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Solution structure of a designed cyclic peptide ligand for nickel and copper ions

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Abstract

Nuclear magnetic resonance (NMR) spectroscopy was used to study a cyclic peptide derived from the amino-terminal copper-and-nickel-binding (ATCUN) motif. The three-dimensional structure of the unliganded peptide in aqueous solution was solved by simulated annealing using distance constraints derived from Nuclear Overhauser Effects. A structural model for the Ni(II)-bound complex was also produced based on NMR evidence and prior spectroscopic data, which are consistent with crystal structures of linear ATCUN complexes. Structural interpolation, or “morphing,” was used to understand the transition of this highly structured cyclic peptide from its unliganded structure to its metal-ion-bound structure.

Keywords

ATCUN; Cyclic Peptide; NMR; Modeling; Ni(II) Binding

1. Introduction

Protein-metal interactions are found throughout nature and control critical biological functions, including protein structure, metal homeostasis, and catalysis. Protein-metal complexes have provided insight and inspiration for chemists interested in developing synthetic compounds with useful metal-binding properties and reactivity. The amino terminal copper-and-nickel-binding (ATCUN) motif is a well-characterized metal-binding motif that was first identified in serum albumin proteins from a variety of species.^{1,2} ATCUN motifs are found in other proteins including neuromidin C,³ human sperm protamine P2a,⁴ and histatins.⁵ The ATCUN motif consists of a free N-terminus followed by a histidine in the third position (Figure 1a.).⁶ This motif has been extensively characterized through UV-vis spectroscopy, NMR, electron paramagnetic resonance (EPR) and X-ray crystallography. All of these techniques have provided excellent structural data indicating

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Supplementary Material NMR acquisition data, processed spectra, chemical shift assignments and coordinate files are available free of charge online. Videos of the morphing analysis are also provided.

that ATCUN motifs use four nitrogens (the N-terminal amine, two backbone amides, and the histidine imidazole) to coordinate Cu(II) or Ni(II) ions in a square planar geometry (Figure 1b).^{7,8}

The wealth of structural data available for ATCUN peptides made it an attractive target for developing macrocyclic ligands with altered metal binding properties. In general, cyclization can potentially alter the thermodynamics and kinetics of metal binding.^{9,10} To study the effects of cyclization on ATCUN peptides, we previously synthesized a series of linear and cyclic ATCUN peptides and characterized them by UV-vis spectroscopy, EPR spectroscopy, cyclic voltammetry, and redox-based reactivity assays.^{11,12} The designed cyclic peptide **1** (Figure 2a) displayed altered metal-binding properties compared to linear analogs. Spectroscopic and mass spectrometric analyses showed that **1** binds Ni(II) or Cu(II) in a 1:1 ratio using a square planar, four-nitrogen coordination set, consistent with ATCUN-like binding. Further, **1** was more potent in assays measuring oxidative DNA cleavage and hydroxyl radical formation.¹¹

To gain further insight into the precise mode of metal coordination by **1**, we sought to apply NMR spectroscopy because of its sensitivity, atomic-level resolution and ability to inform structural models without the need to form crystals. There have been a number of NMR studies on the interaction between ATCUN peptides and transition metal ions,^{13–16} providing precedent and context for the examination of **1** by NMR. Since square-planar-coordinated Cu(II) is paramagnetic, Ni(II) was chosen a suitable metal ion for probing the binding mode of **1** by NMR. Many previous studies have shown a similar coordination geometry for ATCUN ligands with either Cu(II) and Ni(II) ions, allowing ready extrapolation to complexes of **1** with Cu(II) and other transition metals.⁶

2. Results and Discussion

2.1. Sample preparation and data collection

1 was synthesized as previously described.¹¹ The sample was purified to >95% purity by RP-HPLC. Lyophilized **1** was dissolved in 500 μ L 9:1 H₂O/D₂O to a concentration of 3 mM. DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid) was added as an internal reference standard. The sample used for experiments on the unliganded structure was at pH 3.8, but for chemical shift comparisons between unliganded and Ni(II)-bound peptide, the unliganded peptide was titrated to pH 9.5 using aqueous NaOH and diluted to 1.5 mM to avoid precipitation at this higher pH. For the Ni(II)-bound sample, one equivalent of NiCl₂ was added to peptide **1**, and complexation was verified by UV-vis spectroscopy. pH was readjusted to 9.5 after addition of NiCl₂ to ensure complete complexation. All NMR experiments were conducted at 25° C. Full 1-D and 2-D spectra are given in Supplementary Information, along with tables of chemical shifts, structural refinement parameters and a list of all distance constraints for both structural models.

