Binding of Copper(II), Nickel(II), Zinc(II), and Cobalt(II) by 3-[(Carboxymethyl)thio]-L-alanine and 3-[(2-Aminoethyl)thio]-L-alanine
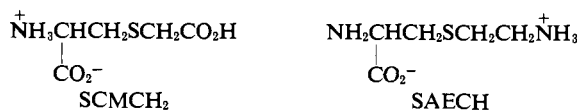
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Abstract: Potentiometric and visible spectral studies of aqueous solutions of Cu(II), Zn(II), Ni(II), and Co(II) with 3-[(carboxymethyl)thio]-L-alanine (SCMCH₂) and 3-[(2-aminoethyl)thio]-L-alanine (SAECH) provide evidence for a variety of complexes. Evidence is presented for complexes such as M(SAECH)²⁺, M(SAECH)₂²⁺, M(SCMCH)⁺, M(SCMC), and M(SCMC)₂²⁻ in which the ligands chelate to the metal ion *via* the amino and carboxylate groups of the α-amino acid residue. Other complexes identified are M(SCMC), M(SAEC)⁺, M(SAEC)-(SAECH)⁺, and M(SAEC)₂; in these, evidence suggests that the sulfur atom is coordinated to the metal ion. The structures of the complexes are found to be especially dependent upon the pH of the solutions. Equilibrium constants for their formation and their visible spectra are discussed in terms of the metal chelate structures and their possible significance to biological systems.

Considering the abundance of naturally occurring amino acids containing thioether and dithioether linkages,[2] it is surprising that only a few studies have been reported concerning the interaction of these aminoacidates with biologically important metal ions. Fernelius, *et al.*,[3–5] were the first to investigate the solution chemistry of transition metal ions with chelating agents containing thioether linkages, *e.g.*, aminoalkyl sulfides and carboxyalkyl sulfides. However, the work was complicated by precipitation of metal hydroxides and metal complexes, "irregularities in the titration curves," and the unusual feature that formation constants for addition of the second ligand were often larger than those for the addition of the first. Later studies[6,7] not only conflicted with those of Fernelius, *et al.*,[3–5] but also with each other.

In this study the Cu(II), Ni(II), Co(II), and Zn(II) complexes of two naturally occurring amino acids, 3-[(carboxymethyl)thio]-L-alanine (SCMCH₂) (found in the urine of cystathioninuric patients[8] and in hydrolyzed hair samples[9]) and 3-[(2-aminoethyl)thio]-L-alanine (SAECH) (extracted from *Rozites coperata*[10]) were investigated. The former amino acid is an effective

antiseborrheic[11] and antiacne agent.[12] The latter has been shown to inhibit the growth of *Bacillus subtilis* and *Escherichia coli*[13] and has been proposed as an intermediate in cystamine formation.[14] Hermann and Lemke[15] previously examined the binding of SAECH by Cu(II) in the low pH region where coordination was proposed to occur through the α-amino and carboxylate groups.

Experimental Section

Reagents. The 3-[(carboxymethyl)thio]-L-alanine (also known as *S*-carboxymethyl-L-cysteine) (SCMCH₂)) and the hydrochloride salt of 3-[(2-aminoethyl)thio]-L-alanine (*S*-aminoethyl-L-cysteine) (SAECH·HCl)) were purchased from Sigma Chemical Co. and were of the highest purity available. They were used without further purification. Solutions of SCMCH₂ were standardized by potentiometric pH titration. Solutions of SAECH·HCl were standardized *via* ion-exchange techniques. Aliquots of the hydrochloride salt were passed through Dowex 50W-X8 strongly acidic cation-exchange resin, and the effluent solutions were titrated with standardized sodium hydroxide.

Baker analyzed Cu(NO₃)₂·3H₂O, Ni(NO₃)₂·6H₂O, Co(NO₃)₂·6-H₂O, and Zn(NO₃)₂·6H₂O were used in the preparations of metal ion solutions. These solutions were standardized *via* ion-exchange techniques[16] as explained above.

Potentiometric Measurements. A Corning Digital 112 Research Model pH meter was used to determine hydrogen ion concentrations in all potentiometric titrations, which were carried out in a double-walled cell of 50-ml capacity. The titration cell was fitted with Corning glass and calomel extension electrodes, a microburet delivery tube, and a nitrogen inlet tube. The temperature of all solutions was maintained at 25.00 ± 0.05° by circulation of thermostated water through the outer jacket of the cell, and the solutions were stirred with a magnetic stirrer. Ionic strengths of all solutions were maintained at 0.10 *M* by the addition of an appropriate amount of 1.0 *M* KNO₃. All titrations were performed in triplicate and at two or three different concentrations.

The glass electrode was calibrated in terms of −log [H⁺] (pH$_c$) according to the procedure of Rajan and Martell[17] using HCl, acetic acid, and NaOH solutions. For the acetic acid titration, the pH$_c$ values were calculated from data tabulated by Harned and Owen.[18]

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Table I. Equilibrium Constants for the Interaction of SCMC^{2-}, SMC$^-$, and SAEC$^-$ with Metal Ions[a]

M^{2+}	SCMC[b]		SMC[c]		SAEC[d]			
	K_1	K_2	$K_1{}^g$	$K_2{}^g$	$K_1{}^e$	K_{1a}	$K_2{}^h$	K_{2a}
Cu^{2+}	8.15 ± 0.01^{e,f}		7.88	6.84	7.22 ± 0.01	−6.30 ± 0.01	6.14 ± 0.02	i
Ni^{2+}	6.22 ± 0.02	4.94 ± 0.02	5.26	4.56	4.38 ± 0.02	−5.57 ± 0.02	3.71 ± 0.02	−9.04 ± 0.02
Co^{2+}	4.90 ± 0.02	3.62 ± 0.02	4.12	3.49	3.46 ± 0.02	−6.94 ± 0.02	2.61 ± 0.01	−9.09 ± 0.01
Zn^{2+}	5.12 ± 0.02	4.14 ± 0.02	4.46	4.06	3.71 ± 0.02	−6.82 ± 0.01		

