	11.90
phenylalanine	0.98	−5.70	0.9	22.2	−0.53	4.88
hydroxyproline	1.37	−7.83	1.6	31.4	−2.65	14.10

Table 6. Ternary Cu(D′ or L′)(L-HyPro) Concentration Formation Constants (I_c = 0.1 M KNO_3)

		β at T/K				
analyte	$\log_{10}$	288.15	298.15	310.15	318.15	333.15
L-proline	β_{1011}	16.52 ± 0.01	16.32 ± 0.03	15.84 ± 0.02	15.57 ± 0.02	15.02 ± 0.03
D-proline	β_{1011}	16.57 ± 0.01	16.37 ± 0.01	15.92 ± 0.03	15.69 ± 0.02	15.17 ± 0.02
L-valine	β_{1011}	15.87 ± 0.01	15.73 ± 0.02	15.25 ± 0.02	14.88 ± 0.01	14.0 ± 0.1
D-valine	β_{1011}	15.82 ± 0.02	15.64 ± 0.02	15.18 ± 0.01	14.75 ± 0.02	13.9 ± 0.1
L-phenylalanine	β_{1011}	15.88 ± 0.01	15.57 ± 0.02	15.14 ± 0.01	14.91 ± 0.01	14.47 ± 0.03
D-phenylalanine	β_{1011}	15.8 ± 0.1	15.46 ± 0.02	15.07 ± 0.02	14.84 ± 0.02	14.43 ± 0.03
L-Leucine	β_{1011}	15.83 ± 0.02	15.69 ± 0.02	15.22 ± 0.03	14.97 ± 0.03	14.4 ± 0.1
D-Leucine	β_{1011}	15.87 ± 0.02	15.68 ± 0.03	15.2 ± 0.1	14.92 ± 0.03	14.3 ± 0.1

Table 7. Enthalpy and Entropy Changes for Stepwise Formation of Ternary Cu(D′ or L′)(L-HyPro) Complexes at 298.15 K (I_c = 0.1 M KNO_3)

analyte	$\Delta_r S_c$ ($J \cdot mol^{-1} \cdot K^{-1}$)	$\Delta_r H_c$ ($kJ \cdot mol^{-1}$)
L-leucine	88.8 ± 0.8	−14.7 ± 0.4
D-leucine	74.5 ± 0.8	−18.9 ± 0.4
L-valine	89.6 ± 0.8	−14.7 ± 0.4
D-valine	81.6 ± 1.3	−16.3 ± 0.4
L-proline	82.9 ± 0.8	−20.0 ± 0.4
D-proline	89.6 ± 0.4	−18.4 ± 0.4
L-phenylalanine	49.0 ± 0.4	−26.0 ± 0.4
D-phenylalanine	59.5 ± 0.8	−22.2 ± 0.4

coordination sites. Using these rules, Bjerrum[37] and Beck[38] have shown for bidentate-ligand binding to Cu^{2+} that $K_{stat} = K_{1,stat}/K_{2,stat} = 8$ when solvent displacement from aquated Cu^{2+} coordination sites is not considered as part of the ideal complexation reaction, so that $\Delta G_{stat} = -5.16$ kJ/mol and $\Delta S_{stat} = R \ln(K_{stat}) = 17.30$ J/mol K. Statistical and residual ligand-effect contributions to $\Delta_r G_B$ and $\Delta_r S_B$ are reported in Table 5. Although statistical effects explain much of the higher stability of the mono-binary complex, nonideal entropy effects ($\Delta_r S_B$) are seen to provide an additional stabilizing effect. This can be explained, at least in part, by the negative hydration entropy ($\Delta S_{hyd} = -455.1$ J/mol K) for the Cu^{2+} ion,[39] which is known to be a strong kosmotrope (i.e., structure maker). Solvent entropy is therefore increased during formation of a mono-binary complex from a bis-binary complex as the reaction involves removal of the hyperstructured coordination shell of water molecules solvating the free copper(II) ion.[40]

