Metal Ion Complexes Containing Dipeptides, Tripeptides, and Biologically Important Zwitterionic Buffers

Hassan A. Azab,* Kholoud M. Abou El-Nour, and Sherif H. Sorror

Chemistry Department, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt

Potentiometric equilibrium measurements have been performed at (25.0 ± 0.1) °C and ionic strength $I = 0.1$ mol·dm^{-3} KNO_3 for the interaction of glycylglycine, glycylhistidine, glycylglycylglycine, glycylhistidylglycine, and Al(III), Ga(III), In(III), and Tl(I) with the biologically important secondary ligands bicine [*N*,*N*-bis(2-hydroxyethyl)glycine], tricine [*N*,*N*,*N*-tris(hydroxymethyl)methylglycine], PIPES [piperazine-1,4-bis(2-ethanesulfonic acid)], HEPES (*N*-[2-hydroxyethyl]piperazine-*N*-[2-ethanesulfonic acid]), and EPPS 4-(2-hydroxyethyl)-piperazine 1-propanesulfonic acid in a 1:1:1 ratio. Ternary complexes of the type Al(III)−glycylglycine−guanosine, Al(III)−glycylglycine−guanosine 5′-monophosphate (GMP), Al(III)−glycylglycylglycine−guanosine, Al(III)−glycylglycylglycine−GMP, Al(III)−glycylhistidine−guanosine, Al(III)−glycylhistidine−GMP, Al(III)−glycylhistidylglycine−guanosine, Al(III)−glycylhistidylglycine−GMP in a 1:1:1 ratio have been investigated. The experimental conditions were selected such that self-association of the nucleotide and their complexes was negligibly small; that is, the monomeric normal and protonated complexes were studied. The formation of various mixed ligand complexes was inferred from the potentiometric titration curves. Initial estimates of the formation constants of the resulting species and the protonation constants of the different peptides and zwitterionic buffers used have been refined with the SUPERQUAD computer program. Confirmation of the formation of ternary complexes of the type M−P−Z in solution has been carried out using differential pulse polarography, square wave voltammetry, cyclic voltammetry, and UV spectroscopic measurements.

Introduction

The interactions of Al(III) with the dipeptide AspAsp and the tripeptide AspAspAsp in aqueous solutions were studied by pH potentiometry and multinuclear ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Their numerous negatively charged COO^- functions allow these ligands to bind Al(III) even in weakly acidic solutions.

Various mononuclear 1:1 complexes are formed in different protonation state. ^{13}C NMR spectroscopy unambiguously proved participation of the COO^- functions in a monodentate or chelating mode in Al(III) binding; however, the terminal -NH_2 group seems to be excluded from the coordination. Depending on the metal ion to ligand ratio, precipitation occurs at pH (5.0 to 6.0). This indicates that the COO^- groups at the low level of preorganization in such small peptides are not sufficient to keep the Al(III) ion in solution and to prevent the precipitation of $Al(OH)_3$ at physiological pH.[1]

Complexes formed with low molecular mass biomolecules are the dynamic or mobile units of Al(III), which may be involved in the absorption and transport processes of this toxic element in organisms. Rubini et al.[2] reviewed the interactions of Al(III), from speciation and structural aspects, with biologically relevant endogenous and exogenous small biomolecules such as inorganic ligands (hydroxide, fluoride, (oligo)phosphates, and silicic acid), amino acids, phosphorylated amino acids, oligopeptides, biophosphates including nucleotides, phosphonates, hydroxamates, and aromatic and aliphatic hydroxycarboxylates.

Kiss et al.[3] discussed the solution state of the neurotoxic Al(III) in biological systems. The importance of the Al(III)−peptide and Al(III)−protein interactions in the various neurodegeneration processes is emphasized and evaluated.

Group III cations exhibit an essentially similar chemical behavior in aqueous solution. Under physiological conditions these cations exist as metal complexes. They are known to bind tightly to human serum transferrin in the blood. The numerous published studies on the interactions of group III metals with transferrin are reviewed, with particular attention being given to the comparative analysis of the binding constants and to the kinetics and mechanisms of metal ion uptake and release. The structural and functional information obtained on these metallotransferrins by advanced physicochemical methods, such as NMR spectroscopy, is presented in light of the recent crystal structures of ferric− and apotrans−ferrin. The biological consequences of binding of aluminum(III) and indium(III) to transferrin are discussed in relation to the relevant roles played by these metal ions in pharmacology and toxicity.[4]

Transition metal complexes of peptides containing monodentate or chelating imidazole side chains have been studied by the combined application of potentiometric and spectroscopic techniques. The results obtained on the complexes of peptides containing C-terminal histidyl residues (Gly3His, Gly4His, and Gly5His) provided clear evidence that both amino and imidazole functions are effective metal binding sites. The formation of various macrochelates were described via the coordination of both termini, but the major species were characterized by 4N-coordination starting from the N-termini. The coordination chemistry of a series of peptide molecules containing bis(imidazolyl) agents revealed that the donor functions of the peptide backbone cannot compete with chelation of the bis(imidazolyl) residue. However, the presence of a terminal amino group promotes amide coordination, while

* Corresponding author. E-mail: azab2@yahoo.com.

10.1021/je060319k CCC: $37.00
Published on Web 01/10/2007

Table 1. Formation Constants for the Binary Complexes of Metal Ions (M) + Peptide (P) at (25.0 ± 0.1) °C and I = 0.1 mol·dm^{-3} (KNO_3)[a]

	log K_1 (P =)			
metal ion	glycylglycine	glycylhistidine	glycylglycylglycine	glycylhistidylglycine
Al(III)	3.02 ± 0.02	3.52 ± 0.02	3.26 ± 0.01	3.01 ± 0.02
Ga(III)	2.87 ± 0.01	4.02 ± 0.01	2.87 ± 0.01	2.24 ± 0.01
In(III)	2.63 ± 0.01	3.39 ± 0.01	2.62 ± 0.01	3.27 ± 0.02
Tl(I)	6.13[b] ± 0.02	5.51[b] ± 0.02	3.56 ± 0.01	3.29 ± 0.01

[a] ± uncertainties refer to 3 times the standard deviation (3σ). [b] Refers to formation constants for protonated complexes: log $K_{M(HP)}$: M + HP = MHP.

Table 2. Formation Constants for the Binary Complexes of Metal Ions (M) + Zwitterionic Buffer (Z) at (25.0 ± 0.1)°C and I = 0.1 mol·dm^{-3} (KNO_3)[a]

	log K_1 (Z =)				
metal ion	HEPES	EPPS	PIPES	Bicine	Tricine
Al(III)	3.26 ± 0.01	3.01 ± 0.01	3.56 ± 0.02	3.52 ± 0.01	3.01 ± 0.01
Ga(III)	1.99 ± 0.01	2.44 ± 0.02	1.59 ± 0.01	2.72 ± 0.01	2.85 ± 0.02
In(III)	0.70 ± 0.01	3.42 ± 0.01	3.28 ± 0.01	3.42 ± 0.01	3.02 ± 0.01
Tl(I)	6.10[b] ± 0.01	8.42[b] ± 0.03	3.30 ± 0.02	3.57 ± 0.02	8.64[b] ± 0.03

[a] ± uncertainties refer to 3 times the standard deviation (3σ). [b] Refers to formation constants for protonated complexes: log $K_{M(HZ)}$: M + HZ = MHZ.

Table 3. Formation Constants for Mixed Ligand Complexes of Metal Ions (M) + Peptide (P) Glycylglycine (GG) + Zwitterionic Buffer (Z) at (25.0 ± 0.1) °C and I = 0.1 mol·dm^{-3} (KNO_3)[a]

metal ion	log $K_{M(P)(HEPES)}$	log $K_{M(P)(EPPS)}$	log $K_{M(P)(PIPES)}$	log $K_{M(Bicine)(P)}$	log $K_{M(Tricine)(P)}$
Al(III)	3.63 ± 0.02	13.33[b] ± 0.02 3.30 ± 0.01	13.25[b] ± 0.03	13.86[b] ± 0.04	13.73[b] ± 0.03
Ga(III)	4.43 ± 0.02	14.35[c] ± 0.04	5.13 ± 0.02	5.43 ± 0.02	3.77 ± 0.02
In(III)	4.00 ± 0.02	6.22 ± 0.02	13.25[c] ± 0.03	4.46 ± 0.02	8.64[c] ± 0.02
Tl(I)	4.00 ± 0.02	14.35[c] ± 0.04	13.25[c] ± 0.03	3.60 ± 0.01	3.42 ± 0.01

[a] ± uncertainties refer to 3 times the standard deviation (3σ). [b] Refers to formation constants for protonated complexes: log $K_{M(HP)(Z)}$: MP + HZ = MPHZ (charges omitted for simplicity). [c] Refers to formation constants for protonated complexes: log $K_{M(HP)(HZ)}$.

