DALTON
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Metal complexes of imidazole ligands containing histamine-like donor sets: equilibrium, solution structure and hydrolytic activity ‡

Ibolya Török,[a] Tamás Gajda,*,†,[b] Béla Gyurcsik,[a] Gábor K. Tóth[c] and Antal Péter[b]

[a] *Biocoordination Chemistry Research Group of the Hungarian Academy of Sciences, A. József University, H-6701 Szeged, P.O. Box 440, Hungary*
[b] *Department of Inorganic and Analytical Chemistry, A. József University, H-6701 Szeged, P.O. Box 440, Hungary*
[c] *Department of Medical Chemistry, A. Szent-Györgyi Medical University, H-6720 Szeged, Dómtér 8, Hungary*

The equilibrium and structural properties of copper(II) and zinc(II) complexes of *N*,*N*′-di-L-histidylethane-1,2-diamine (dhen) and those of the strongly related histamine have been characterized by pH-metric and spectroscopic (UV/VIS, CD, EPR and NMR) methods. In both dhen systems a dimeric M_2L_2 species is dominant near the physiological pH, having bis(histamine-like) $2N_{im}$, $2NH_2$ co-ordination. The MLH_{-2} complex, also formed in both systems above pH 10, has different structures with the two metal ions. A hydroxo mixed-ligand complex is formed in the case of zinc(II), while the base-consuming processes are assigned to metal-promoted deprotonation of amide nitrogens in the copper(II) system. Between these two dominant species (pH 7–10) tetrameric complexes are formed in each case (as suggested by the CD, EPR and NMR results), with the participation of imidazole-N^1 (pyrrolic) nitrogen in the co-ordination. The catalytic activity of the zinc(II)-containing systems towards the hydrolysis of uridine 2′,3′-cyclic monophosphate as nuclease model has been examined. The zinc(II)–histamine complexes efficiently catalyse the hydrolysis. A kinetic study performed at different pH, concentrations and metal-to-ligand ratios, combined with the equilibrium data, revealed three reaction pathways involving Zn(OH), ZnL and ZnL(OH) complexes as active species, in order of activity ZnL ≪ Zn(OH) < ZnL(OH).

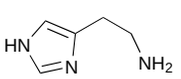
The imidazole moiety plays a particular role in the active centre of a large number of (metallo)proteins. Model compounds mimicking the main features of active sites have long been used to characterize better the relation between structure and function of these enzymes. In this respect, many imidazole-containing ligands (*e.g.* peptides,[1–6] pseudo-peptides,[7–10] polyamines[11–13]) have been studied from a co-ordination chemical point of view. The results revealed the exceptional co-ordinating ability of the imidazole ring and also that its position in a peptide chain[1–10] is a determining factor regarding the structure of the complexes formed. These studies were mostly devoted to mimicking structural features of imidazole-containing active centres, and paid less attention to functional mimicking. In case of most (pseudo)peptides studied, however, the metal-promoted deprotonation of amide nitrogen(s), in the presence of Cu^{II}, Ni^{II} or Zn^{II}, occurs near the physiological pH, which is particularly rare in metalloproteins.

The presence of an imidazole moiety in the active centre of proteins is often related to hydrolytic reactions, even in the absence of metal ions. One of the most abundant class of hydrolytic enzymes are the phosphatases, which hydrolyse phosphate ester bonds. They have extremely widespread use in biological systems: (i) reparation and hydrolysis of DNA and RNA, (ii) transfer of energy (ATP), (iii) metabolic processes (glucolysis, synthesis of amino acids), (iv) control of the blood sugar level and (v) phosphorylation–dephosphorylation (one of the most important controls of the cell functions).

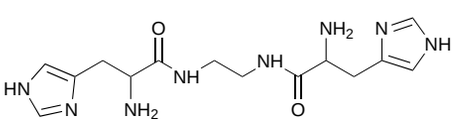
In zinc(II)-containing nucleases, metal-bound imidazole rings are always present. Recently, great efforts have been made to develop efficient artificial nucleases.[14–24] Most of the nuclease-model studies[14–20] were made to attain greater hydrolysis rates, using metal ions (*e.g.* lanthanoids, Co^{III}), ligands (most of them polyaza-macrocycles) and test compounds (activated aryl phosphate esters) which do not reflect the active centre of nucleases and native substrates. Only a few metal complexes have been published, having structural similarities with the native enzymes.[13,21–23] Labile metal complexes, containing bidentate ligands, are generally considered less active than rigid ones in hydrolysing phosphate esters, however, good hydrolytic activity was reported in certain cases against activated (aryl)-phosphate esters.[14]

Here we report the synthesis and co-ordination properties of *N*,*N*′-di-L-histidylethane-1,2-diamine (dhen), which contains two N-terminal histidyl residues separated by an ethylene spacer. Our aim was to design a peptide-like ligand having strong metal-ion binding ability near the physiological pH, without the participation of amide nitrogen(s) in the co-ordination. Combined pH-metric and spectroscopic (UV/VIS, CD, EPR and ^{1}H NMR) methods were used to determine the equilibrium properties and solution structure of copper(II) and zinc(II) complexes formed with dhen and those of the strongly related histamine. The proximity of the two histamine-like donor sets results in very strong metal-binding ability, which indeed prevents co-ordination of amide nitrogen at physiological pH.

The zinc(II) complexes of dhen and histamine were also tested as nuclease models towards hydrolysis of the non-



Histamine (hist)



N,*N*′-Di-L-histidylethane-1,2-diamine (dhen)

† E-Mail: tamas.gajda@chem.u-szeged.hu
‡ *Non-SI unit employed*: G = 10^{-4} T.

activated uridine 2′,3′-cyclic monophosphate (2′,3′-cUMP), having real biological relevance.[24] Although the equilibrium and structural studies showed that the Zn^{II}–dhen complexes, in spite of the ligand structure, cannot be entirely regarded as dimers of zinc(II)–histamine species, their comparison is interesting due to the probable differences between mono- and poly-metallic systems. The results showed that the labile zinc(II)–histamine complexes are able efficiently to hydrolyse phosphate diester bonds.

Experimental

Materials

Copper(II) and zinc(II) perchlorate (Fluka) solutions were standardized complexometrically. pH-Metric titrations were performed using Titrisol NaOH standard solution (Merck). Histamine dihydrochloride (Fluka), HEPES, 2-morpholinoethanesulfonic acid (mes) and 2-cyclohexylethanesulfonic acid (ches) (Aldrich) were used without further purification.

Synthesis of *N*,*N*′-di-L-histidylethane-1,2-diamine (dhen)

L-Histidine hydrochloride hydrate (31.45 g, 150 mmol) was dissolved in a mixture of acetone (80 cm^3), water (40 cm^3) and $Me_3COC(O)OC(O)OCMe_3$ (76 cm^3, 330 mmol) with stirring. After 2 h at room temperature the acetone was evaporated and the residual aqueous phase extracted with *n*-hexane. The remaining solution was distributed between 10% aqueous $KHSO_4$ solution and ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated. The oily product [N^a,N^{im}-di(*tert*-butoxycarbonyl)-L-histidine **I**] was homogeneous by TLC investigations.

