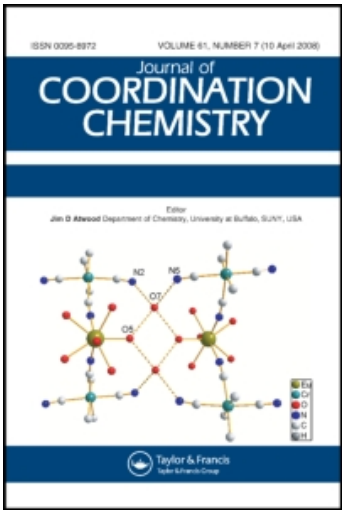

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Coordination properties of 6-aminopenicillanic acid: binary and ternary complexes involving biorelevant ligands

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COORDINATION PROPERTIES OF 6-AMINOPENICILLANIC ACID: BINARY AND TERNARY COMPLEXES INVOLVING BIORELEVANT LIGANDS

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Formation equilibria of copper(II) complexes of 6-aminopenicillanic acid (APA) and the ternary complexes Cu(APA)B (B = glycine, alanine, valine, isoleucine, phenylalanine, proline, hydroxyproline, serine, threonine, ornithine, histidine, methionine, glycylglycine and inosine) were investigated at 25°C and 0.1 M ionic strength. The speciation of the complexes was resolved. Values of $\Delta\log K$, $\log X$ and $\log\beta_{stat}$ indicate a large enhancement of the stability of the mixed ligand complexes. The effects of temperature and organic solvent on the dissociation constant of APA and the formation constant of Cu(APA) were studied and thermodynamic parameters were calculated. The solid complex of $Cu(APA)Cl\cdot 2H_2O$ was separated and identified by elemental analysis and infrared spectroscopy. In the complex APA is coordinated to copper(II) through the amino group and β-lactam carbonyl oxygen. Absorption spectra of the binary complexes of copper(II) and APA were also investigated.

Keywords: 6-Aminopenicillanic acid; Effect of solvent; Effect of temperature; Stability constant; Ternary complexes

INTRODUCTION

Most pharmaceuticals, including 6-aminopenicillanic acid (APA), contain electron donor groups that can bind naturally occurring metal ions [1]. Essential trace metal ions such as zinc and copper are present in too low concentrations in blood plasma to significantly influence the bioavailability of these drugs. The belief that antibiotic action is related to the ability of these compounds to form complexes with metal ions has stimulated investigations of the complexing properties of antibiotics as ligands.

The involvement of multimetal–multiligand equilibria in biological systems is well known [2]. We have therefore studied the interaction of Cu^{II} with APA in the presence of ligands of biological significance, namely amino acids, glycylglycine and inosine, which may be useful in elucidating the mechanism of actions of this class of drugs

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and in understanding the driving forces leading to the formation of such complexes in biological systems. We have also investigated the complex-formation equilibria in solvents of lower polarity, which represent biological conditions.

Current work in our laboratories is focused on the study of metal complexes of amino acids, peptides and DNA constituents [3–6]. We report here a quantitative study of the formation equilibria of binary and ternary complexes of copper(II) with APA and the amino acids glycine, alanine, valine, isoleucine, phenylalanine, proline, hydroxyproline, serine, threonine, ornithine, histidine and methionine, and also glycylglycine and inosine. The effects of organic solvents on the dissociation constant of APA and on the formation constants of Cu^{II}-APA complexes are discussed. The thermodynamic parameters ΔH, ΔS and ΔG are calculated from the temperature dependence of the equilibrium constants.

EXPERIMENTAL

Reagents

Fresh solutions of APA (obtained from Across Organics Chemical Company) were prepared daily to ensure the stability of the APA during measurements in aqueous media [7–9]. Solutions of APA gave consistent potentiometric results as long as they were kept in the refrigerator for more than 2 weeks. Outside the refrigerator, the potentiometric results were only consistent for 2 days. The amino acids, glycylglycine and inosine were provided by Sigma Chemical Company. Copper(II) chloride was provided by BDH. The copper content of the solutions was estimated by complexometric titration with standard EDTA solution [10].

Synthesis of $Cu(APA)Cl \cdot 2H_2O$

A mixture of APA (0.216 g, 1 mM) and $CuCl_2 \cdot 2H_2O$ (0.170 g, 1 mM) in 20 mL distilled water was stirred for 2 h. The resulting precipitate was filtered and washed thoroughly with water, ethanol and diethyl ether. The dark green precipitate (0.310 g, 88.6%) of $Cu(APA)Cl \cdot 2H_2O$ was dried under vacuum. Anal. Calcd. for $C_8H_{11}O_3N_2SCuCl \cdot 2H_2O$ (%) (MW 350.28): C, 27.43; H, 4.32; N, 8.00, S, 9.15. Found: C, 27.39; H, 4.38; N, 7.90, S, 9.08.

Instruments

Potentiometric titrations were performed with a Metrohm 686 titroprocessor equipped with a 665 Dosimat (Switzerland-Herisau). The titroprocessor and electrode were calibrated with standard buffer solutions, potassium hydrogen phthalate (pH 4.008) and a mixture of KH_2PO_4 and Na_2HPO_4 (pH 6.865) at 25°C. IR spectra were measured on a 8001-PC FTIR Shimadzu spectrophotometer using KBr pellets. TGAs were performed in a N_2 atmosphere with a TGA-50 Shimadzu instrument. UV–Vis spectra were recorded on a Shimadzu UV-2101 spectrophotometer.

Procedure and Measurements

Acid dissociation constants of the ligands were determined by titrating 40 mL of ligand solution (1.25×10^{-3} M). Formation constants of the binary complexes were determined by titrating 40 mL of the solution containing copper(II) (0.625×10^{-3} M) and APA (0.625×10^{-3}, 1.25×10^{-3} or 2.5×10^{-3} M). Formation constants of the ternary complexes were determined by titrating equimolar solution mixtures of copper(II) ion, APA and the other ligand (amino acids, glycylglycine and inosine), each of concentration 1.25×10^{-3} M.

All titrations were carried out in a purified N_2 atmosphere. pH readings were corrected in order to read the p[H] of the free hydrogen ion concentration by titrating a known concentration of standard HCl solution with standard NaOH solution under the same experimental conditions. Titrations were performed at different temperatures in aqueous or organic solvent–water solutions of different compositions. A constant ionic strength of 0.1 M (adjusted with $NaNO_3$) was used. NaOH solutions were standardized each time with standard potassium hydrogen phthalate solution. pK_w values in organic solvent–water solutions were determined as described previously [6].