Table 4. Formation Constants for the Mixed Ligand Complexes of Metal Ions (M) + Peptide (P) Glycylhistidine (GH) or Glycylhistidylglycine (GHG) + Zwitterionic Buffer (Z) at (25.0 ± 0.1) °C and I = 0.1 mol·dm-3 (KNO3)[a]

metal ion	log $K_{M(GH)(Tricine)}$	log $K_{M(GHG)(Tricine)}$
Al(III)	4.16 ± 0.02	14.62[c] ± 0.02
Ga(III)	3.50 ± 0.01	8.63[b] ± 0.02
In(III)	3.77 ± 0.01	14.62[c] ± 0.03
Tl(I)	16.01[c] ± 0.04	12.34[c] ± 0.04

[a] ± uncertainties refer to 3 times the standard deviation (3σ). [b] Refers to formation constants for protonated complexes: log $K_{M(HP)(Z)}$ or log $K_{M(P)(HZ)}$: MP + H + Z = MPHZ. [c] Refers to formation constants for protonated complexes: log $K_{M(HP)(HZ)}$: MP + H + HZ = MHPHZ.

imidazole residues act as additional donor sites or bridging ligands.[5]

Most reports of metal-binding peptides and peptidomimetics focus on metal−peptide complexes utilizing peptide side chains or the amino, imidazole, carboxylate, sulfhydryl, indole, or phenol groups as metal-binding sites. All these studies have been carried out in the presence of biologically important zwitterionic buffers, which have been used for controlling the pH values to the physiological pH range of (6.0 to 8.0). The participation of these buffer ligands in the formation of different types of ternary complexes containing peptides and metal ions has not taken into consideration during the biomedical or toxicological studies using these peptides in presence of metal ions like Al(III), Ga(III), In(III), or Tl(I). This will affect the properties of these peptides in various ways when they are used as substrates. To the best of our knowledge, no data have been reported in the literature for the ternary systems of the type metal M−P−Z (where M = Al(III), Ga(III), In(III) or Tl(I); Z = bicine, tricine, HEPES, EPPS, or PIPES; and P = glycylglycine, glycylhistidine, glycylglycylglycine, or glycylhistidylglycine). This prompted us to investigate the ternary complexes of these systems. The study of these systems may lead to guidelines for the synthesis of possible antitumor drugs.

As medical technology continues to develop, there is an increasing need for metal complexes to become available for use as clinical in vivo imaging agents. Two imaging techniques that use metal complexes and are becoming widely used in clinical practice are magnetic resonance imaging (MRI) and single photon emission computed tomography (SPECT).[6] A further imaging technique that is beginning to receive increased attention is positron emission tomography (PET). This method uses computational data analysis to develop the image created from the pair of γ-rays generated 180° apart that result from the annihilation of the emitted position.

For SPECT imaging, much of the development has been carried out using technetium complexes containing the ^{99m}Tc isotope. Other metal ions can, however, be used for imaging applications. Two such ions are Ga(III) and In(III). As a result, there is an increasing interest in the use of new complexes of gallium and indium as radiopharmaceuticals. These two metal ions can be used in clinical applications as the radioactive isotopes ^{67}Ga, ^{68}Ga, ^{111}In, and ^{113}In. Complexes of ^{68}Ga, which is a positron emittor, can be used for PET imaging. The other isotopes of gallium and indium are γ-ray emittors that can be used in standard imaging techniques. This prompted us to investigate the ternary systems of the type Ga(III)−P−Z and In(III)−P−Z. These systems can be considered as models for the development of imaging agents for several proteins, containing the peptides under investigation, using radioactive gallium and indium.

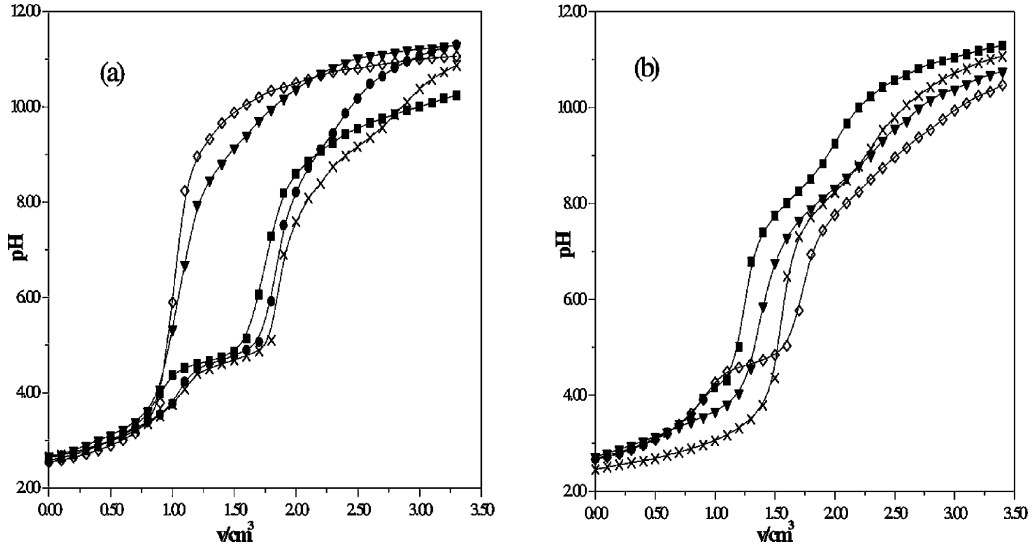

Figure 1. pH against volume of 0.0373 mol·dm^{-3} KOH at I = 0.1mol·dm^{-3} KNO_3 and t = (25.0 ± 0.1)°C for (a) Al(III) + glycylglycylglycine (GGG) + guanosine system: ◇, 4 × 10^{-3} mol·dm^{-3} HNO_3 + 1 × 10^{-3} mol·dm^{-3} guanosine; ▼, 4 × 10^{-3} mol·dm^{-3} HNO_3 + 1 × 10^{-3} mol·dm^{-3} GGG; ■, 4 × 10^{-3} mol·dm^{-3} HNO_3 + 1 × 10^{-3} mol·dm^{-3} guanosine + 1 × 10^{-3} mol·dm^{-3} Al(III); ●, 4 × 10^{-3} mol·dm^{-3} HNO_3 + 1 × 10^{-3} mol·dm^{-3} GGG + 1 × 10^{-3} mol·dm^{-3} Al(III); ×, 4 × 10^{-3} mol·dm^{-3} HNO_3 + 1 × 10^{-3} mol·dm^{-3} guanosine + 1 × 10^{-3} mol·dm^{-3} GGG + 1 × 10^{-3} mol·dm^{-3} Al(III). (b) 4 × 10^{-3} mol·dm^{-3} HNO_3 + 1 × 10^{-3} mol·dm^{-3} M + 1 × 10^{-3} mol·dm^{-3} glycylglycylglycine (GGG) + 1 × 10^{-3} mol·dm^{-3} tricine system: ◇, M = Al(III); ▼, M = Ga(III); ■, M = In(III); ×, M = Tl(I)

Experimental Section

Materials and Solutions. All materials used in the present investigation were of A R Grade. The peptides were purchased from Sigma Chemical Co. They are as follows: glycylglycine (GG) ($C_4H_8N_2O_3$), glycylglycylglycine (GGG) ($C_6H_{11}N_3O_4$), glycylhistidine (GH) ($C_8H_{12}N_4O_3$), glycylhistidylglycine (GHG) ($C_{10}H_{15}N_5O_4$). They were used without purification.

Reagent grade bicine [*N*,*N*-bis(2-hydroxyethyl)glycine], tricine [*N*,*N*,*N*-tris(hydroxylmethyl)methylglycine], PIPES [piperazine-1,4-bis(2-ethanesulfonic acid)], HEPES (*N*-[2-hydroxyethyl] piperazine-*N*-[2-ethanesulfonic acid]), and EPPS [4-(2-hydroxyethyl)piperazine-1-propane sulfonic acid], guanosine ($C_{10}H_{13}N_5O_5$), and GMP (guanosine 5′-phosphoric acid disodium salt pentahydrate) ($C_{10}H_{12}N_5O_8Na_2$) were purchased from Sigma Chemical Co. Potentiometric pH titrations have been carried out to verify/determine the purity, especially for acidic/basic contaminants of the used peptides and zwitterionic buffers. The purity of the zwitterionic buffers averages 99.5 % with standard deviation of 0.05 %.

Metal salts used in this work are $Al(NO_3)_3$·$9H_2O$, $Ga(NO_3)_3$, $InCl_3$·$4H_2O$, and TlCl·$4H_2O$ and were purchased from Merck p.a. Stock solutions of metal nitrates or chlorides were prepared by dissolving precisely weighed amounts of the salt in bidistilled water. The concentrations of the metal ion stock solutions were determined complexometrically by ethylene diamine tetraacteic acid disodium salt (EDTA).[7]

Nitric acid and KOH were from Merck p.a. Stock solutions were prepared using bidistilled, CO_2-free water. The concentration of KOH was determined by titrations with a standard solution of potassium hydrogen phthalate (Merck AG). HNO_3 solutions were prepared and standardized potentiometrically with tris(hydroxymethyl) aminomethane.

