



Cite this: *New J. Chem.*, 2017,
41, 7253

Received 20th January 2017,
Accepted 6th June 2017

DOI: 10.1039/c7nj00254h

rsc.li/njc

Interaction of the Zn(II)-cyclen complex with aminomethylphosphonic acid: original simultaneous potentiometric and ^{31}P NMR data treatment[†]

Ingrida Rostášová,^a Mária Vilková,^b Zuzana Vargová,^b Róbert Gyenes,^{cd} Miroslava Litecká,^a Vojtěch Kubíček,^b Ján Imrich^b and Ivan Lukeš^c

The interaction of aminomethylphosphonic acid (H_2amp) with the Zn(II)-cyclen complex and the formation of ternary complexes was studied by potentiometry and ^{31}P NMR titrations. Data evaluation of each of the methods separately was found to be insufficient. Thus, data obtained from the both methods were simultaneously treated with the computer program OPIUM. The determined stability constants indicated only weak ($\log K \sim 3$) coordination of aminomethylphosphonic acid through the phosphonate group. The chelating coordination mode with the participation of the amine group was not confirmed. In excess of Zn(II)-cyclen, the formation of dinuclear complexes, in which two Zn(II)-cyclen units are bridged by a phosphonate group, was observed and their presence was confirmed by mass spectrometry. Such a coordination motif is typical for phosphonates in the solid state and also for phosphatases.

Introduction

More than 300 enzymes containing Zn(II) in their centers have been described. To understand the role of Zn(II) as well as other transition metal ions in mechanistic pathways of the metallo-enzymes, model complexes have been recently designed, synthesized and studied. The coordination site of the Zn(II) ion in the enzymes is formed by side chains of amino acids such as histidine or aspartate and its geometry results from the secondary or tertiary structure of the protein. It is very difficult to prepare such a coordination site artificially and, thus, in the models the coordination vicinity of the donor atoms is simplified.^{1–3} A number of ligands have been utilized and tested. Most of them are based on an aza-macrocyclic core^{4–6} and among them, cyclen is commonly used.

Investigation of the Zn(II)-cyclen system has been mostly focused on the recognition of small organic molecules

occurring in organisms,^{5–8} on nucleic acids, which have been summarized previously,⁹ and also on phosphates.^{10,11} In our group, we have studied the interaction of the Zn(II)-cyclen system with amino acids¹² and dipeptides.¹³ The high affinity of amino acids for the Zn(II)-cyclen system can be explained by significant non-bonding contributions (hydrophobic and/or π - π stacking).¹² The study of dipeptide interaction indicated carbonyl-carboxylate chelate binding in the acidic range and carbonyl-amine chelate or only terminal amino group coordination in the slightly alkaline range.¹³

This paper continues with our investigation of the Zn(II)-cyclen interaction with bioactive molecules. Here, we deal with interaction with a phosphorus analogue of glycine, *i.e.* aminomethylphosphonic acid (H_2amp).

Natural aminophosphonates have been found in many different organisms, from prokaryotes to eubacteria and fungi, mollusks, insects and others.^{14–17} They play an important role in the interaction with many metalloenzymes. Many natural as well as synthetic aminophosphonates bind strongly in the active site of enzymes and act as inhibitors of their function.¹⁸ The aminomethylphosphonate binding mode toward metal ions has been extensively studied and summarized.^{19–21} Generally, differences in basicity, charge, the electron-releasing effect and the size of relevant donor groups between aminophosphonates and aminocarboxylates result in different complexing properties. Aminophosphonates offer the possibility of monodentate coordination of the ligand *via* the phosphonate group, forming stable protonated complexes. In the case of chelate formation,

^a Department of Inorganic Chemistry, Faculty of Science, P. J. Šafárik University, Moyzesova 11, SK-041 54 Košice, Slovak Republic.

E-mail: zuzana.vargova@upjs.sk

^b NMR Laboratory, Faculty of Science, P. J. Šafárik University, Moyzesova 11, SK-041 54 Košice, Slovak Republic

^c Department of Chemistry, Faculty of Education, J. Selye University, Bratislavská cesta 3322, SK-945 01 Komárno, Slovak Republic

^d Department of Inorganic Chemistry, Faculty of Science, Charles University, Hlavova 2030/8, CZ-128 43 Prague 2, Czech Republic

[†] Electronic supplementary information (ESI) available. CCDC 1528770. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c7nj00254h



the thermodynamic stability of complexes of aminophosphonates is usually higher than that of aminocarboxylates, which is caused, at least in the first approximation, by the enhanced basicity of the ligands.

Investigation of metal–phosphonate systems is often hampered by the formation of insoluble complexes and, thus, also the aminomethylphosphonate complexing ability towards Zn(II) has not attracted much attention.²² Protonated $[\text{Zn}(\text{Hamp})]^+$, neutral $[\text{Zn}(\text{amp})]$ and anionic $[\text{Zn}(\text{OH})(\text{amp})]^-$ species were identified and their coordination modes were proposed.

Here, we report the results of the study of ternary complexes of the Zn(II)–cyclen system with aminomethylphosphonic acid. The system was studied by a combination of potentiometry, ^1H , ^{13}C , ^{31}P NMR titration and mass spectrometry.

Experimental

Reagents and solutions

All solutions were prepared using deionized water. Aminomethylphosphonic acid (H₂amp) was synthesized according to the previously described procedure.²³ Cyclen was obtained from CheMatech (France). Zn(NO₃)₂·4H₂O was purchased from Lachema. The others chemicals were of analytical purity.