The general four-component equilibrium can be written as follows (charges are omitted for simplicity):

$$l(\mathrm{Cu}) + p(\mathrm{A}) + q(\mathrm{B}) + r(\mathrm{H}) \rightleftharpoons (\mathrm{Cu})_l(\mathrm{A})_p(\mathrm{B})_q(\mathrm{H})_r \quad (1)$$

where l, p, q and r are the stoichiometric coefficients corresponding to Cu^{II}, APA, the secondary ligand and proton, respectively.

The overall formation constants are defined as:

$$\beta_{lpqr} = \frac{[(\mathrm{Cu})_l(\mathrm{A})_p(\mathrm{B})_q(\mathrm{H})_r]}{[Cu]^l[\mathrm{A}]^p[\mathrm{B}]^q[\mathrm{H}]^r}. \quad (2)$$

Calculations were performed using the MINIQUAD-75 program [11]. Stoichiometries and stability constants of the complexes formed were determined by trying various composition models. The model selected gave the best statistical fit and was chemically consistent with the titration data without giving any systematic drifts in the magnitudes of various residuals, as described elsewhere [11]. The fitted model was tested by comparing the experimental titration data points and the theoretical curve calculated from the values of the acid dissociation constant of the ligand and the formation constants of the corresponding complexes. Tables I and II list the formation constants obtained using the MINIQUAD-75 program. The concentration distribution diagrams were obtained using the SPECIES program [12].

RESULTS AND DISCUSSION

Acid–base Equilibria of APA

6-aminopenicillanic acid ($HL^{\pm}$) is protonated to give H_2L^+ species. Analysis of the potentiometric titration curve of APA (Fig. 1a) yielded two pK_a values. The lowest pK_a value (at 25°C) is attributed to a carboxylic group ($pK_{a2} = 4.74$, at 25°C) and

TABLE I Binary and ternary formation constants of Cu^{2+}, APA and ligand at 25°C and 0.1 M ionic strength

System	*M*	L_1	L_2	H^a	$\log \beta^b$	$pK_a{}^c$
APA						
	0	1	0	1	4.74(0.01)	4.74
	0	1	0	2	6.52(0.05)	1.78
Cu-APA						
	1	1	0	0	4.14(0.03)	
	1	1	0	−1	0.21(0.02)	3.93
	1	1	0	−2	−7.82(0.06)	8.03
	1	2	0	0	7.83(0.01)	
Glycine						
	0	0	1	1	9.61(0.02)	9.61
	0	0	1	2	11.93(0.03)	2.32
Cu-glycine						
	1	0	1	0	8.10(0.01)	
	1	0	1	−1	0.67(0.03)	7.43
	1	0	1	−2	−10.13(0.01)	10.80
	1	0	2	0	14.78(0.01)	
Cu-(APA)-glycine						
	1	1	1	0	13.50(0.03)	
	1	1	1	1	17.80(0.04)	4.30
	1	1	1	−1	6.09(0.07)	7.41
Alanine						
	0	0	1	1	9.69(0.03)	9.69
	0	0	1	2	11.89(0.04)	2.20
Cu-alanine						
	1	0	1	0	8.05(0.01)	
	1	0	1	−1	0.30(0.03)	7.75
	1	0	1	−2	−10.16(0.01)	10.46
	1	0	2	0	14.70(0.01)	
Cu-(APA)-alanine						
	1	1	1	0	13.42(0.04)	
	1	1	1	1	17.63(0.04)	4.21
	1	1	1	−1	6.20(0.05)	7.22
Valine						
	0	0	1	1	9.58(0.02)	9.58
	0	0	1	2	11.71(0.04)	2.13
Cu-valine						
	1	0	1	0	7.97(0.02)	
	1	0	1	−1	1.68(0.03)	6.29
	1	0	1	−2	−10.10(0.04)	11.49
	1	0	2	0	14.82(0.02)	
Cu-(APA)-valine						
	1	1	1	0	13.48(0.03)	
	1	1	1	1	17.80(0.04)	4.32
	1	1	1	−1	6.22(0.03)	7.26
Isoleucine						
	0	0	1	1	9.76(0.02)	9.76
	0	0	1	2	12.23(0.03)	2.47
Cu-isoleucine						
	1	0	1	0	8.20(0.01)	
	1	0	1	−1	1.13(0.04)	7.05
	1	0	1	−2	−9.62(0.01)	10.55
	1	0	2	0	15.06(0.01)	
Cu-(APA)-isoleucine						
	1	1	1	0	13.75(0.03)	
	1	1	1	1	18.25(0.02)	4.5
	1	1	1	−1	6.35(0.05)	7.4
Phenylalanine						
	0	0	1	1	9.12(0.02)	9.12
	0	0	1	2	11.01(0.03)	1.89
Cu-phenylalanine						
	1	0	1	0	7.78(0.01)	
	1	0	1	−1	−0.93(0.05)	7.71

(*continued*)

TABLE I Continued

System	*M*	L_1	L_2	H^a	$\log \beta^b$	$pK_a{}^c$
	1	0	1	−2	−10.88(0.01)	10.68
	1	0	2	0	14.40(0.01)	
Cu-(APA)-phenylalanine						
	1	1	1	0	12.63(0.03)	
	1	1	1	1	17.25(0.03)	4.62
	1	1	1	−1	5.27(0.10)	7.36
Proline						
	0	0	1	1	10.52(0.03)	10.52
	0	0	1	2	12.03(0.03)	1.51
Cu-proline						
	1	0	1	0	8.78(0.01)	
	1	0	1	−1	1.32(0.09)	7.46
	1	0	1	−2	−10.35(0.09)	11.67
	1	0	2	0	15.83(0.03)	
Cu-(APA)-proline						
	1	1	1	0	14.75(0.03)	
	1	1	1	1	19.20(0.03)	4.45
	1	1	1	−1	7.49(0.06)	7.26
Hydroxyproline						
	0	0	1	1	9.40(0.02)	9.40
	0	0	1	2	11.39(0.03)	1.99
Cu-hydroxyproline						
	1	0	1	0	7.64(0.01)	
	1	0	1	−1	0.04(0.03)	7.60
	1	0	1	−2	−10.11(0.01)	10.15
	1	0	2	0	13.47(0.01)	
Cu-APA-hydroxyproline						
	1	1	1	0	13.40(0.04)	
	1	1	1	1	18.01(0.05)	4.61
	1	1	1	−1	5.71(0.06)	7.89
Serine						
	0	0	1	1	9.14(0.02)	9.14
	0	0	1	2	11.40(0.03)	2.26
Cu-serine						
	1	0	1	0	7.87(0.01)	
	1	0	1	−1	1.08(0.02)	6.79
	1	0	1	−2	−9.31(0.01)	10.39
	1	0	2	0	14.28(0.01)	
Cu-(APA)-serine						
	1	1	1	0	12.13(0.03)	
	1	1	1	1	16.75(0.04)	4.62
	1	1	1	−1	5.20(0.05)	6.93
Threonine						
	0	0	1	1	9.06(0.02)	9.06
	0	0	1	2	11.03(0.03)	1.97
Cu-threonine						
	1	0	1	0	8.16(0.02)	
	1	0	1	−1	1.53(0.05)	6.63
	1	0	1	−2	−8.62(0.03)	10.15
	1	0	2	0	14.67(0.03)	
Cu-(APA)-threonine						
	1	1	1	0	12.70(0.02)	
	1	1	1	1	16.92(0.05)	4.22
	1	1	1	−1	6.00(0.06)	6.70
Ornithine						
	0	0	1	1	10.58(0.02)	10.58
	0	0	1	2	19.44(0.03)	8.86
	0	0	1	3	21.39(0.03)	1.95