Apparatus and Procedure. Potentiometric pH measurements were made on the solutions in a double-walled glass vessel at (25.0 ± 0.1) °C with a commercial Fisher combined electrode, and a magnetic stirrer was used. A Fisher Accumet pH/Ion meter model 825 MP was used. Purified nitrogen was bubbled through the solutions during titrations, and the titrant KOH solution was added by an automatic dispenser.

The test solutions were titrated with standard CO_2-free KOH. The electrodes were calibrated in both the acidic and alkaline regions by titrating 0.01 mol·dm^{-3} nitric acid with standard potassium hydroxide under the same experimental conditions. The concentration of free hydrogen ion, C_{H^+}, at each point of the titration is related to the measured E of the cell by the Nernst equation:

$$E = E^\circ + Q \log C_{H^+} \quad (1)$$

where E° is a constant that includes the standard potential of the glass electrode and Q is the slope of the glass electrode response. The value of E° for the electrode was determined from a Gran plot derived from a separate titration of nitric acid with a standard KOH solution under the same temperature and medium conditions as those for the test solution titration. The results so obtained were analyzed by the nonlinear least squares computer program ESAB2M[8] to refine E° and the autoprotolysis constant of water, K_W. During these calculations K_W was refined until the best value for Q was obtained. The results obtained indicated the reversible Nernstian response of the glass electrode used.

The solutions titrated can be presented according to the following scheme: HNO_3 (4 × 10^{-3} mol·dm^{-3} or 2 × 10^{-3} mol·dm^{-3}) + peptide (1 × 10^{-3} mol·dm^{-3} or 5 × 10^{-4} mol·dm^{-3}) (a); HNO_3 (4 × 10^{-3} mol·dm^{-3} or 2 × 10^{-3} mol·dm^{-3}) + peptide (1 × 10^{-3} mol·dm^{-3} or 5 × 10^{-4} mol·dm^{-3}) + M(1 × 10^{-3} mol·dm^{-3} or 5 × 10^{-4} mol·dm^{-3}) (b); HNO_3 (4 × 10^{-3} mol·dm^{-3} or 2 × 10^{-3} mol·dm^{-3}) + zwitterionic buffer ligand (1 × 10^{-3} mol·dm^{-3} or 5 × 10^{-4} mol·dm^{-3}) (c); HNO_3 (4 × 10^{-3} mol·dm^{-3} or 2 × 10^{-3} mol·dm^{-3}) + zwitterionic buffer ligand (1 × 10^{-3} mol·dm^{-3} or 5 × 10^{-4} mol·dm^{-3}) + M (1 × 10^{-3} mol·dm^{-3} or 5 × 10^{-4} mol·dm^{-3}) (d); HNO_3 (4 × 10^{-3} mol·dm^{-3} or 2 × 10^{-3} mol·dm^{-3}) + peptide (1 × 10^{-3} mol·dm^{-3} or 5 × 10^{-4} mol·dm^{-3}) + zwitterionic buffer ligand (1 × 10^{-3} mol·dm^{-3} or 5 × 10^{-4} mol·dm^{-3}) + M (1 × 10^{-3} mol·dm^{-3} or 5 × 10^{-4} mol·dm^{-3}) (e).

A constant ionic strength was obtained with 0.1 mol·dm^{-3} KNO_3, and the total volume was kept at 10.0 cm^3. At least four

Table 5. Formation Constants for the Mixed Ligand Complexes of Metal Ions (M) + Peptide (P) Glycylglycylglycine (GGG) + Zwitterionic Buffer (Z) at (25.0 ± 0.1) °C and I = 0.1 mol·dm^{-3} (KNO_3)[a]

metal ion	log $K_{M(Tricine)(P)}$	log $K_{M(Bicine)(P)}$	log $K_{M(P)(PIPES)}$	log $K_{M(P)(EPPS)}$	log $K_{M(P)(HEPES)}$
Al(III)	3.50 ± 0.02	4.25 ± 0.02	13.08[c] ± 0.02	13.51[c] ± 0.02	3.77 ± 0.02
Ga(III)	4.00 ± 0.02	3.85 ± 0.02	13.08[c] ± 0.02	3.40 ± 0.01	14.58[c] ± 0.03
In(III)	5.90 ± 0.02	8.53[b] ± 0.02	3.37 ± 0.02	4.30 ± 0.02	4.16 ± 0.02
Tl(I)	4.91 ± 0.01	9.55[b] ± 0.03	13.08[c] ± 0.03	3.40 ± 0.01	6.62 ± 0.02
		3.39 ± 0.03			

[a] ± uncertainties refer to 3 times the standard deviation (3σ). [b] Refers to formation constants for protonated complexes: log $K_{M(HP)(Z)}$: MP + H + Z = MHPZ. [c] Refers to formation constants for protonated complexes: log $K_{M(HP)(HZ)}$: MP + H + HZ = MHPHZ.

Table 6. Formation Constants for Al(III) + Peptide (P) + Guanosine (GS) or Guanosine 5′-Monophosphate (GMP) in 1:1:1 Ratio at (25.0 ± 0.1) °C and I = 0.1 mol·dm^{-3} (KNO_3)[a]

log $K_{Al(III)(GS)}$ or log $K_{Al(III)(GMP)}$		
metal ion	guanosine	GMP
Al(III)	3.26 ± 0.02	3.49 ± 0.02

log $K_{Al(III)(P)(GS)}$ or log $K_{Al(III)(P)(GMP)}$				
	glycylglycine		glycylglycylglycine	
peptide	guanosine	GMP	guanosine	GMP
Al(III)	3.77 ± 0.02	8.30 ± 0.03	5.51 ± 0.02	7.46 ± 0.03

	glycylhistidine	HGlycylhistidine	glycylhistidylglycine	HGlycylhistidylglycine
peptide	HGuanosine	HGMP	HGuanosine	HGMP
Al(III)	6.50 ± 0.03	14.70 ± 0.04	6.00 ± 0.04	14.40 ± 0.05

[a] ± uncertainties refer to 3 times the standard deviation (3σ).

titrations were performed for each system. For both ligand protonation and metal complex formation equilibria, data were collected over the largest possible pH interval, although a number of experimental points were frequently discarded for the final stability constant calculations, especially within the range where the complexation observed was insignificant.

Initial estimates of the formation constants of ternary complexes and the stability constants of the binary 1:1 complexes have been refined using the SUPERQUAD computer program.[9] During this refinement, the stepwise stability constant

$$K_{M(P)(Z)} = \frac{[M_p(P)_q(Z)_r]}{[M_pP_q][Z]^r} \quad (2)$$

which refers to the addition of Z to the binary complex $M_p(P)_q$. The overall complexation reaction involving protonation is

$$pM + qP + rZ + sH \rightleftharpoons M_p(P)_q(Z)_r(H)_s \quad (3)$$

$$\beta_{pqrs} = \frac{M_p(P)_q(Z)_r(H)_s}{[M]^p[P]^q[Z]^r[H]^s} \quad (4)$$

in which P = the fully deprotonated form of the peptide (glycylglycine, glycylhistidine, glycylglycylglycine, or glycylhistidylglycine); Z = the zwitterionic buffer ligands (bicine, HEPES, EPPS, PIPES, or tricine); M = Al(III), Ga(III), In(III), or Tl(I). p, q, r, and s are the moles of M, P, Z, and H in M_p, $(P)_q$, $(Z)_r$, and $(H)_s$, respectively. All side reactions due to metal ion hydrolysis especially for Al(III), Ga(III), or In(III)[10−12] have been included in the calculations.

Electrochemical Measurements. Cyclic voltammetry, square wave voltammetry, and differential pulse voltammetry measurements are collected using an E.G and G Princeton applied research, potentiostat/galvanostat model 263 with a single-compartment voltammetric cell equipped with a glassy carbon (GC) working electrode (area = 0.1963 cm^2) embedded in a resin, a Pt-wire counter electrode, and an Ag/AgCl reference electrode. In a typical experiment, a sample volume of 25 cm^3 containing the free metal ion (5×10^{-4} or 1.5×10^{-3}) mol·dm^{-3} M (a); (5×10^{-4} or 1.5×10^{-3}) mol·dm^{-3} M + (5×10^{-4} or 1.5×10^{-3}) mol·dm^{-3} glycylhistidylglycine (GHG) or glycylglycylglycine (GGG) (b); (5×10^{-4} or 1.5×10^{-3}) mol·dm^{-3} M + (5×10^{-4} or 1.5×10^{-3}) mol·dm^{-3} zwitterionic buffer (Z = HEPES) (c); or (5×10^{-4} or 1.5×10^{-3}) mol·dm^{-3} M + (5×10^{-4} or 1.5×10^{-3}) mol·dm^{-3} GGG + (5×10^{-4} or 1.5×10^{-3}) mol·dm^{-3} (Z = HEPES) (d) was used. The ionic strength of the studied solutions was adjusted at 0.1 using a KNO_3 or at 0.05 using a KCl solutions.

