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POTENTIOMETRIC STUDY OF COMPLEXES FORMED BETWEEN (S)-α-AMINO-3-HYDROXY-5-METHYL-4-ISOXAZOLEPROPANOIC ACID AND SOME TRANSITION METAL IONS

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ABSTRACT. Potentiometric study has been carried out on (S)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid [AMPA] in the presence of transition metal ions to measure the thermodynamic stabilities of its complexes; and hence give an insight into its possible role in binding metal ions. The nitrogen donor atom of AMPA is alanine-like with the addition of a hydroxy-isoxazole ring. The first complex [CuHL], which is fully formed by pH 4 is proposed to be with {N,O} bonding which results in the formation of a stable five membered chelate ring. The [CuL] species has some enhanced stability which suggest some form of tridentate co-ordination through {H_2N, COO^-, O^-}. When this changes to [CuL_2], one of these bonds must be broken if Cu(II) is to have a maximum co-ordination number of four. Zn and Cd formed very similar comlexes with AMPA, these were [ZnHL], [ZnL], [ZnL_2] and [CdHL], [CdL], [CdL_2], respectively. [ZnL] and [ZnL_2] are significantly more stable than alanine, suggesting tri-dentate co-ordination. [CdHL] complex is again very important suggesting some bidentate chelation.

KEY WORDS: (S)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid, Transition metal ions, Thermodynamic stability constants, Potentiometry, Speciation

INTRODUCTION

(S)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid [AMPA] belongs to a family of peptide-like molecules all of which are thought to be important in the central nervous system (CNS). AMPA was synthesised first by Hansen and Krogsgaad-Larsen [1] and is a potent and selective agonist of the putative, excitatory neurotransmitter L-glutamic acid (L-Glu) [2].

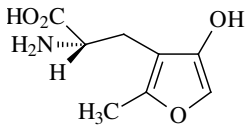

Figure 1. (S)-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid [AMPA].

Structurally, AMPA (Figure 1) may be considered as an analogue of glutamic acid. Other CNS receptors for excitatory amino acids that have been a subject of a similar study include: N-methyl-D-aspartic acid (NMDA); quisqualic acid and kainic acid (Figure 2, 3, and 4, respectively) [3].

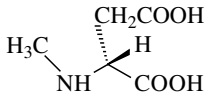

Figure 2. N-Methyl-D-aspartic acid (NMDA).

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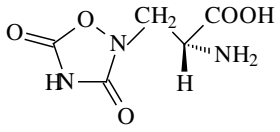


Figure 3. Quisqualic acid.

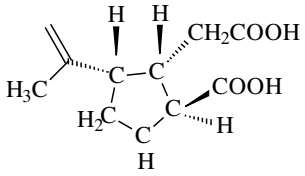


Figure 4. Kainic acid.

L-glutamic acid is a neuroexcitatory amino acid found in the CNS but up to until recently there was still doubt as to whether it is actually a neurotransmitter used by the CNS and hence has been investigated using the structurally similar quisqualic acid [4]. No further attempt will be made to discuss the medicinal aspects of these bio-active compounds as that is beyond the scope of our present study.

The co-ordination chemistry of the neurologically active amino acids is studied because of the importance of metal ion binding at receptors [5, 6]. Co-ordination of a neurotoxin to a metal ion in the cerebrospinal fluid may prevent a toxin acting on a receptor or from promoting an imbalance in the speciation of the amino acids normally present in the CNS. The co-ordination at a receptor site may actually involve a metal ion, hence a knowledge of the stability of metal ion complexes is vital in the understanding of their biological action.

The results from a recent study [3] of the amino acids kainic acid, NMDA, and quisqualic acid show that all are capable of forming stable complexes with Cu^{2+}, Ni^{2+} and Zn^{2+}. The complexes formed with Cu^{2+} were the most stable, while those of Zn^{2+} were the weakest. The complexation modes in their complexes were found to resemble those of their simpler amino acid analogues.

α-Kainic acid, [(2S,3S,4S)-2-carboxy-4-isopropenyl-3-pyrrolidinethanoic acid], is a conformationally restricted analogue of L-Glu and may act as an agonist to glutamate receptors. Quisqualic acid, [3-(3,5-dioxo-1,2,4-oxadiazolidin-2-yl)-L-alanine], is also structurally similar to glutamic acid. This similarity arises from the presence of an acidic hydrogen in the structure -CO-NH-CO-, which is separated from the α-amino group by a distance similar to that of the γ -carboxyl group of L-Glu.