2.2. Chemical shift changes upon Ni(II) complexation

First, we sought to monitor Ni(II) complexation at pH 9.5 using NMR spectroscopy. Prior studies have shown characteristic chemical shift changes for the ATCUN motif upon Ni(II)

binding.^{13–16} Specifically, increased shielding of H_α protons is typically observed for positions immediately adjacent to Ni(II)-coordinating amide nitrogens.

Changes in chemical shifts upon Ni(II) complexation are shown in Figure 2b. The H_α and H_β protons of the histidine residue experience upfield shifts upon Ni(II) coordination, indicating that both the His amide and N_ε nitrogens are coordinating Ni(II). The aspartate H_α was not observed due to its proximity to the water peak, which led to its suppression. However, the aspartate H_β protons show downfield shifts of 0.839 and 0.409 ppm, indicating a distinct change in their electronic environment upon chelation of Ni(II). Interestingly, all of the protons of the lysine residue except for one of the H_ε protons shift dramatically downfield upon Ni(II) binding. Downfield shifts for amino-terminal H_α protons upon metal complexation have been observed before,^{14,15} but the other protons of the N-terminal residue are not typically altered. While the effect of heavy atoms on chemical shift remains difficult to predict,¹⁷ deshielding of these protons is consistent with being conformationally restricted and being held in the vicinity of the Ni(II) ion. These results provide evidence that the lysine sidechain is in proximity to the Ni(II) in the bound structure.

In contrast to the lysine residue, the leucine protons show little to no chemical shift change, indicating a lack of perturbation upon Ni(II) binding. This was expected based on the spectroscopic data that implicated an ATCUN-like binding mode for **1**, but this analysis provides a higher-resolution means of ruling out any contributions by this residue.

Titration experiments involving the addition of NiCl₂ to a 1.5 mM solution of **1** show clear transitions for protons with large chemical shift changes. For instance, the peaks corresponding to the aromatic His-H_{ε1} and His-H_{δ2} protons of unliganded **1** diminish as NiCl₂ is added, and two new resonances in the aromatic region appear (Figure 2c). Both protons experience an upfield shift upon Ni(II) binding, as previously observed for histidine-containing, metal-binding peptides.^{13–16} In all cases the relative volumes of the peaks correspond to the ratio of Ni(II) to **1**. Thus, these data directly demonstrate the transition from unliganded to Ni(II)-bound peptide.

2.3. Structural models of unliganded and Ni(II)-bound peptide **1**

To gain more insight into the mode of Ni(II) complexation, structural models for the unliganded and Ni(II) bound peptide were produced. Complete 2D COSY, TOCSY, NOESY, ROESY and ¹H-¹³C HSQC spectra (shown in Supplementary Information) were obtained for unliganded **1**, and these experiments were performed at pH 3.8 in order to observe all exchangeable protons. 54 distance constraints were derived from NOEs, and simulated annealing was performed using CNSsolve.^{18,19} Custom patches for the CNSsolve software were developed to create topology and parameter files for **1**. Specifically, we introduced representations of the sidechain-linked lysine and the sidechain-modified aspartate. The structural model of unliganded **1** had no NOE distance constraint violations after 5 rounds of annealing and 2 rounds of refinement.

For the structural model of Ni(II)-bound peptide **1**, additional modifications to topology and parameter files were necessary to incorporate the Ni(II) atom and deprotonated amino acids, as described by Gasmi et al.¹⁶ To restrain the Ni(II)-bound peptide to a square planar

complex (as evidenced by UV-vis and EPR spectroscopic data),^{11,12} distance and dihedral constraints from crystal structures of Ni(II)-bound ATCUN peptides were used.^{8,20–22} Specifically, bond length restraints of 1.8–1.93 Å for amide and imidazole Ni–N bonds and of 1.9–1.95 Å for the amine Ni–N distance were applied. An improper torsion angle of the four nitrogen atoms in complex with Ni(II) was set to $0 \pm 10^\circ$ which allowed for deviations from ideal square planar geometry.⁸ Finally, the torsional angle for the imidazole N $^\delta$, C $^\gamma$, and C $^\delta$ and the Ni(II) was defined as $180 \pm 15^\circ$ to bring the imidazole into the same plane as the other nitrogen ligands.