[a] All equilibrium constants are in log K units, at 25.0° and 0.10 (KNO₃) ionic strength. [b] Log of the protonation constants: $K_1' = 8.89 \pm 0.01$, $K_2' = 3.36 \pm 0.02$, and $K_3' = 1.99 \pm 0.02$. [c] Log of the protonation constant: $K_1' = 8.73$, ref 20. [d] Log of the protonation constants: $K_1' = 9.67 \pm 0.02$, $K_2' = 8.32 \pm 0.02$, and $K_3' = 1.70 \pm 0.01$. [e] Formation of [M(HL)]. [f] Log $K_{1a} = -2.97 \pm 0.01$. [g] Values from ref 20. [h] For Cu(II), [Cu(HL)₂$^{2+}$], eq 6; for Co(II), Ni(II), [M(HL)L$^+$], eq 3. [i] Log $K_{1a}' = -7.82 \pm 0.01$ and log $K_{2a}' = -9.27 \pm 0.01$.
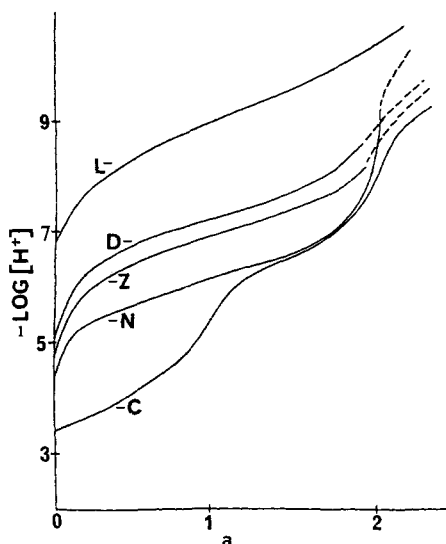
Figure 1. Potentiometric titration curves for 6.40 × 10⁻³ M SAECH·HCl (L) and 1:1 SAECH·HCl to Cu(II) (C), Ni(II) (N), Co(II) (D), and Zn(II) (Z) solutions. Dashed lines indicate precipitate formation and a is moles of base per mole of ligand.
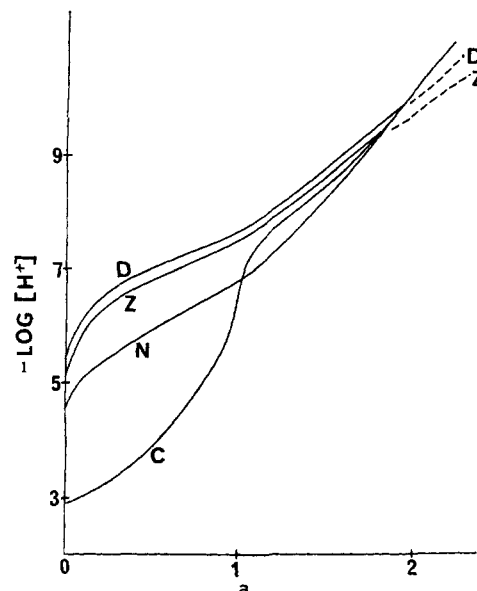
Figure 2. Potentiometric titration curves for 2:1 SAECH·HCl (6.40 × 10⁻³ M) to Cu(II) (C), Ni(II) (N), Co(II) (D), and Zn(II) (Z) solutions. Dashed lines indicate precipitate formation and a is moles of base per mole of ligand.

Equilibrium constants were calculated (see Results) on an IBM 360–65 digital computer using data from 20 to 80% of a particular buffer region.

Visible Spectra. In order to measure visible spectra simultaneously with pH, a potentiometric titration cell was constructed with solution inlet and outlet tubes. A peristaltic pump (Manostat, New York, N. Y.) was used to circulate the solution from the titration cell to a flow-through quartz cell (Precision Cells, Inc., Hicksville, N. Y.). All spectra (350–800 nm) were recorded on a Beckman DB-G grating spectrophotometer. The cell compartment of the instrument was maintained at the same temperature as that of the potentiometric cell by the circulation of thermostated water from the same water bath. All measurements of 1:1 (2.5 × 10⁻² and 1.25 × 10⁻³ M metal ion) and 2:1 ligand to metal solutions (1.0 × 10⁻² and 5.0 × 10⁻³ M metal ion) were taken at 25.00 ± 0.05° and an ionic strength of 0.10 M.

Nmr Studies. Proton nmr spectra were obtained with a Varian Associates Model A-60 spectrometer on 0.5 M solutions of SAECH·HCl in 98% D₂O using sodium 2,2-dimethyl-2-silapentane-5-sulfonate (0.00 ppm to TMS) as an internal standard. Appropriate amounts of 0.25 M NaOD, prepared by the addition of Na to 98% D₂O, were added, and the nmr spectra were recorded at ambient temperature.

Results

Potentiometric Data. The SAECH·HCl titration curve (Figure 1) consists only of a high pH buffer zone from which values of K_1' and K_2' were calculated. The K_3' value was obtained from titration data for a 1:1 solution of HClO₄ and SAECH·HCl. The ligand titration curve for SCMCH₂ is shown in Figure 3. Values of K_2' and K_3' (Table I) were calculated from

data in the low pH buffer zone. Data from the higher buffer zone were used to calculate K_1'.