Potentiometric studies have been carried out on AMPA in the presence of transition metal ions to measure the thermodynamic stabilities of its metal complexes; and hence give an insight into its possible role in binding metal ions. The nitrogen donor of AMPA is alanine-like with the addition of a hydroxy-isoxazole ring. As an amino acid AMPA would be expected to form stable complexes with transition metal ions with the possibility of unexpected stabilities as a result of interaction or steric hindrance between the metal ions and the hydroxy-isoxazole substituent on the amino acid backbone.

EXPERIMENTAL

The ligand AMPA was sufficiently water-soluble. Titrations involved an ionic background of 0.1 mol dm^{-3} KNO_3, metal ion concentration of 1.5 x 10^{-3} mol dm^{-3}, and a metal to ligand ratio of 1:2. Stability constants for the complexes of H^+ and Cu^{II}, Ni^{II} and Cd^{II} were calculated from titrations carried out using total volumes of 2 cm^3. Alkali was added from a 0.100 cm^3 micrometer syringe which had been calibrated by weight titrations and titrations of standard materials.

The pH-metric titrations were performed at 25 °C using the MOLSPIN automatic titration system with a micro – combined glass-calomel electrode calibrated in hydrogen ion concentration using HNO_3 [7]. Titrations were performed in triplicate and the SUPAQUAD computer program was used for stability constants calculations [8]. Standard deviations quoted refer to random errors only. They are, however, a good indication of the importance of a particular species in the equilibrium.

RESULTS AND DISCUSSION

Protonation constants

To confirm the purity of the ligand AMPA as supplied (fully protonated form represented as $[H_3L]^+$), the cumulative protonation constants and the initial amount of reagents (T_L, T_H /mmol) were refined simultaneously using SUPERQUAD. Then three experimental curves were appended together and, after the refinement process, gave a good fit between experimental and theoretical curves. The calculated results are shown in Table 1. The data for alanine and quisqualic acid are included for comparison.

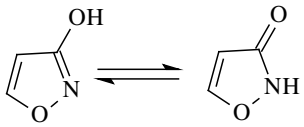
Table 1. Protonation constants for AMPA, quisqualic acid and alanine.

Ligand	log β		
	HL	H_2L	H_3L
AMPA [H_3L]	9.710(2)	14.852(3)	16.899(8)
Alanine[b] [H_2L]	9.80	12.27	
Quisqualic acid[c] [H_2L]	8.54(1)	12.58(1)	-10.412[a]

	Stepwise constants		
Ligand	log K (HL)	log K (H_2L)	log K (H_3L)
AMPA	9.71	5.14	2.05
Alanine[b]	9.80	2.47	
Quisqualic acid[c]	8.54	4.04	

[a]interpreted as deprotonation reaction, L = $[H_{-1}L] + H^+$. [b] ref. [9]. [c] ref. [3]. log K (HL) = log K (H + L → HL); log K (H_2L) = log K (HL + L →H_2L); log K (H_3L) = log K (H_2L + H →H_3L). The values in parentheses are the standard deviations computed by SUPERQUAD and refer to random error only.

The hydroxy-isoxazole ring exists in a tautometric equilibrium and in aqueous solutions, the hydroxy tautomer is more favoured [10].



AMPA has a free amino-N terminal and consequently the first protonation constant, log K(HL) = 9.71, refers to protonation of this amino nitrogen. The next stepwise protonation constant, log $K(H_2L)$, corresponds to protonation of the *semi-phenolic* group of the isoxazole ring. The final stepwise protonation, log $K(H_3L)$ is attributed to the protonation of the terminal carboxyl group, which occurs at low pH. It was possible to include another constant about pH 11 but this was doubtful since it could have resulted from the *mathematics* of SUPERQUAD calculations. It was an insignificant species below pH 10.5 and was therefore excluded from any further calculations.

Copper(II) complexes

The protonation constants were kept constant for the second stage of refinement in which the potentiometric data of the solutions containing Cu(II):L ratios of 1:~2.7 were processed in order to explore the binary complexes. The ligand concentration was also held at that calculated with the protonation constants.

The copper(II) complex formation constants for AMPA are given in Table 2. Also included in the table are the literature values for alanine and quisqualic acid to enable direct comparisons to be made. The species distribution diagram (Cu:L, 1:2) in which AMPA is compared with the simpler, well characterised alanine is shown in Figure 6.

Table 2. Copper(II) complex formation constants for quisqualic acid, AMPA and alanine.

	log β				
Ligand	CuHL	CuL	CuL_2	$CuH_{-1}L_2$	$CuH_{-2}L_2$
AMPA	13.696(12)	9.848(5)	15.279(6)	4.949(10)	
Quisqualic acid[b]		7.62	13.52(2)	4.0(1)	-7.3(2)
Alanine[c]		8.13	14.92		

[b] ref. [3]. [c] ref. [7]. The values in parentheses are the standard deviations computed by SUPERQUAD and refer to random error only.