The 30 lowest energy structural models of unliganded **1** and Ni(II)-bound **1** are shown in Figure 3. Not surprisingly, the Ni(II)-bound structure showed extremely good coherence, with RMSD of 0.045 Å for heavy atoms. Only the side chain of the leucine residue shows any mobility in this model of the Ni(II)-**1** complex. More surprisingly, the unliganded model also showed tight coherence, with a heavy-atom RMSD value 0.147 Å. The ring is clearly quite constrained even without the metal bound, with some mobility for the leucine and histidine sidechains.

2.4. Interpolation between unliganded and Ni(II)-bound models

The surprising degree of structure for unliganded **1** led us to examine more closely the implied transition between unliganded **1** and Ni(II)-bound **1**. To help visualize this transition, we applied a distance-interpolation, or “morphing,” algorithm to show which atoms and torsional angles of **1** experience the most relative movement upon Ni(II) complexation. Videos of this morphing analysis are provided as Supplementary Information, and frames from the analysis are shown in Figure 4.

Care should be taken when drawing conclusions from this kind of morphing analysis, since it only takes into account the spatial translation of atoms and not the overall energies of proposed intermediate structures along this trajectory. Still, it does effectively demonstrate that the unliganded structure of **1** is quite similar to the Ni(II)-bound structure, and that it can transition to the metal-bound conformation through the rotation of very few torsional angles. Specifically, the His χ_1 angle (the side chain torsional angle of the C $_\alpha$ –C $_\beta$ bond) must rotate roughly 130° , the His χ_2 angle (the side chain torsional angle of the C $_\beta$ –C $_\gamma$ bond) must rotate roughly 130° , and the Asp ϕ angle must rotate roughly 160° . These three rotations are most critical for the alignment of the ATCUN motif within **1**. The His side chain rotations bring the imidazole into position, and the Asp ϕ angle rotation has the effect of flipping the relative orientation of the Lys–Asp amide bond, bringing this amide nitrogen into position. This indirect visualization of the transition between a well-structured, unliganded peptide and its metal-bound complex is uniquely informative, and should assist in the future design of related peptide-metal complexes.

3. Conclusion

The structures of unliganded **1** and Ni(II)-bound **1** were analyzed using NMR spectroscopy. Proton chemical shift changes confirm that Ni(II) is coordinated in a square planar geometry by the N-terminal amine, the imidazole, and the two amide nitrogens in between. No involvement of the leucine sidechain or other potential coordination groups was indicated.

Using these data, and extrapolating from our own spectroscopic data and published data on ATCUN-metal complexes, we derived a model for the Ni(II)-**1** complex. In addition, a solution structure of unliganded **1** in water at pH 3.8 was determined using NOE-derived distance constraints, and revealed a high degree of structure even in the absence of metal ion. This allowed visualization of the transition between unliganded and metal-bound peptide, and a window into the dynamic process of metal-peptide complexation. Given the power of metal-peptide complexes as asymmetric catalysts,²³ we anticipate that these structural data will be useful for the rational design of desirable metallopeptide catalysts.

4. Experimental

All NMR experiments were conducted at 25° C. 1D, COSY, TOCSY, NOESY, ROESY, and ¹H-¹³C HSQC experiments were conducted on a Bruker Avance 500 MHz spectrometer (Tufts University Department of Chemistry, Medford, MA). For the unliganded peptide, water suppression was achieved through presaturation or gradient suppression with irradiation at the O1 frequency of 2350.38 Hz. All 1D proton and 2D ¹H-¹H spectra had a spectral width of 6009.615 Hz, and with a 2 s recycle delay. The COSY spectrum had acquisition times of 170 ms and 21 ms in the F2 and F1 dimensions respectively. Signal averaging of 8 scans resulted in a total collection time of 1 hour and 16 minutes. The TOCSY spectrum had acquisition times of 170 ms and 21 ms in the F2 and F1 dimensions respectively. A TOCSY mixing time of 60 ms and signal averaging of 16 scans resulted in a total collection time of 2 hours and 36 minutes. The NOESY spectrum had an O1 frequency of 2350.61 Hz and acquisition times of 170 ms and 21 ms in the F2 and F1 dimensions respectively. A NOESY mixing time of 400 ms and signal averaging of 64 scans resulted in a total collection time of 11 hours and 54 minutes. ¹H-¹³C HSQC spectra were collected with a spectral width of 6009.615 Hz along the F2 dimension and 41666.664 Hz along F1. A recycle delay of 2.0 s was used between successive scans, and acquisition times of 170 ms and 3 ms along F2 and F1, respectively. A total time of 2 hours and 21 minutes was required for spectral collection due to signal averaging of 64 scans.