(*continued*)

TABLE I Continued

System	*M*	L_1	L_2	H^a	$\log \beta^b$	$pK_a{}^c$
Cu-ornithine						
	1	0	1	0	11.72(0.10)	
	1	0	1	−1	1.75(0.01)	9.97
	1	0	2	0	15.79(0.03)	
	1	0	1	1	17.89(0.01)	6.17
Cu-(APA)-ornithine						
	1	1	1	0	16.69(0.05)	
	1	1	1	1	22.57(0.03)	6.08
Histidine						
	0	0	1	1	9.41(0.02)	9.41
	0	0	1	2	15.59(0.02)	6.18
	0	0	1	3	17.39(0.03)	1.80
Cu-histidine						
	1	0	1	0	10.61(0.01)	
	1	0	1	1	14.02(0.05)	3.41
	1	0	1	−1	5.42(0.03)	5.21
	1	0	1	−2	−1.60(0.02)	7.02
	1	0	2	0	18.62(0.01)	
	1	0	2	1	24.25(0.01)	5.63
Cu-(APA)-histidine						
	1	1	1	0	16.36(0.03)	
	1	1	1	1	21.11(0.04)	4.75
Methionine						
	0	0	1	1	9.12(0.01)	9.12
	0	0	1	2	11.37(0.01)	2.25
Cu-methionine						
	1	0	1	0	7.73(0.01)	
	1	0	1	−1	−0.97(0.03)	8.70
	1	0	2	0	14.46(0.02)	
Cu-(APA)-methionine						
	1	1	1	0	12.29(0.07)	
	1	1	1	1	17.24(0.04)	4.95
	1	1	1	−1	4.92(0.04)	7.37
Glycylglycine						
	0	0	1	1	8.10(0.01)	8.10
	0	0	1	2	11.30(0.01)	3.20
Cu-glycylglycine						
	1	0	1	0	5.63(0.02)	
	1	0	1	−1	1.35(0.02)	4.28
	1	0	1	−2	−8.05(0.03)	9.40
	1	0	2	−1	4.32(0.03)	
Cu-(APA)-glycylglycine						
	1	1	1	0	12.63(0.03)	
	1	1	1	1	17.11(0.04)	4.48
	1	1	1	−1	5.21(0.05)	7.42
Inosine						
	0	0	1	1	8.81(.01)	8.81
Cu-inosine						
	1	0	1	0	4.61(0.02)	
	1	0	1	−1	−2.08(0.03)	6.96
	1	0	1	1	11.02(0.04)	6.41
	1	0	2	0	9.02(0.03)	
	1	0	2	1	15.62(0.05)	6.60
Cu-(APA)-inosine						
	1	1	1	0	13.72(0.06)	
	1	1	1	1	21.54(0.08)	7.82

[a] M, L_1, L_2 and H are the stoichiometric coefficients corresponding to Cu^{2+}, APA, the other ligands and H^+, respectively. The coefficient −1 refers to a proton loss. [b] Standard deviations are given in parentheses. Sums of squares of residuals are less than 5e–7. [c] The pK_a of the ligands, protonated species, aquo complexes or peptide NH.

TABLE II Formation constants of APA at different compositions of water–organic solvent ratios at 25°C and 0.1 M ionic strength

System	*Solvent* (%)	*L*	*H*	$\log \beta$[a]	pK_{a1}[b]	pK_{a2}[c]
Dioxane	25	1	1	4.77(0.01)	4.77	3.00
		1	2	7.77(0.01)		
	37	1	1	4.76(0.01)	4.76	3.50
		1	2	8.26(0.01)		
	50	1	1	4.84(0.01)	4.84	3.80
		1	2	8.64(0.01)		
	62.5	1	1	5.03(0.002)	5.03	4.00
		1	2	9.03(0.003)		
	75	1	1	5.31(0.01)	5.31	4.24
		1	2	9.55(0.01)		
Ethanol	25	1	1	4.70(0.01)	4.70	2.50
		1	2	7.20(0.02)		
	37	1	1	4.65(0.01)	4.65	3.10
		1	2	7.75(0.01)		
	50	1	1	4.69(0.02)	4.69	3.52
		1	2	8.21(0.02)		
	62.5	1	1	4.80(0.02)	4.80	3.76
		1	2	8.56(0.02)		
	75	1	1	4.97(0.03)	4.97	4.02
		1	2	8.99(0.02)		
Methanol	25	1	1	4.56(0.02)	4.56	2.43
		1	2	6.99(0.05)		
	37	1	1	4.57(0.02)	4.57	2.87
		1	2	7.44(0.03)		
	50	1	1	4.63(0.02)	4.63	3.22
		1	2	7.85(0.03)		
	62.5	1	1	4.77(0.02)	4.77	3.48
		1	2	8.25(0.02)		
	75	1	1	4.93(0.02)	4.93	3.62
		1	2	8.55(0.02)		
Acetonitrile	25	1	1	4.38(0.01)	4.38	2.18
		1	2	6.56(0.01)		
	37	1	1	4.35(0.01)	4.35	2.51
		1	2	6.86(0.01)		
	50	1	1	4.48(0.01)	4.48	2.87
		1	2	7.35(0.01)		
	62.5	1	1	4.61(0.01)	4.61	3.06
		1	2	7.67(0.02)		
	75	1	1	4.78(0.04)	4.78	2.08
		1	2	7.86(0.07)		

[a]Standard deviations are given in parentheses. Sums of squares of residuals are less than 5e–7. [b]pK_a of NH_3^+. [c]pK_a of COOH.

the highest one to the ammonium nitrogen atom ($pK_{a2} = 4.74$, at 25°C). These values are in close agreement with the literature values [13] ($pK_{a1} = 1.82$ and $pK_{a2} = 4.84$) at 37°C and 0.1 M $NaNO_3$.