Compound **I** (50 mmol) was dissolved in absolute chloroform (50 cm^3). After cooling to 0 °C, dicyclohexylcarbodiimide (8.66 g, 42 mmol) and 1-hydroxy-1*H*-benzotriazole (5.67 g, 42 mmol) were added. The reaction mixture was stirred for 10 min at 0 °C and ethane-1,2-diamine (1.13 cm^3) was added. After stirring for 2 h at room temperature the precipitated dicyclohexyl urea was filtered off, the residue was evaporated, dissolved in ethyl acetate and extracted with 5% $KHCO_3$ and 5% $KHSO_4$ solutions. The organic layer was dried over Na_2SO_4 and evaporated. The oily residue was crystallized from ethyl acetate–hexane. The yield of *N*,*N*′-bis[N^a,N^{im}-di(*tert*-butoxycarbonyl)-L-histidyl]ethane-1,2-diamine **II** was 2.1 g (57%).

Compound **II** (1.1 g) was treated with 1.5 M HCl in acetic acid (10 cm^3) at 0 °C. After 30 min the acetic acid was evaporated and the residue triturated with diethyl ether. The crystalline product was filtered off and recrystallized from methanol–diethyl ether. Yield: 0.51 g, 71%. The structure and purity was demonstrated by mass and NMR spectroscopy and HPLC. HPLC: Lichrosorb 7RP18 column, 0.8 cm^3 min^{-1}, 0.1% CF_3CO_2H in water as eluent, isocratic t_R = 8.2 min. ^{1}H NMR (in water): δ 8.645 ($C^2_{im}H$), 8.410 (NH_{amide}), 7.378 ($C^5_{im}H$), 4.208 (CH_{hist}), 3.314 ($CH_{2,hist}$) and 3.251 ($CH_{2,en}$).

pH-Metric measurements

The co-ordination equilibria were investigated by potentiometric titrations in aqueous solution (0.1 M NaCl and 298 ± 0.1 and 363 ± 1 K) in an automatic titration set including a Dosimat 665 (Metrohm) autoburette, an Orion-710A precision digital pH-meter and an Orion ROSS 8103BN type combined glass electrode. The experimental procedure at 298 K was described earlier.[8–10]

At 363 K the pH-meter was calibrated with hepes buffer (0.05 M) having known p*K* (6.6)[25] at this temperature and an ideal nernstian function was assumed. The value pK_w = 12.07 was used[26] as the autoprotolysis constant of water at 363 K. Owing to experimental difficulties, the stability constants determined at this temperature have lower accuracy than is usual for pH-metric studies.

The protonation and complex stability constants were calculated as the average of 4(2) and 8(4) independent titrations at 298 (363 K) (*ca.* 80 data points per titration), respectively. The metal-to-ligand ratios were varied from 2:1 to 1:2 (1:1 to 1:8 in case of histamine), with metal-ion concentrations between 2×10^{-3} and 1×10^{-2} M. The pH metric data were evaluated by the PSEQUAD computer program.[27]

Electronic absorption and CD measurements

The UV/VIS spectra were recorded on a Hewlett-Packard 8452A diode-array spectrophotometer. The individual spectra of copper(II) complexes formed were calculated by the PSEQUAD computer program. The CD spectra were recorded on a Jobin Yvon CD6 spectropolarimeter in the wavelength interval 230 to 800 nm. The metal-ion concentration was 5×10^{-3} M in cells with 0.1 and 1 cm optical pathlengths in the UV and VIS spectral regions, respectively. The CD data are given as the differences in molar absorptions between left and right circularly polarized light, normalized to the metal-ion concentration in M^{-1} cm^{-1} units.

EPR measurements

The EPR spectra were recorded on a JEOL-JES-FE 3X spectrometer at X-band with 100 kHz field modulation at room temperature. Manganese(II)-doped MgO powder served as field standard. The copper(II) concentration was 5×10^{-3} M. The EPR parameters were calculated by a recently developed computer program[28] able to treat the spectra of several (but preferably two) coexisting species.

NMR measurements

The ^{1}H NMR measurements were performed on a Bruker AM-400 spectrometer, operating at room temperature. The pH readings in the mixture of 2% D_2O–98% water were uncorrected for the isotopic effect. The chemical shifts are given as relative shifts from dss (the sodium salt of 4,4-dimethyl-4-silapentane-1-sulfonate) using 1,4-dioxane as internal reference (3.7 ppm from dss).

Kinetic measurements

Kinetic data were measured by hydrolysing 2′,3′-cUMP in the presence of zinc(II)–histamine and –dhen complexes at 363 K and analysing the unchanged substrate and products (uridine 2′-phosphate, uridine 3′-phosphate and a small amount of uridine) by high-performance liquid chromatography (HPLC). The pH of the reaction solutions was measured at 298 K, prior to initiation of the hydrolysis, and extrapolated at 363 K with the aid of the known p*K* value of HEPES at 363 K, while those of ches and mes at 363 K were determined by us (7.8 ± 0.05 and 5.4 ± 0.05, respectively). The initial substrate concentration was 5×10^{-4} M. Ten aliquots of the reaction solutions were periodically taken in each run. The progress of the reaction was stopped by cooling it in an ice-bath and the sample was injected into the HPLC column. The chromatographic separations were carried out on a Waters HPLC system, consisting of an M-600 low-pressure gradient pump, an M-996 photodiode-array detector and a Millenium 2010 Chromatography Manager data system (Waters Chromatography). The column was a Lichrospher 100 RP-18 (150 × 4 mm inside diameter), 5 μm particle size (Merck). The mobile phase was acetate buffer (0.025 M, pH 4.3, containing 0.1 M ammonium chloride) using a 0.4 cm^3 min^{-1} flow rate, while the UV detection was carried out at 260 nm. The hydrolysis was followed for about three half-lives by observing the disappearance of uridine 2′,3′-cyclic monophosphate and in all cases exhibited excellent pseudo-first-order kinetics. The rate constants (k_{obs}) were obtained