The 1:1 and 2:1 SAECH·HCl to Cu(II), Ni(II), Co(II), and Zn(II) titration curves are shown in Figures 1 and 2, respectively. For Co(II), Ni(II), and Zn(II) it was necessary to assume that [M^{2+}], [MHL^{2+}], and [ML$^+$] (where L = SAEC$^-$) (eq 1 and 2) were present in the 1:1 and low pH regions of the 2:1 SAECH₂$^+$ to metal ion solutions. The 1:1 Cu(II) to SAECH₂$^+$ solution also followed eq 1 and 2.

$$K_1 = [\text{MHL}^{(2+x)+}]/[\text{M}^{2+}][\text{HL}^{x+}] \qquad (1)$$

$$K_{1a} = [\text{H}^+][\text{ML}^{(1+x)+}]/[\text{MHL}^{(2+x)+}] \qquad (2)$$

$$K_1 = [CL + CS - AL(C + D)]/[(TM - CL + C \cdot AL)AL]$$

where $C = K_2'K_3'[\text{H}^+]^2 + K_2'[\text{H}^+] + 1 + 1/(K_1'[\text{H}^+])$, $D = 2K_2'K_3'[\text{H}^+]^2 + K_2'[\text{H}^+] - 1/(K_1'[\text{H}^+])$, $CS = [\text{Cl}^-] + [\text{OH}^-] - [\text{Na}^+] - [\text{H}^+]$, $AL = (CL + A \cdot CS)/(A \cdot D + C)$, CL = total ligand concentration, TM = total metal concentration, $A = 1 + [\text{H}^+]/K_{1a}$, K_1', K_2', K_3' = ligand protonation constants, and [HL] = NH₂CH(COO$^-$)CH₂SCH₂CH₂NH₃$^+$. Values of K_1 and K_{1a} were determined via iterative programs and are listed in Table I.

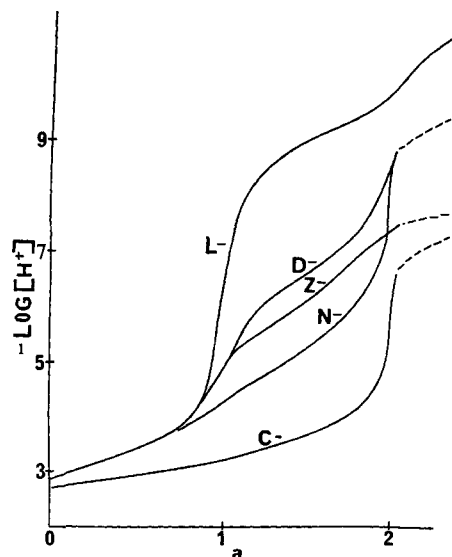The higher pH buffer region (above $a = 1$) of the 2:1

Figure 3. Potentiometric titration curves for 5.50×10^{-3} M SCHCH$_2$ (L) and 1:1 SCMCH$_2$ to Cu(II) (C), Ni(II) (N), Co(II) (D), and Zn(II) (Z) solutions. Dashed lines indicate precipitate formation and a is moles of base per mole of ligand.

SAECH·HCl to Ni(II) and Co(II) systems were solved by assuming [ML$^+$], [M(HL)L$^+$], and [ML$_2$] to be present in solution (eq 3 and 4). By combining

$$K_2 = [\text{M(HL)L}^+]/[\text{ML}^+][\text{HL}] \tag{3}$$

$$K_{2a} = [\text{ML}_2][\text{H}^+]/[\text{M(HL)L}^+] \tag{4}$$

charge and mass balance equations, the following relationship was derived.

$$K_{2a} = [TM - AL \cdot C(1 + K_2 AL)][\text{H}^+]/[K_2 C(AL)^2]$$

where AL is obtained from the quadratic

$$K_2 C(AL)^2 + (C + D)AL + (-CS - 2TM) = 0$$

and C, D, CS, and TM are defined as above.

Values of K_2 and K_{2a} were determined *via* iterative programs and are listed in Table I.

Data from the buffer region of the 2:1 SAECH·HCl to Cu(II) titration curve were found to follow eq 5 and 6.

$$K_1 = [\text{CuHL}^{2+}]/[\text{Cu}^{2+}][\text{LH}] \tag{5}$$

$$K_2 = [\text{Cu(HL)}_2{}^{2+}]/[\text{CuHL}^{2+}][\text{LH}] \tag{6}$$

Bjerrum's method[19] was used to calculate the K_1 and K_2 values which are found in Table I.

Deprotonation constants of [Cu(HL)$_2{}^{2+}$] were calculated (eq 7 and 8) in the upper buffer region according to Bjerrum's method.[19]

$$K_{1a}' = [\text{Cu(HL)L}^+][\text{H}^+]/[\text{Cu(HL)}_2{}^{2+}] \tag{7}$$

$$K_{2a}' = [\text{CuL}_2][\text{H}^+]/[\text{Cu(HL)L}^+] \tag{8}$$

Titration curves for 1:1 and 2:1 SCMCH$_2$ to Co(II), Ni(II), Zn(II), and Cu(II) are shown in Figures 3 and 4, respectively. The 1:1 and 2:1 SCMCH$_2$ to Ni(II), Zn(II), and Co(II) consist of a low pH buffer region equivalent to that of the ligand solution (no metal complexation) and a higher pH buffer zone (in which complexation occurs) terminated by an inflection point at $a = 2$. In this latter region values (Table I) of K_1 (for

(19) J. Bjerrum, "Metal Ammine Formation in Aqueous Solutions," P. Haase and Son, Copenhagen, 1957.
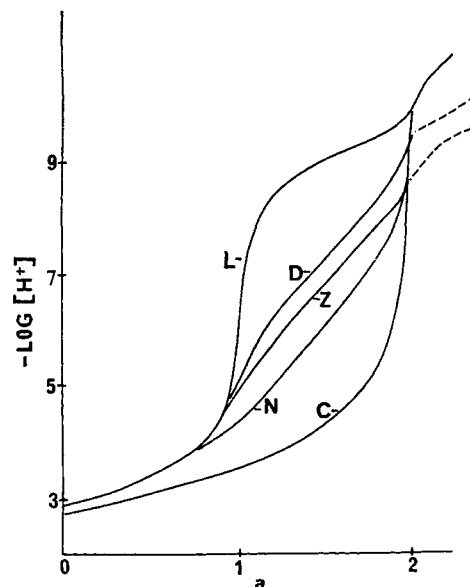
Figure 4. Potentiometric titration curves for 5.50×10^{-3} M SCMCH$_2$ (L) and 2:1 SCMCH$_2$ to Cu(II) (C), Ni(II) (N), Co(II) (D), and Zn(II) (Z) solutions. Dashed lines indicate precipitate formation and a is moles of base per mole of ligand.

