# Conformational change in the thiazole and oxazoline containing cyclic octapeptides, the patellamides. Part 1. $Cu^{2+}$ and $Zn^{2+}$ induced conformational change †

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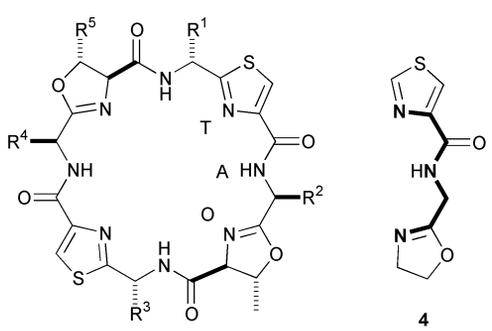
Conformational change during the binding of $Cu^{2+}$ and $Zn^{2+}$ to the thiazole and oxazoline containing cyclic octapeptides, the patellamides, is examined by a combination of experimental and theoretical methods. Circular dichroism and NOE-restrained molecular dynamics studies indicate that upon complexing with one equivalent of $Cu^{2+}$, patellamide C undergoes a change in conformation which pre-organises a second $Cu^{2+}$ binding site, and that the binding of a second $Cu^{2+}$ induces no further conformational change. The binding of $Zn^{2+}$ induces little conformational change in patellamide C. A restrained conformational search shows that the conformational change induced by the addition of one equivalent of $Cu^{2+}$ to patellamide C is an intrinsic design feature of the system. Electronic structure calculations indicate that the patellamides provide an ideal coordination environment for $Cu^{2+}$. On the basis of the evidence gathered, it can be proposed that $Cu^{2+}$ is the biologically relevant metal for the patellamides.

## Introduction

The thiazole (Thz) and oxazoline (Oxn) containing cyclic octapeptide patellamides (*e.g.* **1**–**3**), isolated from the Indo-Pacific ascidian (seasquirt) *Lissoclinum patella*, are known to bind both $Cu^{2+}$ and $Zn^{2+}$.[1–5] The presence of thiazole and oxazoline rings has not yet been identified in other natural metal complexes or metalloproteins. One of these compounds, patellamide C (**1**), on complexing to one equivalent of Cu(II) forms a pre-organised binding site for a second Cu(II). Such a co-operative 'binary switching' ligand has not previously been described. The studies described below strongly implicate Cu(II) as the biologically relevant metal for this ligand.



**4**

**1**, patellamide C $R^1$=$CH_2Ph$; $R^2$=$CHMe_2$; $R^3$=Me; $R^4$=CHMeEt; $R^5$=Me
**2**, ascidiacyclamide, $R^1$=$R^3$=$CHMe_2$; $R^2$=$R^4$=CHMeEt; $R^5$=Me
**3**, patellamide A, $R^1$=$R^3$=$CHMe_2$; $R^2$=$R^4$=CHMeEt; $R^5$=H

The patellamide backbone can adopt either an open or closed conformation in solution depending on $R^1$–$R^4$ and solvent in the absence of metal ions.[6] If the side chains are symmetrically disposed ($R^1$ = $R^3$, $R^2$ = $R^4$; *e.g.* **2**, **3**) then it will adopt the 'open' conformation **B** in polar solvents (Fig. 1b) which has a positive maximum in the circular dichroism (CD) spectrum at 210 nm. An asymmetrical disposition of sidechains ($R^1 \neq R^3$, $R^2 \neq R^4$; *e.g.* **1**) results in the adoption of the 'closed' conformation **A** (Fig. 1a), which has a positive maximum at 250 nm in its CD spectrum.

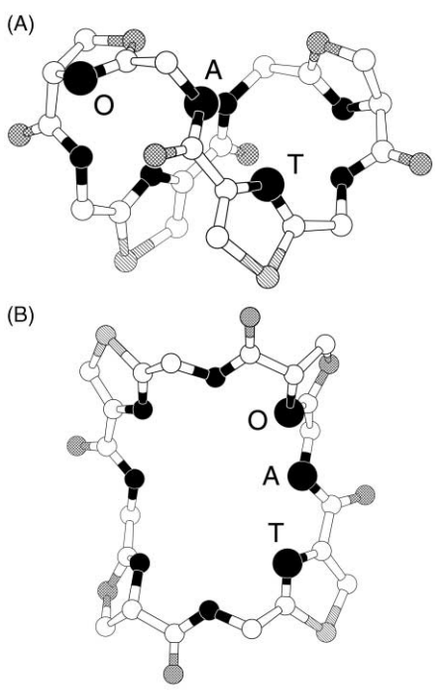



**Fig. 1** Conformations of the patellamide skeleton indicating the 'TAO' motif. (**A**) The 'figure of eight' or folded conformation. (**B**) The 'square' or open conformation.