Binary Complexes of the Cu^{II}-APA System

The potentiometric data of the Cu^{II}-APA system provide a good fit assuming the formation of the species $Cu(APA)^+$ (110), $Cu(APA)_2$ (120), the monohydroxo Cu(APA)(OH) (11–1) and the dihydroxo $[Cu(APA)(OH)_2]^-$ (11–2) (Scheme 1).

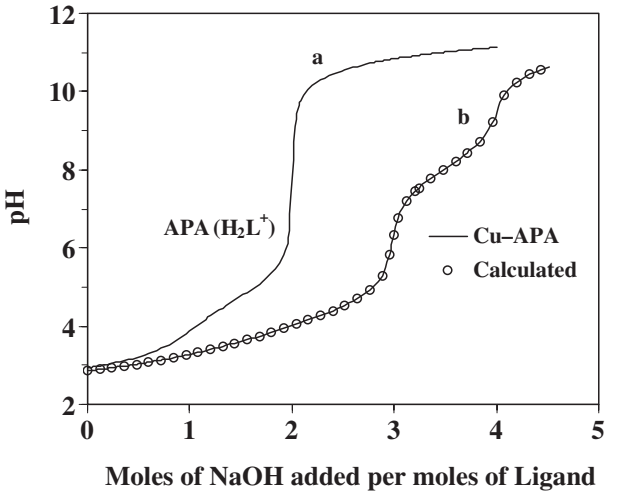
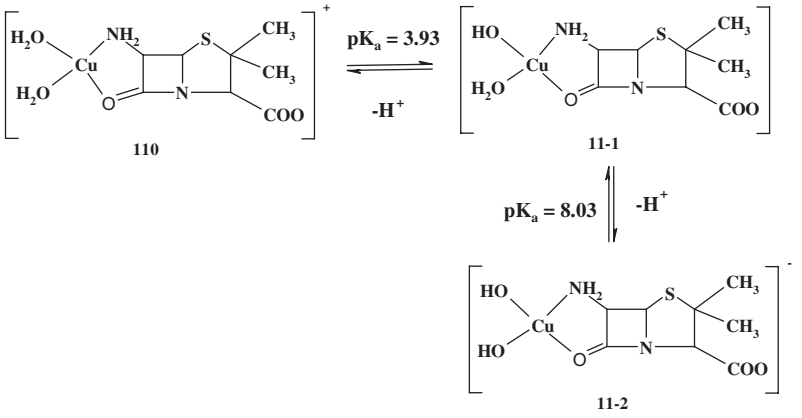


FIGURE 1 Potentiometric titration curves for the Cu^{II}-APA system: (a) APA (H_2L^+); (b) Cu^{II}-APA (H_2L^+) (1 : 2).



SCHEME 1 Acid–base equilibria of the $[Cu(APA)(H_2O)_2]^+$ complex.

The good fit between the experimental and theoretical curves (Fig. 1b) indicates the validity of the complex formation model.

From the concentration distribution curves (Fig. 2), the copper(II) aquo complex (100) and the $Cu(APA)^+$ species (110) predominate at low pH and the 110 species has a maximum concentration of 37.45% at pH 3.5. The $Cu(APA)_2$ species (120) exists at lower concentration (maximum concentration of 26.3% at pH 4.3). The mono-hydroxo species Cu(APA)(OH) (11–1) dominates in the pH range 4–8, that is, it is the main species in the physiological pH range (maximum concentration of 95.5% at pH 6.4). The dihydroxo species $[Cu(APA)(OH)_2]^-$ (11–2) prevails above about pH 8.

The infrared spectrum of APA (Fig. 3) exhibits bands at 2654 and 1774 cm^{-1} assigned to NH_3^+ and β-lactam carbonyl stretching, respectively [14,15].

In the spectrum of the Cu(APA) complex (Fig. 4), the NH_3^+ band disappeared, and was replaced by NH_2 stretching at 3310 cm^{-1} (compared to Cu-glycinate at

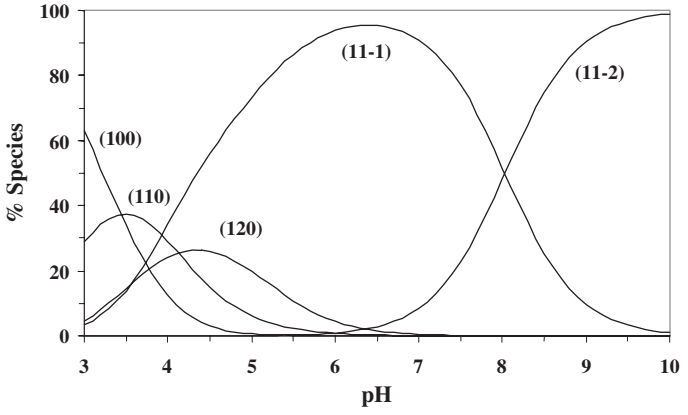


FIGURE 2 Concentration distribution of various species as a function of pH in the Cu-APA system; at concentrations of 0.0625 and 1.25 mmol L^{-1} for Cu^{+2} and APA, respectively.

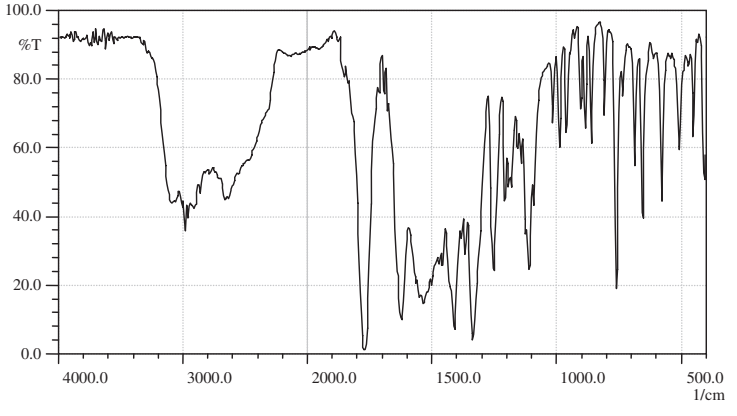


FIGURE 3 Infrared spectrum of 6-aminopenicillanic acid (APA).