M(SCMC)) and K_2 (for M(SCMC)$_2{}^{2-}$) as defined by eq 9 and 10 were calculated according to Bjerrum's method.[19]

$$K_1 = [\text{ML}^{(2+x)+}]/[\text{M}^{2+}][\text{L}^{x+}] \tag{9}$$

$$K_2 = [\text{ML}^{(2+2x)+}]/[\text{ML}^{(2+x)+}][\text{L}^{x+}] \tag{10}$$

Precipitates, probably metal hydroxides, formed above $a = 2$ in the 1:1 and 2:1 SCMCH$_2$ to Co(II) and Zn(II) systems and the 1:1 SCMCH$_2$ to Ni(II) solution.

The 1:1 and 2:1 SCMCH$_2$ to Cu(II) curves consist of a long buffer zone terminated by a sharp inflection at $a = 2$. Bjerrum's method[19] could not be used for the 1:1 SCMCH$_2$ to Cu(II) solutions, and it was necessary to assume that [Cu^{2+}], [CuHL$^+$], and [CuL] (where L = SCMC^{2-}) were present in solution (eq 1 and 2). By combining the appropriate mass and charge balance equations, the following equation was derived.

$$K_{1a} = [-CL - CS + AL(A + B)][\text{H}^+]/$$
$$[CS + 2CL - AL(2A + B)]$$

where AL is obtained from the quadratic, $AL^2[K_1 K_1' A \cdot [\text{H}^+]] + AL(B + 2A) - CS - 2CL = 0$: $A = K_1' K_2' \cdot K_3'[\text{H}^+]^3 + K_1' K_2'[\text{H}^+]^2 + K_1'[\text{H}^+] + 1$, $B = -2 - K_1'[\text{H}^+] + K_1' K_2' K_3'[\text{H}^+]^3$, K_1', K_2', and K_3' are ligand protonation constants, $CS = [\text{OH}^-] - [\text{H}^+] - [\text{Na}^+]$, and $CL =$ total ligand concentration. Values of K_1 and K_{1a} were determined *via* iterative programs and are tabulated in Table I.

Visible Spectra. Visible spectral data for the Ni(II), Co(II), and Cu(II) complexes of SAEC$^-$ and SCMC^{2-} are tabulated in Table II. Only the solution spectra of 1:1 and 2:1 SAECH·HCl to Ni(II) are shown (Figure 5). The 1:1 SAECH·HCl to Ni(II) solution spectra consist of two peaks which shift to higher energy upon addition of base. The appearance of three isosbestic points indicates that only two metal complexes are present in the region $a = 0$ to $a = 2$. The color change in this region was from green to blue.

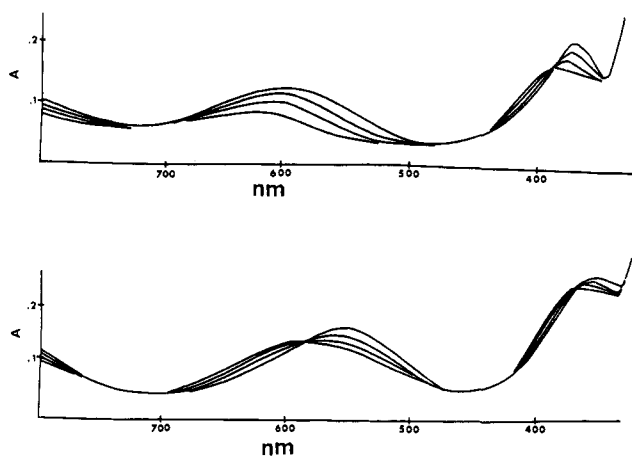At $a = 2$, 1 mol of SAECH·HCl was added to the [Ni(SAEC)$^+$] chelate solution. No change in intensity

Figure 5. Visible spectra of 1:1 (top, from $a = 0.3$ to 1.9) and 2:1 (bottom, from $a = 1$ to 2) SAECH·HCl to Ni(II) solutions.

Table II. Visible Spectra of Metal Chelates of SAEC⁻ and SCMC²⁻

	[MSAEC⁺]		[M(SAEC)₂]		[M(SCMC)₂²⁻]	
	λ_{max}, nm	ϵ_{max}, M^{-1} cm⁻¹	λ_{max}, nm	ϵ_{max}, M^{-1} cm⁻¹	λ_{max}, nm	ϵ_{max}, M^{-1} cm⁻¹
Cu²⁺	675	39	604ᵃ	87	605ᵇ	86
Ni²⁺	608	10	554	21	579	21
	368	23	350	44	367	34
Co²⁺	482	36	470ᶜ	84	495	23

ᵃ [Cu(SAECH)₂²⁺], λ_{max} 604 nm, ϵ 87 M^{-1} cm⁻¹. ᵇ [CuSCMC], λ_{max} 710 nm, ϵ 32 M^{-1} cm⁻¹. ᶜ The 470 nm peak is a shoulder on more intense ultraviolet band.

or position of the peak at 608 nm was observed; this indicates that the added SAECH·HCl does not coordinate to the metal ion, and only Ni(SAEC)⁺ is present at this point. The peak at 368 nm was shifted to slightly higher energy and increased in intensity on adding the SAECH·HCl. This is due to a ligand absorption which tails into the visible.

Additions of base to the now 2:1 SAECH HCl to Ni(II) solution resulted in further shifts to high energy and increases in intensity. The color of the solution changed from blue to purple in this region (2–4 mol of base per mole of metal ion). Three isosbestic points were observed indicating that only two metal complexes are in equilibrium.