Cyclic Voltammetry. The solution was purged with nitrogen for 120 s, and then the potential was scanned at different scan rates in the respective potential windows for each metal ion.

Square Wave Voltammetry. The samples were analyzed as in cyclic voltammetry, but at the scan rate = 36.6 mV·s^{-1}. The pulse height was 25 mV, and the scan increment was dE = 2.0 mV.

Differential Pulse Voltammetry. The samples were analyzed also as in cyclic voltammetry but at the scan rate = 36.6 mV·s^{-1}. The pulse height was 25 mV, the pulse width = 50 s, and the scan increment was 2.0 mV.

Spectrophotometric Measurements. The ultraviolet (UV) spectra of the solutions of the binary and ternary complexes were scanned on a Perkin-Elmer spectrophotometer model (Perkin-Elmer UV−Visible automatic recording spectrophotometer with a 1-cm quartz cell). The required volume of the stock metal ion salt is mixed with that of the ligand solution, keeping the total concentration of each to be 1×10^{-5} mol dm^{-3}. The ionic strength of each solution was adjusted to 0.1 mol·dm^{-3} KNO_3. All the studied solutions were diluted with bidistilled water, after the pH adjustment to the required value using diluted solutions of either HNO_3 or KOH. The binary complex solutions in 1:1 ratio were scanned against bidistilled water as a blank in a 1-cm quartz cell.

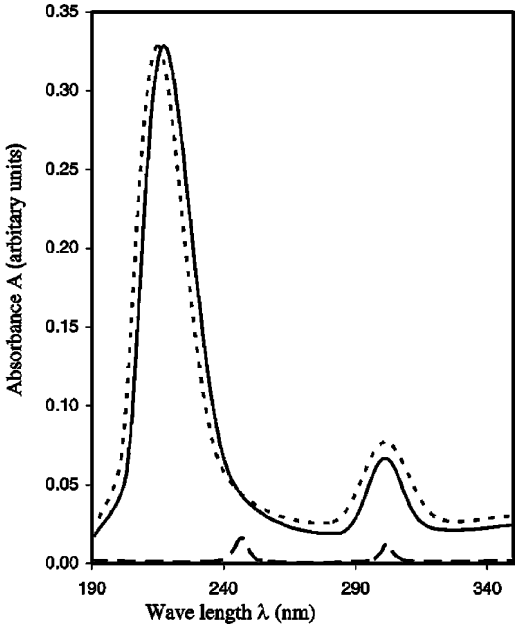

Figure 2. UV absorbance spectra for the Al(III) + glycylglycylglycine (GGG) + HEPES system at $I = 0.1$ mol·dm^{-3} KNO_3 and $t = (25.0 \pm 0.1)$ °C: —, 1×10^{-5} mol·dm^{-3} Al(III) + 1×10^{-5} mol·dm^{-3} GGG (pH 5.5); ···, 1×10^{-5} mol·dm^{-3} Al(III) + 1×10^{-5} mol·dm^{-3} HEPES (pH 4.5); - - -, 1×10^{-5} mol·dm^{-3} Al(III) + 1×10^{-5} mol·dm^{-3} GGG + 1×10^{-5} mol·dm^{-3} HEPES (pH 5.8).

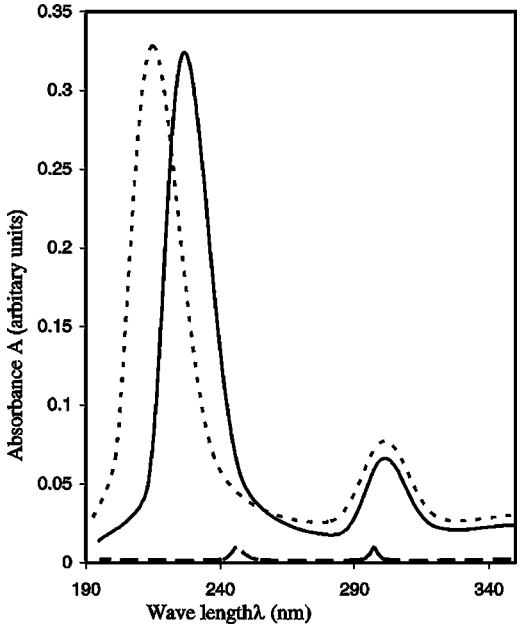

Figure 3. UV absorbance spectra for the Al(III) + glycylhistidylglycine (GHG) + HEPES system at $I = 0.1$ mol·dm^{-3} KNO_3 and $t = (25.0 \pm 0.1)$ °C: —, 1×10^{-5} mol·dm^{-3} Al(III) + 1×10^{-5} mol·dm^{-3} GHG (pH 5.3); ···, 1×10^{-5} mol·dm^{-3} Al(III) + 1×10^{-5} mol·dm^{-3} HEPES (pH 4.5); - - -, 1×10^{-5} mol·dm^{-3} Al(III) + 1×10^{-5} mol·dm^{-3} GHG + 1×10^{-5} mol·dm^{-3} HEPES (pH 5.2).

The ternary complex solutions were prepared in 1:1:1 ratio keeping the concentration of each species to be 1×10^{-5} mol·dm^{-3} and at 0.1 mol·dm^{-3} KNO_3. The desired pH value is maintained using HNO_3 or KOH diluted solutions. Each ternary complex solution was scanned against the binary complex containing the metal ion and the primary ligand corresponding to each system.

Results and Discussion

The second protonation constants determined at (25.0 ± 0.1) °C for HEPES (log $K_2 = 8.0 \pm 0.02$), bicine (log $K_2 = 8.30 \pm 0.02$), tricine (log $K_2 = 8.40 \pm 0.02$), PIPES (log $K_2 = 6.82 \pm 0.02$), and EPPS (log $K_2 = 8.36 \pm 0.02$) were in good agreement with those found in the literature.[13−15]

The protonation constant values for glycylglycine (log $K_1 = 3.14 \pm 0.02$, log $K_2 = 8.22 \pm 0.02$), glycylhistidine (log $K_1 = 2.50 \pm 0.02$, log $K_2 = 6.76 \pm 0.02$), glycylglycylglycine (log $K_1 = 3.11 \pm 0.02$, log $K_2 = 7.88 \pm 0.02$), and glycylhistidylglycine (log $K_1 = 3.04 \pm 0.02$, log $K_2 = 6.48 \pm 0.02$, log $K_3 = 7.99 \pm 0.02$). The stability constants of their binary metal complexes were determined from the titration curves, and the results were found to agree well with those reported in the literature.[16] The plus/minus values refer to statistically determined uncertainties at 95 % intervals of the reported values.

In Tables 1 to 6 , the values of refined stability constants of all the binary and ternary complexes formed in solution in this study are listed. Calculation of pM for metal-ion buffers containing excess of complexing agent is straightforward,[11] using Schwarzerbach's[17] α-coefficient method. It is necessary to know the pH of the solution, the pK_a values of the ligand, and the stability constants of the metal complexes. If the metal ion undergoes significant hydrolysis, as in our case of Al(III) or In(III), the appropriate constants are also included.

Stability constants (β'_n) are defined in terms of the equilibrium between a metal complex and its components, except that the free ligand concentration is replaced by total concentration of all ligand species not actually complexed to the metal, and the free metal ion term includes hydrolyzed metal ion and metal ion bound to other complexing species. Calculation of β'_n can by done by

$$\beta'_n = \frac{\beta_n}{\alpha_M \cdot (\alpha_L)^n} \tag{5}$$

where

$$\alpha_M = ([M] + [MOH] + [M(OH)_2] + ...)/[M] \tag{6}$$

Many metal ions hydrolyze to form polynuclear species so that α_M would be concentration-dependent, but in the presence of excess strong ligand it is usually sufficient to consider only the formation of mononuclear species. Under these conditions, α_M reduced to

$$\alpha_M = 1 + 10^{(pH - pK_1)} + 10^{(2pH - pK_1 - pK_2)} + ... \tag{7}$$

where pK_1, pK_2, ... are the successive pK_a values for the loss of a proton from a hydrated metal ion. The metal ion hydrolysis constants published in the IUPAC stability constants data base and other sources have been used.[18,19] All the possible hydrolytic species resulting from the formation of the different hydroxy complexes including different metal ions have been taken into consideration during the SUPERQUAD calculations. Initial estimates of the stability constants of different normal and protonated binary and ternary complexes formed in solution have been refined with the SUPERQUAD computer program.[9]