Concentration Formation Constants and Thermodynamics for Ternary Complexes. Stereoselectivity is observed in ternary complexes containing an L- or D-amino acid, Cu^{2+}, and the chiral selector L-HyPro (Table 6). To fix ideas, we focus on the energetics of the Cu^{2+}(Phe)(L-HyPro) complex. In aqueous solution, Cu(II) is tetragonally coordinated by four water molecules and by two additional water molecules positioned axially and further away from the copper. Replacement of the tetragonally coordinated water molecules with two chemically dissimilar bidentate amino acid ligands is known to lock the backbone of each ligand into the Cu^{2+} coordination plane. For the solid Cu(Phe)(L-HyPro) ternary complex, Chen et al.[41] have reported that the rigid planar structure of the coordinated backbone of D-Phe results in an unfavorable steric interaction between the phenyl ring and the α-carboxylate group; from this it may be inferred that there is a decrease in stability of the hetero-ternary complex relative to the homo-ternary complex. Subtle differences in the spatial geometry of the homo- and hetero-ternary complexes can therefore lead to differences in the respective enthalpy and entropy of complex formation.[42] As shown in Table 6, the difference between the concentration equilibrium ternary formation constants for the homo- and hetero-complexes is typically small. Nevertheless, Davankov[43,44] and others[45−47] have shown that the resulting Gibbs energy difference ($\delta\Delta_r G_C = \Delta_r G_{Cu(L')(L-HyPro)} - \Delta_r G_{Cu(D')(L-HyPro)}$) can be used to separate amino acid racemates by chiral ligand-exchange chromatography (CLEC) or by various electrophoretic modes of chiral chromatography. Our results indicate that in general, the preference of the L-HyPro ligand for either the L- or D-enantiomer of a given amino acid is maintained with a change in solution temperature. However, for leucine the stereoselectivity of the L-HyPro ligand is seen to invert with increasing temperature, suggesting that the distribution and energetics of accessible conformational states for each complex have unique dependence on temperature.

Stepwise ternary formation constants for each enantiomer are defined by the following complexation reactions:

$$\text{Cu(L-HyPro)} + \text{D′ or L′} \overset{K_1}{\rightleftharpoons} \text{Cu(L-HyPro)(D′ or L′)}$$

Both stepwise ternary formation constants can be computed from the standard formation constant data reported in Tables 3 and 6. For example, the overall reaction equilibria defining complexation of the L′ enantiomer with the Cu(L-HyPro) complex is given by

$$Cu^{2+} + L^- + \text{L-HyPro}^- \overset{\beta_{1011}}{\rightleftharpoons} \text{Cu(L′)(L-HyPro)}$$

$$Cu^{2+} + \text{L-HyPro}^- \overset{\beta_{1001}}{\rightleftharpoons} \text{Cu(L-HyPro)}^+$$

The stepwise ternary formation constant K_t is therefore given by

$$K_t = \frac{\beta_{1011}}{\beta_{1001}} \tag{15}$$

Table 8. Thermodynamic Properties for the Conversion Reaction $\frac{1}{2}CuA_2 + \frac{1}{2}CuB_2 \overset{K_M}{\rightleftharpoons} Cu(A)(B)$ at T° = 298.15 K (I_c = 0.1 M KNO_3)

analyte	$\log_{10} K_m$	$\Delta_r G^\circ_m$ ($kJ \cdot mol^{-1}$)	$\log_{10} K_r$	$\Delta_r H^\circ_m$ ($kJ \cdot mol^{-1}$)	$\Delta_r S^\circ_m$ ($J \cdot mol^{-1} \cdot K^{-1}$)	ΔS°_r ($J \cdot mol^{-1} \cdot K^{-1}$)
L-valine	0.54	−3.06	0.23	13.4	54.9	49.0
D-valine	0.46	−2.64	0.15	11.7	47.3	41.5
L-proline	0.48	−2.76	0.18	2.9	18.4	13.0
D-proline	0.54	−3.06	0.24	4.6	25.1	19.3
L-phenylalanine	0.56	−3.22	0.26	2.9	20.1	14.7
D-phenylalanine	0.45	−2.60	0.15	0.9	10.9	5.0

Stepwise ternary formation constants calculated using eq 15 were analyzed according to the van't Hoff equation (eq 1) to determine complexation thermodynamics. As shown in Figure 3, curvature is observed in the resulting van't Hoff plots, indicating a nonzero heat capacity change $\Delta_r C_p^\circ$ for complexation of each amino acid enantiomer with the Cu(L-HyPro)$^+$ complex. The Kirchoff equation provides the temperature dependence of the Gibbs energy change for any such reaction:

$$\frac{\Delta_r G_c(T)}{RT} = -\ln K_t = \frac{\Delta_r H_c^\circ}{RT} + \frac{\Delta_r C_p^\circ}{RT}(T - T^\circ) - \frac{\Delta_r S_c^\circ}{R} - \frac{\Delta_r C_p^\circ}{R}\ln\left(\frac{T}{T^\circ}\right) \quad (16)$$