Table 1 Logarithmic formation constants for copper(II) and zinc(II) complexes of dhen and histamine at $I = 0.1$ M (NaCl), 298 K [363 K]; $\beta_{pqr} = [M_pL_qH_r]/[M]^p[L]^q[H]^r$, with estimated errors in parentheses (last digit)

dhen			Histamine		
pqr	Cu^{II}	Zn^{II}	*pqr*	Cu^{II}	Zn^{II}
011	7.773(4) [6.31(5)]		011	9.763(1) [7.94(5)]	
012	14.560(4) [11.88(5)]		012	15.844(2) [12.79(5)]	
013	19.916(5) [16.24(5)]		—	—	
014	24.517(5) [19.91(5)]		—	—	
112	20.81(1)	—	111	12.85(3)	10.87(4) [—]
111	16.77(1)	12.33(1) [11.0(1)]	110	9.48(1)	5.15(1) [4.5(1)]
220	27.83(2)	16.47(2) [15.0(1)]	121	21.48(3)	—
42-4	16.29(4)	−2.29(6) [1.3(1)]	120	15.98(1)	9.97(1) [8.6(1)]
42-5	—	−11.56(9) [−5.3(1)]	11-1	—	−3.04(2) [−2.0(1)]
42-6	—	−21.15(9) [−12.2(1)]	12-1	5.19(1)	—
11-2	−6.12(1)	−11.77(5) [−8.8(1)]	22-2	7.02(1)	—
Number of experimental points	579	462 [218]	Number of experimental points	721	391 [326]
Fitting parameter (cm^3)	0.006	0.007 [0.013]	Fitting parameter (cm^3)	0.006	0.005 [0.008]

from plots of the peak area ratio: [substrate]/([substrate] + [product]), A_t, *vs.* time by non-linear least-squares fitting using the standard exponential model $A_t = A_0 \exp(-k_{obs}t)$.

Results and Discussion

Histamine complexes

Although much data have been published concerning the formation constants of copper(II)– and zinc(II)–histamine complexes,[29,30] we redetermined them for comparison since dhen contains two histamine-like donor sets. The values (Table 1) agree well with the earlier results.

dhen complexes

Although the protonation constants of dhen (Table 1) show relatively strong overlapping of the steps, pK_1 and pK_2 can be mostly assigned to the imidazole rings, pK_3 and pK_4 to the amino groups. The two imidazole rings of dhen have surprisingly low pK values (4.62 and 5.32), compared to histamine (6.08), but are closer to the corresponding values of histidine methyl ester[31] (His-OMe, pK = 5.01) or the structurally similar *N*,*N*′-bis(imidazol-4-ylmethyl)ethane-1,2-diamine[11] (bimeda, pK = 4.26 and 3.21). The amino groups of dhen are also less basic compared to those of the analogous *N*,*N*′-diglycylethane-1,2-diamine[32] (dgen, pK = 7.48 and 8.22), due to the electron-withdrawing effect of the imidazole rings.

The titration curves of copper(II)- and zinc(II)-containing systems are shown in Fig. 1. In both cases 1 : 2, 1 : 1 and 2 : 1 metal-to-ligand ratios were used. The metal-promoted deprotonations of imidazolium and ammonium groups are closely overlapping and are complete at pH 5–6, suggesting the strong complexing ability of this ligand. At higher pH further deprotonations occurred during which very slow complex-formation processes were detected in case of metal excess ([M] : [L] = 2 : 1), up to pH 7.5 and 9 (for Cu^{II} and Zn^{II}, respectively), at which precipitation started. Thus, in the evaluation of pH-metric data, only the systems with 1 : 1 and 1 : 2 metal-to-ligand ratios were used.

A ^{1}H NMR study was carried out to get a better insight into the complexes formed in Zn^{II}–dhen system and also to distinguish between the several models to describe the pH-metric titration curves. Fig. 2 shows the imidazole region of the Zn^{II}–dhen system at different pH and metal-to-ligand ratios. The important line broadening of the C^2H signals between pH 4 and 7 suggests the co-ordination of N^3 nitrogens in the complexes formed. In presence of metal excess (Fig. 2A) new peaks appeared gradually at above pH 6.6, signalling the formation of new complex(es), in slow equilibrium on the NMR time-scale. The formation of the latter complex(es) is complete at pH 7.6. Since no precipitation was observed below pH 9, this observation suggests the formation of oligonuclear complex(es) with 2 : 1 metal-to-ligand ratio. The peaks belonging to these oligonuclear complexes also appeared in 1 : 1 and 1 : 2.5 systems. Their formation parallels the observed extra deprotonation (Fig. 1A) at any metal-to-ligand ratio. The intensity of the two sets of peaks is almost equal in equimolar solution between pH 8 and 11, suggesting the unique formation of oligonuclear species under these conditions, too. In the case of a ligand excess, the intensity of the peaks belonging to the oligonuclear species decreases at higher pH, showing the formation of new complexes.

The question arises as to the structure of the oligonuclear species. The base consumption detected above pH 6 could be assigned to three different processes: (i) deprotonation of co-ordinated water molecules, (ii) metal-promoted deprotonation of amide or (iii) imidazole N^1 nitrogens. It is interesting to analyse the shift of the proton signals of dhen during these complex-formation processes. The peak of the imidazole C^2H proton was shifted nearly 1.4 ppm, the C^5H only 0.3 ppm, upfield during the complexation compared to the signals of neutral imidazole (the C^2H proton exchanges easily with deuterium in D_2O, which can help to assign the signals). At the same time, the methylene protons of the ethylenediamine part (Fig. 3), directly linked to the amide nitrogens, are weakly influenced. The formation of hydroxo mixed-ligand complexes alone [zinc-bound water deprotonation(s) beside the already co-ordinated amino and N^3-imidazole nitrogens] cannot result in such an important shift of the imidazole protons. The zinc(II)- or diamagnetic nickel(II)-promoted deprotonation of amide nitrogens in imidazole-containing peptides generally results also in kinetically stable complexes.[1,9] The chemical shifts of the imidazole protons are, however, weakly influenced during these processes, while those of the protons near to the amide nitrogen are considerably altered. In our case the observed changes are just the opposite (see above), thus the ^{1}H NMR behaviour should correspond to a fundamental change in the co-ordination of the imidazole rings. Earlier results showed that deprotonation of imidazole N^1 (pyrrolic) nitrogen in diamagnetic nickel(II) complexes[9,10] is accompanied by very similar shifts to those observed in present case. The 1.4 ppm upfield shift of the imidazole C^2H upon a base-consuming process could only be assigned to the metal-promoted deprotonation of imidazole nitrogen N^1 in this case, too. The imidazolate anions, formed in this way, act as bidentate bridging ligands. This is in line with the higher inequivalence of the histidyl $C^\beta H_2$ signals in the oligonuclear complex compared to those of free dhen (Fig. 3), showing more fixed imidazole rings. Moreover,