† Electronic supplementary information (ESI) available: further calculational details. See http://www.rsc.org/suppdata/p2/b2/b201823n/

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**Table 1** CD data for complexation of 0.025 mM **1** and **3** in MeOH with 0–2 equivalents of $M^{2+}$ ($\Delta A/10^{-3}$ cm$^{-1}$ mol$^{-1}$)

| | 210 nm | | | 250 nm | | |
|---|---|---|---|---|---|---|
| | 0 equiv. | 1 equiv. | 2 equiv. | 0 equiv. | 1 equiv. | 2 equiv. |
| **1**–$Cu^{2+}$ | 0.185 | 0.226 | 0.230 | 0.151 | 0.014 | 0.012 |
| **1**–$Zn^{2+}$ | 0.192 | 0.203 | 0.210 | 0.156 | 0.147 | 0.145 |
| **3**–$Cu^{2+}$ | 0.183 | 0.224 | 0.223 | 0.017 | 0.009 | 0.006 |
| **3**–$Zn^{2+}$ | 0.174 | 0.216 | 0.242 | 0.014 | −0.016 | −0.019 |

CD and mass spectrometric (MS) studies have shown that patellamide C (**1**) is selective for Cu(II) in the presence of Zn(II) and does not bind to Ni(II) or Co(II).[7] The present work deals with the difference in conformational behaviour between Cu(II) and Zn(II) binding of the patellamides. In the X-ray diffraction crystal structure of the dicopper carbonate complex of **2**,[3] the two Cu(II) ions bind to the nitrogens of the thiazole, amide and oxazoline groups which we have named the TAO motif (**4**). The Cu(II) adopts a distorted square pyramidal coordination, with the equatorial plane containing the TAO nitrogens and an O donor from the bridging carbonate and apical coordination from a water O. The TAO motif is pre-organised to accept Cu(II) in uncomplexed **2**, which adopts the open conformation (**B**) in solution and the crystalline state (Fig. 1).[8]

## Results and discussion

Titrations of compound **3** with 0–2 equivalents of Cu(II) or Zn(II) and compound **1** with 0–2 equivalents of Zn(II) result in only very small changes in their CD spectra at both the 250 and 210 nm positive maxima indicating little change in conformation.[7] In combination with the MS data this shows the formation of a di-copper complex with the retention of the open conformation (**B**) for **3** (Table 1)[7] with 2 equivalents of Cu(II) and no complex formation and the retention of the closed conformation (**A**) in the case of **1** with Zn(II). This in stark contrast to the behaviour of **1** with Cu(II) where a switch from a closed conformation (**A**)[9] to an open conformation (**B**) occurs on binding to Cu(II); this has been confirmed by CD studies which show the presence of only two conformations.[7] These CD spectra indicate that addition of only 1 equivalent of Cu(II) to **1** (Table 1) causes the observed change in conformation. This conformational change pre-forms a second empty Cu(II) binding site, with the addition of a second equivalent of Cu(II) causing no further conformational change.

MS studies indicated a binding preference for the Ala(Thz)-Ile(Oxn) (left) TAO site in **1**.[7] To obtain the structure of **1** complexed with one equivalent of Cu(II), NOE restrained molecular dynamics calculations were used. A sample of **1** in $CD_3OD$ was prepared in an inert atmosphere and one equivalent of Cu(II) was added as $CuCl_2$. Because of the fast relaxation caused by the Cu(II), selective 1D-NOE spectroscopy with short mixing times[10] was used (26 NOEs; only weak and medium correlations were observed due to the geometry of **1**). Most of the NOEs were found to be on the Val (right) side of **1**. This suggested that the Cu(II) was bound to the Ala(Thz)-Ile(Oxn) TAO (left) binding site with its paramagnetic effects making it impossible to detect the NOEs to that portion of the molecule. The 3D structure of **1**–$Cu^{2+}$ was calculated using XPLOR 3.851;[11] during the calculations no specific Cu(II) binding model was used. The lowest energy ensemble consistent with the observed NOEs contained 18 structures, had only five minor NOE violations and an energy of 23.3 kcal mol$^{-1}$. The structures in the ensemble all had the same backbone conformation (RMSD = 0.00 ± 0.00 Å; Fig. 2, Table 2). When the structure of the complex **1**–$Cu^{2+}$ was compared to the X-ray crystal structure of **2**,[8,12] an overlay of the TAO motif (**4**) on the Val (right) side was found to fit within typical experimental error (RMSD = 0.36 Å, Table 2, Fig. 3). This indicates that upon binding one equivalent of Cu(II), the structure of **1** changes from the closed conformation to the open conformation and in doing so makes a second binding site available for binding to another Cu(II).