The quality of the fit during this refinement was judged by the values of the sample standard deviations and goodness of fit χ^2 (Pearson's test). At $\sigma_E = 0.1$ mV (0.001 pH error) and $\sigma_V = 0.005$ mL, the values of σ in different sets of titrations were between 1.0 and 1.7 and χ^2 was between 12.0 and 13.0. The scatter of residuals ($E_{obs} - E_{calc}$) versus pH was reasonably random, without any significant systematic trends, thus indicating a good fit of the experimental data of the expected model

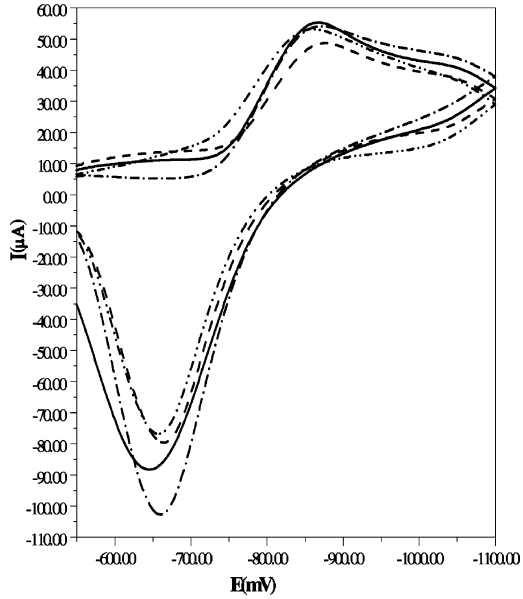

Figure 4. Cyclic voltammograms for the Tl(I) + glycylglycylglycine(GGG) + HEPES system at $I = 0.05$ mol·dm^{-3} KNO_3, pH 6.0, scan rate = 150 mV·s^{-1}, and $t = (25.0 \pm 0.1)$ °C: —, 1.5×10^{-3} mol·dm^{-3} Tl(I); - · -, 1.5×10^{-3} mol·dm^{-3} Tl(I) + 1.5×10^{-3} mol·dm^{-3} HEPES; - - -, 1.5×10^{-3} mol·dm^{-3} Tl(I) + 1.5×10^{-3} mol·dm^{-3} GGG; - · · -, 1.5×10^{-3} mol·dm^{-3} Tl(I) + 1.5×10^{-3} mol·dm^{-3} HEPES + 1.5×10^{-3} mol·dm^{-3} GGG.

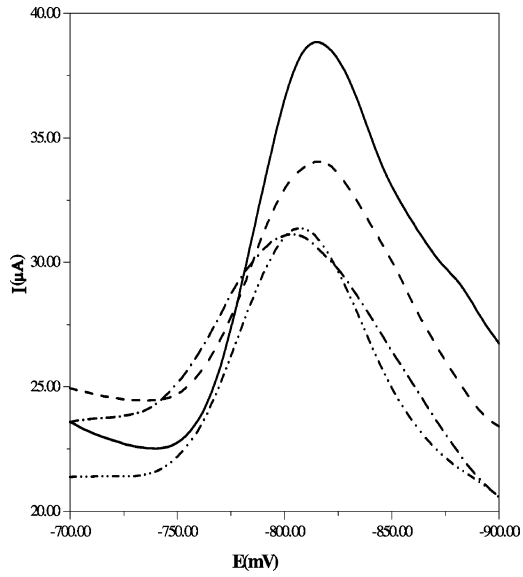

Figure 5. Square wave polarograms for the Tl(I)+ glycylglycylglycine (GGG) + HEPES system at $I = 0.05$ mol·dm^{-3} KNO_3, pH 6.0, scan rate = 100 mV·s^{-1}, frequency = 30 Hz, and $t = (25.0 \pm 0.1)$ °C: —, 1.5×10^{-3} mol·dm^{-3} Tl(I); - · -, 1.5×10^{-3} mol·dm^{-3} Tl(I) + 1.5×10^{-3} mol·dm^{-3} HEPES; - - -, 1.5×10^{-3} mol·dm^{-3} Tl(I) + 1.5×10^{-3} mol·dm^{-3} GGG; - · · -, 1.5×10^{-3} mol·dm^{-3} Tl(I) + 1.5×10^{-3} mol·dm^{-3} HEPES + 1.5×10^{-3} mol·dm^{-3} GGG.

systems under our experimental conditions taking into consid eration all the possible hydrolysis processes taking part at different pH values.

Representative titration curves for the ternary systems of the types Al(III) + GGG + guanosine and M + GGG + tricine are given in Figure 1. In aqueous solution below pH 7.0, Al(III) did not give rise to any definite changes in the UV spectra of Al(III)−GGG as indicated in Figure 2. This shows that, even if it does bind to the GGG tripeptide, Al(III) does not have an effect on the backbone conformation of this peptide functionality. However, in the case of UV spectra of Al(III)−GHG (as shown in Figure 3) Al(III) resulted in significant spectral effects,

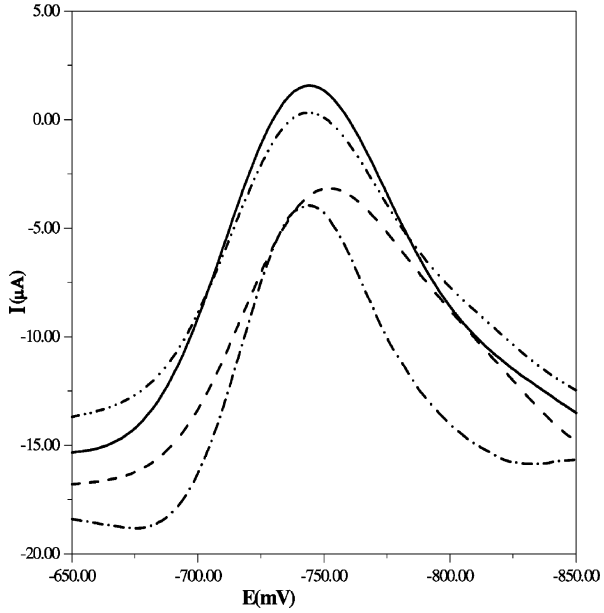

Figure 6. Differential pulse polarograms for the Tl(I) + glycylglycylglycine (GGG) + HEPES system at $I = 0.05$ mol·dm^{-3} KNO_3, pH 6.0, scan rate = 10 mV·s^{-1}, and $t = (25.0 \pm 0.1)$ °C: —, 1.5×10^{-3} mol·dm^{-3} Tl(I); - · -, 1.5×10^{-3} mol·dm^{-3} Tl(I) + 1.5×10^{-3} mol·dm^{-3} HEPES; - - -, 1.5×10^{-3} mol·dm^{-3} Tl(I) + 1.5×10^{-3} mol·dm^{-3} GGG; - · · -, 1.5×10^{-3} mol·dm^{-3} Tl(I) + 1.5×10^{-3} mol·dm^{-3} HEPES + 1.5×10^{-3} mol·dm^{-3} GGG.

clearly indicating conformation changes in the tripeptide GHG. This behavior may confirm the participation of the histidyl residue in Al(III) binding, and the Al(III) binding abilities of the GHG were stronger than those of GGG tripeptide.

The UV spectra shown in Figure 2 confirms the formation of the ternary complex of the type Al(III) + GGG + HEPES as was observed by pH-potentiometric titration. The most interesting observation in this titration is the dissolution of the precipitate of pH 7.02 as the pH increases to 8.50, due to the formation of soluble hydroxy complex.

The same feature has been observed in Figure 3, which confirms the formation of the ternary complex of the type Al(III) + GHG + HEPES. The above-mentioned results lead us to claim the possibility of using the binary complex Al(III)-HEPES for the dissolution of possible Al(III) + GGG precipitate at physiological pH (around 7.40).

Taking into consideration the therapeutic activity of taurine, we may consider substituted taurine (HEPES) as a base for the development of a drug with possible activity in neurodegenerative diseases. Biomolecules containing such functions may be involved in the uptake and transport processes of Al(III).

The practically irreversible binding of Al(III) in biological systems, which can be rather rare, has been observed during our potentiometric titration of the system Al(III) + GGG + GMP. Our results confirm the presence of such sluggish metal ion character of Al(III) in biological systems. It may occur, as observed in our results as the pH increases, in molecular aggregates when the exchange reactions of Al(III) are slowed down because of the formation of hydrolyzed oxo or hydroxo-bridged Al clusters wrapped around by biological compounds.