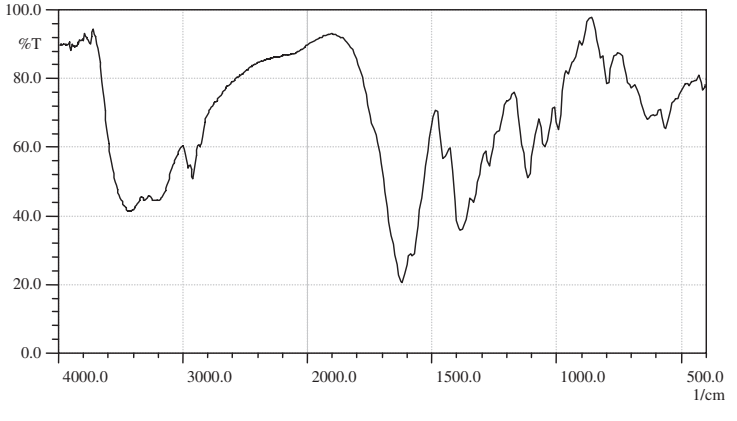


FIGURE 4 Infrared spectrum of Cu(APA) complex.

ca 3320 cm^{-1}) [14]. The β-lactam carbonyl band shifted to lower frequency. The bands correspond to coordinated β-lactam carbonyl (stretching), NH_2 (bending), and COO^- (stretching) groups appeared as a broad band between 1750 and 1550 cm^{-1}. This broad band features a shoulder at *ca* 1710 cm^{-1} and two maxima at 1620 and 1582 cm^{-1}, indicating that APA coordinates to copper(II) through both amino and β-lactam carbonyl groups. The presence of two coordinated water molecules is confirmed by the appearance of a broad band at *ca* 3420 cm^{-1} [14]. Thermogravimetric analysis of the Cu-APA complex confirms the presence of two coordinated water molecules.

Electronic absorption spectra of aqueous copper(II) and a solution of copper(II) and APA prepared at *ca* pH 3.9 and in different molar ratios were compared. The broad band at 805 nm of the free copper(II) solution ($^2B_{1g} \leftarrow {}^2A_{1g}$ transition) [16] undergoes a blue shift to 724 and 690 nm in the spectra of solution mixtures of Cu^{II} and APA with molar ratios of 1 : 1 and 1 : 2 (Cu^{2+} : APA), respectively. The first shift indicates the coordination of one APA and the second shift is due to the coordination of the second APA molecule.

Ternary Complexes of Cu^{II}, APA and Other Ligands

The enhancement of the stability of the mixed ligand complexes can be explained as follows.

(1) According to Sigel [17,18], the relative stability of a ternary complex CuAB (1110) compared to its binary complex MA (1100) or MB (1010) can be expressed quantitatively by:

$$\Delta \log K = \log \beta_{1110} - (\log \beta_{1100} + \log \beta_{1010}).$$

CuA (1 : 1) has fewer coordination sites (labile water molecules) than the aquated Cu^{2+} ion complex. Consequently, the secondary ligands (B) are expected to bind to the CuA complex with a smaller stability constant than with an aquated metal ion. Therefore, $\Delta \log K$ should be negative and generally have a value between -0.5 and -2.0 [17,19] depending on the geometry of the complex. For Cu^{+2} ions, usually having a coordination number of four, the expected $\Delta \log K$ value would be -0.6. The values of $\Delta \log K$ (Table III) are between 0.12 and 4.97, indicating an enhancement of stability of the mixed ligand complexes. Positive values are considered as evidence of enhanced stability as a result of intermolecular ligand–ligand interactions, hydrogen bonding, the π-back donation effect and/or hydrophobic effects. The abnormally high positive $\Delta \log K$ values for the ternary complexes of inosine may be due to extensive intermolecular ligand–ligand interactions.

(2) The quantitative stabilization of ternary complexes can be expressed in terms of their disproportionation constant X:

$$\log X = 2 \log \beta_{1110} - (\log \beta_{1200} + \log \beta_{1020})$$

TABLE III $\Delta \log K$ values of the ternary complexes of Cu^{II}, APA and ligand at 25°C and 0.1 M ionic strength

Ligand	$\Delta \log K$[a]	$\log X$[b]	$\log \beta_{1110}$ (*experiment*)	$\log \beta_{stat}$[c]
Glycine	1.26	4.39	13.5	11.61
Alanine	1.23	4.31	13.42	11.57
Valine	1.37	4.31	13.48	11.63
Isoleucine	1.41	4.61	13.75	11.75
Phenylalanine	0.71	3.03	12.63	11.42
Proline	1.83	5.84	14.75	12.13
Hydroxyproline	1.62	5.50	13.40	10.95
Serine	0.12	2.15	12.13	11.36
Threonine	0.40	2.90	12.70	11.55
Ornithine	0.74	10.15	16.60	11.83
Histidine	1.61	6.27	16.36	13.53
Methionine	0.42	2.29	12.29	11.45
Inosine	4.97	10.59	13.72	8.73

[a] $\Delta \log K = (\log \beta_{1110} - \log \beta_{1100} - \log \beta_{1010})$. [b] $\log X = (2 \log \beta_{1110} - \log \beta_{1200} - \log \beta_{1020})$. [c] $\log \beta_{stat} = (\log 2 + 1/2 \log \beta_{1200} + 1/2 \log \beta_{1020})$.

The value for $\log X$ expected from statistical reasons is +0.6 [18–20] for all geometries. The values of $\log X$ in Table III are $\gg 0.6$, indicating marked stabilities of the ternary complexes.

(3) The stability of the ternary complexes investigated can also be calculated using a statistical method [17,20] according to the equation:

$$\log \beta_{stat} = \log 2 + \frac{1}{2} \log \beta_{1200} + \frac{1}{2} \log \beta_{1020}.$$

The values of $\log \beta_{stat}$ for the mixed ligand complexes are shown in Table III. The large differences ($\log \beta_{1110} - \log \beta_{stat}$) indicate that the CuAB system is more stable than both CuA_2 and CuB_2.

