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Cd^{2+} and Zn^{2+} Interactions with Amino Acids N-Substituted by a Sulfonic Group. Effect of the Additional Ligand 2,2'-Bipyridine on the Metal-Induced Amide Deprotonation

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The interaction of *N*-(phenylsulfonyl)glycine and *N*-(tolylsulfonyl)glycine (L) with Cd^{2+} and Zn^{2+} was investigated through dc polarography, pH-metric titrations, and ^1H NMR spectroscopy. 2,2'-Bipyridine as additional ligand lowers the pK_a of Cd^{2+}-promoted deprotonation of the sulfonamide nitrogen (from 8 in the binary system to 7.6 in the presence of the heteroaromatic base), as previously observed for the Cu^{2+} ion, and, most of all, enables the Zn^{2+} ion to substitute for the sulfonamide nitrogen bound hydrogen of these ligands. It is known that Zn^{2+} is ineffective in such a substitution in binary Zn^{2+}-L systems.

Introduction

The ability of Cu^{2+}, Pd^{2+}, and Cd^{2+} ions to substitute for a sulfonamide nitrogen bound hydrogen in amino acids N-protected by a sulfonic group at acidic or slightly alkaline pH is well documented.[1-3] It has been previously shown that 2,2'-bipyridine further decreases the pK_a value of metal-promoted sulfonamide nitrogen deprotonation in the corresponding ternary Cu^{2+} systems,[4] while it exerts an opposite effect on amide deprotonation in analogous ternary complexes with peptides.[5] Our aim is to investigate whether 2,2'-bipyridine maintains this property also in the presence of Cd^{2+} and Zn^{2+}. We here report the pH-metric, polarographic, and ^1H NMR investigation of the ternary systems M-bpy-L, where M = Zn^{2+} or Cd^{2+} and L = *N*-(phenylsulfonyl)glycine or *N*-(tolylsulfonyl)glycine (*N*-tosylglycine). Zn^{2+} normally does not promote either peptide or sulfonamide nitrogen deprotonation;[6] the species [Zn(Gly-His)], in which the ligand acts as a tridentate through the terminal amino group, the deprotonated peptide nitrogen, and one imidazole nitrogen of the histidine side chain,[7] is the only known complex in which a deprotonated amide nitrogen is involved in Zn^{2+} coordination. Moreover, it is a fact that several deprotonated amides bind to the Zn^{2+} ion in carbonic anhydrase, where three histidine residues coordinate the metal.[8] Even if additional protein-ligand interactions play a key role in the mechanism of binding, the present ternary systems, involving an amide and an aromatic N-donor ligand such as 2,2'-bipyridine, may be useful to obtain further insight into this kind of interaction.

Experimental Section

Materials. *N*-(Phenylsulfonyl)glycine and *N*-tosylglycine (bsgly and tsgly hereafter) were twice recrystallized before use.

pH-Metric Analysis. Potentiometric titrations of the binary systems M-L were carried out by dissolving the $[M(LO)_2(H_2O)_4]$ crystalline complex obtained as in refs 3 and 6, up to a 1×10^{-3} M metal ion concentration, and by adding amounts of concentrated ligand solution in order to obtain ligand-to-metal molar ratios from 2:1 to 8:1. Stoichiometric amounts of $HClO_4$ were then added to neutralize the starting metal-bound ligand, and the ionic strength was adjusted to 0.1 M with $NaClO_4$. Carbonate-free sodium hydroxide standardized against phthalate was used as a titrant. Measurements were performed on a fully automatic Orion 960 Autochemistry system having a Ross 8102 c combination electrode and operating under nitrogen atmosphere at 25 ± 0.1 °C. This procedure was followed for both metal ions and both ligands. The same experimental conditions were used for the pH-metric analysis of the ternary systems M-bpy-L. A bpy:metal ratio of 1:1 was used throughout.