The Co(II)–SAECH·HCl system behaved similarly to the corresponding Ni(II) solution in that the addition of 1 mol of SAECH·HCl to a 1:1 Co(II)–SAECH·HCl solution at $a = 2$ resulted in no increase in band intensity or position. The total color change was from pink to red-brown in the 2:1 SAEC to Co(II) system. No isosbestic points were observed as the Co(II) peak shifted to higher energy and increased in intensity. In the final few spectra, above 3.2 mol of base per mole of metal ion, the Co(II) peak was observed as only a shoulder on a more intense ultraviolet band.

The 2:1 SAECH·HCl–Cu(II) spectra indicated complete formation of a diprotonated [Cu(SAECH)₂²⁺] chelate at $a = 1$. From $a = 1$ to $a = 2$, the peak at 604 nm did not increase in intensity or shift to a lower wavelength, indicating no change in the coordination sphere of Cu(II). However, there was a shift to lower energy in a ligand ultraviolet band which tails into the

visible region suggesting deprotonation of the amino groups.

The 1:1 SAECH·HCl and SCMCH₂ to Cu(II) solution spectra showed both a shift to higher energy and an increase in intensity upon addition of base. No isosbestic points were observed.

Spectra of the 1:1 and 2:1 SCMCH₂ to Ni(II) and Co(II) solutions both show shifts to lower wavelengths and increases in intensity upon addition of base. Unlike the Ni(II) and Co(II) SAECH·HCl spectra, the addition of 1 mol of ligand to the 1:1 M²⁺ to ligand solution at $a = 2$ resulted in both peak shifts and an increase in band intensity, indicating that 1:1 and 2:1 species are present in these solutions.

Nmr Studies. The nmr spectrum of SAECH·HCl in D₂O consisted of a multiplet at 3.25 ppm and a triplet at 4.00 ppm; the latter is assigned to the hydrogen at the chiral center. Between $a = 0$ and $a = 1$, moles of base per mole of SAECH·HCl, the triplet shifted from 4.00 to 3.50 ppm; this was accompanied by a slight reordering of the multiplet, which is apparently due to the effects of deprotonation on the methylene α to the chiral center. From $a = 1$ to $a = 2$, no change was observed in the position of the triplet. These data indicate that the second protonation constant of SAEC, K_2', can be assigned to the protonation of the amino group adjacent to the chiral carbon.

Discussion

Protonation Constants. The K_1', K_2', and K_3' values (Table I) of SAEC⁻ are assigned to protonation of the S-ethylamino group, the amino group of the alanine moiety, and the carboxylate group, respectively, based on the nmr results and the similarity of K_2' with the K_1' value for S-methylcysteine (SMCH) (log $K_1' = 8.73$).[20]

$$\overset{+}{N}H_3CHCH_2SCH_3$$
$$|$$
$$CO_2^-$$
$$SMCH$$
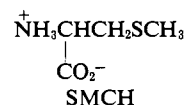
The K_1', K_2', and K_3' values of SCMC²⁻ are assigned to protonation of the amino group, the –SCH₂COO⁻ residue, and the carboxylate of the alanine moiety, respectively, based on the protonation constants of dithiodiacetic acid (log $K_1' = 3.81$ and log $K_2' = 2.88$)[21] and S-methylcysteine (Table I).
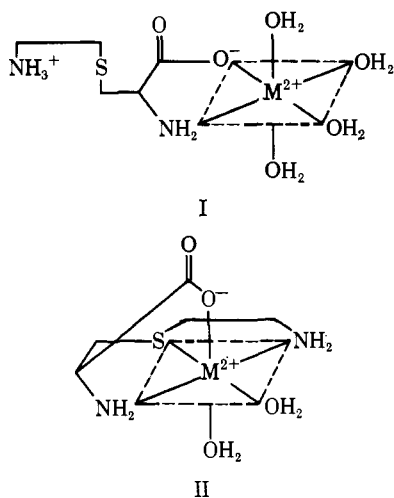
The protonation constant of the α-amino group is dependent upon the charge on the ligand, i.e., log K of SCMC²⁻ (8.89) is larger than that (8.73) of SMC⁻, which is larger than that (8.32) of SAECH. The dependence of protonation constants upon the charge of the ligand has been noted before in the larger K_1' values of dipeptides as opposed to those of their ester derivatives.[22]

1:1 SAEC Metal Chelates. The 1:1 SAECH·HCl to Cu(II), Ni(II), Co(II), and Zn(II) systems follow eq 1 and 2 as shown by potentiometric and visible spectral data. The proposed structures of the metal chelates are indicated in I (low buffer region for Cu(II)) and II (high buffer region for Cu(II)). Structure I is similar to that suggested[20] for M(SMC)⁺ complexes of these same metal ions. The proposed lack of sulfur coordination

(20) G. R. Lenz and A. E. Martell, *Biochemistry*, **3,** 745 (1964).
(21) K. Suzuki, C. Karaki, S. Mori, and K. Yamasaki, *J. Inorg. Nucl. Chem.*, **30,** 167 (1968).
(22) R. Nakon and R. J. Angelici, *Inorg. Chem.*, **12,** 1269 (1973).

O

OH₂

NH₃⁺ S

O⁻

M²⁺

OH₂

OH₂

NH₂

OH₂

OH₂

I

O

O⁻

S

NH₂

M²⁺

NH₂

OH₂

OH₂

II

The proposed structure for [M(HL)L⁺] is shown in III; [ML₂] is similar to III except with the uncoordinated

O

O⁻

S

NH₂

M²⁺

NH₂

O⁻

NH₂

NH₃⁺ S

O

III

in the SMC⁻ complexes has been subsequently confirmed by nmr studies.[23] The K_1 values for SAECH are 0.88–0.66 log units lower than those of SMC⁻, indicating that the positive NH_3^+ of SAECH has reduced the coordinating ability of this ligand.