Ternary Complexes of Cu^{II}, APA and Amino Acids

The potentiometric data of the ternary complexes involving simple amino acids are best fitted assuming formation of the species with stoichiometric coefficients 1110, 1111 and 111–1. The pK_a values of the protonated complex 1111 ($pK_a = \log \beta_{1111} - \log \beta_{1110}$) for glycine, alanine, valine, isoleucine, phenylalanine, proline, hydroxyproline, serine and threonine are in the range 4.22–4.62. The pK_a values of free APA are 1.78 and 4.74 and those of glycine are 2.32 and 9.61. The pK_a of the protonated complex 1111 probably corresponds to the ammonium group of monocoordinated glycine. The lowering of the pK_a of glycine upon coordination through COO^- at low pH has been reported [21]. The concentration distribution diagram of the glycine complex is shown in Fig. 5. The protonated complex 1111 is formed in the acidic pH range and prevails with formation of 48.9% at *ca* pH 3.4. The protonated complex undergoes deprotonation forming complex species 1110, reaching a maximum concentration of 59.6% at *ca* pH 5.8. Further deprotonation of the species 1110 gives the species 111–1, which corresponds to deprotonation of a coordinated water molecule in the axial position,

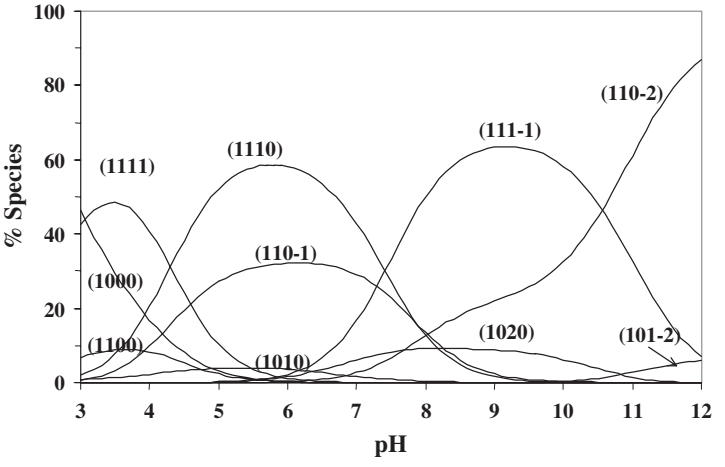


FIGURE 5 Concentration distribution of various species as a function of pH in the Cu-APA-glycine system; at concentrations of 1.25 mmol L^{-1} for Cu^{+2}, APA and glycine.

with a pK_a value of 7.41. This species predominates with a maximum formation of 60% at *ca* pH 9. This finding is confirmed by the color change from blue to purple at higher pH. The high acidification of the axially coordinated water molecule may indicate involvement of the sulfur atom of APA in chelation at higher pH as reported previously [7].

Serine and threonine have an alcohollato group, which was reported to participate in transition metal ion complex formation. The species 1110 undergoes proton ionization with pK_a values of 6.93 and 6.70 for Cu(APA)serine and Cu(APA)thereonine complexes, respectively. These values are in fair agreement with those for Cu(APA)-simple amino acid complexes (pK_a 7.41 in the case of glycine). This indicates that the alcoholic OH group is not involved in the formation of ternary complexes (111–1).

Ornithine and histidine form the ternary complexes 1110 and 1111. The formation constant values of the 1110 species are higher than those of the α-amino acids, indicating that chelation occurs by the two amino groups for ornithine and by both amino and imidazole groups for histidine. The pK_a values of the protonated species 1111 are 6.08 for Cu(APA)ornithine and 4.75 for Cu(APA)histidine. These values correspond to a protonated amino group of ornithine and a protonated imidazole group of histidine. At higher pH the coordination sites are shifted: for ornithine to the two amino groups and for histidine to the amino and imidazole groups.

Ternary Complexes of Cu^{II}, APA and Glycylglycine

Glycylglycine, taken as a representative of peptides, is found to form ternary complexes with stoichiometric coefficients 1110, 1111 and 111–1. The stability constant of the 1110 species is in the same order of the ternary complexes of the amino acids, indicating that glycylglycine is bidentate. The pK_a of the Cu(APA)glycylglycine (1110) is 7.42 ($\log \beta_{1110} - \log \beta_{111-1}$), which is very close to the corresponding values for Cu(APA)amino acids [7.41 for Cu(APA)glycine]. The species 111–1 corresponds to deprotonation of a H_2O molecule in the axial position as for Cu(APA)glycine and

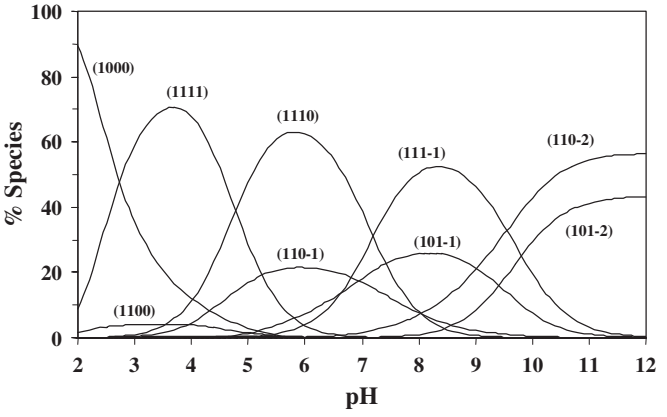


FIGURE 6 Concentration distribution of various species as a function of pH in the Cu-(APA)-glycylglycine system; at concentrations of 1.25 mmol L^{-1} for Cu^{+2}, APA and glycylglycine.

does not induce peptide ionization. The concentration distributions of different species as a function of pH are plotted in Fig. 6.

Ternary Complexes of Cu^{II}, APA and Inosine

The data show formation of the ternary complexes with stoichiometric coefficients 1110 and 1111. Metal-ion speciation in Fig. 7 indicates that the 1111 complex is formed in acidic pH range, which corresponds to the $N_{(7)}$ coordinated species, while $N_{(1)}$ is still protonated. The pK_a of the protonated complex ($\log \beta_{1111} - \log \beta_{1110}$) is 7.87, which compares favorably with the pK_a of $N_{(1)}H$ of inosine (8.81) if acidification of the $N_{(1)}H$ site by coordinated Cu^{II} is considered. At higher pH, the 1110 species is formed with a maximum concentration of 45% at *ca* pH 8.5.

Solvent Effect

The solvent effect on the acid dissociation constants of a ligand [22] can be summarized as follows.

(i) As the solvent dielectric constant decreases, the pK_a of the ligand increases and *vice versa*.
(ii) On decreasing the extent of hydrogen bonding in water by an organic solvent, the proton-accepting properties of the water increases, and consequently the pK_a of the ligand decreases.
(iii) Increasing proton solvation by an organic solvent is accompanied by a decrease in the pK_a of ligand.