Potentiometric titration

Potentiometric measurements were accomplished using a PHM 220 pH-meter, an ABU 901 autoburette and a GK 2401 B combination electrode (Radiometer) in a glass vessel (10 cm³) thermostatted at 25 ± 0.1 °C at an ionic strength of $I = 0.1$ M (KNO₃). An inert atmosphere was ensured by constant flow of nitrogen gas. Precision calibration was accomplished using 0.028 M HNO₃ and 0.192 M KOH in the pH range 1.8–12.0, with the pH-meter yielding E values. The relation between E and $-\log[\text{H}^+]$ can be described as:

$$E = E^0 - S(-\log[\text{H}^+]) + j_a[\text{H}^+] + j_b(\text{p}K_w/[\text{H}^+]),$$

where E^0 presents the standard potential of the electrodes and the contribution of inert ions to the liquid-junction potential. S corresponds to the Nernstian slope and $j_a[\text{H}^+]$ and $j_b[\text{OH}^-]$ are the contributions of H⁺ and OH[–] ions, respectively, to the liquid-junction potential. The pK_w value was 13.78.

The protonation constants of the ligands (H₂amp or cyclen) were determined at $c_L = 0.004$ M. For the determination of the stability constants in the binary systems, the metal:ligand molar ratios were 1:1, 1:2, 1:4 for the Zn(II)–amp system and 1:1 for the Zn(II)–cyclen system ($c_{\text{Zn}} = 0.004$ M). In the ternary system, the Zn:cyclen:amp ratio was 1:1:1 ($c_{\text{Zn}} = 0.004$ M). Due to slow complexation kinetics (4 to 5 hours were required to reach equilibrium), the binary system Zn(II)–cyclen and the ternary system were studied by the out-of-cell method with an equilibration time a 3 days.²⁴ Three titrations were performed for each system and each ratio. Typical titration curves are shown in Fig. S4 (ESI[†]).

^1H , ^{13}C and ^{31}P NMR characterization

^1H , ^{13}C and ^{31}P NMR spectra were recorded on a Varian VNMRS 600 MHz spectrometer (resonance frequency for ^1H = 599.870 MHz,

^{13}C = 150.836 MHz, and ^{31}P = 242.836 MHz). Samples were prepared in H₂O and measured with a D₂O insert containing 0.5% *t*-BuOH (1.25 ppm) as a reference. ^1H spectra were measured using a PRESAT pulse sequence. NMR measurements were accomplished for free H₂amp, for the binary system Zn(II)–amp and for the ternary system Zn(II)–cyclen–amp. The initial concentration of individual species was 0.05 M for both binary and ternary systems. Cyclen was used in 10% excess. For the ternary system, Zn(II) and cyclen solution were mixed and left for 4 h at RT before H₂amp solution addition. The pH was adjusted with 0.1 M aq. NaOH and 0.1 M aq. HNO₃.

^{31}P NMR titration

The stock solution of the Zn(II)–cyclen complex was prepared by dissolving cyclen in Zn(NO₃)₂ solution. To assure full complexation, the ligand was used in 10% excess and the solution was heated at 80 °C overnight. The aliquot part of the stock solution was mixed with an appropriate amount of H₂amp solution to reach H₂amp–Zn(II) ratios of 2:1 to 1:12. The concentration of H₂amp in each sample was $c_{\text{amp}} = 20$ mM. For each ratio, several samples were prepared in the pH range 4–10 (adjusted with 0.1 M aq. NaOH or 0.1 M HNO₃). Finally, ^{31}P NMR spectra were recorded.

Data treatment

The protonation constants and the stability constants of the complexes in the binary systems (Zn(II)–cyclen and Zn(II)–amp) were determined from potentiometry data. The stability constants of the ternary complexes were determined by simultaneous treatment of potentiometry data and data from ^{31}P NMR titration. All data were processed using the program OPIUM.²⁵ The protonation constants of ligands β_{110} , β_{210} and β_{310} are concentration constants and are defined as $\beta_{110} = [\text{HL}]/[\text{H}][\text{L}]$, $\beta_{210} = [\text{H}_2\text{L}]/[\text{H}]^2[\text{L}]$, $\beta_{310} = [\text{H}_3\text{L}]/[\text{H}]^3[\text{L}]$, ($\text{p}K_3 = \log \beta_{110}$, $\text{p}K_2 = \log \beta_{210} - \log \beta_{110}$, $\text{p}K_1 = \log \beta_{310} - \log \beta_{210}$). The stability constants in binary systems are defined as $\beta_{\text{pqr}} = [\text{H}_p\text{L}_q\text{M}_r]/[\text{H}]^p[\text{L}]^q[\text{M}]^r$. In the ternary system, the stability constants are defined as $\beta_{\text{pqrs}} = [\text{H}_p\text{L}_q^1\text{L}_r^2\text{M}_s]/[\text{H}]^p[\text{L}]^q[\text{L}]^r[\text{M}]^s$ (L^1 = cyclen and L^2 = amp).

Mass spectrometry

Stock solutions of ZnCl₂, H₂amp and cyclen were mixed to reach $c_{\text{Zn}} = c_{\text{cyclen}} = 1$ mM and $c_{\text{amp}} = 1\text{--}10$ mM or $c_{\text{amp}} = 1$ mM and $c_{\text{Zn}} = c_{\text{cyclen}} = 1\text{--}10$ mM. A solution of NMe₄OH was added to reach pH ~ 7 and ESI-MS spectra were measured on a Bruker Esquire 3000 apparatus in the positive mode.