Polarographic Analysis. Investigation of the binary systems M-bsgly (M = Cd^{2+}, Zn^{2+}) was carried out on aqueous solutions prepared as described above with a 1×10^{-4} M metal ion concentration and ligand-to-metal molar ratios ranging from 2:1 to 20:1. The same metal ion concentration was used for the ternary systems, with metal:bpy:ligand molar ratios from 1:1:2 to 1:1:20. The pH of the solutions was adjusted by adding small amounts of concentrated aqueous NaOH. $NaClO_4$ was used as base electrolyte, and the ionic strength was kept constant (I = 0.1 M). Polarographic and voltammetric measurements were carried out with an Amel 472 Multipolarograph at 25 ± 0.1 °C. A saturated calomel electrode (SCE) was used as the reference electrode, and a platinum sheet, as the counter electrode. All the $E_{1/2}$ values are referred to the SCE. An Amel 337 pH meter equipped with an Ingold HA 405-60-K1 pH combination electrode was used for pH measurements. Analysis of the electronic transfer processes was performed by using metal ion concentrations from 5×10^{-5} to 5×10^{-4} M (with the same metal:L and metal:bpy:L molar ratios) and dropping times of 1, 2, 3, 4, and 5 s. The reversibility of the processes was determined by semilogarithmic analysis of the polarographic waves and by voltammetric measurements carried out at 100 mV/s with a hanging mercury drop electrode. Reversible and quasi-reversible reduction processes characterize the binary systems M-L and the ternary systems M-bpy-L, respectively. The reversible $E_{1/2}$ values for quasi-reversible processes were determined according to Matsuda and Ayabe.[9] The semilogarithmic analysis of the quasi-reversible processes shows two slopes: one reversible relative to the lower part of the polarographic wave and the other irreversible for the upper part; the reversible $E_{1/2}$ value is determined by extrapolation of the reversible slope to $\log (i/(i_d - i)) = 0$.[10]

^1H NMR Analysis. Proton NMR spectra were obtained on a Varian XL-200 spectrometer operating at 200.057 MHz. Typical parameters were as follows: spectral bandwidth, 2.4 kHz; pulse width, 9 μs (50° pulse); pulse delay, 2 s; collected number of scans, 15-50. Spectra were run in D_2O (the residual water signal was suppressed by a presaturation pulse from the decoupler) and are referenced to tetramethylsilane.

Results and Discussion

pH-Metric Data. The pH-metric titrations of *N*-(phenylsulfonyl)glycine and *N*-tosylglycine in aqueous solution in the presence of 10^{-3} M Cd^{2+} ion carried out with different ligand-to-metal molar ratios invariably show two titration steps with apparent pK_a's of 3.6 ± 0.2 and 8.0 ± 0.2 (Figure 1a, curve I). The low metal ion concentration allowed us to obtain a complete titration curve, with no metal hydroxide precipitation.[3] The first step corresponds to the dissociation of the carboxylic group, while the second step can be attributed to the Cd^{2+}-promoted deprotonation of the sulfonamide nitrogen of the ligands, as previously

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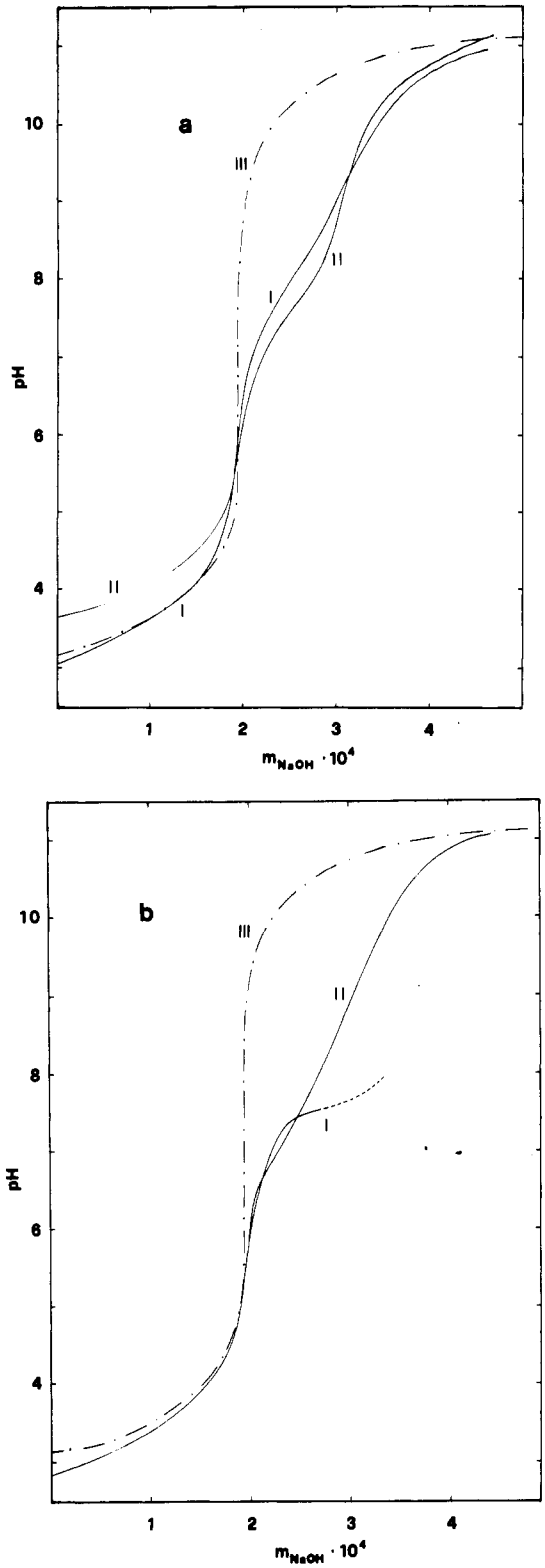