The trend of increasing stability with increasing negative charge on the ligand is shown by the K_1 values for the series of ligands SAECH, SMC⁻, and SCMC²⁻ with Zn(II); it is quite certain that the sulfur atom in these complexes does not coordinate to the Zn(II). The difference in stability between [Zn(SAECH)²⁺] and [Zn(SMC)⁺] is ∼0.7 log units while that between [Zn(SMC)⁺] and [Zn(SCMC)] is also ∼0.7 log units.
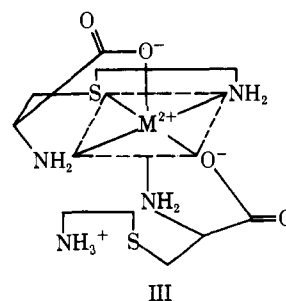
Coordination of sulfur[21,24,25] and the terminal amino group in complex II is supported by the large decrease in the ionization constant for I going to II (log K_{1a} = −5.57 to −6.94 log units). These values compare with −9.67 log units for proton ionization of the S-CH₂-CH₂NH₃⁺ group in the uncoordinated ligand. Further evidence for sulfur and terminal amino group coordination is provided by the ν_{max} values for [Cu(SAEC)⁺] (675 nm) and [Ni(SAEC)⁺] (608 nm) which are considerably lower than those of complexes such as glycinato-copper(II) (725 nm)[26] and glycinatonickel(II) (650 nm) in which only amino and carboxylate donors are present.

The K_1 values follow the Irving–Williams series. With the exception of Cu(II) which has a lower K_{1a} value than expected, the K_{1a} values also follow the Irving–Williams order. The low value for Cu(II) is apparently due to the removal of a carboxylate group from the square plane of Cu(II) upon sulfur and terminal amino group coordination. Whether or not the carboxylate group binds to a weak axial site of Cu(II), the reaction should occur less readily than with Ni(II), which has six essentially equivalent coordination positions.

2:1 SAEC Metal Chelates. Potentiometric and visible spectral data indicate that the 2:1 SAECH·HCl to Ni(II) or Co(II) systems follow eq 1 and 2 in the region $a = 0$ to $a = 1$. Above $a = 1$, the Ni(II) and Co(II) systems followed eq 3 and 4 as shown by the potentiometric results.

amino group deprotonated. Due to observed isosbestic points in the visible spectra (Figure 5) of [Ni-(SAEC)⁺] and SAECH·HCl, only two complexes are in equilibrium. One of these is clearly Ni(SAEC)⁺. The potentiometric results indicate that the other is really a mixture of [M(HL)L⁺] and ML₂. If these latter complexes have structure III and structure III with a deprotonated amino group, respectively, they will both have the same set of donor groups bound to Ni(II) and, therefore, should have the same visible spectra (see [Cu(SAECH)₂²⁺] and Cu(SAEC)₂ below). Deprotonation of the S-ethylamino group should not affect the visible spectra if the coordination sphere of the metal ion remains unchanged. Thus, the visible spectra and potentiometric data can be readily interpreted in terms of the presence of these three complexes. It is unlikely that the deptotonation of [M(HL)L⁺] to give [ML₂] (eq 4) involves a rearrangement of the donor groups, such as to four amino and two sulfur donors, because then three different sets of donor groups attached to the metal ions would be present in solution, and no isosbestic points should be observed.

The 2:1 SAECH·HCl to Cu(II) system follows eq 5 and 6 and the structure of the Cu(SAECH)²⁺ and Cu(SAECH)₂²⁺ chelates are shown in IV and V and are

O

OH₂

NH₃⁺ S

O⁻

Cu²⁺

OH₂

OH₂

NH₂

OH₂

OH₂

IV

O

OH₂

NH₃⁺ S

O⁻

Cu²⁺

NH₂ S NH₃⁺

NH₂

O⁻

O

OH₂

V
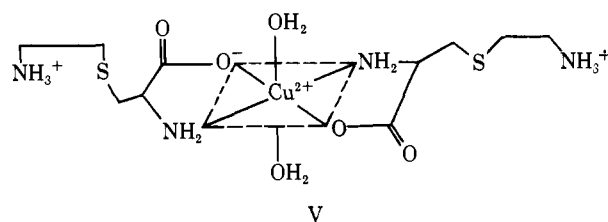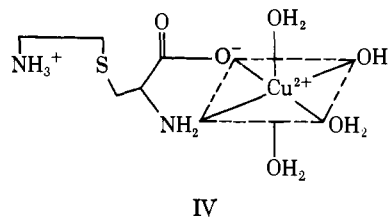
the same as those reported by Hermann and Lemke.[15] Their values of K_1 and K_2 are very similar to our values given in Table I. Similarly, Hawkins and Perrin[6] investigated the Cu(II) complexes of S-(2-aminoethyl-dithio)-L-alanine and postulated structures of the 1:1 and 2:1 chelates to be similar to IV and V, respectively. Fish, et al.,[27] recently reported nmr, esr, ir, Raman, and CD data for the related bis(S-β-(2-pyridylethyl)-L-

(23) D. F. S. Natusch and L. J. Porter, *J. Chem. Soc. A*, 2527 (1971).

(24) E. Campi, G. Ostacoli, N. Cibrario, and G. Saini, *Gazz. Chim. Ital.*, **91**, 361 (1961).

(25) K. Kahmann, H. Sigel, and H. Erlenmeyer, *Helv. Chim. Acta*, **47**, 1754 (1965); **48**, 295 (1965).

(26) C. J. Nunez and G. L. Eichhorn, *J. Amer. Chem. Soc.*, **84**, 901 (1962).

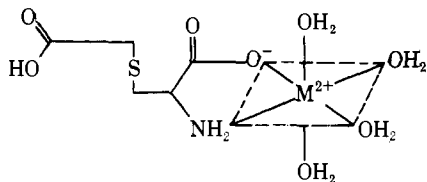(27) R. H. Fish, J. J. Windle, W. Garfield, and J. R. Schener, *Inorg. Chem.*, **12**, 855 (1973).

cysteinato)copper(II) chelate. The data strongly supported aminoacidato–metal bonding as shown in V.

