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The crystal structures of glycylglycine and glycine complexes of *cis,cis*-1,3,5-triaminocyclohexane–copper(II) as reaction intermediates of metal-promoted peptide hydrolysis

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The glycylglycine and glycine complexes of *cis,cis*-1,3,5-triaminocyclohexane–copper(II), model reaction intermediates of peptide hydrolysis by Cu^{II}–triamine complexes, have been synthesized and characterized by X-ray crystallography.

There has been great interest in designing artificial metallopeptidases that hydrolyze unactivated amides under mild conditions.[1,2] Up to now a variety of metal complexes of Cu^{II},[1,3] Zn^{II},[3c,d] Ni^{II},[3c,d] Pd^{II},[4] Co^{III}[2,5] and Ce^{IV}[6] have been used to study the hydrolysis of amides. Recently Burstyn and coworker[1] reported the discovery that a macrocyclic copper(II) complex, Cu[9]aneN_3, can hydrolyze both the unactivated dipeptide glycylglycine and proteins at near physiological pH. Although no reaction mechanism has been shown for Cu[9]aneN_3, in such a reaction, the activation of the carbonyl group of the amide by the metal centre seems to play an important role, and it is necessary to get information about the reaction intermediate to advance the hydrolysis reaction. We have been studying some copper(II)–triamine complexes which effectively promote the hydrolytic cleavage of phage DNA,[7] as well as peptide bonds. To understand the mechanism of the hydrolytic reaction of peptides by Cu^{II}–triamine complexes, we present here the crystal structures of glycylglycine and glycine complexes of *cis,cis*-1,3,5-triaminocyclohexane–copper(II) as model reaction intermediates.

We have investigated the hydrolysis of peptide with *cis,cis*-1,3,5-triaminocyclohexane–copper(II) complex[7] **1** by HPLC and found that glycylglycine is hydrolyzed to glycine when incubated with **1**.† The hydrolysis of glycylglycine (Gly-Gly) (2 mM) to glycine (Gly) with **1** (2 mM) at 70 °C and pH 8.1 ± 0.1 (50 mM HEPES) is *ca.* 18% for 24 h, which is similar to that of Cu[9]aneN_3, and the conversion is dependent on metal complex concentration, reaction time and solution pH.

The tetraphenylborate salts of $[Cu(tach)(gly\text{-}gly)]^+$ **2** and $[Cu(tach)(gly)]^+$ **3** (tach = *cis,cis*-1,3,5-triaminocyclohexane, gly = glycine anion, gly-gly = glycylglycine anion) were synthesized by stirring a mixture of $CuSO_4.5H_2O$ (1 mmol), tach (1 mmol) and Gly-Gly (1 mmol) for **2** or Gly (1 mmol) for **3** in MeOH–water at pH 8 (in the presence of $NaBPh_4$) in 40% yield for **2** and 60% yield for **3**, respectively. Blue crystals of the two complexes were grown by slow evaporation of a MeOH–water solution and the structures were characterized by X-ray crystallography.‡

The crystal structures (Figs. 1 and 2) of complexes **2** and **3** both show a five co-ordinate copper centre ligated to the three face capping nitrogens of the tach ligand. In each case the copper co-ordination geometry is square pyramidal distorted along the apical Cu(1)–N(3) bond with a distance of 2.232(3) Å in **2** and 2.152(6) Å in **3**. The other Cu–N distances, Cu(1)–N(1) and Cu(1)–N(2), are 1.990(3) and 2.015(2) Å in **2** and 2.019(6) and 2.042(6) Å in **3**, respectively, as in other copper-tach complexes.[8] The peptide (gly-gly) is coordinated through the terminal NH_2 group and the carbonyl oxygen atom from the same glycine residue. The carbonyl oxygen atom O(1) is here prefered to the peptide nitrogen N(5) as a metal binding site at pHs where the amide proton is not dissociated, as in the tripeptide complexes of $Cu(gly\text{-}gly\text{-}gly)^+$,[9] $Cu(gly\text{-}leu\text{-}tyr)^+$,[10] and $Zn(gly\text{-}gly\text{-}gly)^+$,[11] or the Co^{III}–tetraamine complex of the glycylglycine *O*-ethyl ester.[12] This mode of co-ordination accounts for the ease of hydrolysis of the dipeptide. The positively charged copper(II) ion renders the carbonyl carbon atom C(8) more susceptible to nucleophilic reagents (OH^-) and the peptide chelate ring is not required to break for hydrolysis to occur, so the NH_2-terminal glycine remains attached to the Cu(tach) residue to form [glycinato–copper(II)–tach] complex

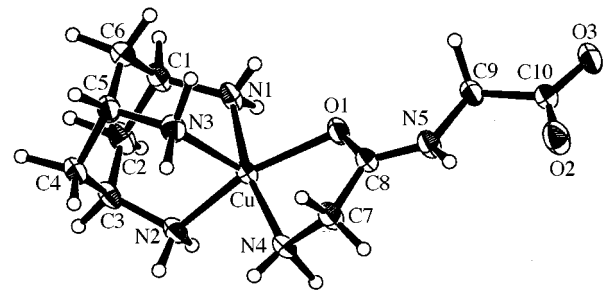


Fig. 1 ORTEP plot with 30% probability thermal ellipsoids showing the structure of the cation of **2**. Selected bond distances (Å) and angles (°): Cu–N(1) 1.990(3), Cu–N(2) 2.015(2), Cu–N(3) 2.232(3), Cu–N(4) 2.017(3), Cu–O(1) 2.039(2), O(1)–Cu–N(1) 89.38(9), O(1)–Cu–N(2) 162.32(10), O(1)–Cu–N(3) 104.52(9), O(1)–Cu–N(4) 80.32(9), N(1)–Cu–N(2) 92.61(9), N(1)–Cu–N(3) 91.5(1), N(1)–Cu–N(4) 167.49, N(2)–Cu–N(3) 93.00(9), N(2)–Cu–N(4) 95.09(9), N(3)–Cu–N(4) 97.9(1).