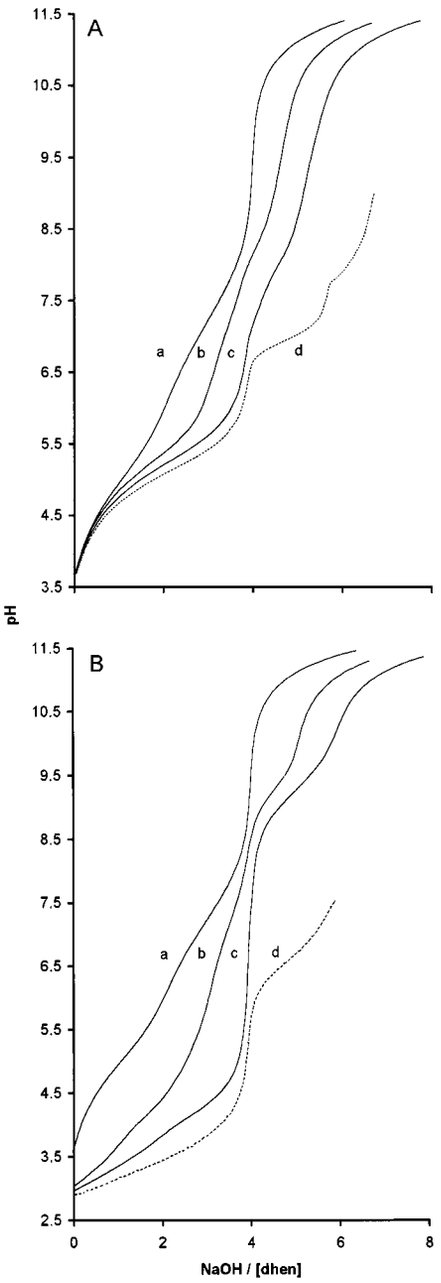


Fig. 1 Titration curves of Zn^{II}–dhen (A) and Cu^{II}–dhen (B) systems at different metal-to-ligand ratios; [L] = 0.0028 M (a) and [M] = 0.0014 (b), 0.0026 (c) and 0.0052 M (d)

the NMR equivalence of imidazole, CH and CH_2 protons in the oligomeric sequence strongly supports an identical chemical environment, and thus the existence of cyclic oligomers. Although, the formation of Zn_2LH_{-2} species cannot be excluded, in this complex the imidazole rings would not be equivalent. Several cyclic, tetranuclear, imidazolate-bridged complexes have been characterized by X-ray diffraction[4,33] and by EPR and NMR spectroscopy.[8,9] By analogy, we propose the formation of $Zn_4L_2H_{-4}$ species in the Zn^{II}–dhen system, having N_{im}, N^-_{im}, NH_2 co-ordination. Taking into account the above facts, the 'best fit' of experimental pH-metric curves was obtained by considering the presence of six species, listed in Table 1. The species distribution curves (Fig. 4) show, that near pH 7, a 4N co-ordination species is dominant. Its composition can be either ZnL or Zn_2L_2, but in both cases the metal ions are co-ordinated by two amino and two imidazole N^3 nitrogens. Although we have no definite evidence, a dimer complex seems to be formed in light of the pH-metric and kinetic data and the results obtained on the Cu^{II}–dhen system (see later). The extra deprotonation and the formation of the tetranuclear $Zn_4L_2H_{-4}$ complex start at pH 7, in agreement with the NMR results (Figs. 2 and 4). The NMR data, however, show the presence of this structure in a wide pH range, where further deprotonations were detected by pH-metry. This can be explained by the formation of hydroxo mixed-ligand complexes ($Zn_4L_2H_{-5}$, $Zn_4L_2H_{-6}$), which does not alter the co-ordination of the imidazole rings or their NMR signals. Above pH 10 the oligonuclear structure disappears and the monomeric $ZnLH_{-2}$ complex forms. Since this complex is in the fast-exchange limit on the NMR time-scale, the two extra deprotonations are probably related with the formation of hydroxo mixed-ligand complexes and not with metal-promoted deprotonation of amide nitrogens.

As expected, the copper(II)-promoted deprotonation of imidazolium and ammonium groups, and the stepwise formation of 2N, 3N and 4N species, take place at lower pH than in case of zinc(II) (Fig. 1). However, in the zinc(II)–histamine system the 'extra' deprotonations were detected at pH 6–7, one unit lower than in the case of copper(II).

Spectroscopic (UV/VIS, CD and EPR) data were collected to have better assignments of the successively formed complexes in the copper(II)–dhen system. On the basis of both pH-metric and spectroscopic studies, a dominant species is formed between pH 5 and 8 at 1:1 and 1:2 metal-to-ligand ratio, which could be either CuL or Cu_2L_2. The d–d transition of this species is centred at 625 nm, which is a relatively high value for a $2NH_2$, $2N_{im}$ co-ordination [*e.g.* for the bis(histamine) complex $\lambda^{d-d}_{max} = 600$ nm]. The EPR parameters determined for this species [$g_0 = 2.101$, $A_0 = 68$ G (Fig. 5b), $g_\parallel = 2.238$, $g_\perp = 2.057$, $A_\parallel = 180$ G] however indicate 4N co-ordination. Moreover, the analogous bis(histamine-like) complex[3,5] $Cu(His–Gly)_2$ (negatively charged complex, charges of complexes omitted for simplicity) has very similar λ^{d-d}_{max} values[3] (630 nm) and EPR parameters[5] ($g_\parallel = 2.247$, $g_\perp = 2.044$, $A_\parallel = 186$ G) which further support the $2NH_2$, $2N_{im}$ co-ordination in the species in question. Fig. 5a presents the room-temperature EPR spectra of the $Cu(hist)_2$ complex ($g_0 = 2.110$, $A_0 = 76$ G). Both the isotropic (Fig. 5b) and anisotropic (not shown) EPR spectra of the Cu^{II}–dhen system are much more broadened than that of the copper(II)–histamine system. The increased linewidth of the isotropic spectra, especially at lower magnetic fields, reflects the increased radius of the complex molecule. Since the monomeric Cu(dhen) complex does not have a significantly higher radius compared to $Cu(hist)_2$, the results suggest the formation of a dimeric Cu_2L_2 species depicted in Scheme 1. In the hypothetical CuL complex an eleven-membered chelate ring would be formed between the two amino groups, which probably could not stabilize the monomer structure. The dimer structure is, however, likely to be preferred by the linear form of the peptide chain.

Starting from acidic pH, the CD spectra of both 1:1 and 1:2 systems show the gradual formation of a positive Cotton effect relating to the copper(II) d–d transition (centred at 674 nm, $\varepsilon = 0.73$ M^{-1} cm^{-1}), assigned to the Cu_2L_2 complex (Fig. 6). Between pH 7 and 10, however, the CD spectra of the 1:1 and 1:2 systems behave very differently, while above pH 10.5 the systems again present identical CD spectra (Fig. 6). On the basis of pH-metric results, the species formed above pH 10 is the monomeric $CuLH_{-2}$. Its d–d transition band (centred at 514 nm) and EPR parameters [$g_0 = 2.080$, $A_0 = 90.4$ G (Fig. 5c), $g_\parallel = 2.178$, $g_\perp = 2.046$, $A_\parallel = 206.6$ G] indicate a very high ligand-field strength in this complex. These facts and the characteristic CD couplet ($\varepsilon = -0.27$ and 0.49 M^{-1} cm^{-1} at 474 and 556 nm, respectively) observed for this species strongly suggest the $2N^-_{amid}$, $2NH_2$ co-ordination in $CuLH_{-2}$ depicted in Scheme 1.