Synthesis of $[\text{Zn}(\text{Hamp})_2] \cdot 4\text{H}_2\text{O}$

Zinc perchlorate hexahydrate (134 mg, 0.360 mmol) in water (10 mL) was slowly added into an aqueous solution (10 mL) of cyclen (62 mg, 0.360 mmol). After standing for 24 hours, an equimolar amount of H₂amp (40 mg, 0.360 mmol) in water (10 mL) was added and the solution was kept at room temperature (pH = 4.5). After five days, very fragile colourless crystals, suitable for X-ray analysis, were obtained.



Table 1 Crystallographic data of the $[\text{Zn}(\text{Hamp})_2] \cdot 4\text{H}_2\text{O}$ complex

	$[\text{Zn}(\text{H}_2\text{O})_4(\text{Hamp})_2]$
Empirical formula	$\text{C}_2\text{H}_{17}\text{N}_2\text{O}_{10}\text{P}_2\text{Zn}$
M_r	356.49
Crystal colour	White
Crystal system	Orthorhombic
Space group	$Pca/21$
Unit cell dimensions	
a (Å)	9.9779(4)
b (Å)	12.0890(5)
c (Å)	10.2249(5)
α (°)	90.00
β (°)	90.00
γ (°)	90.00
V_c (Å ³)	1233.36
Molecules per cell, Z	4
D_{calc} (g cm ⁻³)	1.920
μ (mm ⁻¹)	2.295
$2\theta_{\text{max}}$	55°
Measured reflections	10 197
Independent reflections	2824
$R_1[I > 2\sigma(I)]$	0.0237
wR_2	0.0489

X-ray crystallography

Single-crystal X-ray measurements of the complex $[\text{Zn}(\text{Hamp})_2] \cdot 4\text{H}_2\text{O}$ was performed on a Nonius Kappa four-circle CCD diffractometer equipped with a Bruker APEX II detector and using graphite monochromated MoK α radiation ($\lambda = 0.71073$ Å). Diffraction data were processed using the diffractometer software.²⁶ The structure model was refined by full-matrix least-squares on F^2 using SHELXL 97.²⁷ All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in their ideal positions and refined isotropically. The structural figures were drawn using the DIAMOND 3.0 software.²⁸ Crystallographic data and processing parameters are given in Table 1.

Results and discussion

Speciation and stability constants

To describe the interaction modes of H₂amp with the Zn(II)-cyclen species, NMR spectra were measured in solutions equilibrated at different pH values. In the ¹H NMR spectra of free H₂amp and those obtained for Zn(II)-amp system, one doublet signal of CH₂ group (coupling to ³¹P with $J = 13.2$ Hz) was observed (Fig. S1 in ESI[†]). Similarly, the ¹³C NMR spectra show a doublet with $J = 141.8$ Hz (Fig. S2 in ESI[†]). In the ³¹P NMR spectra, one signal of the phosphorus nucleus (coupling from ¹H) was noted (Fig. S3 in ESI[†]).

In the ternary system, the changes in the ¹H NMR spectra of cyclen (Fig. 1) are similar to those previously reported.^{12,24} The signal of free cyclen disappears at pH 3.5–4 due to Zn(II) complexation. The two multiplets of the Zn(II)-cyclen complex remain unchanged over the whole pH range, which indicates the same coordination mode of cyclen in all species. In the ternary system, H₂amp shows a characteristic pH dependence of δ_{H} and δ_{C} , however, the differences from free H₂amp and from the binary Zn(II)-amp system are not significant and, so,

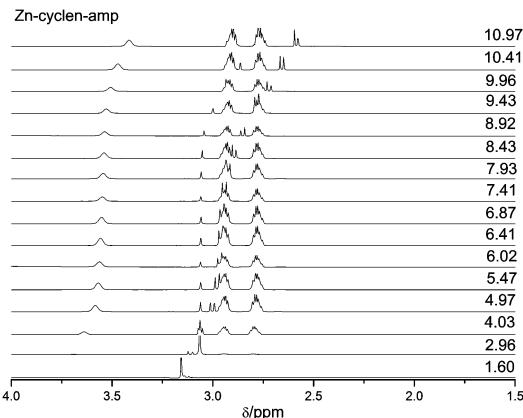


Fig. 1 pH changes of the ¹H NMR spectra of the ternary Zn(II)-cyclen-amp system. $c_{\text{amp}} = c_{\text{Zn}} = 0.05$ M, $c_{\text{cyclen}} = 0.06$ M, 25 °C.

they do not provide clear hints of the coordination mode (Fig. 2A and B). Both ¹H and ¹³C NMR spectra show similar trends as observed in systems with glycine (Gly),¹² in which analogous species $[\text{Zn}(\text{cyclen})(\text{HGly})]^{2+}$ and $[\text{Zn}(\text{cyclen})(\text{Gly})]^+$ were determined. More information could be obtained from ³¹P NMR data (Fig. 2C). The changes observed for free H₂amp are consistent with previous results.²⁹ The decrease of the δ_{P} at pH 5–6 indicates intramolecular interaction of the deprotonated phosphonate group with the protonated amino group. The largest changes in the ³¹P NMR shift are caused by deprotonation of the amino group at pH > 9. The binary system Zn(II)-amp could not be studied at pH above 7 due to the precipitation of the polymeric hydroxido species. However, a significant decrease of the δ_{P} at pH 5–6 could be observed. Comparison with distribution diagrams (Fig. S6 in ESI[†]) shows that the change is associated with deprotonation of the amino group as result of *N,O*-chelate coordination of the ligand. So, the decrease of the δ_{P} results from the formation of a cyclic structure.