Figure 1. Neutralization curves for bsgly in the presence of Cd^{2+} (a) and Zn^{2+} (b). In both cases, curves I and II refer to the binary system M^{2+}–bsgly and to the corresponding ternary system with 2,2′-bipyridine, respectively; curve III refers to free ligand. $m_{bsgly} = 2 \times 10^{-4}$, $m_{M^{2+}} = 10^{-4}$, $m_{bpy} = 10^{-4}$ (m = number of moles); $c_{NaOH} = 0.097$ M, $c_{M^{2+}} = 10^{-3}$ M; $V_i = 0.1$ dm^3. The dashed line in curve I of part b refers to the beginning of zinc hydroxide precipitation.

demonstrated by ^{113}Cd NMR spectroscopy.[3] Since the number of moles of NaOH needed to obtain the second equivalent point coincides with that of the Cd^{2+} ion, only one bidentate amino acid molecule appears to bind the metal ion. In the presence of Zn^{2+} ion, for all the ligand-to-metal molar ratios investigated, only one step appears with an apparent pK_a of 3.3 ± 0.2, relative to the

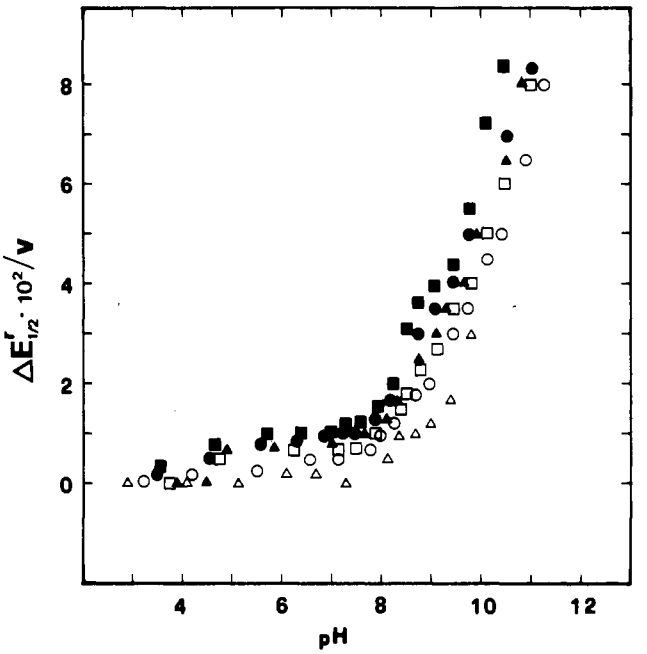

Figure 2. Plots of $\Delta E^r_{1/2}$ vs pH at increasing bsgly concentration. $[Cd^{2+}] = 1 \times 10^{-4}$ M. Bsgly concentrations ([L], M): ((Δ) 2×10^{-4}; (○) 4×10^{-4}; (□) 6×10^{-4}; (▲) 8×10^{-4}; (●) 10^{-3}; (■) 2×10^{-3}. $\Delta E^r_{1/2} = E^r_{1/2}(M) - E^r_{1/2}(C)$, where $E^r_{1/2}(M)$ is the reversible half-wave potential of the free metal ion and $E^r_{1/2}(C)$ that of the complexed metal ion. Dropping time = 1 s.

dissociation of the carboxylic group. This is invariably followed by zinc hydroxide precipitation at pH about 7.5 (Figure 1b, curve I).

The presence of 2,2′-bipyridine in a 1:1 molar ratio with the metal ion induces some changes in the above pH-metric behavior, particularly as far as the Zn^{2+} systems are concerned. Indeed, the pH-metric curves of the ternary systems Zn^{2+}–L–bpy show two titration steps ($pK_{a1} = 3.4 \pm 0.2$; $pK_{a2} = 7.8 \pm 0.2$) (Figure 1b, curve II). The first one nearly coincides with the single step observed in the binary systems corresponding to the titration of the carboxylic group of the amino acid ligand; the additional step, which requires a number of moles of NaOH corresponding to that of the metal ion, may be attributed to the formation of the [Zn(bpy)(LNO)] species. The titration curves of the ternary systems Cd^{2+}–L–bpy (Figure 1a, curve II) are similar to those of the corresponding binary systems, except for the pK_{a2} values that each turn out to be 0.4 unit lower.