AMPA would be expected to co-ordinate in an alanine-like fashion as the primary donor centres are identical. The ligand is regarded as $[H_3L]^+$ when fully protonated, hence [CuHL] has therefore lost two protons. These could be either from {COOH, OH} or {COOH, NH_3^+}. If they were from {COOH, NH_3^+} then [CuHL] would resemble [CuL] of alanine. If log K(CuHL): 13.696 is corrected for the protonation of the semi-phenolic isoxazole group, *i.e.* log K = log K(CuHL) – log $K(H_2L)$, then the corresponding value of log K : 8.56 is almost identical to that of log K(CuL)-alanine: 8.13. The first complex, [CuHL], which is fully formed by pH 4 is therefore proposed to be with {N, O} bonding which results in the formation of a stable five membered chelate ring. The co-ordination centres in [CuHL] are as shown in Figure 5.

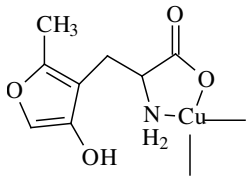


Figure 5. Proposed bonding scheme in [CuHL] complex.

The [CuL] species is more stable than [CuL] with alanine and extends to higher pH. This enhanced stability might also suggest some form of tridentate co-ordination through {H_2N, COO^-, O^-}. When this changes to [CuL_2], one of these bonds must be broken if Cu(II) is to have a maximum co-ordination number of four. Hence [CuL_2]-AMPA must be similar to [$Cu(Ala)_2$] since they have virtually identical stability constants.

At higher pH (>9), mixed hydroxy complexes probably begins to form, *e.g.* [$CuH_{-1}L_2$]. This was not a significant species in the calculations until near pH 10.

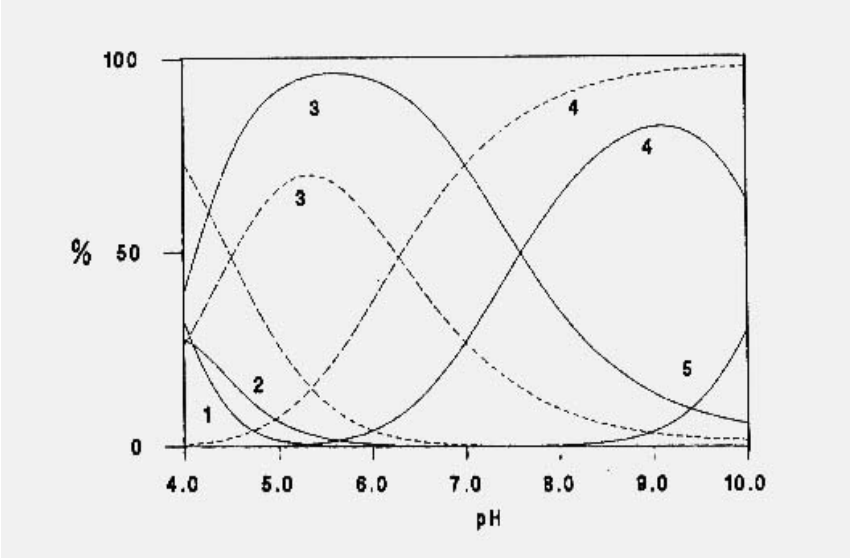


Figure 6. The species distribution diagrams of Cu(II)-AMPA [1:2]. 1, Cu(II); 2, CuHL; 3, CuL; 4, CuL_2; 5, $CuH_{-1}L_2$. --- Cu(II)-alanine [1:2].

Zn(II) complexes

Zn(II) complexes could only be fitted reliably up to pH 8.5 and gave the species; [ZnHL], [ZnL], and [ZnL_2]. The Zn(II)-AMPA complex formation constants were as shown: log β(ZnHL) = 12.54(57), log β(ZnL)= 6.00(53), and log β(ZnL_2) =10.24(78).

The errors shown reflects the complications that arise during Zn(II) titrations, *i.e.* the possibility of hydrolysis, precipitation, possible formation of polynuclear species, and possible

formation of tetrahedral and octahedral complexes, all of which may contribute to the deviations observed between the measured and calculated values.

As with all other amino acids, there was extensive hydrolysis above pH 8.5 with possible polynuclear complex formation. [ZnL_3] could not be detected. [ZnL] and [ZnL_2] are significantly more stable than alanine, suggesting some tri-dentate co-ordination. The [ZnHL] species is also very important, suggesting bidentate chelation. The species distribution curves are shown in Figure 7.

The formation of hydroxy complexes and their subsequent precipitation at high pH causes problems in interpreting any data above pH 8.