**Table 2** Distances in Å between thiazole ($N_T$), amide ($N_A$) and oxazoline ($N_O$) nitrogens in bound and free patellamides

| Structure | Method | $N_T$–$N_A$ | $N_A$–$N_O$ | $N_O$–$N_T$ |
|---|---|---|---|---|
| **1**–$Cu^{2+}$ | NOE-MD[a] | 2.84 | 2.93 | 5.04 |
| **1**/restraints | MC[b] | 2.90 | 2.73 | 4.71 |
| **2** | XRD[c] | 2.82 | 2.79 | 4.80 |
| **2**–$Cu_2CO_3$ | XRD[c] | 2.59 | 2.64 | 4.01 |
| **4**–$Cu^{2+}(MeOH)_2$ | *ab initio*[d] | 2.66 | 2.62 | 4.14 |
| **4**–$Cu^{2+}(H_2O)_2$ | *ab initio*[d] | 2.65 | 2.62 | 4.11 |

[a] NOE restrained molecular dynamics calculations. [b] Monte-Carlo conformational search. [c] Published X-ray crystal structure. [d] *ab initio* Electronic structure calculations.

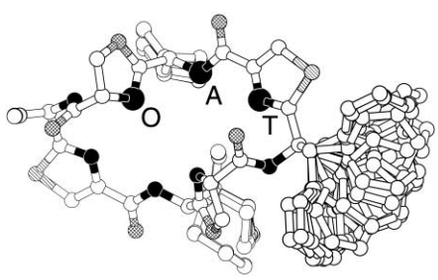


**Fig. 2** A cluster of 18 low energy structures of **1**–$Cu^{2+}$ indicating the TAO motif.

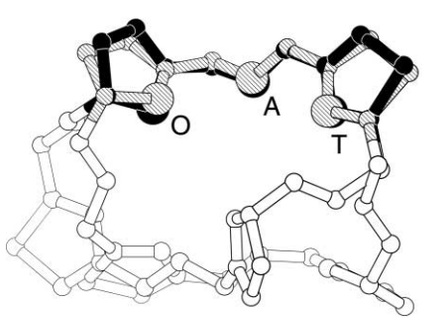


**Fig. 3** Overlay of the minimum energy conformation of **1**–$Cu^{2+}$ (black) and the crystal structure of ascidiacyclamide **2** (hatched).

NOE restrained molecular dynamics calculations on **1** with 2 equivalents of Zn(II) in $CD_3OD$, using 25 NOE restraints gave a structure with one NOE violation, an energy of 28.4 kcal mol$^{-1}$ and a backbone RMSD of 0.00 ± 0.00 Å for the 38 lowest energy structures (Fig. 4). In qualitative terms, after addition of 2 equivalents of Zn(II), **1** appears to adopt a slightly expanded version of the closed conformation **A** (Fig. 5); however, these changes are maybe within experimental error. Previous studies have suggested that Zn(II) may complex with the patellamides, but the present study suggests that little conformational change is involved.[4]

A Monte-Carlo conformational search of the coordinate space for **1** in which the Ala(Thz)-Ile(Oxn) TAO site was restrained into the copper-bound conformation was conducted

