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Structures and reactivity of synthetic zinc(II) complexes resembling the active sites and reaction intermediates of aminopeptidases†

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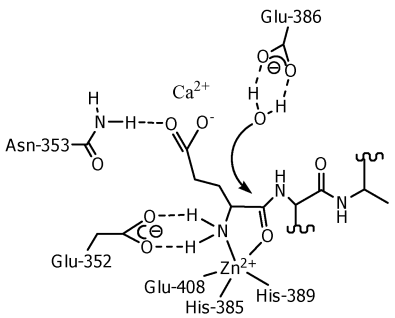
Herein we report the first crystallographic characterization and hydrolysis of a synthetic zinc(II) complex that resembles the active site and reaction intermediates proposed for aminopeptidases.

There is great interest in designing metal complexes that resemble the active site of metallopeptidases and capable of hydrolysing unactivated peptide bonds.[1] Such complexes not only could be useful in elucidating fundamental aspects of the enzyme chemistry but also find important applications in protein sequencing. Metallopeptidases typically require a zinc(II) ion with a co-ordination environment consisting of a glutamate and two histidine residues.[2] In addition, active-site residues participate in substrate binding and/or co-operate with the zinc(II) ion towards facilitating the amide hydrolysis reaction, but the effects associated with the second co-ordination sphere are not well understood.[3] An example of zinc metallopeptidase for which this co-ordination environment has been proposed is aminopeptidase A (APA), which specifically cleaves *in vitro* the N-terminal glutamyl or aspartyl residue from peptide substrates (Scheme 1).[4]

Several metal complexes have been used as synthetic models for metallopeptidases. Substitutionally inert metal complexes of Co(III) have demonstrated that amide hydrolysis can be promoted through activation of the carbonyl and/or intramolecular attack of a metal bound hydroxide.[5] Complexes of Cu(II) and Zn(II) were also used to study the mechanism and reaction intermediates of metal-promoted amide-bond hydrolysis.[6] Because of the great stability of peptide bonds, however, these studies generally involved activated amides or amide tethered to the ligand. Recently, it has been shown that Pd(II) complexes spontaneously bind to the side chains of methionine and histidine residues and effect hydrolytic cleavage of short peptides.[7] To our knowledge the only metal complexes of biologically relevant Cu(II) and Zn(II) ions that are able to cleave unactivated peptides and/or proteins have been two Cu(II) complexes of two N_3 ligands.[8,9] Thus to date, no synthetic zinc(II) complex with double nitrogen and single carboxylate ligation has been reported. In this Communication, we report the structure of two zinc(II) complexes that resemble quite faithfully the zinc(II) ligation and several of the peripheral active site features of APA.



Scheme 1

Furthermore, we demonstrate that these complexes have the ability to hydrolyse the unactivated dipeptide glycylglycine (Gly-Gly) to glycine (Gly).

Perchlorate salts of $[(LH^+)_2Zn_2(Gly\text{-}Gly)_2]^{4+}$ **1** and $[(LH^+)Zn(Gly)]^{2+}$ **2** (L = 1-methyl-4-(6-amino-2-pyridinylmethyl)-piperazine; Gly = glycine anion, Gly-Gly = glycylglycine anion) were assembled by stirring a mixture of equimolar amounts of $Zn(ClO_4)_2\cdot 6H_2O$ and Gly-Gly for **1** and Gly for **2** in methanol. Crystals of the two complexes were grown by slow evaporation of a MeOH–water solution at room temperature and their structures were determined by X-ray crystallography.‡

In the crystal structures of **1** and **2** the zinc(II) centre is in a co-ordination environment between square pyramidal and trigonal bipyramidal (Figs. 1(i) and 2(i)).§ Whereas **1** adopts a dimeric structure of formulation $[(LH^+)_2Zn_2(Gly\text{-}Gly)_2](ClO_4)_4\cdot 2H_2O$ (Fig. 1(ii)), **2** is a $[(LH^+)Zn(Gly)](ClO_4)_2$ 'polymer'(Fig. 2(ii)). The Zn–N distances of Zn–N(1) 2.185(3) Å and Zn–N(2) 2.085(3) Å for **1** are slightly shorter than those of **2**, (Zn–N(1) 2.1996(17) Å and Zn–N(2) 2.0957(16) Å) presumably because Gly is a slightly stronger ligand than Gly-Gly. As expected, Zn–N distances in **1** and **2** are longer than those of the tetrahedral complex $(L)Zn(Cl)_2$.[10] Binding provided by L mimics the bis-histidine zinc-ligation in aminopeptidases. Binding of the Gly and Gly-Gly through the COO^- group mimics glutamate binding to the zinc(II) centres of aminopeptidases. In **1**, the peptide (Gly-Gly) is co-ordinated through the terminal NH_2 group and the carbonyl amide oxygen atom. This mode of co-ordination was recently suggested to account for the ease of hydrolysis of Gly-Gly with a *cis*,*cis*-1,3,5-triaminocyclohexane–copper(II) complex[9] (tach-Cu(II)), and also resembles the peptide binding mode proposed for APA.[11,12] Remarkably, the positioning of a water molecule O(1W) in the crystal structure of **1** would be suitable for attack on the carbonyl carbon atom C(3G) (Fig. 1, O(1W)···C(3G) 3.050 Å), which should be activated for