Derived from methionine and cysteine metabolism, taurine (2-aminoethanesulfonic acid) is known to play an important role in numerous physiological functions. While conjugation of bile acids is perhaps its best-known function, this accounts for only a small proportion of the total body pool of taurine in humans. Other metabolic actions of taurine include detoxification,

Table 7. (Δ log K_M)[a] for Metal Ions (M) + Peptide (P) + Zwitterionic Buffer (Z) Ternary Complexes at (25.0 ± 0.1) °C and I = 0.1 mol·dm^{-3} KNO_3

	Δ log K_M (Z =)										
	HEPES		EPPS		PIPES		Bicine		Tricine		
metal ion	GG	GGG	GG	GGG	GG	GGG	GG	GGG	GG	GGG	GH
Al(III)	+0.37	+0.24		+1.24						+0.51[b]	+1.15
Ga(III)	+2.44	+2.01		+1.44	+3.54		+2.71	+0.68	+0.92		+0.65
In(III)	+3.30	+5.20	+2.80			+0.09	+1.83[b]	+1.68[b]		+1.54[b]	+0.75

[a] Δ log K_M = log $K_{M(III)(P)(Z)}$ − log $K_{M(III)(Z)}$; Z = HEPES, EPPS, or PIPES. [b] Δ log K_M = log$K_{M(III)(Z)(P)}$ − log $K_{M(III)(P)}$; Z = Bicine or Tricine.

Table 8. Spectroscopic Characteristics of 1 × 10^{-5} mol·dm^{-3} Ga(III), Its Binary and Ternary Complexes with HEPES and Glycylglycylglycine (GGG) or Glycylhistydylglycine (GHG) at I = 0.1 mol·dm^{-3} KNO_3 and at 25 °C

	λ/nm			ϵ/cm^{-1}·mol^{-1}·dm^3		
system	λ_1	λ_2	λ_3	ϵ_1	ϵ_2	ϵ_3
Ga(III)	209.6	301.1		38847	7616	
Ga(III) + HEPES (pH 4.3)	209.0	301.1		39862	7418	
Ga(III) + HEPES (pH 6.5)	208.3	300.8		40000	7303	
Ga(III) + HEPES (pH 8.0)	210.2	300.8		40000	9476	
Ga(III) + GGG	207.8	301.1		40000	7689	
Ga(III) + GHG	206.7	301.1		40000	7554	
Ga(III) + HEPES + GGG	204.5	230.9	263.3	297	261	377
Ga(III) + HEPES + GHG	229.4	263.0		258	3580	

membrane stabilization, osmoregulation, and modulation of cellular calcium levels.

Clinically, taurine has been used in the treatment of a wide variety of conditions, including cardiovascular diseases, epilepsy and other seizure disorders, macular degeneration, Alzheimer's disease, hepatic disorders, and cystic fibrosis. An analog of taurine, acamprosate, has been used as a treatment for alcoholism. The above-mentioned discussion prompted us to try to investigate the interactions of substituted taurines EPPS, HEPES, and PIPES with trivalent metal ions Al(III), Ga(III), or In(III) and peptides (GG, GGG, GH, or GHG). Our results demonstrate the possibility of dissolving the precipitate of Al(III)−peptides by increasing pH until the formation of soluble hydroxy complexes in the physiological pH range (about 7.40). This may indicate the possibility of using these interesting substituted taurines (EPPS, HEPES, PIPES) as drugs for some diseases including Al(III)−peptide precipitates.

Examination of the different formation constant values listed in Table 4 clearly reveals that the order of the stability constants of the different ternary complexes in the systems [M + P + Z] [where M = Al(III) or In(III), P = GH or GHG, Z = tricine] in terms of peptides generally follows the trend of GHG > GH. This may be attributed to the combination of both carboxylate oxygen and nitrogen donor atoms during the formation of the ternary complexes containing GHG in the presence of strongly coordinating imidazole of histidyl residues.

Our pH-potentiometric titration data confirm the formation of ternary complexes of the type Al(III) + GGG + guanosine and Al(III) + GGG + GMP in solution as models for Al(III)−peptide−nucleotide or nucleobase systems. These also can be considered as models for the interesting biological systems (Al(III)−protein−DNA).

Similar in size to the natural activator Mg^{2+}, Al(III) may act by substituting for Mg^{2+} in vital processes.[20,21] For example, in tubulin polymerization to microtubules, Al(III) is more than 10^7 times as effective as Mg^{2+}. Mg^{2+} serves as a natural activator by binding to the phosphates of guanosine triphosphate (GTP), and Al(III) displaces Mg^{2+} at this site. Virtually all ATP-associated reactions use Mg^{2+}, and Al(III) potentially interferes in these processes.

Our results in the present investigation indicate the possibility of other types of Al(III) interference in biological processes through its interaction with the phosphates of GMP. Reliable stability constants for Al(III) binding to nucleoside phosphates are rare, and remedying this deficincy stands as an important need in Al(III) biochemistry.

Like Mg^{2+}, Ca^{2+},[22] and the lanthanides,[23] Al(III) binds to all nucleoside phosphates predominantly at the phosphate groups. The only basic phosphate group (pK_a > 6.0) is the terminal one. Since the nucleoside triphosphates exhibit similar phosphate pK_a values, as do the nucleoside disphosphates and nucleoside monophosphates, the equilibrium constants reported in our invetigation may be expected and applied for other nucleoside monophosphates (AMP).

Although the free nucleoside phosphate ligands are minimally stacked under the conditions of this study, the charge neutralization provided by binding of Al(III) should promote base stacking. Stacking between two ligands bound to a single Al(III) may abet formation of 2:1 complexes. The extent of stacking in Al(III) complexes of GMP remains to be determined by NMR spectroscopy along the lines employed for other diamagnetic metal ions.[24]

From Table 6 the formation constant values for the mixed ligand 1:1:1 systems of the type Al(III)−P−GMP or guanosine with respect to peptide can be arranged as follows: GH > GHG > GG > GGG for Al(III)−GMP−P ternary systems and GH > GHG > GGG > GG for Al(III)−guanosine−P ternary systems.

From Tables 3 to 5, the formation constant values for the mixed ligand 1:1:1 systems can be arranged as follows: Ga(III) > In(III) > Al(III) for M(III)−GG−HEPES systems, Al(III) > Ga(III) > In(III) for M(III)−GG−EPPS systems, Al(III) > In(III) > Ga(III) for M(III)−GH−tricine systems, In(III) > Ga(III) > Al(III) for M(III)−GGG−HEPES systems, In(III) > Ga(III) for M(III)−GGG−bicine systems, In(III) > Al(III) for M(III)−GGG−tricine systems.

The observed orders may be attributed to different types of interactions depending on metal ion's geometrical behaviors during the formation of binary and ternary complexes in solution. The trend may be due to different hydrolysis behaviors of trivalent metal ions used in aqueous solutions and the possible formation of mixed hydroxo species.

The Δ log K_M values are given in Table 7for the different ternary complexes formed in this study. Positive values of Δ log K_M indicate enhancement of the ternary complex formation through interligand interactions possibly hydrogen bond formation.

It is reported that for metal complexes with a series of structurally related ligands, a linear relationship holds between the stability constant of the complex and the acid dissociation constant of the ligand.[25] The importance of these plots is that they afford a means of estimating the formation constants of complexes that have not yet been determined.

Table 9. Voltammetric Characteristics of 5×10^{-4} mol·dm^{-3} Al(III) or Ga(III), Their Binary and Ternary Complexes with HEPES and Glycylhistidylglycine (GHG) at (25 ± 0.1) °C and I = 0.1 mol·dm^{-3} KNO_3

system	E_{pc}/mV	i_{pc}/μ**A**	D_{ox}/cm^2·s^{-1}	α	K_o/cm·s^{-1}	$-\Delta G°_{25}$/J·mol^{-1}
Al(III)	1108	172.2	7.98×10^{-11}	0.43	1.5×10^{-13}	7.3×10^4
Al(III) + HEPES	1110	152.5	7.08×10^{-11}	0.38	1.13×10^{-12}	6.82×10^4
Al(III) + GHG	1210	117.7	3.98×10^{-11}	0.40	7.68×10^{-14}	7.48×10^4
Al(III) + HEPES + GHG	1086	129.2	4.1×10^{-11}	0.48	1.99×10^{-14}	7.82×10^4
Ga(III)	1158	57.07	1.12×10^{-11}	0.31	1.37×10^{-12}	6.77×10^4
Ga(III) + HEPES	620	8.35	1.46×10^{-13}	0.51	2.43×10^{-12}	6.63×10^4
Ga(III) + GHG	1214	46.02	6.64×10^{-12}	0.34	1.12×10^{-13}	7.39×10^4
Ga(III) + HEPES + GHG	634	8.081	1.78×10^{-13}	0.39	2.19×10^{-11}	6.08×10^4