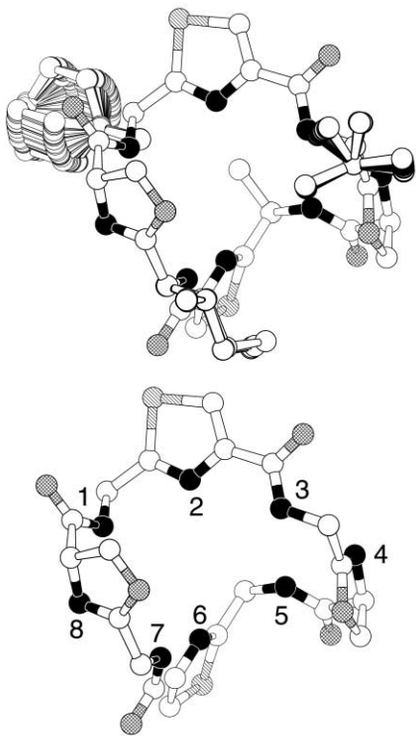


**Fig. 4** Ensemble of the 38 lowest energy structures of Zn(II) bound **1** (top) and the minimum energy structure showing residue numbers for clarity (bottom).

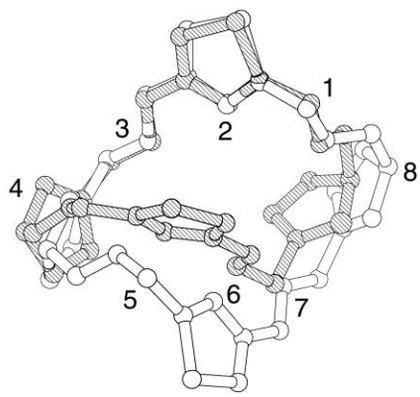


**Fig. 5** Overlay of Zn(II) bound and uncomplexed (hatched) **1** (heavy atoms only) with residue numbers shown at the amide nitrogen for clarity. The models were overlaid using the backbone atoms of the three residues which gave the lowest RMSD (1, 2 and 3; RMSD = 0.20 Å).

using MacroModel.[13] Again, no specific Cu(II) binding model was employed, so that the effect on Phe(Thz)-Val(Oxn) of restraining Ala(Thz)-Ile(Oxn) in the Cu(II) bound form could be studied. The lowest energy conformation found during the search (5000 steps) again showed pre-organisation of the Phe-(Thz)-Val(Thz) TAO site. This showed good overlay of the TAO motif (**4**) on the Val (right) side of the structure with the crystal structure determined for **2** (RMSD = 0.09 Å, Table 2). The conformational search indicates that the pre-organisation of a second TAO binding site is an integral conformational feature of the patellamides.

*ab initio* Electronic structure calculations were performed at the ROHF level on **4**–Cu(II) using the PC GAMESS version[14] of the GAMESS (US) QC package.[15] The SBKJC effective core potential basis set with additional heavy atom polarisation functions was employed for all calculations.[16–18] The initial structures had the metal in an octahedral coordination geometry composed of the three co-planar TAO nitrogens with the remaining sites occupied by the oxygen atoms of 3 solvent molecules ($H_2O$ and MeOH to simulate both the natural and

**Table 3** Selected distances and angles for structures from electronic structure calculations in comparison to those determined from a published X-ray crystal structure

| | **2**–$Cu_2CO_3$[a] | **4**–$Cu^{2+}(MeOH)_2$ | **4**–$Cu^{2+}(H_2O)_2$ |
|---|---|---|---|
| Angles/deg | | | |
| $N_T$–Cu–$N_A$ | 80.7 | 81.7 | 82.2 |
| $N_O$–Cu–$N_A$ | 81.3 | 81.6 | 82.1 |
| Distances/Å | | | |
| $N_T$–$N_A$ | 2.590 | 2.659 | 2.652 |
| $N_A$–$N_O$ | 2.640 | 2.619 | 2.616 |
| $N_O$–$N_T$ | 4.069 | 4.141 | 4.109 |
| $N_T$–Cu | 2.101 | 2.119 | 2.098 |
| $N_A$–Cu | 1.925 | 1.940 | 1.931 |
| $N_O$–Cu | 2.052 | 2.066 | 2.051 |

[a] Published X-ray crystal structure.[3]

experimental conditions experienced by the metal). During the course of both optimisations the solvent ligand below the TAO plane was ejected resulting in a distorted square pyramidal coordination environment (Fig. 6). The results from the