In the ternary system, the pH dependence of δ_{P} is different; δ_{P} remains constant up to pH 8. The increase at higher pH results from deprotonation of the amino group. The absence of the δ_{P} drop at pH 5–6 indicates that H₂amp should be coordinated only through the phosphonate group in the ternary system. The phosphonate group might be coordinated in a bidentate mode as suggested by Kimura³⁰ or a monodentate mode with additional interactions with cyclen amine groups through hydrogen bonds.

The determination of the stability constants of the ternary complexes requires detailed and precise characterization of all subsystems under identical conditions. So, the protonation constants of the ligands (cyclen and H₂amp) as well as the stability constants in the binary systems (Zn(II)-amp and Zn(II)-cyclen) had to be redetermined (Tables S1–S4 in ESI[†]).

The protonation constants of H₂amp as well as the stability constants of the complexes with Zn(II) (Tables S1 and S2 in ESI[†]) were found to be close to those previously reported.^{22,31} The complexes in the Zn(II)-amp system are formed in the pH range 4–10. The distribution diagrams are shown in Fig. S5 and S6 (ESI[†]). The dominant species at pH 7–8 is the $[\text{Zn}(\text{amp})]$ complex.



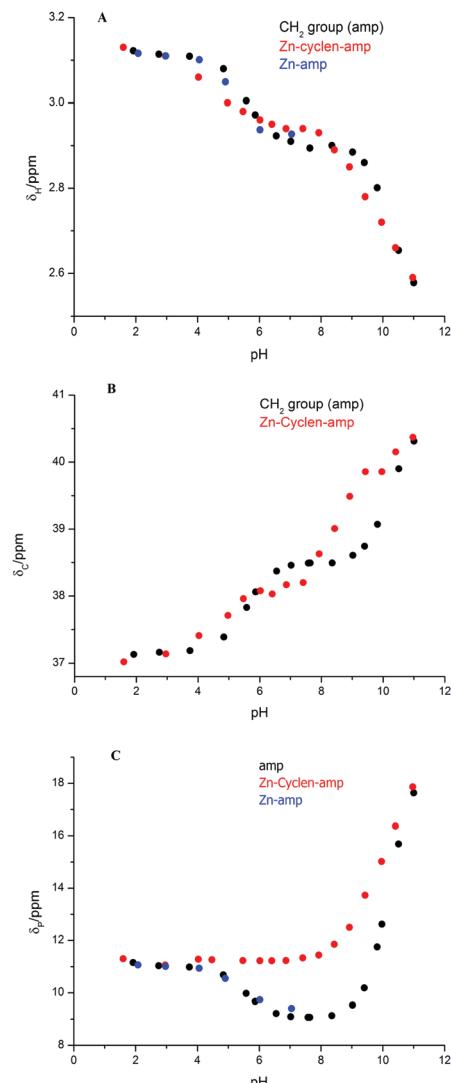


Fig. 2 pH dependence of the ^1H (A), ^{13}C (B) and ^{31}P (C) NMR shifts of H_2amp . Free H_2amp (black), binary $\text{Zn}(\text{II})$ -amp system (blue) and ternary $\text{Zn}(\text{II})$ -cyclen-amp (red) system. $c_{\text{amp}} = c_{\text{Zn}} = c_{\text{cyclen}} = 0.05 \text{ M}$, 25°C .

The protonated complex $[\text{Zn}(\text{Hamp})]^+$ is present in low abundance (about 10%). The derived value of induced deprotonation $\log \beta_{111} - \log \beta_{011} = 6.03$ is significantly lower than the pK value of free amp due to the high stability of the complex. It indicates the formation of the N,O -chelate. Above $\text{pH} \sim 8$, the formation of the hydroxido-species $[\text{Zn}(\text{amp})(\text{OH})]^-$ and less abundant $[\text{Zn}(\text{amp})_2]^{2-}$ is observed.

Because of the slow formation kinetics of macrocyclic complexes, the “out-of-cell” method was used for potentiometric titration of the binary system $\text{Zn}(\text{II})$ -cyclen. The protonation constants and stability constants of the complexes (Tables S3 and S4 in ESI†) are in very good agreement with those previously reported.²⁴ The distribution diagrams are shown in Fig. S7 and S8 in the ESI.† The dominant species in the pH range 5–7 is non-protonated $[\text{Zn}(\text{cyclen})]^{2+}$. The protonated complex $[\text{Zn}(\text{Hcyclen})]^{3+}$ was identified in low abundance at $\text{pH} \sim 4$. In the alkaline range, the coordinated water molecule is

deprotonated forming hydroxido complexes $[\text{Zn}(\text{cyclen})(\text{OH})]^+$ and $[\text{Zn}(\text{cyclen})(\text{OH})_2]$.

The ternary system $\text{Zn}(\text{II})$ -cyclen-amp was studied by a combination of potentiometry and ^{31}P NMR. Each of the methods separately was found to be insufficient. The data obtained from potentiometry have shown the low stability of the ternary complexes; and mostly, the formation of the complexes is not associated with a change in the protonation state of the ligands (e.g. coordination of the deprotonated phosphonate). ^{31}P NMR titration is more suitable as it allows utilizing some components in a large excess. It leads to a higher abundance of the ternary complexes and better determination of their stabilities. However, the studied system contains many species differing in their protonation states and NMR titration could hardly distinguish between these species. So, only the combination of both techniques gave reliable speciation as well as stability constants of the ternary system. Data were treated with the program OPIUM²⁵ that allows simultaneous treatment of data obtained by various analytical techniques.