The visible spectra of [Cu(SCMC)$_2$$^{2-}$], [Cu(SAECH)$_2$$^{2+}$], [Cu(SAEC)$_2$], and bisprolinatocopper(II)[28] are equivalent (Table II), indicating the donor groups (amino and carboxylate) are the same in these metal complexes. There is no indication of S binding to Cu(II) in the 2:1 SAECH·HCl to Cu(II) system, indicating the preference of Cu(II) for aminoacidate binding over sulfur and terminal amino group coordination.
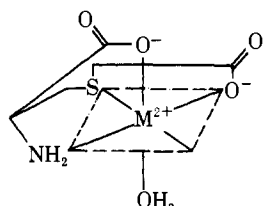
Potentiometric and visible spectral data (no changes in λ_{max} or ϵ_{max} of the 604 mm peak) from $a = 1$ to $a = 2$ in the 2:1 SAECH·HCl to Cu(II) solutions indicate a stepwise deprotonation of the S-terminal amino groups with no subsequent change in the coordination sphere.

The 2:1 SAECH·HCl to Zn(II) solutions behave in the same manner as did the corresponding Ni(II) and Co(II) systems from $a = 0$ to $a = 1$. However, above $a = 1$, Zn(II) solutions did not follow eq 5 and 6. The potentiometric data also did not conform to simple deprotonation of free SAECH$_2$$^+$ either. Therefore, some interaction of SAECH with the [ZnSAEC$^+$] chelate must occur. Due to the low affinity of Zn(II) for thioether sulfur,[23,24] formation of Zn(SAECH)$_2$$^{2+}$ or Zn(SAECH)(SAEC)$^+$ is postulated above $a = 1$. Although no constants were calculated due to the complex equilibria involved, it appears that Zn(SAECH)$_2$$^{2+}$ forms and then undergoes stepwise deprotonation as was observed in the 2:1 SAECH·HCl to Cu(II) system.

SCMC Metal Chelates. Potentiometric and visible spectral data suggest that in 1:1 SCMCH$_2$ to Cu(II) solutions the following species (VI and VII) are present.


VI

VII

The similarity of K_1 values for [CuSCMCH$^+$] and [CuSMC$^+$] suggests that the donor groups are the same. Since Lenz and Martell[20] have shown that the sulfur is not coordinated in CuSMC$^+$, it is probably not coordinated in VI either. As discussed previously, a large difference between the K_1 values of the Cu(II) chelates of SMC$^-$ and SCMC^{2-} would be expected if the S-carboxymethyl group were not protonated.

Coordination of the thioether sulfur in VII is supported by the low ionization constant for VI going to VII (log $K_{1a} = -2.97$). This value compares with -3.66 for the ionization constant of the -SCH$_2$COO$^-$ group in the uncoordinated ligand. Further evidence

(28) D. P. Graddon and L. Munday, *J. Inorg. Nucl. Chem.*, **23**, 237 (1961).

for sulfur coordination is provided in the lower λ_{max} value (710 nm) for [CuSCMC] than those for [Cu(glycine)$^+$] (725 nm)[26] or [Cu(alanine)$^+$] (720 nm).[29] Molecular models indicate a great deal of strain exists in VII if the carboxylate of the alanine moiety is coordinated to the metal ion. The K_{1a} values for the formation of M(SAEC)$^+$ complexes of Cu(II) and Ni(II) and the visible spectra of these complexes indicate coordination of the carboxylate group of the alanine moiety; coordination of this group in the M(SCMC) complexes is much less certain although structure VII is drawn with this geometry.
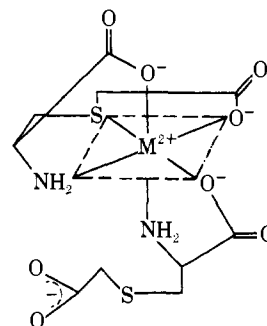
Although formation constants were not obtained for the 2:1 SCMCH$_2$ to Cu(II) system due to the presence of a variety of protonated metal chelates, the visible data (Table II) suggest that [Cu(SCMC)$_2$$^{2-}$] has structure VIII. Its spectrum is very similar to those of other
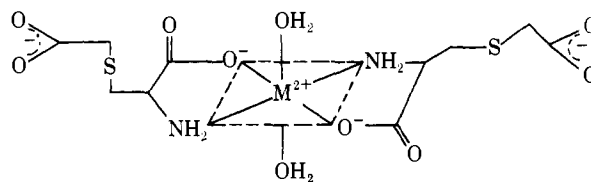
VIII

bis(aminoacidato)copper(II) complexes,[28] in which only amino and carboxylate groups coordinate. Structure VIII has also been found[27] for the related bis(S-β-(2-pyridylethyl)-L-cysteine)copper(II) complex.

Visible spectral data (Table II) strongly indicate coordination of sulfur in both [Ni(SCMC)] (VII) and [Ni(SCMC)$_2$$^{2-}$] (IX). The 1:1 solution of SCMC^{2-} to

IX

Ni(II) at $a = 2$ has a λ_{max} at 615 nm which is considerably lower than that of corresponding 1:1 solutions of glycine to Ni(II), λ_{max} 650 nm,[26] and alanine to Ni(II), λ_{max} 640 nm.[28] The [Ni(SCMC)$_2$$^{2-}$] absorbs at 579 nm while 2:1 solutions of glycine and alanine to Ni(II) absorb at 616[26] and 610 nm,[29] respectively. Further evidence supporting sulfur coordination is the lack of precipitate formation above $a = 2$ and the lack of hydrolysis of [Ni(SCMC)$_2$$^{2-}$] at moderately high pH values (Figure 4). The corresponding bisglycinato-nickel(II) chelate does undergo disproportionation[30] and hydrolysis[31] above $a = 2$.