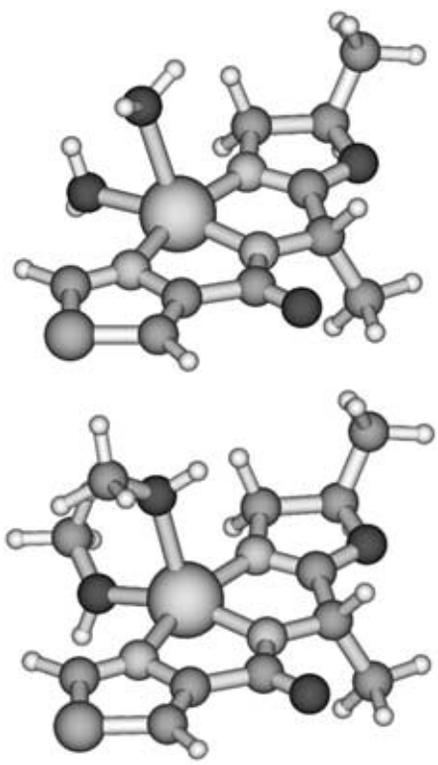

**Fig. 6** Output geometry from electronic structure calculations for **4**–$Cu^{2+}(H_2O)_2$ (top) and **4**–$Cu^{2+}(MeOH)_2$ (bottom).

calculation in MeOH showed good overlap with the TAO motif in the X-ray crystal structure of **2**–$Cu_2CO_3$ (RMSD = 0.085 Å, Table 2), indicating that Thz-(C=O)–NH–$CH_2$-Oxn provides the ideal geometry for complexing to Cu(II). The Cu(II) geometry calculated is almost identical to that obtained from the crystal structure.[3] Selected bond angles and distances for **4** complexed with $Cu^{2+}$ in $H_2O$ and MeOH are given in Table 3 in comparison to those measured from the crystal structure for **2**–$Cu_2CO_3$[3] showing excellent correspondence between experimental and theoretical values.

## Conclusions

The above studies suggest that the conformational change observed on the addition of one equivalent of $Cu^{2+}$ to patellamide C, creating a second $Cu^{2+}$ binding site, is an intrinsic

design feature of these compounds. Electronic structure calculations show that the coordination environment present in these molecules is ideal for $Cu^{2+}$. It can therefore be proposed that Cu(II) is the biologically relevant metal in this unusual natural product. In addition, the binary switching nature of the patellamides on complexing to Cu(II) could prove to be useful in the creation of novel molecular devices.

## Experimental

### NOE restrained molecular dynamics

Restraints were derived from T-ROESY[19] or 1D selective NOE[10] spectra and classified as weak, medium or strong. ROE's were quantified by contour counting. Restrained molecular dynamics calculations were carried out with XPLOR 3.851[11] with a force field with repulsive non-bonded terms. No dielectric term was included in the calculations to take account of solvent effects. *ab initio* Simulated annealing calculations (YASAP 3.0: 120 ps total time simulated annealing from 2000 to 100 K, 200 steps minimisation)[20,21] were used to calculate structures from a starting conformation with randomised $\phi$ and $\psi$ angles. Sum averaging[22] was used for all methyl, methylene, and aromatic ring atom pairs. Assignment of prochiral groups was achieved by floating assignment and swapping of prochiral groups.[23,24] A reduced set of non-bonded interactions and a reduced representation of the amino acid side chains was used during the conformation search phase.[25] During all stages of the simulated annealing calculation the temperature was maintained by coupling to a heat bath[26] with a coupling frequency of 10 $ps^{-1}$. From each ensemble the lowest energy structures were refined using a simulated annealing with slow cooling protocol (600 ps, cooling from 1500 to 100 K, 4000 steps of minimisation). The lowest energy structures from each ensemble were selected to represent their structures. The overlay and display of structures was achieved using Molmol.[27] Figures were created using DINO[28] and rendered using POV-Ray™.[29]

### Monte-Carlo conformational searching

The input structures were drawn in by hand and the peptide backbone kept planar until the beginning of the run. Restraints were added (as described in the electronic supplementary information) to simulate the presence of a bound Cu(II). 5000 steps of Monte-Carlo conformational searching were followed by (up to) 5000 energy minimisation steps within the MM2* force field.[13] The generalised Born solvent accessible area (GB/SA) continuum solvent model was used to simulate the chloroform environment in which the NMR values were obtained.[30] Cut-offs for non-bonded interactions were employed (12 Å for electrostatics and 7 Å for van der Waals interactions) giving a distance-dependent dielectric environment for the solute molecule.

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