The equimolar solution, between pH 7 and 10, showed the formation of new complex(es) having characteristically different CD spectra compared to those of Cu_2L_2 and $CuLH_{-2}$ (Fig. 6). Matrix rank analysis[34] of the data obtained from

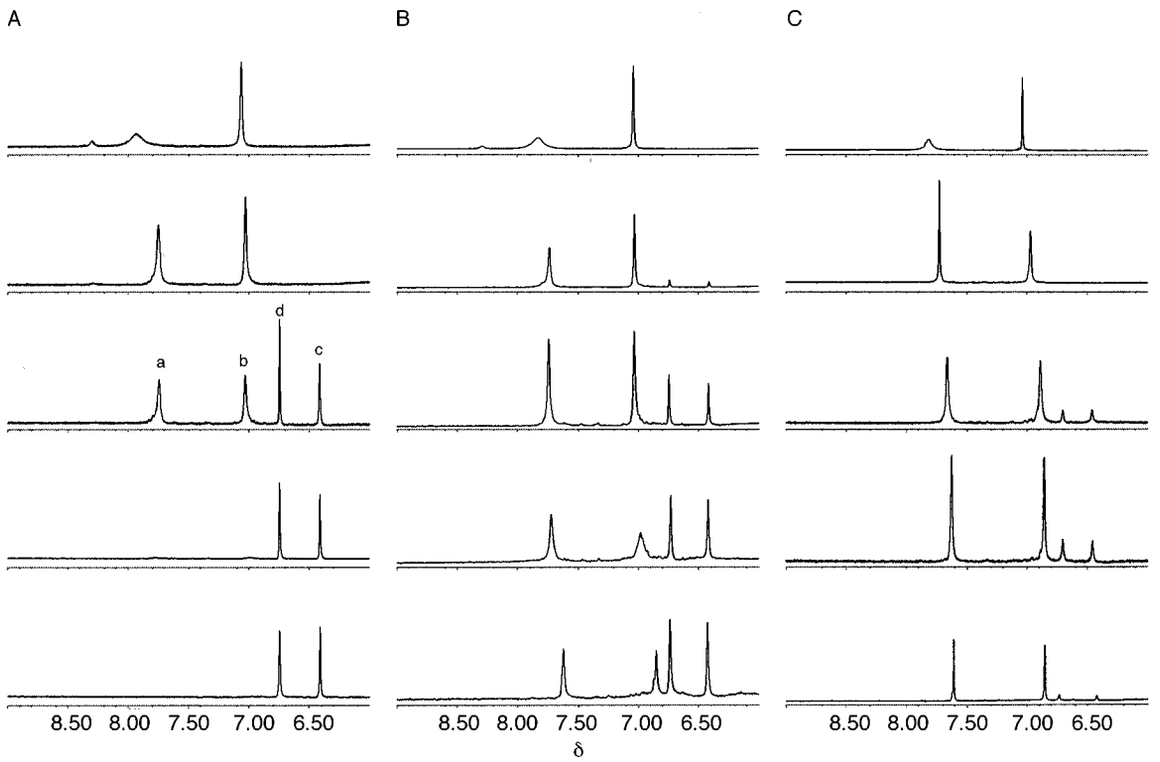


Fig. 2 The imidazole region of the ^{1}H NMR spectra in the Zn^{II}–dhen system; C^2H and C^5H signals are labelled 'a' and 'b' in the fast-exchanging, 'c' and 'd' in the slow-exchanging species, respectively. A [M] : [L] = 2 : 1, pH 5.40, 6.62, 7.09, 7.64 and 9.35 (from top to bottom); B [M] : [L] = 1 : 1, pH 5.78, 7.24, 7.73, 8.31 and 10.70; C [M] : [L] = 1 : 2.5, pH 6.30, 7.64, 8.80, 9.36 and 10.94

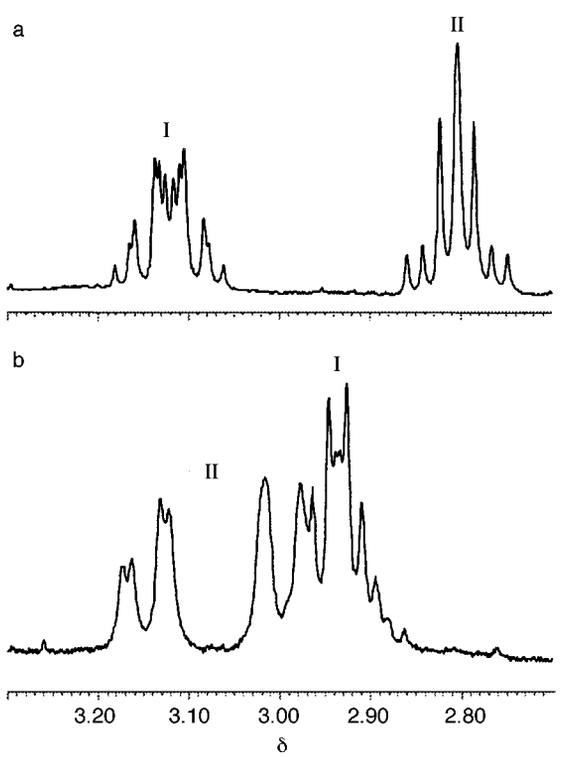


Fig. 3 Proton NMR spectra of the methylene protons of the ethylenediamine (I) and histidyl part (II) of free dhen at pH 10.5 (a) and in the Zn^{II}–dhen 2 : 1 system at pH 9.35 (b)

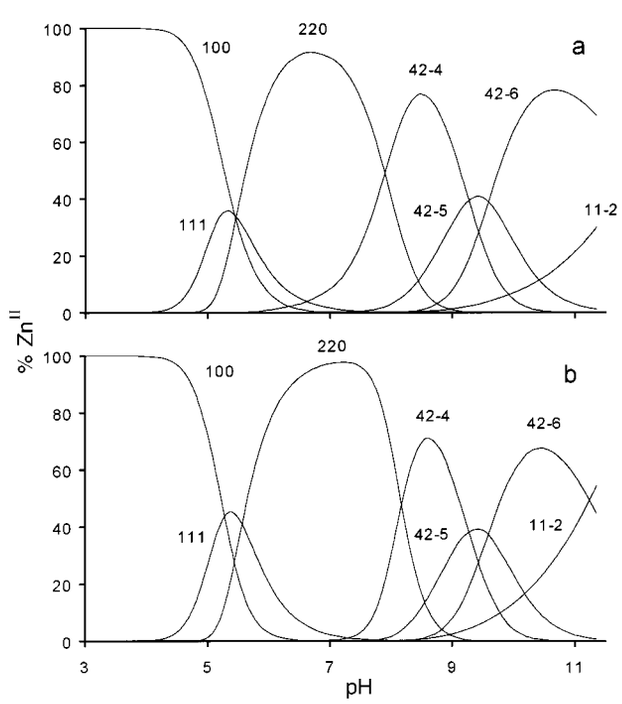


Fig. 4 Species distribution curves of the Zn^{II}–dhen system (I = 0.1 M, T = 298 K): a, 1.1[Zn] = [L] = 0.003 M; b, 1.05[Zn] = 0.5[L] = 0.0015 M