Nonlinear fitting of eq 16 to each data set shown in Figure 3 therefore provides an estimate of $\Delta_r H_c^\circ$ and $\Delta_r S_c^\circ$ from the tangent to the slope at 298.15 K (T°), and an estimate of $\Delta_r C_p^\circ$ from the local curvature. The resulting thermodynamic data for stepwise formation of each ternary complex are reported in Table 7. To our knowledge, these are the first enthalpy and entropy data reported for ternary Cu^{2+} – mixed amino acid complexes. Although the uncertainties in taking the second derivative of our formation constant data are too large to report quantitative estimates of $\Delta_r C_p^\circ$, the concave shapes of all van't Hoff plots in Figure 3 indicate that addition of an unlike amino acid to the Cu(L-HyPro$^+$) complex results in a negative change in heat capacity. Heat capacity changes for binding of uncharged or analytes in aqueous solution are thought to mainly arise from solvent effects.[48] Nonpolar dehydration generally results in a decrease in heat capacity, while an increase in C_p accompanies polar dehydration.[49,50] Thus, dehydration of the relatively polar zwitterionic backbone (i.e., dehydration of the α-amino and carboxylate groups) of the amino acid complexing to Cu(L-HyPro) would be expected to increase C_p. In the formation of mono- and bis-binary complexes, this effect is balanced by expected decrease in heat capacity due to desolvation of the kosmotropic Cu^{2+} ion. As a result, formation of these these complexes is seen to result in no change in heat capacity. The observed net decrease in C_p during formation of all stepwise mixed chelate complexes suggests that additional effects contribute to $\Delta_r C_p^\circ$ when unlike amino acids chelate Cu(II), but the source of these additional contributions is unknown.

Comparison of the stability of the (mixed) ternary complex to those of the parent bis-binary complexes is facilitated by defining an equilibrium constant K_m for the conversion reaction:[34]

$$\frac{1}{2}\mathrm{CuA_2} + \frac{1}{2}\mathrm{CuB_2} \overset{K_m}{\rightleftharpoons} \mathrm{Cu(A)(B)}$$

K_m is then given by

$$K_m = \frac{[\mathrm{Cu(A)(B)}]}{\sqrt{[\mathrm{Cu(A)_2}]}\sqrt{[\mathrm{Cu(B)_2}]}} = \frac{\beta_{1011}}{\sqrt{\beta_{\mathrm{Cu(A)_2}}\beta_{\mathrm{Cu(B)_2}}}} \quad (17)$$

and the enthalpy charge for the reaction is given by

$$\Delta H_m = \Delta H_{\mathrm{Cu(A)(B)}} - \frac{1}{2}\Delta H_{\mathrm{Cu(A)_2}} - \frac{1}{2}\Delta H_{\mathrm{Cu(B)_2}} \quad (18)$$

Dissection of $\Delta_r G_m$ ($= -RT \ln K_m = -RT \ln K_{stat} - RT \ln K_r$) into its enthalpic and entropic contributions, including the associated statistical (s) and residual (r) effects, is shown in Table 8 for one aliphatic (valine), one cyclic