Polarographic Data. The polarographic behavior of the binary system Cd^{2+}–bsgly is similar to that previously reported for the Cd^{2+} ion interacting with tsgly.[3] A single polarographic wave, reversible, bielectronic, and diffusion-controlled, is observed in the pH range 3–11. The pH dependence of the reversible $\Delta E^r_{1/2}$ values is shown in Figure 2. Up to pH 4, the $E_{1/2}$ value is independent of ligand concentration and coincides with that relative to the reduction of the solvated Cd^{2+} ion. Upon an increase in pH, the half-wave potential becomes concentration-dependent and shifts toward more negative values by following two titration steps. The diffusion current (i_d) remains nearly constant from acidic pH up to pH 10: the decrease observed at higher pH values is due to metal hydroxide precipitation. This polarographic behavior is fully consistent with the above pH-metric titration, so the first and the second polarographic step may be assigned to the reduction of complexes resulting from the ligand acting as a simple carboxylate and N,O-bidentate, respectively. The overall stability constants (β) calculated by the method of Shaap and McMasters[11] are reported in Table I. The Lingane plots[12] (Figure 3) show that the [Cd(LNO)] species prevails for most ligand-

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Table I. log β of Complexes Prevailing at Different pH's[a]

ternary species	pH	log β[b]	log $K^{M(bpy)}_{M(bpy)(L)}$[c]	binary species[d]	pH	log β
$[Zn(bpy)(LO)]^+$	<8	8.96	3.66	*e*		
		(8.82)	(3.52)			
[Zn(bpy)(LNO)]	>8	11.98	6.68	*e*		
		(11.86)	(6.56)			
$[Cd(bpy)(LO)]^+$	<8	7.18	2.93	*e*		
		(7.02)	(2.77)			
[Cd(bpy)(LNO)]	>8	9.90	5.65	[Cd(LNO)]	8–9.5	5.06
		(9.79)	(5.54)			
				$[Cd(LNO)_2]^{2-}$	>9.5	7.66
				$[Cd(LNO)(OH)]^-$	>9.5	8.95
				$[Cd(LNO)_2(OH)]^{3-}$	>9.5	12.02

[a] Overall stability constants (β) are relative to the equilibrium M^{2+} + bpy + nLO$^-$ (or LNO^{2-}) $\rightleftharpoons$ $[M(bpy)(LO)_n]^{2-n}$ {or $[M(bpy)(LNO)_n]^{2-2n}$}. [b] Values refer to bsgly species; those in parentheses, to tsgly species. [c] The β values for $[Zn(bpy)]^{2+}$ and $[Cd(bpy)]^{2+}$ species are 5.3 and 4.25, respectively.[16] [d] The β values of the Cd^{2+}–tsgly system are reported in ref 3. [e] See text.

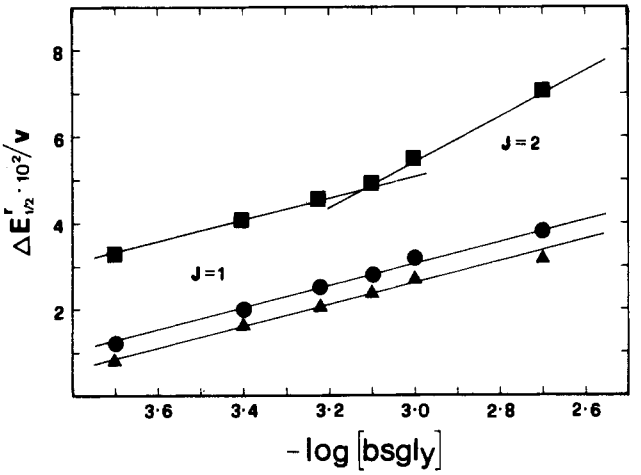

Figure 3. Plots of $\Delta E^r_{1/2}$ vs –log [bsgly] for the binary system Cd^{2+}–bsgly at different pH values: (▲) pH 8.5; (●) pH 9; (■) pH 10.

to-metal ratios and pH values. The presence of the $[Cd(LNO)_2]^{2-}$ species is observed only at pH values higher than 9.5 and for ligand-to-metal ratios greater than 10:1. No stability constants are reported for the carboxylate complexes: due to the small $\Delta E^r_{1/2}$ values of the first polarographic step ($\Delta E^r_{1/2}$(max) = 10 mV), we can only roughly estimate these log β values as being about a few units, as previously observed for the analogous system with tsgly.[3] The polarographic behavior of bsgly and tsgly interacting with the Zn^{2+} ion is characterized by a quasi-reversible wave showing a unique pH-titration step that is closely similar to the first step of the binary Cd^{2+} systems described above. This means that in this case only carboxylate complexes are formed, with a stability comparable to that of the analogous Cd^{2+} complexes. At pH values higher than 7.5, zinc hydroxide precipitation occurs. The ability of the Cd^{2+} ion, unlike the Zn^{2+} ion,[6] in promoting sulfonamide nitrogen deprotonation in this class of ligands was previously indicated by ^{113}Cd NMR spectroscopy.[3] The present data nicely support this previous finding. The stability constants of the species [M(LNO)] and $[M(LNO)_2]^{2-}$ with the same ligands and with Cu^{2+} and Pd^{2+} ions[2,4] were found higher by factors of about 10^4 and 10^{17}, respectively, in agreement with the relative stability of complexes with the same metals and with simple amino acids.[13-15]