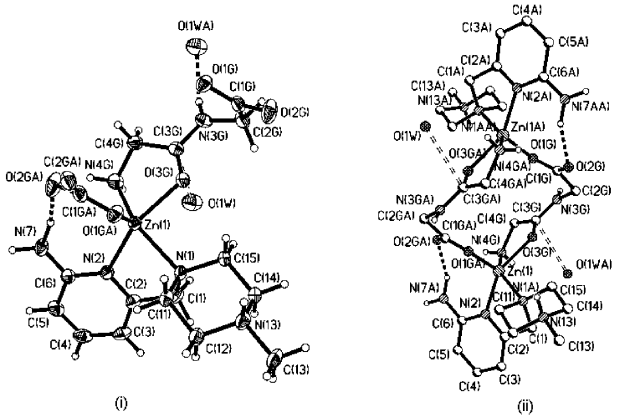


Fig. 1 (i) Thermal ellipsoid plot (50% probability) showing the zinc(II) co-ordination environment of **1**; (ii) structure of the $[(LH^+)_2Zn_2(Gly\text{-}Gly)_2]^{4+}$ cation with water molecules close to the amide bond. Selected bond lengths (Å) and angles (°): Zn(1)–N(1) 2.185(3), Zn(1)–N(2) 2.085(3), Zn–N(4G) 2.099(3), Zn–O(1GA) 1.952(2), Zn–O(3G) 2.094(2), O(1GA)–Zn–N(2) 112.98(11), O(1GA)–Zn–N(4G) 115.76(13), O(1GA)–Zn–O(3) 94.14(10), O(1GA)–Zn–N(1) 98.61(10), N(2)–Zn–O(3G) 151.52(11), N(2)–Zn–N(4G) 95.38(11), N(2)–Zn–N(1) 78.78(10), O(3G)–Zn–N(4G) 79.54(11), O(3G)–Zn–N(1) 89.08(10), N(4G)–Zn–N(1) 144.29(13).

† Electronic supplementary information (ESI) available: Experimental details (synthesis, characterization and hydrolysis studies). See http://www.rsc.org/suppdata/cc/b3/b314089j/

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nucleophilic attack by the zinc(II) centre. Moreover, like in the case of Gly-Gly hydrolysis with the tach-Cu(II) complex, the peptide chelate ring would not be required to be broken for hydrolysis to occur, as shown by the retention of this chelation motif in crystal structure of **2**. Thus Gly is chelated to one zinc(II) ion *via* the nitrogen atom and oxygen atom of the carboxylate, and bridges to the next zinc *via* the other oxygen atom. To our knowledge the only other example with this polymeric chain structure is [Cu(Gly)-$(NO_3)(H_2O)$].[13] The positioning of the NH_2 group of the ligand in **2** is such that forms H-bonding with both of the oxygens of the carboxylate group of Gly (N(7)···O(1G) 3.059 Å, N(7)···O(2G) 3.225 Å). Similarly, the amino group of the ligand in **1** is H-bonded to one of the oxygens of the zinc(II) bound carboxylate group of Gly-Gly (N(7)···O(2G) 2.957 Å). In principle, these H-bonding interactions may assist peptide binding and orient the bound peptide.