Table 10. Voltammetric Characteristics of 1.5×10^{-3} mol·dm^{-3} Tl(I), Its Binary and Ternary Complexes with HEPES and Glycylglycylglycine (GGG) at (25 ± 0.1) °C and I = 0.05 mol·dm^{-3} KNO_3

system	$-E_{pc}$/mV	$+E_{pa}$/mV	$E_{pc} - E_{pa}$/mV	i_{pc}/μ**A**	i_{pa}/μ**A**	D_{ox}/cm^2·s^{-1}	D_{red}/cm^2·s^{-1}	α	K_0/cm·s^{-1}	$-\Delta G°_{25}$/J·mol^{-1}
Tl(I)	874	642	232	66.38	−95.42	3.52×10^{-7}	7.28×10^{-7}	0.55	9.08×10^{-4}	1.74×10^4
Tl(I) + HEPES	874	654	220	64.17	−114.21	3.29×10^{-7}	1.04×10^{-6}	0.54	1.13×10^{-3}	1.68×10^4
Tl(I) + GGG	886	662	224	58.59	−89.55	2.74×10^{-7}	6.41×10^{-7}	0.49	8.56×10^{-4}	1.75×10^4
Tl(I) + HEPES + GGG	868	652	216	56.49	−93.35	2.55×10^{-7}	6.97×10^{-7}	0.51	9.86×10^{-4}	1.72×10^4

The relation between basicity of ligands and the stability constants of complexes has been extensively discussed.[26−28] The ligands of stronger basicity produce more stable complexes, in other words, ligands that strongly combine with protons form stable complexes with metal ions. According to the above-mentioned discussion, the stability of M(III)−zwitterionic buffer reported in this investigation according to the basicity of the ligands should be bicine > tricine > EPPS > HEPES > PIPES. The stability constant values of M(III)−zwitterionic buffer do not obey this rule due to the presence of different factors affecting the coordination mode of these metal ions by zwitterionic buffers and also due to their great tendency for hydrolysis.

The stability constant values of Tl(I) complexes with zwitterionic buffers HEPES, EPPS, PIPES, bicine, and tricine follow the above-mentioned basicity effect as indicated in Table 2. Even for the protonated Tl(I) complexes formed in the case of Tl(I)−tricine, Tl(I)−EPPS, and Tl(I)−HEPES this trend has been observed, and the stability constant values follow the order Tl(I)−tricine > Tl(I)−EPPS > Tl(I)−HEPES. So great care must be taken during toxicological studies using tricine, HEPES, or EPPS buffers in the presence of Tl(I) ions. Also, great reservation should be exercised in employing the biologically important zwitterionic buffer ligands HEPES, EPPS, PIPES, bicine, and tricine in aqueous solutions in systems containing Al(III), Ga(III), In(III), or Tl(I) ions and the peptides GG, GH, GGG, or GHG. The likelihood for the formation of ternary complexes is also rather high, as was demonstrated in the present study. This will affect the properties of these peptides in various ways when they are used as substrates. To our knowledge, no data for the ternary complexes of the biologically important buffers HEPES, EPPS, PIPES, bicine, and tricine with the peptides GG, GH, GGG, or GHG are available in the literature for comparison.

Taking into consideration the factors that affect metal−peptide interactions, including different conformational parameters, as well as factors associated with the trivalent metal ion chemistry, one can account for the different trends observed for the stability constants of the different ternary complexes of the type M(III) + P + Z. Via the formation of mixed−ligand complexes certain ligand−ligand associations and interactions may be favored. Thus, distinct structures may be created in a way that involves only small changes from an energetic point of view.

Absorption spectra of the investigated complexes of In(III) with peptides were measured in the range of (190 to 350) nm, and the existence of the usual d−d bands and CT bands (between 200 and 230 nm) was observed in all cases. Deprotonation and coordination of the two amide nitrogens of tripeptides GGG and GHG resulted in the complex formation reactions with investigated metal ions. This complex formation has been confirmed by a small shift of the band due to the free metal ions in solution.

As shown in Tables 3 to 5, the ternary complexes of In(III) are more stable than those of Ga(III) with the exception of the systems containing GGG. An additional factor favoring higher stability of the Ga(III)−GG−Z ternary systems is the fact that binding during the formation of these complexes (Ga(III)−GG−HEPES, Ga(III)−GG−EPPS, Ga(III)−GG−PIPES, and Ga(III)−GG−tricine) involves the formation of a six-membered chelate ring. It has been shown that six-membered chelate rings form more stable chelates with small metal ions and have lower affinity for larger metal ions (the effective ionic radius for octahedral In(III) is 0.80 Å while that of Ga(III) is 0.62 Å). Also, on the hard acid soft acids and bases (HSAB) scale, a very basic negative oxygen donor has higher affinity for a hard metal ions, such as Ga(III), than for a less hard metal ion, such as In(III). Accordingly, a less basic negative oxygen donor such as carboxylate groups, in the systems containing peptides GGG or GHG, binds more to the harder Ga(III) ion which may result in the higher stability of the former complexes. Confirmation of this behavior will be done using 2D NMR studies at several pH values.

Confirmation of the formation of binary and ternary complexes of the type M−peptide−Z, where M = Al(III), In(III), Ga(III), or Tl(I); Z = HEPES; peptide = GGG or GHG, in solution has been carried out using cyclic voltammetry (CV), differential pulse polarography (DPP), and square wave voltammetry (SWV) (see Table 8).

Figures 4 to 6 show the electrochemical behavior (CV, SWV, and DPP) of the ternary complexes of the type Tl(I)−GGG−HEPES investigated in this study. All the voltammetric diagrams confirm the formation of different binary and ternary complexes that has been found using potentiometric titrations.

It is quite interesting to observe that changing the frequancy from (30 to 60) Hz resulted in a quite clear change in the shape of the SWV of the ternary complex formed in solution, which may be attributed to changing the mechanistic behavior of the electrochemical reduction of the resulting ternary complex at the glassy carbon electrode. The reversibility of the electrochemical reaction of binary or ternary complexes in the system Tl(I)−HEPES under investigation has been investigated using CV.

The peak separation between the anodic and cathodic peaks is more than 30 mV. These values indicate that the electrochemical reduction in the case of free Tl(I) ions, Tl(I) binary, or Tl(I) ternary complexes under investigation is quasi-reversible at the glassy carbon electrode. The different electrochemical characteristics and some kinetic parameters of the systems under investigation are given in Tables 9 and 10. The CV response for the binary and ternary complexes containing Tl(I) on glassy carbon electrode reveal a one electron reduction process with the following electrochemical features in the scan rate (ν) range of (150 to 300) $mV \cdot s^{-1}$: $i_{p,a}/i_{p,c}$ ratio decrease by increasing ν; ($\Delta E_p = E_{p,c} - E_{p,a}$) increase by increasing ν with slopes in the range (0.459) mV for $E_{p,c}$ (i_p vs $\nu^{1/2}$ plots) Tl(I) + HEPES, Tl(I) + GGG and Tl(I) + HEPES + GGG, respectively, which agrees very well with the theory for a typical quasi-reversible process.

Kinetic parameters for the above-mentioned binary and ternary M−peptide−HEPES complexes have been calculated with the aim to probe their electron transfer ability when used as basis for biosensors for the electrochemical determination of the two peptides, GGG and GHG. So the results obtained in this study concerning the electrochemical reduction and kinetic parameters calculations for the ternary systems of the type M−GGG−HEPES can be considered as basis for the future development of novel biosensors for the trace determination of these biologically important peptides.

We have carried out an exhustive determination of ΔE_p values at different scan rates finding a linear behavior between ΔE_p and the square root of the scan rate for the systems Tl + HEPES, Tl + GGG, and Tl + HEPES + GGG, respectively, which agrees very well with the theory for a typical quasi-reversible process. In fact, according to this theory, the kinetic parameter ψ varies lineary with $\nu^{1/2}$, and ΔE_p approaches linearity for small ψ. Consequently, in this zone ΔE_p should vary linearly with $\nu^{1/2}$.

Under our condition of quasi-reversibility for the electrochemical reduction of the binary and ternary systems under investigation, it may be possible to study the kinetics of the electrode reaction. The separation of the peak potentials, ΔE_p, should be a measure of the standard rate constant for the electron transfer. These ΔE_p values were introduced in the working curve described by Nicholson[29] for obtaining the transfer parameter, ψ, and then the standard heterogeneous charge-transfer rate constant (K^o) for the electron-transfer process at the glassy carbon electrode according to the following equation:

$$\psi = \frac{(D_o/D_R)^{\alpha/2} K^o}{D_o \pi \nu (nF/RT)^{1/2}} \quad (8)$$

where D_o and D_R are the diffusion coefficients for oxidized and reduced species, respectively, and can be calculated from

$$i_p = (2.69 \times 10^5) n^{3/2} A D_o^{1/2} \nu^{1/2} C_o \quad (9)$$

where ν is the scan rate and A is the surface area of the electrode. To convert experimentally determined values of ψ to K^o, good agreement between the values of the kinetic parameters calculated using this method and those calculated by the Butler equation is obtained.[30] This work can be considered as a continuation of the auther's work in the field of bioinorganic chemistry.[31−35]

Stability constants are the key to an understanding of equilibria in solution. They are therefore fundamental to work in industrial chemistry, environmental studies, medicinal and analytical chemistry, oceanograhpy, and chemical education. Our results in this paper are essential prerequisites to working in these fields as well as speciation in any field of quantitative science related to biological fluids containing di- or tripeptides; zwitterionic buffers; and the metal ions Al(III), Ga(III), In(III), or Tl(I).