Potentiometry was performed using the “out-of-cell” method with equimolar concentrations of all components. ^{31}P NMR titration was performed at various pH values 4–10 and at amp:Zn ratios from 2:1 to 1:12. In each sample, cyclen was used in 10% molar excess over $\text{Zn}(\text{II})$ to assure full complexation. The results show significant differences between the ^{31}P NMR spectra of free H_2amp and those measured in the presence of the $\text{Zn}(\text{II})$ -cyclen complex (Fig. 3). The stability constants presented in Table 2 and Table S5 in the ESI† represent the best model obtained from simultaneous fitting of the potentiometry and NMR data.

The distribution diagrams (Fig. 4) show that the only ternary species $[\text{Zn}(\text{cyclen})(\text{Hamp})]^+$ is formed in significant abundance. This species is present in the pH range 4–9. Its abundance ranges from 60% at equimolar concentrations of all components to >90% at a 5-fold excess of $\text{Zn}(\text{II})$ and cyclen. At higher pH, the ternary complex undergoes deprotonation yielding $[\text{Zn}(\text{cyclen})(\text{amp})]$. However, its abundance does not exceed 20% even at a 5-fold

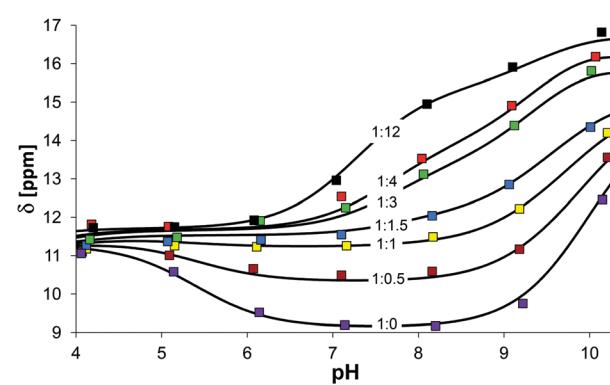


Fig. 3 Ternary system $\text{Zn}(\text{II})$ -cyclen-amp. Dependence of the ^{31}P NMR chemical shift of H_2amp on pH for various amp:Zn ratios (the numbers in the plot give the ratio for each series; $c_{\text{amp}} = 20 \text{ mM}$, 25°C). In each sample, cyclen was used in 20% molar excess over $\text{Zn}(\text{II})$. The curves were constructed using the ^{31}P NMR shifts of individual species calculated simultaneously with the determination of stability constants.



Table 2 Protonation and stability constants of the ternary complexes in the Zn(II)-cyclen-amp system (25 °C)

Equilibrium	$\log K$
$[\text{Zn}(\text{cyclen})]^{2+} + \text{Hamp}^- \rightarrow [\text{Zn}(\text{cyclen})(\text{Hamp})]^+$	3.05
$[\text{Zn}(\text{cyclen})]^{2+} + \text{amp}^{2-} \rightarrow [\text{Zn}(\text{cyclen})(\text{amp})]$	3.3
$[\text{Zn}(\text{cyclen})(\text{amp})] + \text{H}^+ \rightarrow [\text{Zn}(\text{cyclen})(\text{Hamp})]^+$	9.8
$[\text{Zn}(\text{cyclen})(\text{amp})] + [\text{Zn}(\text{cyclen})]^{2+} \rightarrow [\{\text{Zn}(\text{cyclen})\}_2(\text{amp})]^{2+}$	3.06
$[\{\text{Zn}(\text{cyclen})\}_2(\text{amp})(\text{OH})]^{2-} + \text{H}^+ \rightarrow [\{\text{Zn}(\text{cyclen})\}_2(\text{amp})]^{2+}$	9.4

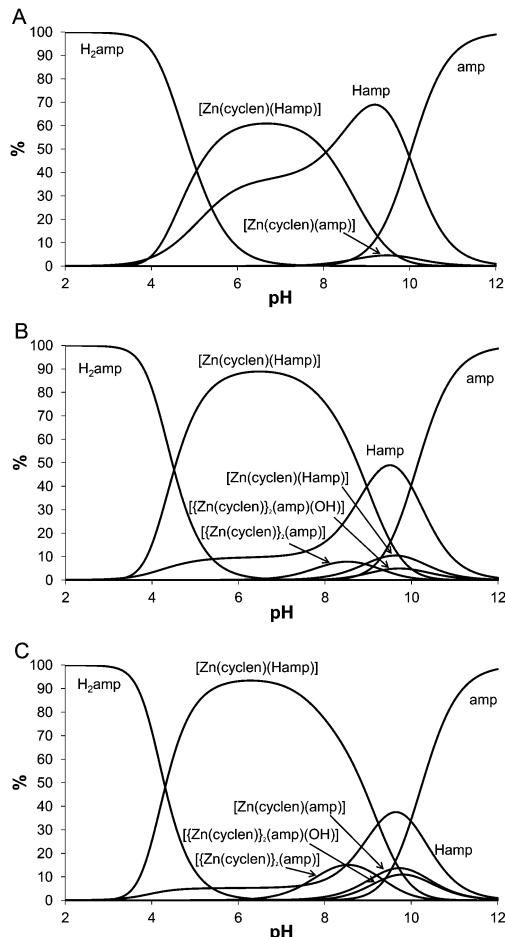


Fig. 4 Distribution of aminomethylphosphonate in the ternary system Zn(II)-cyclen-amp (A: $c_{\text{amp}} = c_{\text{cyclen}} = c_{\text{Zn}} = 4 \text{ mM}$; B: $c_{\text{amp}} = 4 \text{ mM}$, $c_{\text{cyclen}} = c_{\text{Zn}} = 12 \text{ mM}$; C: $c_{\text{amp}} = 4 \text{ mM}$, $c_{\text{cyclen}} = c_{\text{Zn}} = 20 \text{ mM}$; $I = 0.1 \text{ M}$, 25 °C).