CD curves in Fig. 6, recorded at several pH in this region, confirmed the presence of only one species beside Cu_2L_2 and $CuLH_{-2}$. This further species formed in relatively high amount in the 1 : 1 system however is only a minor species in the case of ligand excess. The above CD and pH-metric results cannot be explained by the formation of a species having a 1 : 2 or 1 : 1 metal-to-ligand ratio, between pH 7 and 10. Only 2 : 1 complexes (Cu_2LH_{-2}, $Cu_4L_2H_{-4}$) can be present in considerably larger quantity in the 1 : 1 compared to the 1 : 2 system. The presence of such an oligomeric complex, was also supported by the loss of EPR intensity in this region, due to the antiferromagnetic interaction between the copper(II) centres. On the basis of the pH-metric results only, it is difficult to draw a distinction between the formation of dinuclear (Cu_2LH_{-2}) and tetranuclear ($Cu_4L_2H_{-4}$) species, having the same protonation states. The CD results, however, favour the tetranuclear complex, since the difference between the CD curves of the 1 : 1 and 1 : 2 systems can be explained only by assuming a tetranuclear species.

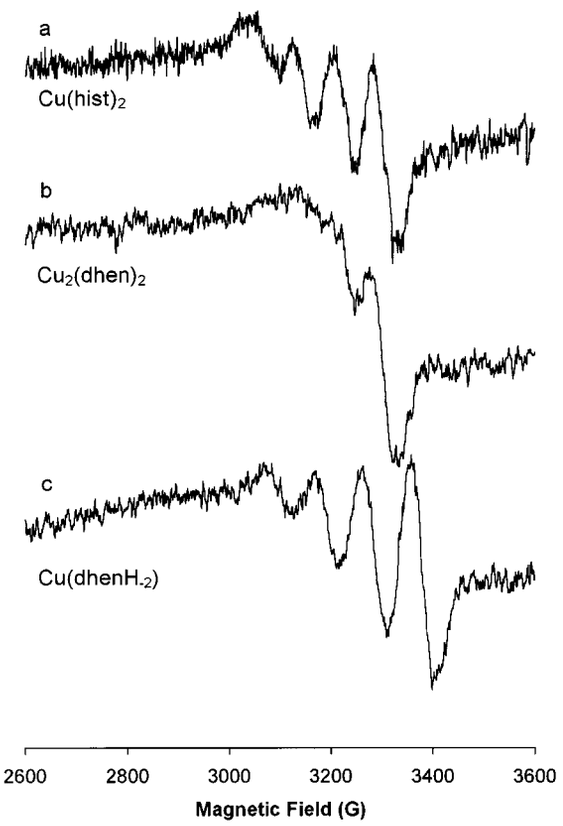
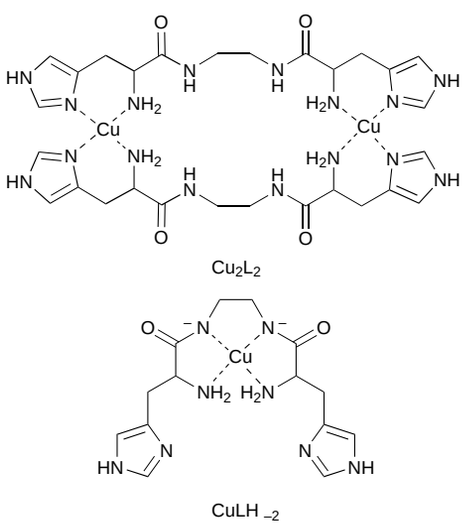


Fig. 5 Isotropic EPR spectra of copper(II)–histamine (a) and –dhen (b,c) systems. [Cu] = 0.004 M; [Cu] : [L] = 1 : 5 (a), 1 : 1 (b,c); pH 8.0 (a), 7.5 (b) and 11.0 (c)



Scheme 1 Proposed structures of the dominant species in the Cu^{II}–dhen system

In conclusion the whole set of pH-metric curves, taking into account the spectroscopic results, was best fitted by considering five complexes: $Cu(H_2L)$, $Cu(HL)$, Cu_2L_2, $Cu_4L_2H_{-4}$ and $CuLH_{-2}$. Their distribution curves are depicted in Fig. 7. In the acidic region protonated complexes are formed, having 2N and 3N co-ordination. The dimeric Cu_2L_2 complex, having bis-(histamine-like) $2N_{im}$, $2NH_2$ co-ordination, is the only species in a wide pH range (5–8), as a consequence of its high stability. The basicity-corrected formation constants of $M(hist)_2$ complexes (*e.g.* for CuL_2 $\log \beta_{120,corr} = \log \beta_{120} - 2 \log \beta_{012} = -15.71$) are several order of magnitude lower than those of the corresponding complexes of any other histamine-like ligands, *e.g.* histidine methyl ester[31] ($\log \beta_{120,corr} = -11.92$), His–Gly[3] ($\log \beta_{120,corr} = -11.61$) or the present dhen [$(\log \beta_{220,corr})^{\frac{1}{2}} = -12.01$].

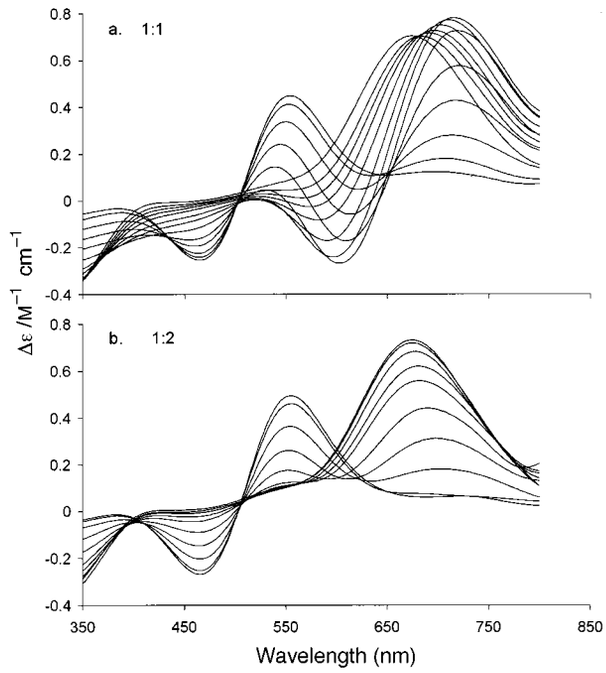


Fig. 6 Effect of pH on the CD spectra of the Cu^{II}–dhen system: a [Cu] = [L] = 0.0025 M, pH 7.43, 8.12, 8.37, 8.58, 8.80, 9.01, 9.18, 9.41, 9.68, 9.87, 10.12, 10.37 and 10.57 (top to bottom at 650 nm); b [Cu] = 0.5[L] = 0.0015 M, pH 7.55, 8.23, 8.61, 8.85, 9.01, 9.25, 9.44, 9.66, 10.04 and 10.46