Addition of 2,2′-bipyridine to the above systems in a 1:1 molar ratio with the metal ion induces significant changes in the $E^r_{1/2}$ values and their pH dependence. For both ternary systems involving Cd^{2+} and Zn^{2+}, two waves are contemporarily present throughout the pH range investigated (Figure 4a,b, respectively). Bsgly and tsgly show closely similar polarographic behaviors; the reported figures refer to the former ligand. For both metals the wave at lower $\Delta E^r_{1/2}$ values (wave I) is fully comparable to that observed for the binary systems; the additional wave at more negative $\Delta E^r_{1/2}$ values (wave II) is quasi-reversible and shows two pH-titration steps. Most likely, waves I and II correspond to the reduction of binary and ternary complexes, respectively. By applying the same arguments mentioned above for the binary systems concerning the assignment of the polarographic waves, we can reasonably consider the first step of wave II as relative to the reduction of the ternary species $[M(bpy)(LO)_{1,2}]$ and the second step as due to the reduction of the species $[M(bpy)(LNO)_{1,2}]$. The coexistence of wave I and wave II indicates that the ternary complexes are in equilibrium with the corresponding binary species over the entire pH range investigated. The $\Delta E^r_{1/2}$ values over the whole pH range investigated are nearly identical for bsgly and tsgly. The overall stability constants for the ternary complexes, calculated according to Shaap and McMasters,[11] are collected in Table I. The Lingane plots (Figure 5) indicate that the species bearing only one deprotonated ligand molecule prevail over the entire pH range and the range of ligand-to-metal ratios investigated. The Zn^{2+} complexes are more stable than the corresponding Cd^{2+} complexes by a factor of 10^2; it is worth noting that the step constants $K^{M(bpy)}_{M(bpy)(LO)}$ and $K^{M(bpy)}_{M(bpy)(LNO)}$ for the Zn^{2+} complexes are higher by a factor of 10 as compared to those of the corresponding Cd^{2+} species, and this is the same difference in stability found between the $[M(bpy)]^{2+}$ complexes.[16] An analogous polarographic behavior was observed for ternary Cu^{2+} complexes with 2,2′-bipyridine and N-protected amino acids.[4] As for the binary systems mentioned above, the stability constants of the Cu^{2+} complexes are higher by a factor of 10^5 and 10^7 as compared to those of the corresponding Zn^{2+} and Cd^{2+} complexes, respectively.

NMR Data. Valuable information on the acid–base equilibria of the amino acid ligands interacting with Cd^{2+} and Zn^{2+} in binary and ternary systems with 2,2′-bipyridine can be obtained from the pH dependence of the ^1H NMR signals. The ^1H NMR spectrum of *N*-(phenylsulfonyl)glycine in D_2O contains the resonance of the methylene group and the signal pattern of the aromatic protons. The sulfonamide nitrogen bound hydrogen is exchanged in these conditions. The pH dependence of the methylene resonance is reported in Figure 6. It clearly shows a sigmoidal pattern due the ionization of the carboxylic group, with an estimated pK_a value of 3.5. The chemical shift decrease above pH 10 is due to the deprotonation of the sulfonamide nitrogen that is known to occur with a pK_a of about 11.4 for the free ligand. The aromatic signals do not show any pH dependence up to pH 10 (not shown). The NMR titration of *N*-tosylglycine yields the same results; the additional resonance from the methyl group bound to the aromatic ring is pH-independent. In the presence of Zn^{2+}, in a 2:1 ligand-to-metal molar ratio, the pH dependence of the methylene resonance of the ligands remains unchanged, while an additional titration step with a pK_a of about 7.6 clearly appears for both ligands interacting with the Cd^{2+} ion (Figure 6). This additional ionization can be safely attributed