Our data (Table IV, Figs. 8 and 9) confirm those of other researchers with respect to the difference in ionization behavior of the carboxyl group compared to that of the $-NH_3^+$ group [23,24], and also show that these differences apply in the four solvents examined. Thus, the –COOH dissociation in APA decreases (i.e. pK_{a1} increases), more or less linearly, with the increase in the concentration of organic solvent (decreasing dielectric constant). The trend of dissociation of $-NH_3^+$ is nonlinear (and less pronounced than in the case of –COOH) and in most cases passes through a maximum

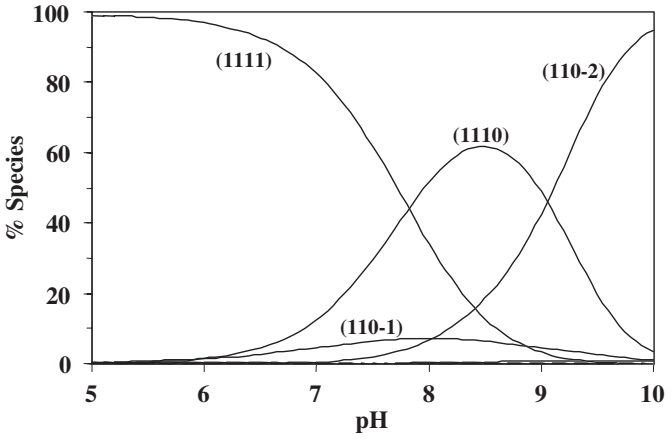


FIGURE 7 Concentration distribution of various species as a function of pH in the Cu-APA-inosine system; at concentrations of 0.0625, 0.0625 and 1.25 mmol L^{-1} for Cu^{+2}, APA and inosine, respectively.

TABLE IV Formation constants of Cu-APA at different concentrations of dioxane at 25°C and 0.1 M ionic strength

% Dioxane	*M*	*L*	*H*	$\log \beta^{a}$
0	1	1	0	4.14(0.03)
12.5	1	1	0	4.08(0.03)
25	1	1	0	4.00(0.03)
37.5	1	1	0	3.97(0.05)
50	1	1	0	3.95(0.07)

[a]Standard deviations are given in parentheses. Sums of squares of residuals are less than 5e–7.

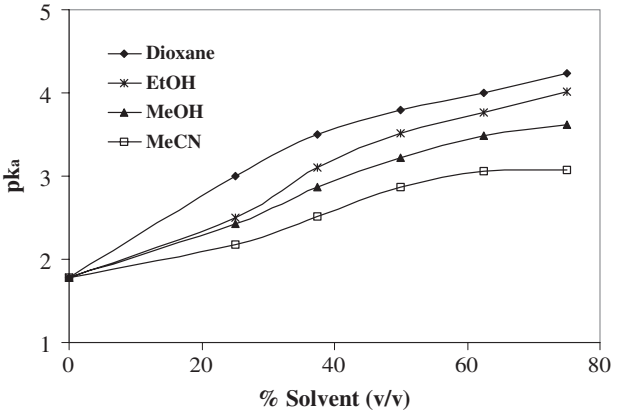


FIGURE 8 Effect of solvent on the pK_a(COOH) of the APA system.

K_{a2} (minimum pK_{a2}). The variation in pK_{a1} for APA (COOH) with solvent composition can be interpreted by using an electrostatic model. In general, the stability of compounds containing O–H increases with increasing organic content of the solvent, due to the decrease in the dielectric constant of the bulk solvents. As the dielectric constant decreases, the ion–ion interaction involving the proton and the anionic oxygen donor of

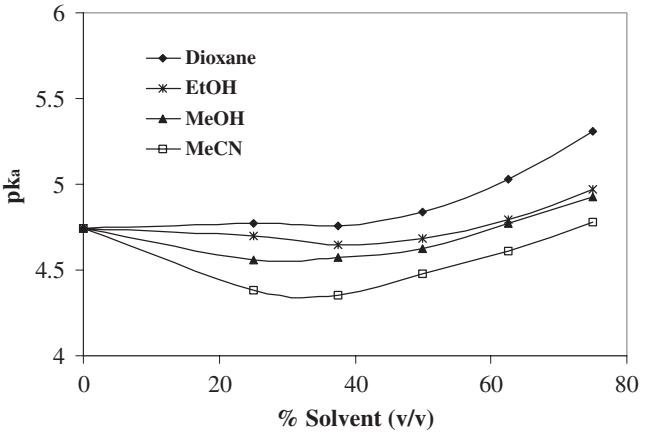


FIGURE 9 Effect of solvent on the $pK_a(NH_3^+)$ of the APA system.

the ligand increases to a greater extent than the ion–dipole interaction between the proton and the solvent molecule. The failure of the cationic acids of $-NH_3^+$ to conform to the electrostatic model has been discussed in terms of relative importance of the solvent effect (ii and iii) and the dielectric effect (i), and the former is assumed to be greater in acids involving $-NH_3^+$ dissociation [25]. The nonelectrostatic contribution to the change in pK_{a2} has been regarded as representing the sum of medium effects for individual ions [23]. Some coworkers [26] have linked what has been regarded as the exceptional behavior of the amine group on protonation to an appreciable amount of interaction with the cosolvent.

Effects (ii) and (iii) overcome the decrease in the dielectric constant (i) up to *ca* 40% (v/v) of organic composition. After *ca* 40% in all solvents studied a larger decrease in the dielectric constant overcomes the other effects of the solvent leading again to a slight increase in pK_{a2}. Comparing the effect on pK_{a2} in the four solvents under investigation (Fig. 9) indicates that the relative pK_{a2} values in different solvents increase on decreasing the dielectric constant: dioxane (2.2) > ethanol (24.3) > methanol (32.6) > acetonitrile (37.5) > water (78.5).

The effect of solvent on the formation of the Cu^{II}-APA complex ($\log \beta_{110}$) was studied in dioxane–water mixtures of different compositions. The solvent effect on the acidity constant of APA indicates that the decrease in basicity of the amino group (K_{a2}) is not as large as for the carboxylate group (K_{a1}). In general, the stability constants of complexes involving carboxylate groups are more affected by decreasing dielectric constants than those involving $-NH_2$ groups. The data in Table V show an insignificant decrease in the stability of the Cu-APA complex on increasing the percentage of the organic solvent. This confirms that the coordination between Cu^{II} and APA is through NH_2 and carbonyl oxygen and not COO^-.

Effect of Temperature on the Acidity Constants of APA and the Complex Formation of Cu^{2+} and APA

The values obtained for ΔH, ΔS and ΔG associated with protonation of APA and its complex formation with Cu^{2+} were calculated from the temperature dependence of the data in Tables VI and VII. The thermodynamic parameters ΔH and ΔS were obtained

TABLE V Formation constants of APA at different temperatures and 0.1 M ionic strength

T (°C)	L	H	$\log \beta$[a]	pK_{a1}[b]	pK_{a2}[c]
15	1	1	4.91(0.01)	4.91	1.34
	1	2	6.25(0.08)		
20	1	1	4.86(0.01)	4.86	1.59
	1	2	6.45(0.05)		
25	1	1	4.74(0.01)	4.74	1.78
	1	2	6.52(0.03)		
30	1	1	4.73(0.01)	4.73	2.03
	1	2	6.76(0.02)		
35	1	1	4.67(0.01)	4.67	2.18
	1	2	6.85(0.02)		

[a]Standard deviations are given in parentheses. Sum of square of residuals are less than 5e–7. [b]pK_a of NH_3^+. [c]pK_a of COOH.