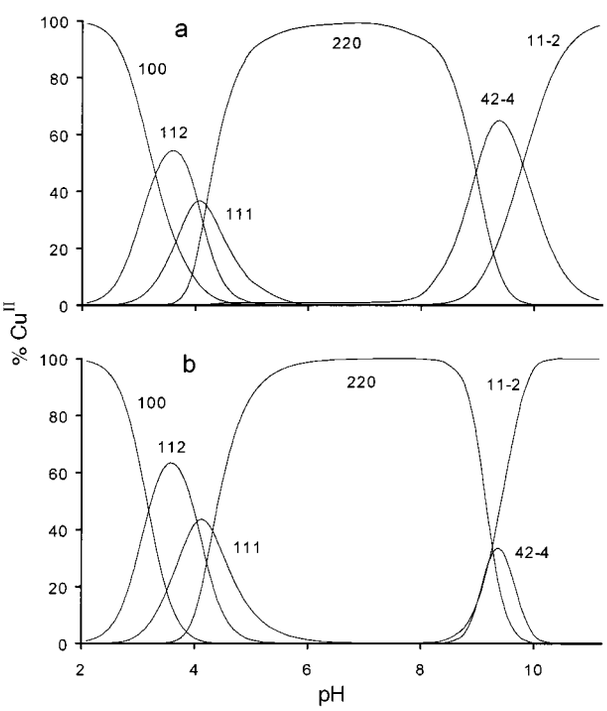


Fig. 7 Species distribution curves of the Cu^{II}–dhen system ($I = 0.1$ M, $T = 298$ K): a, [Cu] = [L] = 0.003 M; b, [Cu] = 0.5[L] = 0.0015 M

The *ca.* 20–30 nm red-shift of the d–d transition of the Cu(His–Gly)$_2$ complex compared to Cu(hist)$_2$ (see above) was explained by assuming that one of the four nitrogen atoms co-ordinates to the metal ion in an axial position.[3] The above-mentioned extra stabilization is, however, inconsistent with this hypothesis and rather suggests axially co-ordinated oxygen donor(s) which may also explain the red-shift of the d–d transition. The high stability of the Cu_2L_2 complex, due to the $2N_{im}$, $2NH_2$ co-ordination, is also reflected by the fact that deprotonation of the amide nitrogens and the formation of $CuLH_{-2}$ take place at two pH units higher compared with those of the analogous

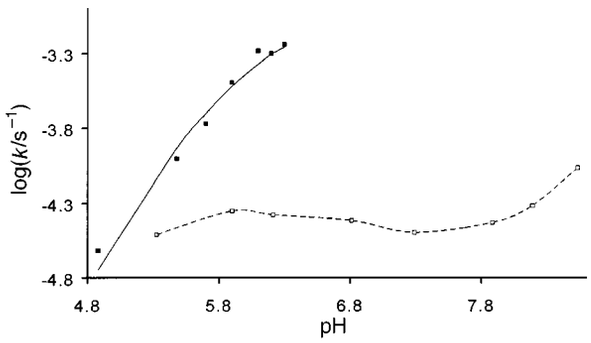


Fig. 8 Effect of pH on hydrolysis of 2′,3′-cUMP by zinc(II)-histamine (■, [Zn] = 0.45[L] = 0.004 M) and Zn^{II}–dhen (□, [Zn] = 0.95[L] = 0.002 M) complexes at 363 K. The solid line is the fitted curve (see text)

N,*N*′-diglycylethane-1,2-diamine complex.[32] The process $M_2L_2 \longrightarrow 2\ MLH_{-2} + 4\ H^+$ could be characterized by nearly equal log *K* values in the Zn^{II}– and Cu^{II}–dhen systems (log *K* = −40.01 and −40.07, respectively). Taking into account the much lower ability of Zn^{II} to replace amide protons, these similar values indicate different deprotonation processes (amide nitrogen and co-ordinated water), leading to the MLH_{-2} species, in case of the two metal ions.

To give a better description of the kinetic measurements performed at 363 K (see later), the equilibrium properties of the zinc(II)–dhen and –histamine systems have also been investigated at this temperature. The formation of the same species was assumed at 363 K as detected at 298 K. The determined stability constants are listed in square brackets in Table 1. Owing to its importance in the kinetic study, the hydrolysis of zinc(II) aqua ion was also studied at 363 K. A solution containing 0.001–0.004 M $Zn(ClO_4)_2$ could be titrated until the formation of 10–5% of Zn(OH) species (without precipitation) and from these data log $\beta_{10\text{-}1} = -6.8(2)$ can be determined.

Kinetic measurements

Although studies with activated phosphate esters [13,14,16,19,20] contributed considerably to the understanding of metal-ion-promoted hydrolysis of phosphoesters, these compounds contain exceptionally good leaving group(s) which probably hydrolyse by a mechanism that is not biologically relevant. During our work the biologically occurring uridine 2′,3′-cyclic monophosphate, an intermediate of RNA hydrolysis,[24] was used as model compound.

The pH profiles of the pseudo-first-order rate constants are shown in Fig. 8. It can be seen that the hydrolytic activity of the zinc(II)–histamine complexes sharply increases between pH 4.8 and 6.4 in parallel with the formation of the N_{im}, NH_2, OH^- co-ordinated Zn(hist)(OH) species. With increasing ligand excess the $Zn(hist)_2$ complex becomes predominant at pH 6.3. In parallel the hydrolysing ability considerably decreases (Fig. 9A), indicating that the bis complex has practically no effect on the hydrolysis.

These facts are consistent with the earlier observation that metal-bonded OH^-, as a nucleophilic agent, has a profound role in the catalytic mechanism. At pH 6.3, several species are in equilibrium, so one cannot extract information about the reaction order nor about a preequilibrium prior the catalytic action (*i.e.* the formation of a catalytically active complex by co-ordination of the substrate). On the basis of the previous investigations using monomeric metal complexes,[13–20] however, a first-order dependence of the hydrolysis on the concentration of the metal complex is a very plausible assumption in this system.