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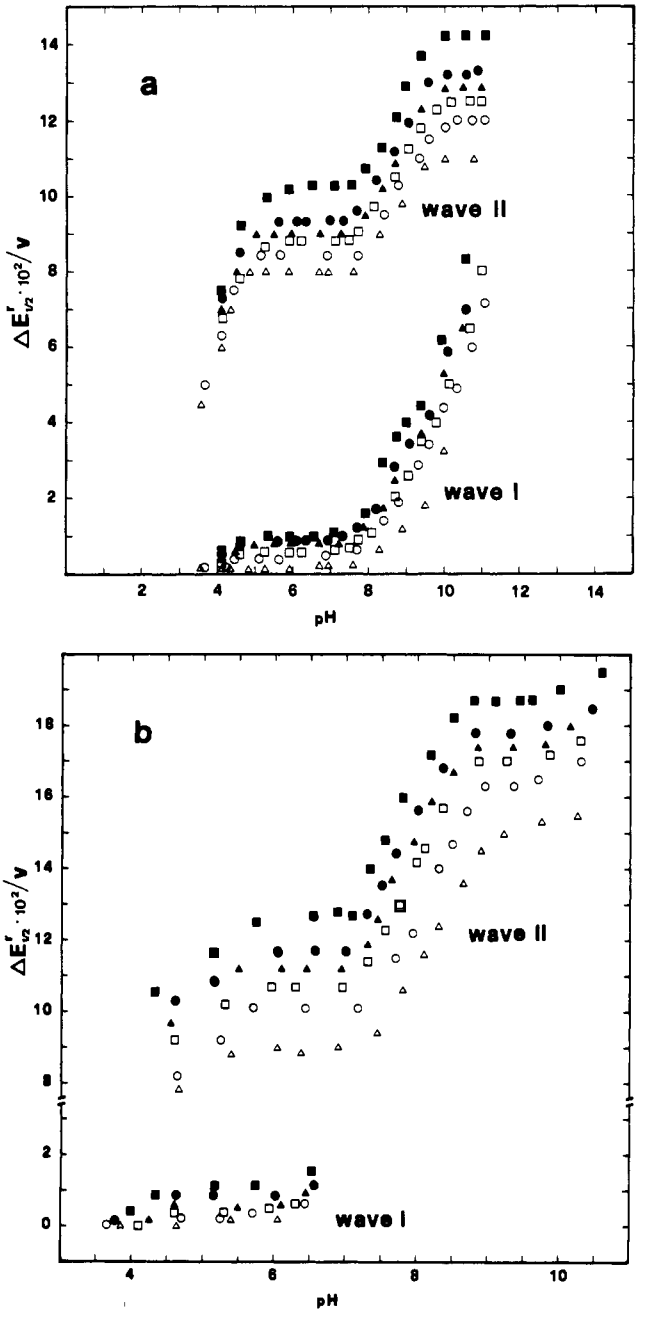

Figure 4. Plots of $\Delta E^r_{1/2}$ vs pH at increasing bsgly concentration for the ternary system M–bpy–bsgly. [M] = [bpy] = 1×10^{-4} M. Metal ion: (a) Cd^{2+}; (b) Zn^{2+}. Bsgly concentrations ([L], M): (△) 2×10^{-4}; (○) 4×10^{-4}; (□) 6×10^{-4}; (▲) 8×10^{-4}; (●) 10^{-3}; (■) 2×10^{-3}. Closely similar qualitative trends are observed for *N*-tosylglycine.

to the metal-induced sulfonamide nitrogen deprotonation of the ligands and, in particular, to the formation of the species [Cd(LNO)] in fast exchange with $[Cd(LO)_2]$. The observed pK_a is fully consistent with the above pH-metric and polarographic data. Addition of 2,2′-bipyridine in a 1:1 molar ratio to the $[M(LO)_2]$ complex dissolved in D_2O at pH 4 causes the splitting of the methylene signal into two resonances of nearly the same intensity. This is consistent with the formation of the complex $[M(bpy)(LO)]^+$ in slow exchange (on the NMR time scale) with the starting species $[M(LO)_2]$, according to the equilibrium

$$[M(LO)_2] + bpy \rightleftharpoons [M(bpy)(LO)]^+ + LO^-$$

Accordingly, one of the methylene signals shows a pH dependence analogous to that of the CH_2 group of the ligand in the binary systems. The coexistence of binary and ternary carboxylate complexes under these conditions nicely fits with the above polarographic data, as well as the formation of species bearing only