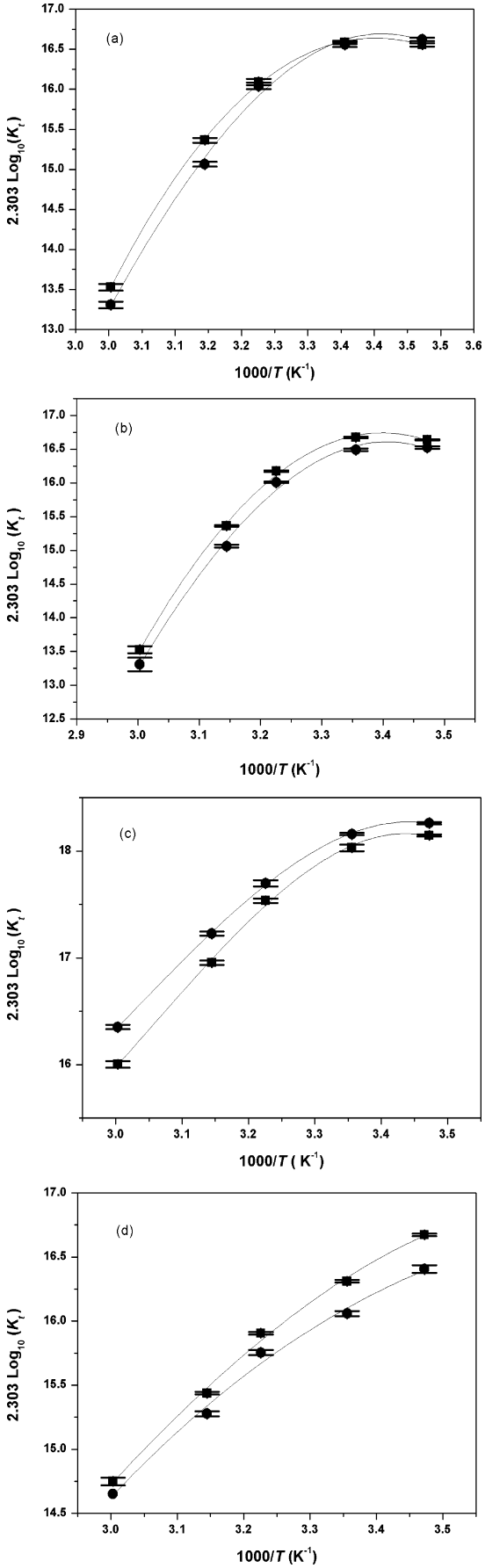

Figure 3. van't Hoff plots for stepwise formation of the ternary complex: Cu(L-HyPro) + D′ or L′ $\overset{K_t}{\rightleftharpoons}$ Cu(D′or L′)(L-HyPro) defined by the equilibrium constant K_t: (a) leucine, (b) valine, (c) proline, (d) phenylalanine (■: L-amino acid, ●: D-amino acid).

(proline), and one aromatic (phenylalanine) amino acid. The data reveal that the mixed ternary chelate complex is always more stable than either of the parent bis-binary complexes. However, the thermodynamics of this hyperstabilizing effect are found to be quite different for the three different classes of amino acid investigated. For valine, conversion of the parent bis-binary complexes into the mixed Cu(Val)(L-HyPro) complex is enthalpically unfavorable. A net increase in entropy therefore drives the conversion reaction. A portion of this gain in entropy hyperstabilizing the mixed chelate complex arises through statistical effects. K_{stat} is equal to 2 for the conversion reaction and therefore stabilizes the mixed chelate complex by ca. 410 cal/mol.

In contrast to aliphatic amino acids, formation of a mixed chelate complex containing two cyclic amino acids via the conversion of the parent $Cu(Pro)_2$ complex is a nearly athermal process, pointing to a more favorable energy of interaction between complexed proline and L-HyPro. In this case, ideal mixing (i.e., statistical) effects provide the main contribution to the hyperstabilization of the mixed complex. Similar complexation thermodynamics are observed for the conversion reaction to form the ternary Cu(Phe)(L-HyPro) complex, where $\Delta_r H_m$ is near zero and $\Delta_r S_m$ again provides the main thermodynamic driving force for the reaction. The origin of the favorable residual complexation entropy is not clear, but may be due to a back-coordination effect where electrons of the t_{2g} orbitals of Cu(II) fill vacant π orbitals of the phenyl ring, allowing close coordination with the aliphatic portion of the cyclic ring of L-HyPro to achieve limited dehydration.[51]

Conclusions

We report the first data characterizing the enthalpy and entropy changes accompanying the formation of ternary Cu^{2+}(D′−L′)(L-HyPro) complexes. Our data show that the energetics and entropy of binary and ternary complexation reactions depend on both solution temperature and the side-chain properties of the participating amino acids. The formation of binary and ternary complexes from the free components is found to be favored both enthalpically, due to bidendate ligand coordination to the planar coordination sites of Cu(II) and, entropically, due to dehydration of the strongly kosmotropic Cu^{2+} ion. When L-HyPro serves as one of the complexing agents, the stability of the mixed ternary complex is greater than either of the parent bis-binary complexes, irrespective of the solution temperature. In general, a gain in entropy drives the conversion reaction. A portion of this entropy gain is due to statistical effects, but significant entropy gains due to nonideal ligand effects are also observed, and their magnitude depends strongly on the side chain of the second amino acid.

We also report the first data defining the temperature dependence of stepwise and concentration equilibrium formation constants for binary and ternary complexes containing Cu^{2+} and L-HyPro. Nonlinear van't Hoff plots are observed for stepwise formation of all ternary complexes, indicating a negative ΔC_p for addition of an unlike amino acid to the $Cu(L\text{-HyPro})^+$ complex. Quantitative values for ΔC_p could not be obtained due to the uncertainty associated with interpreting second derivatives of primary data, pointing to the need for calorimetric studies of mixed chelate complex formation. However, for the L-HyPro ligand, the nonlinear dependence of ternary complex stability on inverse temperature has obvious implications on the optimization of racemate separations by CLEC.

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