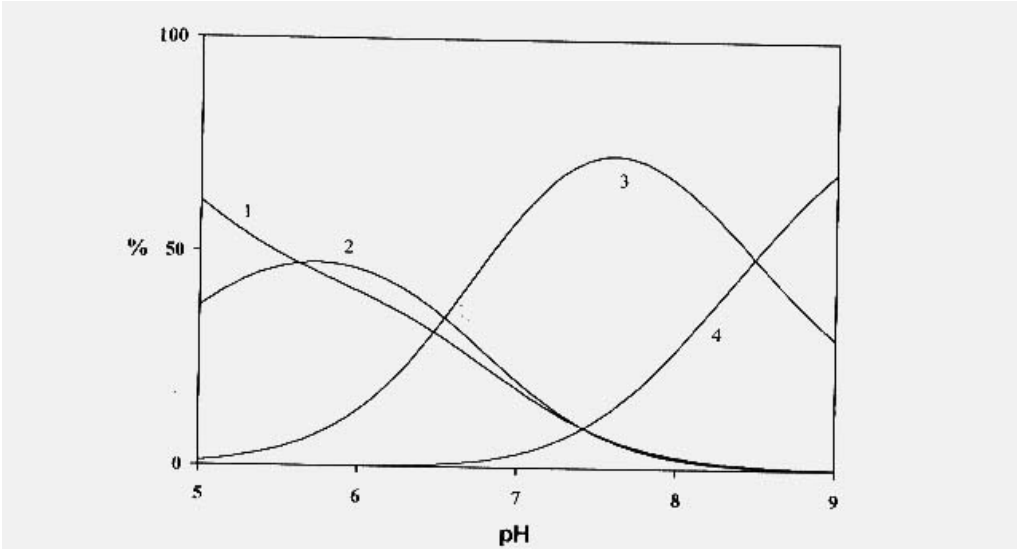


Figure 7. The species distribution diagram of Zn(II)-AMPA [1:2.5]: 1, Zn(II); 2, ZnHL; 3, ZnL; 4, ZnL_2.

Cd(II) complexes

The speciation of Cd(II) with AMPA was very similar to that of Zn(II) with the stability constants as shown: log β(CdHL) = 12.398(11), log β (CdL) = 4.802(7), log β(CdL_2) = 7.774(12), and log β($CdH_{-1}L_2$) = -1.939(8). The species distribution curves are shown in Figure 8.

A [$CdH_{-1}L_2$] species appears to be present near pH 10. This may be a mixed hydroxy complex [$Cd(OH)L_2$]. Cd(II)-AMPA complexes are comparatively stable and AMPA appears to be behave as a potentially tridentate ligand and so appear to form N,O,O-bonded [CdL] and [CdL_2] tridentate complexes. The [CdHL] complex is again very important suggesting some bidentate chelation. There was no evidence for [CdL_3] but this could not be detected with alanine until ratios of approaching 20:1 L:Cd were used [11].

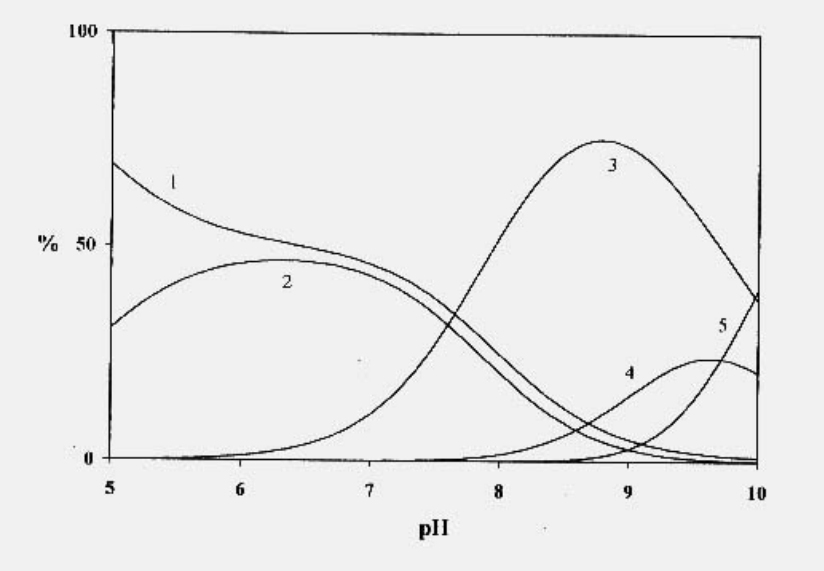


Figure 8. The species distribution diagrams of Cd(II)-AMPA [1:2.5]: 1, Cd(II); 2, CdHL; 3, CdL; 4, CdL_2; 5, $CdH_{-1}L_2$.

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