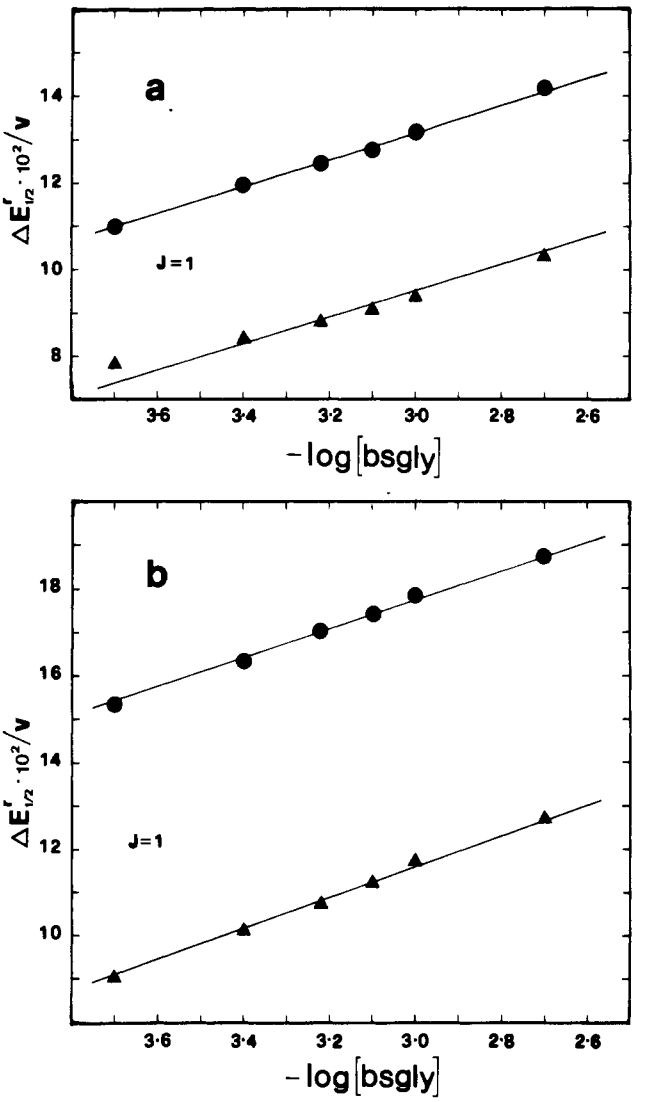

Figure 5. Plots of $\Delta E^r_{1/2}$ vs −log [bsgly] for wave II of the ternary system M–bpy–bsgly: (a) M = Cd^{2+}; (b) M = Zn^{2+}; (▲) pH 6.5; (●) pH 9.5.

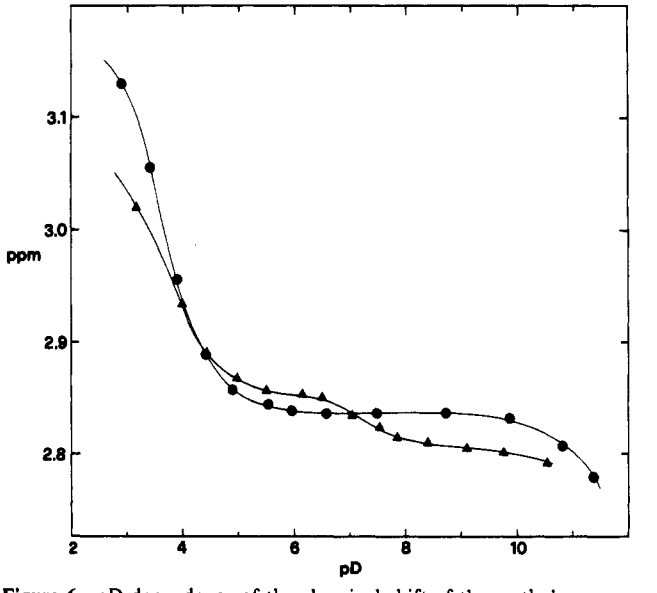

Figure 6. pD dependence of the chemical shift of the methylene resonance in the ^1H NMR spectrum of bsgly in D_2O: (●) for free ligand; (▲) in the presence of the Cd^{2+} ion in a 2:1 ligand-to-metal molar ratio. T = 23 °C; $c_{Cd^{2+}} = 10^{-3}$ M.

one molecule of amino acid ligand bound to the metal. Up to pH 6.2, no changes are observed for the signal pattern of the aromatic

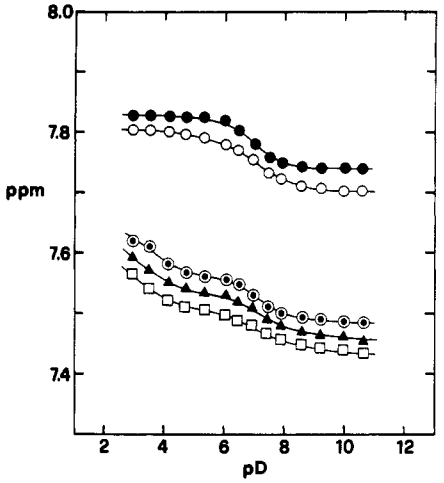

Figure 7. pD dependence of the chemical shift of the multiplet for the equivalent protons 3 and 3′ of 2,2′-bipyridine[19] in the ^{1}H NMR spectrum of the ternary system Cd^{2+}–bpy–bsgly in a 1:1:2 molar ratio. The other signals of 2,2′-bipyridine are either less resolved or overlapped with those of the aromatic ring of bsgly. Analogous patterns are obtained in the presence of the Zn^{2+} ion ($c_{Zn^{2+}} = c_{Cd^{2+}} = 10^{-3}$ M).