TABLE VI Formation constants of Cu-APA at different temperatures and 0.1 M ionic strength

T (°C)	M	L	H	$\log \beta$[a]
15	1	1	0	4.58(0.03)
	1	2	0	8.25(0.02)
	1	1	−1	0.68(0.02)
	1	1	−2	−7.53(0.06)
20	1	1	0	4.33(0.03)
	1	2	0	7.98(0.02)
	1	1	−1	0.70(0.02)
	1	1	−2	−7.30(0.05)
25	1	1	0	4.14(0.03)
	1	2	0	7.83(0.01)
	1	1	−1	0.21(0.02)
	1	1	−2	−7.82(0.06)
30	1	1	0	4.09(0.05)
	1	2	0	7.81(0.02)
	1	1	−1	0.35(0.03)
	1	1	−2	−7.74(0.09)
35	1	1	0	3.88(0.07)
	1	2	0	7.78(0.02)
	1	1	−1	0.23(0.03)
	1	1	−2	−7.71(0.11)

[a]Standard deviations are given in parentheses. Sums of squares of residuals are less than 5e–7.

TABLE VII Thermodynamic parameters for the equilibria of Cu^{II} and APA complexes[a]

Equilibrium[b]	ΔH (kJ mol^{-1})	ΔS (J K^{-1} mol^{-1})	ΔG (kJ mol^{-1})
APA			
(1) $L^- + H^+ \rightleftharpoons LH$	−20.8(0.2)	21.8(1.1)	−27.3(1.2)
(2) $LH + H^+ \rightleftharpoons LH_2^+$	71.7(0.3)	275.0(6.4)	−10.2(0.5)
Cu-APA			
(3) $Cu^{2+} + L^- \rightleftharpoons CuL^+$	−56.2(1.2)	−108.1(5.5)	−23.9(0.6)
(4) $CuL^+ + L^- \rightleftharpoons CuL_2$	17.8(0.3)	131.0(4.1)	−21.3(0.5)
(5) $CuL^+ + OH^- \rightleftharpoons CuL(OH)$	13.2(0.5)	240.1(6.2)	−58.4(0.8)
(6) $CuL(OH) + OH^- \rightleftharpoons [CuL(OH)_2]^-$	15.4(0.4)	165.5(3.1)	−33.9(0.6)

[a]Standard deviations are given in parentheses. [b]Stepwise formation constants.

by a linear least-squares fit of $\ln K$ versus $1/T$ ($\ln K = -\Delta H/RT + \Delta S/R$) leading to an intercept at $\Delta S/R$ and a slope of $-\Delta H/R$.

The results are summarized in Table VII and can be interpreted as follows.

(1) The protonation reaction of APA can be represented:

$$L^- + H^+ \rightleftharpoons LH^{\pm} \tag{3}$$

$$LH^{\pm} + H^+ \rightleftharpoons LH_2^+. \tag{4}$$

The thermodynamic processes accompanying the protonation reactions are:

(i) the neutralization reaction, which is an exothermic process;
(ii) desolvation of ions, which is an endothermic process;
(iii) the change in the configuration and the arrangements of the hydrogen bonds around the free and protonated ligands.

Reaction (3) is exothermic ($\Delta H = -20.77\,\mathrm{kJ\,mol^{-1}}$) (Table VII). Reaction (4), the protonation of the zwitterion ($LH^{\pm} + H^+ \rightarrow LH_2^+$), is endothermic ($\Delta H = 71.7\,\mathrm{kJ\,mol^{-1}}$). The zwitterion (containing NH_3^+ and COO^- groups) is more solvated than LH^{2+}. Consequently this contributes more to the endothermic process upon protonation ($\Delta H = +71.7\,\mathrm{kJ\,mol^{-1}}$). The large positive entropy change ($\Delta S = 275.0\,\mathrm{J\,K^{-1}\,mol^{-1}}$) indicates a release of ordered water molecules and the breaking of hydrogen bonds. A positive ΔH and large ΔS leading to a net negative ΔG for the protonation and complexation reaction of copper ions and organic phosphates were found previously by Kramer-Schnabel and Linder [27].

(2) The complex formation reactions of Cu^{II} with APA can be represented by Equations (5)–(8)

$$Cu^{2+} + L^- \rightleftharpoons CuL^+ \tag{5}$$

$$Cu^+ + L^- \rightleftharpoons CuL_2 \tag{6}$$

$$CuL^+ + OH^- \rightleftharpoons CuL(OH) \tag{7}$$

$$CuL(OH) + OH^- \rightleftharpoons [CuL(OH)_2]^-. \tag{8}$$

Reaction (5) is exothermic, but Reaction (6), which involves complexation of the second APA, is less favored and is an endothermic reaction. As explained above, the contribution of the large solvation of the left-hand side of Reaction (6) contributes more to the endothermic reaction. The entropy change is again large ($\Delta S = 131.0\,\mathrm{J\,K^{-1}\,mol^{-1}}$), which indicates a release of ordered water molecules around CuL^+ and L^-. The net contribution to the free energy is negative ($\Delta G = -21.3\,\mathrm{kJ\,mol^{-1}}$).

(3) The formation constants for the hydrolyzed species of CuL(OH) and $CuL(OH)_2^-$ are found to be endothermic with a large change in entropy, indicating a release of ordered water molecules in the bulk of the solution, which contributes more to the total free energy change of reactions; ΔG values are -58.4 and $-33.9\,\mathrm{kJ\,mol^{-1}}$ for CuL(OH) and $CuL(OH)_2^-$, respectively.

CONCLUSIONS

The present investigation describes formation equilibria of Cu^{II} complexes with 6-aminopenicillanic acid and other ligands of biological significance such as amino acids, glycylglycine and inosine. From a combination of stability constant data of these complexes, it should in principle be possible to calculate the equilibrium distribution of 6-aminopenicillanic acid forms in biological fluids.

These results may have important biological applications. The structural flexibility of bioactive 6-aminopenicillanic acids as well as their capacity to coordinate via two donor sites may favor the formation of ternary complexes of Cu^{II} with various ligands occurring *in vivo*. In particular, such mixed-ligand coordination is likely to occur with bacterial nucleic acid. The earlier observation that copper-tetracycline complexes can bind DNA where tetracycline itself cannot [28] is in line with our results, in which Cu-APA inosine complex formation is favored, as reflected in the positive values of $\Delta \log k$, $\log X$ and $\log \beta_{stat}$.

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