The zinc(II)–dhen complexes are less active and their hydrolytic activity is practically independent of pH (Fig. 8). The slope of the log k_{obs} *vs.* log c_T plot (0.47, Fig. 9B, dashed line),

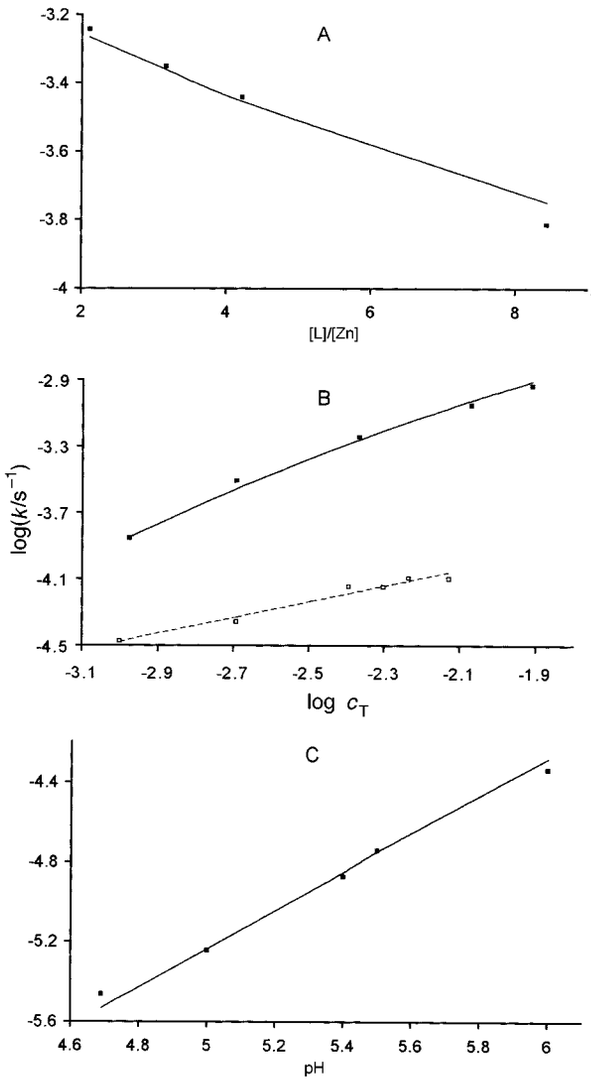


Fig. 9 Pseudo-first-order rate constants for the hydrolysis of 2′,3′-cUMP promoted by zinc(II) complexes (A, B) and Zn^{II} alone (C); solid lines are the fitted curves (see text): A effect of the metal-to-ligand ratio, [Zn] = 0.004 M; B effect of total concentration of Zn^{II}, [Zn] = 0.45[L], for the zinc(II)–histamine (■) system and [Zn] = 0.95[L] for the Zn^{II}–dhen (□) system; C effect of pH for Zn^{II}-promoted hydrolysis (data from ref. 18)

measured at pH 5.9, is considerably smaller than that of the zinc(II)–histamine system. Since between pH 5.5 and 6.1 the Zn_2L_2 species is predominant, the ≈0.5 value may suggest that (i) a dimer complex forms under these conditions and (ii) there is some co-operativity between the two metal ions in the dimer structure. The hydrolytic activity decreases during the 'extra' deprotonations observed by pH-metry. This fact further supports that these deprotonations cannot be attributed to the formation of simple mixed-ligand hydroxo complexes, where an important pH dependence would be detected. The increasing activity above pH 7.5 is related to the formation of the monomeric hydroxo mixed-ligand $ZnLH_{-2}$ species.

The zinc(II)–histamine system accelerates the hydrolysis of 2′,3′-cUMP by about three orders of magnitude compared to the autohydrolysis ($k_{obs,auto} = 2.4 \times 10^{-6}\ s^{-1}$ at pH 6.3). This activity is nearly equal to that of the Zn^{II}–[12]$aneN_3$ complex, measured under similar conditions, which is known as the most active zinc(II)-containing accelerator of 2′,3′-cUMP hydrolysis.[17,18] This observation shows that, in spite of the general opinion, substitution-labile zinc(II) complexes containing bidentate ligands are also able to catalyse efficiently the hydrolysis of phosphate diester bonds.

Imidazole is reported to enhance phosphate ester hydrolysis,

acting as a general base.[35] Histamine may behave in a similar manner. The hydrolysis of 2′,3′-cUMP in the presence of 0.01 M histamine ($k_{obs} = 1.9 \times 10^{-6}$ s^{-1}, pH 6.3, 363 K), however, does not show any acceleration compared to the autohydrolysis. Consequently, the presently observed hydrolytic activity is entirely due to the zinc(II) complexes formed.

In the zinc(II)–histamine system five species are present in the studied pH range: Zn_{aq}, Zn(OH), ZnL, ZnL_2 and ZnL(OH) (charges omitted). Thus the above assignment of the observed catalytic effect to the ZnL(OH) species can only be a first approximation. The kinetic data, obtained at different pH (Fig. 8), concentrations and metal-to-ligand ratios (Fig. 9A and 9B), combined with the concentration distribution of different species provided by the equilibrium data, however, can be used to determine the individual contributions of all species to the observed hydrolysis rate (k_{obs}), based on equation (1).

$$k_{obs} = k_1[Zn] + k_2[Zn(OH)] + k_3[ZnL] + k_4[ZnL_2] + k_5[ZnL(OH)] \quad (1)$$

To obtain an exact value for k_2, the experimental data reported in ref. 17 concerning the log k_{obs} *vs.* pH profile of the uncomplexed zinc(II)-containing system (Fig. 9C), measured under identical conditions, were taken into account. Evaluation of the whole set of data resulted in the following individual second-order rate constants: $k_1 \approx 0$, $k_2 = 0.19 \pm 0.01$, $k_3 = (7 \pm 3) \times 10^{-3}$, $k_4 \approx 0$ and $k_5 = 0.59 \pm 0.02$ M^{-1} s^{-1}. The fitting of the experimental data is shown by the solid lines in Figs. 8 and 9. From these data the following conclusions can be drawn: (i) the species containing metal-bound hydroxide ion are the most active towards hydrolysis of phosphate ester bonds, which can be interpreted by the intramolecular nucleophilic catalysis of metal-bonded hydroxide in the Zn^{II}–L–OH–phosphate ester mixed-ligand complex;[14,16,17] (ii) the three-fold higher activity of ZnL(OH) compared to Zn(OH) should be explained by the π-acceptor property of the co-ordinated imidazole ring,[36] which enhances the stability of mixed-ligand complexes with O-donor ligands; (iii) among the other three species (Zn_{aq}, ZnL and ZnL_2), only ZnL has some activity (zinc-bound water may act in a similar way to zinc-bound hydroxide, however its considerably smaller nucleophilicity results in much lower catalytic activity[13]), which can be explained also in terms of the higher affinity of ZnL to form mixed-ligand complexes compared to Zn_{aq}. This explanation does not hold, however, for ZnL_2 (which would be more active than ZnL). This contradiction can be explained by the peculiar feature of histamine, which enforces a tetrahedral structure in its ZnL_2 complex,[37] preventing the formation of mixed-ligand complexes (ZnL is reported to have mostly octahedral structure).

Acknowledgements

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