excess of Zn(II) and cyclen over H₂amp. The low abundance of the [Zn(cyclen)(amp)] complex is given by the competition of the hydroxide anion. Water molecules coordinated to the metal ion in the [Zn(cyclen)]²⁺ complex are deprotonated at pH ~ 8. At higher pH, the coordination of the hydroxide anion dominates over the formation of ternary complexes which leads to an increasing abundance of free monoprotonated Hamp (Fig. 4A) and low abundance of the [Zn(cyclen)(amp)] complex.

At an excess of [Zn(cyclen)]²⁺ over H₂amp, dinuclear complexes with stoichiometries $[\{\text{Zn}(\text{cyclen})\}_2(\text{amp})]^{2+}$ and $[\{\text{Zn}(\text{cyclen})\}_2(\text{amp})(\text{OH})]^{2-}$ are formed. However, their abundance is low (< 20%) even at a 5-fold excess of the Zn(II)-cyclen complex over H₂amp (Fig. 4C).

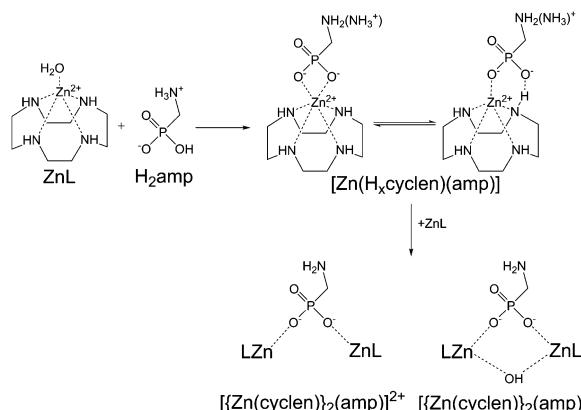
The formation of the ternary complexes is also evidenced by the ESI-MS spectra of the Zn(II)-cyclen-amp system (Fig. S9, ESI[†]). Under equimolar conditions, the dominant detected ion is $[\text{Zn}(\text{cyclen})\text{Cl}]^+$ ($m/z = 271$) and a minor signal of $[\text{Zn}(\text{cyclen})(\text{Hamp})]^+$ ($m/z = 346$) was observed. At a 10-fold excess of H₂amp, the latter signal becomes dominant indicating an increased abundance of the ternary complex in the system. At a 10-fold excess of the Zn(II)-cyclen complex, the detected signal centered at $m/z = 581$ confirms the formation of the dinuclear species $[\{\text{Zn}(\text{cyclen})\}_2(\text{amp})]$.

The speciation is similar to that previously reported in ternary systems of Zn(II)-cyclen with glycine and alanine except for the dinuclear species.¹² On the other hand, such species in which an amino acid connects two [Zn(cyclen)]²⁺ units was observed in the solid-state structure of $[\{\text{Zn}(\text{cyclen})\}_2(\text{picolinic acid})]^+$.²⁴ The value of the protonation constant of the ternary complex ($\log K = 9.8$) is close to the first protonation constant of H₂amp ($\log K = 9.99$). So, $[\text{Zn}(\text{cyclen})(\text{Hamp})]^+$ is protonated on the amino group. The constants describing the coordination of non-protonated amp²⁻ and monoprotonated Hamp⁻ to [Zn(cyclen)]²⁺ are 3.3 and 3.05, respectively. Similar values indicate negligible role of the amino group in the coordination. As suggested above, monodentate or bidentate coordination only through the phosphonate group could be expected in both species. It would point to the bidentate coordination of deprotonated phosphonate in both $[\text{Zn}(\text{cyclen})(\text{Hamp})]^+$ and $[\text{Zn}(\text{cyclen})(\text{amp})]$ species as expected by Kimura³⁰ or to the oxygen interaction of the O-P group with the NH group of cyclen.

The positive charge localized on the protonated amino group in $[\text{Zn}(\text{cyclen})(\text{Hamp})]^+$ does not allow the approach of the additional [Zn(cyclen)]²⁺ unit. So, the dinuclear species are formed only after amine deprotonation. The constants describing the coordination of the first and the second [Zn(cyclen)]²⁺ unit are also similar (3.3 and 3.06, respectively). It is in agreement with the high ability of the phosphonate groups for the bridging of the metal ions. Deprotonation of the coordinated water molecule leads to the dinuclear hydroxido species. The corresponding protonation constant ($\log K = 9.4$) is lower than that of the amino group in $[\text{Zn}(\text{cyclen})(\text{Hamp})]^+$. It shows a preferential coordination of the hydroxide anion that does not allow the N,O-bidentate coordination of H₂amp. It might also indicate that the hydroxide anion also bridges the two Zn(II) ions. The suggested structures of the ternary species are shown in Scheme 1, and the structures of amp and the Zn(II)-cyclen species identified in the studied systems are shown in Scheme S1 (ESI[†]).