Literature Cited

(1) Kilyen, M.; Forgo, P.; Lakatos, A.; Dombi, G.; Kiss, T.; Kotsakis, N.; Salifoglou, A. Interaction of Al(III) with the peptides AspAsp and AspAspAsp. *J. Inorg. Biochem.* **2003**, *94* (3), 207−213.

(2) Rubini, P.; Lakatos, A.; Champmartin, D.; Kiss, T. Speciation and structural aspects of interactions of Al(III) with small biomolecules. *Coord. Chem. Rev.* **2002**, *228* (2), 137−152.

(3) Kiss, T.; Kilyén, M.; Lakatos, A.; Evanics, F.; Körtvélyesi, T.; Dombi, G.; Majer, Z.; Hollósi, M. Interactions of Al(III) with a neurofilament heptapeptide fragment: AcLysSer ProValValGluGly. *Coord. Chem. Rev.* **2002**, *228* (2), 227−236.

(4) Harris, W. R.; Messori, L. A comparative study of aluminum(III), gallium(III), indium(III), and thallium(III) binding to human serum transferring. *Coord. Chem. Rev.* **2002**, *228* (2), 237−262.

(5) Sóvágó, I.; Várnagy, K.; Odblac, K. Metal complexes of peptides containing monodentate or chelating imidazole nitrogen donors: factors influencing the coordination of amide groups and imidazole side chains. *Comm. Inorg. Chem.* **2002**, *23* (2), 149−178.

(6) Sun, Y; Anderson, C. J.; Pajeau, T. S.; Reichert, D. E.; Hancock, D. R. Indium(III) and gallium(III) complexes of bis(aminoethanethiol) ligands with different denicities: stabilities, molecular modeling, and in vivo behavior. *J. Med. Chem.* **1996**, *39*, 458−470 (and referances cited therein).

(7) Welcher, F. J. *The Analytical Uses of Ethylenediaminetetraacetic Acid*; Van Nostrand: Princeton, 1965.

(8) De Stefano, C.; Princi, P.; Rigano, C.; Sammartano, S. Computer analysis of equilibrium data in solution. ESAB2M: An improved version of the ESAB program. *Ann. Chim.* (*Rome*) **1987**, *77*, 643−675.

(9) Gans, P.; Sabatini, A.; Vacca, A. SUPERQUAD: An improved general program for computation of formation constants from potentiometric data. *J. Chem. Soc., Dalton Trans.* **1985**, 1195−1200.

(10) Martell, A. E.; Sillen, L. G. *Stability Constants of Metal Ion Complexes*; The Chemical Society: London, 1971.

(11) Ringbom, A. *Complexation in Analytical Chemistry*; Wiley−Inter-Sciences; New York, 1963.

(12) Perrin, D. D.; Dempsey, B. *Buffers for pH and Metal Ion Control*; Chapman and Hall; London, 1979.

(13) Good, N. E.; Winget, G. D.; Winter, W.; Connoly, T. N.; Izawa, S.; Singh, R. M. M. Hydrogen ion buffers for biological research. *Biochemistry* **1966**, *5*, 467−477.

(14) Ferguson, W. J.; Braunschwelger, W. R.; Smith, J. R.; McCormic, J. J.; Wasmann, C. C.; Jarvis, N. P.; Bell, D. H.; Good, N. E. Hydrogen ion buffers for biological research. *Anal. Biochem.* **1980**, *104*, 300−310.

(15) Smith, R. M.; Martell, A. E.; Motekaitis, R. J. Critically selected stability constants of metal complexes. *NIST Data Base 46, version 8.0*; NIST: Gaithersubrg, MD, 2004.

(16) Burger, K. Biocoordination chemistry, coordination equilibria in biologically active systems. In *Metal Complexes of Peptides and Their Derivatives*; Burger, K., Ed.; Ellis Horwood Series in Inorganic Chemistry; Ellis Horwood: New York, 1990 (and references cited therein).

(17) Schwarzenbach, G. *Complexometric Titrations*; Interscience; New York, 1957.

(18) SC Database; Royal Society of Chemistry; IUPAC: 2004; http://www.Acadsoft.Co.uk/.

(19) Cornelis, R.; Caruso, J.; Crews, H.; Heumann, K. *Handbook of Elemental Speciation: Techniques and Methodology*; Wiley: New York, 2003.

(20) Martin, R. B. *Metal Ions Biol. Syst.* **1988**, *24*, 1.

(21) Macdonald, T. L.; Martin, R. B. Aluminum ion in biological systems. *Trends Biochem. Sci.* **1988**, *13* (1), 15−19.

(22) Sigel, H.; Tribolet, R.; Malini-Balakrishnan, R.; Martin, R. B. Comparison of the stabilities of monomeric metal ion complexes formed with adenosine 5′-triphosphate (ATP) and pyrimidine-nucleoside 5′-triphosphate (CTP, UTP, TTP) and evaluation of the isomeric equilibria in the complexes of ATP and CTP. *Inorg. Chem.* **1987**, *26* (13), 2149−2157.

(23) Shyy, Y. J.; Tsai, T. C.; Tsai, M. D. Metal−nucleotide interactions. 3. Oxygen-17, phosphorus-31, and proton NMR studies on the interaction of scandium(III), lanthanum(III), and lutetium(III) with adenosine 5′-triphosphate. *J. Am. Chem. Soc.* **1985**, *107* (12), 3478−3484.

(24) Scheller, K. H.; Hofstetter, F.; Mitchell, P. R.; Pris, B.; Sigel, H. Macrochelate formation in monomeric metal ion complexes of nucleoside 5′-triphosphates and the promotion of stacking by metal ions. Comparison of the self-association of purine and pyrimidine 5′-triphosphates using proton nuclear magnetic resonance. *J. Am. Chem. Soc.* **1981**, *103* (2), 247−260.
(25) Bjerrum, J. On the tendency of the metal ions toward complex formation. *Chem. Rev.* **1950**, *46* (2), 381−401.
(26) Powell, H. K. J.; Curtis, N. F. Free energy, enthalpy, and entropy of formation for copper(II) complexes with some C(1)-substituted 1,2-diaminoethanes. *J. Chem. Soc. A* **1967**, 1441−1444.
(27) Yasuda, M.; Suzaki, K.; Yamasaki, K. Stability of copper and nickel chelates of some phthalic acid derivatives. *J. Phys. Chem.* **1956**, *60* (12), 1649−1651.
(28) Roy, R. N.; Roy, L. N.; Jordan, S.; Weaver, J.; Dalsania, H.; Kuhler, K.; Hagerman, H.; Standaert, J. Thermodynamics of the second dissociation of a substituted aminopropanesulfonic acid (TAPSO) from 5 °C to 55 °C. *J. Chem. Eng. Data* **1997**, *42* (3), 446−448.
(29) Nicholson, R. S. Theory and application of cyclic voltammetry for measurement of electrode reaction kinetics. *Anal. Chem.* **1965**, *37*, 1351.
(30) Gosser, Jr., D. K. *Cyclic Voltammetry: Simulation and Analysis of Reaction Mechanisms*; VCH Publishers: New York, 1993.
(31) Azab, H. A.; Deghaidy, F. S.; Orabi, A. S.; Farid, N. Y. Comparison of the effectiveness of various metal ions on the formation of the ternary complexes containing adenosine 5′-mono-, 5′-di-, and 5′-triphosphate and some zwitterionic buffers for biochemical and physiological research. *J. Chem. Eng. Data* **2000**, *45*, 709−715.
(32) Azab, H. A.; Orabi, A. S.; Abdel-Salam, E. T. Role of biologically important zwitterionic buffer secondary ligands on the stability of the mixed ligand complexes of divalent metal ions and adenosine 5′-mono-, 5′-di-, and 5′-triphosphate. *J. Chem. Eng. Data* **2001**, *46*, 346−352.
(33) Anwar, Z. M.; Azab, H. A. Ternary complexes in solution. Comparison of the coordination tendency of some biologically important zwitterionic buffers toward the binary complexes of some transition metal ions and some amino acids. *J. Chem. Eng. Data* **1999**, *44*, 1151−1157.
(34) Anwar, Z. M.; Azab, H. A. Role of biologically important zwitterionic buffer secondary ligands in the stability of the ternary complexes containing some metal ions and guanosine 5′-monophosphate, inosine 5′-monophosphate and cytidine 5′-monophosphate. *J. Chem. Eng. Data* **2001**, *46*, 34−40.
(35) Anwar, Z. M.; Azab, H. A. Ternary complexes formed by trivalent lanthanide ions, nucleotides, and biological buffers. *J. Chem. Eng. Data* **2001**, *46*, 613−618.

Received for review July 17, 2006. Accepted December 2, 2006.

JE060319K