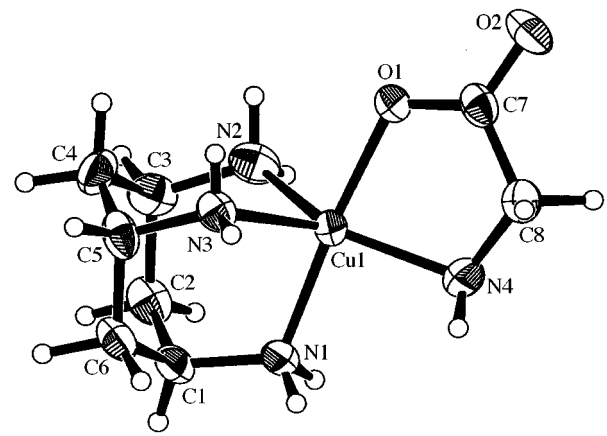
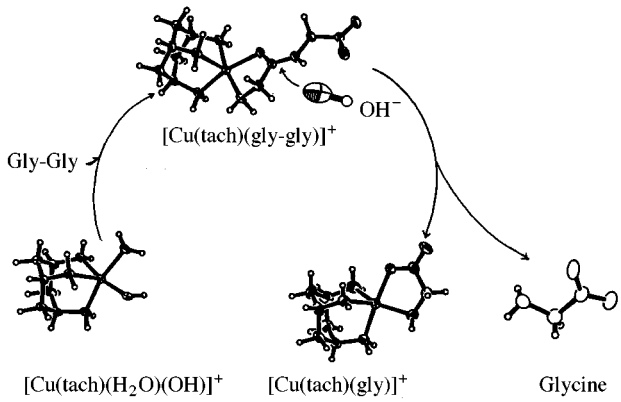


Fig. 2 ORTEP plot with 30% probability thermal ellipsoids showing the structure of the cation of **3**. Selected bond distances (Å) and angles (°): Cu–O(1) 1.974(4), Cu–N(1) 2.019(6), Cu–N(2) 2.042(6), Cu–N(3) 2.152(6), Cu–N(4) 2.007(6), O(1)–Cu–N(1) 173.6(2), O(1)–Cu–N(2) 87.5(2), O(1)–Cu–N(3) 97.0(2), O(1)–Cu–N(4) 82.6(2), N(1)–Cu–N(2), 92.8(3), N(1)–Cu–N(3) 89.4(2), N(1)–Cu–N(4) 93.2(2), N(2)–Cu–N(3) 92.7(2), N(2)–Cu–N(4) 141.4(2), N(3)–Cu–N(4) 125.5(2).



Scheme 1

ion, as shown in Fig. 2. Thus the complex cation of **2**, $[Cu(tach)(gly\text{-}gly)]^+$, is an intermediate of the hydrolysis of the N-terminal peptide bond promoted by Cu^{II}–triamine complex, and is clear evidence for the activation of the C=O group by the metal centre.

On the basis of the intermediate structure a reasonable mechanism may be postulated for the peptide hydrolysis at near physiological pH, as shown in Scheme 1. The initial step involves the rapid replacement of the water molecule by the N-terminal amino group of the peptide, which becomes chelated to the copper(II) ion. The carbonyl group is then activated to attack by external OH^-. The mechanism is supported by the fact that the rate of hydrolysis increases with increasing hydroxide concentration.§

Experiments to prove this mechanism *via* solution chemistry studies and kinetic measurements of the hydrolysis of peptides by Cu^{II}–triamine complexes are presently being conducted.

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Notes and references

† Glycylglycine (2 mM) was dissolved in water and incubated with **1** (2 mM) at pH 8.1 ± 0.1 (50 mM HEPES) and 70 °C for 24 h. Samples were analyzed in a JASCO Model FP-920 Intelligent Fluorescence Detector.

‡ *Crystal data* for **2**: $C_{35}H_{46}N_5O_4BCu$, M = 675.14, triclinic, space group $P\bar{1}$, a = 10.238(4), b = 18.026(7), c = 10.065(3) Å, α = 92.29(3), β = 113.71(3), γ = 101.13(3)°, U = 1654(1) Å^3, λ = 0.71069 Å, Z = 2, D_c = 1.355 g cm^{-3}, μ = 0.707 mm^{-1}, 7778 reflections measured, 5756 observed [$I > 3.00\ \sigma(I)$]. Solution by direct methods with SIR92, expanded using Fourier Techniques with DIRDIF94. Full-matrix least-square refinement on F with all non-hydrogen atoms anistropic and hydrogens included but not refined. Final R and Rw values on observed data were 0.050 and 0.051.

For **3**: $C_{32}H_{39}N_4O_2BCu$, M = 586.04, orthorhombic, space group $P2_12_12_1$, a = 9.879(2), b = 30.374(9), c = 9.769(2) Å, U = 2931(1) Å^3, λ = 0.71069 Å, Z = 4, D_c = 1.328 g cm^{-3}, μ = 0.781 mm^{-1}. 3808 reflections measured, 2857 observed [$I > 3.00\ \sigma(I)$]. Solution methods and refinements as for **2**. Final R and Rw values on observed data were 0.052 and 0.051. CCDC 182/1212. See http://www.rsc.org/suppdata/cc/1999/881/ for crystallographic files in .cif format.

§ The pH dependency is independent on the pK_a curve of $[Cu(tach)(H_2O)_2]^{2+}$ [ref. 7(*b*)], indicating that the attack of internal coordinated OH^- can be neglected.

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