Chaberek and Martell[32] have shown that imino-

(29) G. L. Eichhorn and J. W. Dawes, *J. Amer. Chem. Soc.*, **76**, 5663 (1954).
(30) D. L. Leussing and D. C. Schultz, *J. Amer. Chem. Soc.*, **86**, 4846 (1964).
(31) L. G. Van Uitert and W. C. Fernelius, *J. Amer. Chem. Soc.*, **76**, 375 (1954).
(32) S. Chaberek and A. E. Martell, *J. Amer. Chem. Soc.*, **74**, 5052, 6021 (1952).

diacetic acid (IMDA) and iminodipropionic acid (IMDPA) are tridentate chelating agents and that it is possible to correlate the difference (Δ) between log K_1 and log K_2 with whether the ligand is bi- or tridentate. The Δ value of the Ni(II) complexes of $SCMC^{2-}$ (1.28 log units) is in between tridentates such as aspartic acid, $IMDA^{2-}$, and $IMDPA^{2-}$ ($\sim$1.8 log units)[33] and bidentates such as glycine, methionine, ethionine, and S-methylcysteine (SMCH) ($\sim$0.75 log units).[33]

Potentiometric and visible spectral data for 1:1 and 2:1 solutions of $SCMCH_2$ to Co(II) suggest that the S is not coordinated to the metal ion in this case. The visible spectra of $[Co(SCMC)_2^{2-}]$ and bisglycinato-cobalt(II)[34] are identical, λ_{max} 495 nm, indicating that the donor groups in the two complexes are the same. The formation of a precipitate above $a = 2$ in the 2:1 $SCMCH_2$ to Co(III) system implies disproportionation. Finally, the K_1 and K_2 values for $SCMC^{2-}$ coordination to Zn(II), which does not bind sulfur, are substantially greater than those of Co(II), indicating that sulfur coordination also does not occur in the Co(II) chelates. These data favor deprotonated VI and VIII as structures for the [Co(SCMC)] and $[Co(SCMC)_2^{2-}]$, respectively.

On the other hand, the difference (Δ) between log K_1 and log K_2 for the Co(II) chelates is midway between those of $IMDA^{2-}$ and $IMDPA^{2-}$[33] and those of glycine, methionine, and SMC.[33] As observed for Ni(II), the Δ value again falls in an intermediate range; it appears that these values are not as useful in structural assignments for the $SCMC^{2-}$ complexes as in the simpler amino acid derivatives.[20]

Potentiometric data indicate that Zn(II) forms 1:1 and 2:1 chelates with $SCMC^{2-}$. The chelating agent probably binds to Zn(II) in the same manner as indicated in deprotonated VI and VIII, with the geometry of Zn(II) possibly being tetrahedral. The Δ value (0.98) for the $SCMC^{2-}$ Zn(II) chelates (Table I) is similar to those for bidentates such as glycine[33] and ethionine[33] and is much smaller than those for the tridentate $IMDA^{2-}$ and $IMDPA^{2- \ 33}$ Zn(II) chelates. These data are in accord with those of Sigel, et al.,[35] who indicated little or no sulfur binding in the Zn(II) complexes of S-ethylacetic acid. The higher values of K_1 and K_2 as compared to those of SMC^- are probably due to the additional negative charge on $SCMC^{2-}$.

The strong evidence for coordination of sulfur in

$SCMC^{2-}$ metal chelates of Cu(II) and Ni(II) and the lack of evidence for such coordination in the Co(II) and Zn(II) complexes can be explained by the greater affinity of Cu(II) and Ni(II) for thioether sulfur. This is in agreement with conclusions of Suzuki and Yamasaki[36] who have shown that the following trend exists for the tendency of divalent metal ions to bind thioether sulfur, Cu(II) > Ni(II) > Co(II) > Zn(II).

In contrast to the $[MSAEC^+]$ complexes where convincing evidence exists for the binding of sulfur to all four metal ions, such evidence is found for only the Cu(II) and Ni(II) chelates of $SCMC^{2-}$. Part of the driving force for sulfur coordination is the formation of a second stable chelate ring with the stronger donor, the terminal amino group, being more effective than the carboxylate group. Similar arguments concerning the formation of a second stable chelate ring have been used by Kaden and Zuberbühler[37] and Nakon and Angelici[22] to explain enhancement of amide proton ionization in metal dipeptide complexes.

Two intriguing features of these metal chelates are the pH dependency of the metal chelate structures and the preference of certain metal ions for certain donor groups. For example, at low pH (3–5) copper(II) binds to equimolar SAECH via the α-amino and carboxylate groups (structure I), whereas at higher pH values it shifts to coordination via both amino groups, the sulfur atom and possibly the carboxylate group (structure II). It is obvious that in metalloproteins where a variety of donor groups exist, pH changes could likewise drastically change the groups bound to the metal ion and greatly alter the activity of the molecule. Also, the replacement of one metal ion by another in such biological systems could involve metal binding at quite different sites depending on the properties of the ion. This was seen in this study in the different binding of $SCMC^{2-}$ by Cu(II) and by Zn(II). The differences were largely related to the different affinities of these metal ions for the thioether sulfur donor atom. Such changes of metal complex structures must be commonplace in metalloprotein chemistry.

(33) L. G. Sillén and A. E. Martell, *Chem. Soc., Spec. Publ.*, No. 17 (1964); No. 25 (1972).
(34) F. Fetrů and F. Jursik, *Z. Chem.*, **8**, 116 (1968).
(35) H. Sigel, R. Griesser, B. Prijs, D. B. McCormick, and M. G. Joiner, *Arch. Biochem. Biophys.*, **130**, 514 (1969).

(36) K. Suzuki and K. Yamasaki, *J. Inorg. Nucl. Chem.*, **24**, 1093 (1962).
(37) Th. Kaden and A. Zuberbühler, *Helv. Chim. Acta*, **54**, 1361 (1971).