For the Ni(II)-bound peptide, water suppression was achieved through presaturation or gradient suppression with irradiation at the O1 frequency of 2350.75 Hz. 1D Proton and 2D ¹H-¹H spectra had a spectra width of 6009.615 Hz, and with a 2 s recycle delay. The COSY spectrum had acquisition times of 170 ms and 21 ms in the F2 and F1 dimensions respectively. Signal averaging of 64 scans resulted in a total collection time of 10 hours and 11 minutes. The TOCSY spectrum was had acquisition times of 170 ms and 21 ms in the F2 and F1 dimensions respectively. A TOCSY mixing time of 60 ms and signal averaging of 32 scans resulted in a total collection time of 5 hours and 11 minutes. For the NOESY spectrum, acquisition times of 170 ms and 21 ms in the F2 and F1 dimensions respectively and a mixing time of 500 ms with signal averaging of 32 scans resulted in a total collection time of 6 hours and 10 minutes. ¹H-¹³C HSQC spectra were collected with a spectral width of 6009.615 Hz along the F2 dimension and 12576.471 Hz along F1 with an O1 frequency of 2350.75 Hz. A recycle delay of 2.0 s was used between successive scans, and acquisition times of 170 ms and 10 ms along F2 and F1, respectively. A total time of 9 hours and 10 minutes was required for spectral collection due to signal averaging of 64 scans.

For the Ni(II) titration experiments, water suppression was achieved through presaturation or gradient suppression with irradiation at the O1 frequency of 2348.61 Hz with a spectral width of 6009.615 Hz and a 2 s recycle delay.

All spectra were processed using the Topspin software package (Bruker) and imported into CCPNMR program for peak assignments and generation of NOE-derived distance constraints.²⁴ Simulated annealing was performed using the CNSsolve software suite.^{25,26} Fifty-four nonredundant distance restraints were used during annealing. An initial high temperature annealing stage of 1000 steps was followed by two slower annealing stages, with 1000 and 3000 steps respectively. Annealing was followed by 10 cycles of a 200-step energy minimization. The resulting 30 lowest energy structures were visualized using PyMOL.²⁷

Morphing was achieved using CNSsolve though the morph_dist.inp file downloaded from http://scottlab.ucsc.edu/hh/figs/morphing/morph_dist.inp.^{28,29} A video of the morph was made using Pymol.²⁷

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(a) Chemical structure of prototypical ATCUN peptide Gly-Gly-His and its four-nitrogen, square-planar complex with Cu(II) or Ni(II). Metal-coordinating nitrogen atoms are shown in blue. (b) Crystal structure of Gly-Gly-His bound to Cu(II).⁸

Figure 2.

(a) Cyclic peptide **1** binds Ni(II) and Cu(II) with altered selectivity and redox reactivity compared to linear ATCUN motifs.¹¹ (b) Chemical shift changes upon Ni(II) binding for protons of **1** at pH 9.5. The H_α of Asp was not observed due to proximity to the water peak. (c) Chemical shift changes were proportional to the amount of Ni(II) added (shown in equivalents relative to **1**).

Figure 3.

(a) Ensemble of 30 lowest-energy structures of unliganded **1**, derived from simulated annealing using NOE-derived distance constraints. (b) Ensemble of 30 lowest-energy structures of Ni(II)-bound **1**, derived from simulated annealing using constraints derived from spectroscopic data and known ATCUN ligands.

Figure 4.

Selected frames from a morphing analysis of the transition between unliganded **1** (shown in tan) and Ni(II)-bound **1** (shown in black). The Ni(II) atom, the Asp side chain, and the Leu residue are omitted for clarity. Key torsional angles that differ between the two structures are highlighted.