Similar systems with amino acids Zn(II)-cyclen-Gly or Ala show stronger coordination ($\log K = 4.1$ and 4.0, respectively).¹² It does not correspond to the basicity of the amino groups of the ligands and so, it also excludes the participation of the amino group in metal binding. If we compare the sterical requirements of phosphonate and carboxylate groups, phosphonate is bulkier and both, P-C and P-O, bonds are longer. Thus, phosphonate occupies more space than carboxylate, which could lead to a preferential square-pyramidal coordination sphere of Zn(II). On the other hand, the O-P-O angle is smaller than in carboxylates





Scheme 1 Suggested structures of species identified in the ternary system Zn(II)-cyclen-amp.

and the bidentate coordination of phosphonate and the formation of an octahedral sphere is also possible.

There are many aminophosphonates and aminophosphinates that are used and studied as enzyme inhibitors in medicine or agriculture. The results presented here show that the interaction of aminophosphonates with the Zn(II) centre of the model complex is rather weak and even weaker interaction could be expected for aminophosphinates. The described coordination interaction is insufficient for effective binding of the inhibitor in the enzyme active site. It indicates that the binding of aminophosphonates and aminophosphinates in the enzyme active centre must be dominantly realized through hydrogen bonds and/or through non-bonding interactions with amino acid side chains. As the geometry of each enzyme active site is unique, it implicates a high specificity of the enzyme-inhibitor interaction for aminophosphonate and aminophosphinate inhibitors. It also explains, *e.g.* the low animal toxicity and the high selectivity of the most utilized herbicide glyphosate (phosphonomethylglycine).

Zn(II)-amp coordination modes in the solid state

Despite many attempts to prepare crystals of the studied complexes, only single crystals of $[\text{Zn}(\text{Hamp})_2] \cdot 4\text{H}_2\text{O}$ were isolated as the substance with the lowest solubility product. The solid-state structure is shown in Fig. S10 in the ESI[†] and the principal bond lengths and angles and the list of possible hydrogen bonds are summarized in Tables S6 and S7 in the ESI.[†] The complex crystallizes in the orthorhombic space group $Pca/2_1$ with four formula units per cell. The central Zn(II) atom coordination sphere is formed by four oxygen atoms of the phosphonate part of the aminomethylphosphonate ligand. The coordination polyhedron around the metal centre can be described as a tetrahedron with bond lengths of Zn–O1 1.915(2), Zn–O3 1.940(2), Zn–O4 1.955(2), Zn–O5 1.933(2) Å and bond angles around 104.9° (Table S6 in ESI[†]). The typical feature of the phosphonate coordination way, bridging two metal ions with two oxygen atoms of the phosphonate group and the formation of a polymeric network, was also found in this structure. As this study shows, the formation of the bridged structure is not only the

result of the crystal packing, but also originates from the Zn(II)-phosphonate interaction in solution. However, this crystal structure was determined previously³² and all parameters and bond lengths of both determinations were found to be very similar.

The resulting structure of the Zn(II)-amp complex is sensitive to reaction conditions as found for the published structures. The analogous reaction between ZnCl_2 and H_2amp upon gentle heating led to a polymeric product in which the coordination sphere was formed with three oxygen atoms from three phosphonates and one chloride anion.³³ The formation of the chelate was observed in the case of derivatives with a substituted amino group. The hydrothermal reaction of (1-phenylethyl) aminomethylphosphonic acid with $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and NaCl in a 1:1:0.5 molar ratio, adjusted to $\text{pH} = 5.7$ at 140°C , afforded crystals in which the unit contains two independent Zn(II) ions, two phosphonate ligands, and one chloride anion. Zn1 possess the O_3N_1 coordination sphere in which one ligand molecule is coordinated in the N,O -chelating mode and two ligand molecules are coordinated through one phosphonate oxygen atom. The coordination sphere around Zn2 is formed by three phosphonate oxygen atoms and one chloride anion.³⁴

Conclusion

The interaction of aminomethylphosphonic acid (H_2amp) with the Zn(II)-cyclen complex was studied as a model for enzyme interactions with phosphonates. The stabilities of the complexes were evaluated by a combination of potentiometry and multinuclear NMR. Aminomethylphosphonic acid binds weakly to the Zn(II)-cyclen complex in both monoprotonated and deprotonated forms. Taking into account both the trends based on the solid-state structures and thermodynamic constants determined in this study, coordination only through the phosphonate group is suggested for both mononuclear species $[\text{Zn}(\text{cyclen})(\text{Hamp})]^+$ and $[\text{Zn}(\text{cyclen})(\text{amp})]$. In dinuclear species $[[\text{Zn}(\text{cyclen})_2(\text{amp})]]^{2+}$, the phosphonate group bridges two $[\text{Zn}(\text{cyclen})]^{2+}$ units. This coordination way is probably more favorable than the formation of $[\text{Zn}(\text{cyclen})(\text{amp})]$. The coordination of the amine group is not expected. However, amine as well as phosphonate could further stabilize the structure through hydrogen-bonds with cyclen nitrogen atoms. A similar interaction was previously reported in ternary systems with amino acids.³⁵ The dinuclear species were not described in the solutions of ternary complexes with amino acids¹² and dipeptides,¹³ which documents the high bridging ability of the phosphonate group. The dinuclear species were reported only for ternary complexes with picolinic and nicotinic acids in the solid state.²⁴ In the alkaline range, the dinuclear hydroxide complex $[[\text{Zn}(\text{cyclen})_2(\text{amp})](\text{OH})]^+$ is formed.

Acknowledgements

Financial support was provided by a grant of the Ministry of the Education of the Slovak Republic (grant number KEGA 002UPJŠ-4/2015).