We have investigated the hydrolysis of **1** by NMR and found that Gly-Gly is hydrolysed to Gly at 70 °C and pH 7 ± 0.1 (50 mM HEPES) (Fig. 3).† Gly-Gly was cleaved at **1** and pH 7 with similar efficiency to copper(II) complexes of 1,4,7-triazacyclononane ([9]aneN_3) and *cis*,*cis*-1,3,5-triaminocyclohexane (tach) at pH 8.1. Unlike previously reported synthetic model complexes of metallopeptidases or peptide cleaving agents, however, **1** utilizes a biologically-relevant metal ion, zinc(II), in a biologically-relevant co-ordination environment and achieves cleavage of an otherwise unactivated peptide bond under mild physiological conditions.[14]

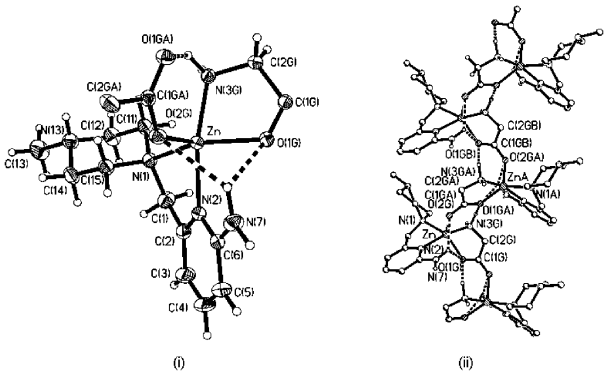

Fig. 2 (i) Thermal ellipsoid plot (50% probability) showing the zinc(II) co-ordination environment of **2**; (ii) The polymeric chain structure of the $[(LH^+)Zn(gly)]^{2+}$ cation. Selected bond lengths (Å) and angles (°): Zn–N(1) 2.1996(17), Zn–N(2) 2.0957(16), Zn–N(3G) 2.0799(17), Zn–O(1G) 2.0645(14), Zn–O(2GA) 2.0020(14), O(2GA)–Zn–O(1) 111.13(6), O(2GA)–Zn–N(3G) 102.08(6), O(1G)–Zn–N(3G) 81.41(6), O(2GA)–Zn–N(2) 94.52(6), O(1G)–Zn–N(2) 90.61(6), N(3G)–Zn–N(2) 163.24(7), O(2GA)–Zn–N(1) 103.88(6), O(1G)–Zn–N(1) 143.95(6), N(3G)–Zn–N(1) 99.79(7), N(2)–Zn–N(1) 78.09(6).

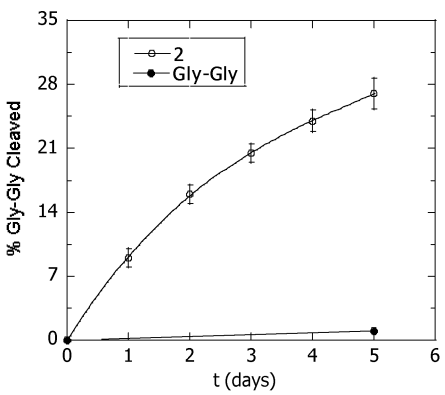

Fig. 3 Extent of hydrolysis of Gly-Gly of **1** and Gly-Gly to Gly at 70 °C and pH 7 ± 0.1 (50 mM HEPES) at different times.

In summary, the structures of two zinc(II) complexes with Gly-Gly and Gly co-ordinated to the zinc(II) centre are reported. These two complexes represent the first crystallographically characterized zinc(II) complexes in which the zinc(II) centre is in co-ordination environment that closely resembles the active site and reaction intermediates proposed for aminopeptidases. Furthermore, hydrolysis of Gly-Gly at the zinc(II)–Gly-Gly complex is achieved at physiological pH. Thus, to our knowledge, this is the first example of an unactivated peptide being hydrolysed by a small biomimetic zinc(II) complex.

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Notes and references

‡ *Crystal data* for **1**·$(ClO_4)_4$·$4H_2O$: $C_{30}H_{60}Cl_4N_{12}O_{26}Zn_2$, M = 1277.44, triclinic, space group $P\bar{1}$, a = 9.0776(10), b = 10.2002(11), c = 14.5845(16) Å, α = 104.467(2), β = 101.407(2), γ = 98.007(2)°, U = 1256.2(2) Å^3, T = 150(2) K, λ(Mo–Kα) = 0.71073 Å, Z = 1, D_c = 1.689 g cm^{-3}, μ = 1.265 mm^{-1}, 11411 reflections measured, 5900 unique, R_{int} = 0.0274 (all data), $R1$ = 0.0657 (all data), $wR2$ = 0.1438 (all data), S = 1.069 (all data), largest difference peak, hole 1.317, −0.550 e Å^{-3}.