protons of bsgly and tsgly as compared to the free ligands. With increasing pH, such a pattern dramatically broadens for both ligands and the signals of 2,2′-bipyridine show a titration pattern with an apparent pK_a of about 7.5 (Figure 7). Such a behavior may indicate the formation of another species, most probably that in which the amino acid ligand acts as a bidentate through the deprotonated sulfonamide nitrogen, in equilibrium with the former:

$$[M(bpy)(LO)]^+ \rightleftharpoons [M(bpy)(LNO)] + H^+$$

The involvement of the deprotonated sulfonamide nitrogen in metal coordination changes the electronic distribution over the complex, so it may well change the shielding of the aromatic protons of both 2,2′-bipyridine and bsgly since, in the latter case, the amino acid moiety is conjugated with the aromatic ring through the SO_2 group.[17] The CH_2 signal of the metal-bound ligand L is, however, not sensitive to sulfonamide nitrogen deprotonation. This is somewhat surprising. An explanation could be that the electronic redistribution due to sulfonamide nitrogen deprotonation in this case also involves the two nitrogens of bpy, so the effect on the CH_2 group may be more complex than that in the above Cd^{2+}–L binary systems and may result in the reciprocal suppression of more effects.

There is a general agreement among the experimental techniques in indicating that for both metal ions the metal-promoted amide deprotonation takes place at lower pH values in the ternary systems as compared to the binary ones. The lowering effect of 2,2′-bipyridine on the pK_{NH} values of this kind of ligands and the corresponding higher stability of the ternary [M(bpy)L] species as compared with the [ML] species were first observed for the Cu^{2+} ion.[4] As for that case, it may be ascribed to a cooperative effect of the π systems of bpy and the amino acid ligands and to the stronger tendency of carboxylate ligands to bind to the $[M(bpy)]^{2+}$ species as compared to the free metal ion[18] (confirmed in this case by the $\Delta E^r_{1/2}$ values of the first step of waves I and II in Figure 4) that strengthens their "anchoring" capability[5] and favors the subsequent closure of the five-membered N,O-chelating ring. The identical $\Delta \log K$ values observed for Cu^{2+} and Cd^{2+} ($\Delta \log K_{Cu} = \Delta \log K_{Cd} = 0.6$) may indeed indicate that the nature of the metal ion plays a secondary role in this effect. The case of the Zn^{2+} ion deserves some comments. Up to now, only one example is known of a deprotonated amide nitrogen coordinated to a Zn^{2+} ion at physiological pH: in the [Zn(Gly-His)] complex the metal is bound to the terminal amino group of the dipeptide, the deprotonated peptide nitrogen, and one imidazole nitrogen of histidine.[7] Also in the present cases, besides the amino acid moiety of L, the Zn^{2+} ion is coordinated to an N-donor aromatic ligand. The presence of such kind of additional ligand could be a necessary requirement for enabling the Zn^{2+} ion to successfully substitute for a proton bound to a peptide or sulfonamide nitrogen.

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Kinetics of Iron Removal from Monoferric and Cobalt-Labeled Monoferric Human Serum Transferrin by Nitrilotris(methylenephosphonic acid) and Nitrilotriacetic Acid

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The kinetics of iron removal from both forms of monoferric transferrin by nitrilotris(methylenephosphonic acid) (NTP) and nitrilotriacetic acid (NTA) have been studied in 0.1 M, pH 7.4 *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonate buffer at 25 °C. The dependence of the observed pseudo-first-order rate constant for removal of iron on the concentration of NTP has been interpreted in terms of two parallel pathways: one that saturates and another that is first order in ligand. For NTP the saturation pathway is more important, while the carboxylate analogue NTA removes iron from both sides entirely by a first-order process. Iron removal from C- and N-terminal monoferric transferrins labeled at the vacant binding site with kinetically inert cobalt(III) has been studied as a model to evaluate cooperativity between the two sites. Cobalt labeling slightly accelerates iron removal by NTP from the C-terminal site and iron removal by NTA from the N-terminal site. The degree of cooperativity is less than that observed previously for iron removal from the C-terminal site by pyrophosphate (Bali, P. K.; Harris, W. R. *J. Am. Chem. Soc.* **1989**, *111*, 4457). Bicarbonate-free Fe–L–Tf ternary complexes are formed with both NTP and pyrophosphate. The pyrophosphate complex is much less stable than the corresponding NTP complex, which may be a factor contributing to the higher first-order rate constant for iron removal by pyrophosphate.

Introduction

The transferrins comprise a family of iron binding proteins that includes serum transferrin, lactoferrin, and ovotransferrin. Several recent reviews of transferrin chemistry are available.[1-6] The characteristic feature of the transferrins is that metal binding

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