References

- 1 T. Heinisch and T. R. Ward, *Eur. J. Inorg. Chem.*, 2015, 3406.
- 2 K. E. Dalle and F. Meyer, *Eur. J. Inorg. Chem.*, 2015, 3391.
- 3 D. Desbouis, I. P. Troitsky, M. J. Belousoff, L. Spiccia and B. Graham, *Coord. Chem. Rev.*, 2012, **256**, 897.
- 4 P. A. Vigato, V. Peruzzo and S. Tamburini, *Coord. Chem. Rev.*, 2012, **256**, 953.
- 5 E. Kimura, *Acc. Chem. Res.*, 2001, **34**, 171.
- 6 S. Aoki and E. Kimura, *Comprehensive Coordination Chemistry*, Elsevier, Amsterdam, 2004, vol. 8, p. 601.
- 7 T. Koike, E. Kimura, I. Nakamura, Y. Hashimoto and M. Shiro, *J. Am. Chem. Soc.*, 1992, **114**, 7338.
- 8 S. Aoki and E. Kimura, *J. Am. Chem. Soc.*, 2000, **122**, 4542.
- 9 S. Aoki and E. Kimura, *Chem. Rev.*, 2004, **104**, 769.
- 10 S. Aoki, K. Iwaida, N. Hanamoto, M. Shiro and E. Kimura, *J. Am. Chem. Soc.*, 2002, **124**, 5256.
- 11 S. Aoki, D. Kagata, M. Shiro, K. Takeda and E. Kimura, *J. Am. Chem. Soc.*, 2004, **126**, 13377.
- 12 Z. Vargová, E. Balentová, M. Walko, L. Arabuli, P. Hermann and I. Lukeš, *J. Mol. Recognit.*, 2011, **24**, 295.
- 13 I. Rostášová, M. Vilková, Z. Vargová, M. Walko, M. Almáši, J. Imrich, P. Hermann and I. Lukeš, *J. Mol. Recognit.*, 2015, **28**, 211.
- 14 B. Nowack, *Water Res.*, 2003, **37**, 2533.
- 15 S. V. Kononova and M. A. Nesmeyanova, *Biochemistry*, 2002, **67**, 184.
- 16 G. DellaCioppa, S. C. Bauer, B. K. Klein, D. M. Shah, R. T. Fraley and G. Kishore, *Proc. Natl. Acad. Sci. U. S. A.*, 1986, **83**, 6873.
- 17 Q. Li, M. J. Lambrechts, Q. Zhang, S. Liu, D. Ge, R. Yin, M. Xi and Z. You, *Drug Des., Dev. Ther.*, 2013, **7**, 635.
- 18 V. P. Kukhar and H. R. Hudson, *Aminophosphonic and Aminophosphinic Acids. Chemistry and Biological Activity*, John Wiley and Sons, New York, 2000.
- 19 T. Kiss, I. Lázár and P. Kafarski, *Met.-Based Drugs*, 1994, **1**, 247.
- 20 T. Kiss, I. Lázár, V. P. Kukhar and H. R. Hudson, *Aminophosphonic and Aminophosphinic Acids. Chemistry and Biological Activity*, Wiley, New York, 2000, p. 285.
- 21 J. Rohovec, P. Vojtíšek, I. Císařová, P. Hermann and I. Lukeš, *J. Chem. Soc., Dalton Trans.*, 1996, 2685.
- 22 M. Wozniak and G. Nowogrocki, *Talanta*, 1979, **26**, 1135.
- 23 R. Tyka and G. A. Hagele, *Synthesis*, 1984, 218.
- 24 Z. Vargová, J. Kotek, J. Rudovský, J. Plutnár, R. Gyepes, P. Hermann, K. Györyová and I. Lukeš, *Eur. J. Inorg. Chem.*, 2007, 3974.
- 25 M. Kývala and I. Lukeš, 1995. International Conference, Chemometrics 95, Pardubice, Česká republika, p. 63, University of Pardubice, Pardubice, 3rd–7th July 1995, the full version of “OPIUM” is available (free of charge) on: <http://web.natur.cuni.cz/~kyvala/opium.html>.
- 26 A. Altomare, G. Cascarano, C. Giacovazzo and A. Guagliardi, *J. Appl. Crystallogr.*, 1993, **26**, 343.
- 27 G. M. Sheldrick, *Acta Crystallogr., Sect. A: Found. Crystallogr.*, 2008, **64**, 112.
- 28 K. Brandenburg, *DIAMOND, Version 2.1e*, Crystal Impact GbR, Bonn, 2000.
- 29 T. G. Appleton, J. R. Hall, A. D. Harris, H. A. Kimlin and I. J. McMahon, *Aust. J. Chem.*, 1984, **37**, 1833.
- 30 T. Koike and E. Kimura, *J. Am. Chem. Soc.*, 1991, **113**, 8935.
- 31 P. Hermann and I. Lukeš, *J. Chem. Soc., Dalton Trans.*, 1995, 2605.
- 32 P. Fenot, J. Darriet, C. Garrigou-Lagrange and A. Cassaigne, *J. Mol. Struct.*, 1978, **43**, 49.
- 33 C. R. Samanamu, E. N. Zamora, J. L. Montchamp and A. F. Richards, *J. Solid State Chem.*, 2008, **181**, 1462.
- 34 X. G. Liu, S. S. Bao, Y. Z. Li and L. M. Zheng, *Inorg. Chem.*, 2008, **47**, 5525.
- 35 Z. Vargová, R. Gyepes, L. Arabuli, K. Györyová, P. Hermann and I. Lukeš, *Inorg. Chim. Acta*, 2009, **362**, 3860.