For **2**·$(ClO_4)_2$: $C_{13}H_{23}Cl_2N_5O_{10}Zn$, M = 545.63, Monoclinic, space group $P2_1/n$, a = 16.0691(10), b = 7.9677(5), c = 16.3441(10) Å, α = 90, β = 96.9230(10), γ = 90°, U = 2077.3(2) Å^3, T = 150(2) K, λ(Mo–Kα) = 0.71073 Å, Z = 4, D_c = 1.745 g cm^{-3}, μ = 1.502 mm^{-1}, 18298 reflections measured, 5145 unique, R_{int} = 0.0260 (all data), $R1$ = 0.0389, $wR2$ = 0.0909 (all data), S = 1.047 (all data), largest difference peak, hole 0.885, −0.504 e Å^{-3}.

§ CCDC 223725–223726. See http://www.rsc.org/suppdata/cc/b3/b314089j/ for crystallographic data in .cif or other electronic format.

1 E. L. Hegg and J. N. Burstyn, *Coord. Chem. Rev.*, 1998, **173**, 133 and references therein.
2 W. N. Lipscomb and N. Sträter, *Chem. Rev.*, 1996, **96**, 2375.
3 R. Krämer, *Coord. Chem. Rev.*, 1999, **182**, 243.
4 (*a*) I. Nagatsu, T. Nagatsu, T. Yamamoto, G. G. Glenner and J. W. Mehl, *Biochim. Biophys. Acta*, 1970, **198**, 255; (*b*) S. Wilk and D. Healy, *Adv. Neuroimmunol.*, 1993, **3**, 195; (*c*) X. Iturrioz, R. Rozenfeld, A. Michaud, P. Corvol and C. Llorens-Cortes, *Biochemistry*, 2001, **40**, 14440.
5 (*a*) P. A. Sutton and D. A. Buckingham, *Acc. Chem. Res.*, 1987, **20**, 357; (*b*) J. T. Groves and L. A. Baron, *J. Am. Chem. Soc.*, 1989, **111**, 5442; (*c*) K. Takasaki, J. H. Kim, E. Rubin and J. Chin, *J. Am. Chem. Soc.*, 1993, **115**, 1157.
6 (*a*) L. Meriwether and F. H. Westheimer, *J. Am. Chem. Soc.*, 1956, **78**, 5119; (*b*) W. A. Connor, M. M. Jones and D. L. Tuleen, *Inorg. Chem.*, 1965, **4**, 1129; (*c*) J. T. Groves and R. J. Rife Chambers, Jr., *J. Am. Chem. Soc.*, 1984, **106**, 630; (*d*) T. H. Fife and T. J. Przystas, *J. Am. Chem. Soc.*, 1986, **108**, 4631; (*e*) J. Chin, V. Jubian and K. Mrejen, *J. Chem. Soc., Chem. Commun.*, 1990, 1326; (*f*) B. F. Duerr and A. W. Czarnik, *J. Chem. Soc., Chem. Commun.*, 1990, 1707; (*g*) L. M. Sayre, K. V. Reddy, A. R. Jacobson and W. Tang, *Inorg. Chem.*, 1992, **31**, 935; (*h*) J. Chin, V. Jubian and K. Mrejen, *J. Am. Chem. Soc.*, 1995, **117**, 7015.
7 T. N. Parac, G. M. Ullmann and N. K. Kostic, *J. Am. Chem. Soc.*, 1999, **121**, 3127 and references therein.
8 E. L. Hegg and J. N. Burstyn, *J. Am. Chem. Soc.*, 1995, **117**, 7015.
9 X. S. Tan, Y. Fujii, T. Sato, Y. Nakano and M. Yashiro, *Chem. Commun.*, 1999, 881.
10 J. C. Mareque Rivas, E. Salvagni, R. Torres Martín de Rosales and S. Parsons, *Dalton Trans.*, 2003, 3339.
11 It is interesting that the proposed H-bonding between Glu-386 and Glu-352 of APA and the nucleophilic water/hydroxide and co-ordinated NH_2 from the peptide substrates (Scheme 1), which are believed to be functionally critical,[12] are mimicked by similar H-bonds to the perchlorate anions in the crystal structure of **1**.
12 G. Vazeux, J. Wang, P. Corvol and C. Llorens-Cortes, *J. Biol. Chem.*, 1996, **271**, 9069.
13 H. O. Davies, R. D. Gillard, M. B. Hursthouse, M. A. Mazid and P. A. Williams, *J. Chem. Soc., Chem. Comm.*, 1992, 226.
14 The half-life of hydrolysis of Gly-Gly at pH 7 and 70 °C is *ca.* 2 years: see A. Radzicka and R. Wolfenden, *J. Am. Chem. Soc.*, 1996, **118**, 6105.