Comparison of Cysteine and Penicillamine Ligands in a Co(II) Maquette

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L-Penicillamine (Pen) has been investigated as a ligand for metalloprotein design by examining the binding of Co(II) to the sequence NH$_2$–KL(Pen)EGG–(Pen)IGl(Pen)GA(Pen)–GGW–CONH$_2$. For comparison, we have studied Co(II) binding to the analogous sequence with Cys ligands, the ferredoxin maquette ligand IGA that was originally designed to bind a [4Fe-4S] cluster. The Co(II) affinity and UV–vis spectroscopic properties of IGA indicate formation of a pseudotetrahedral tetrathiolate ligated Co(II). In contrast, IGA-Pen showed formation of a pseudotetrahedral complex with Co(II) bound by three Pen ligands and an exogenous H$_2$O. EXAFS data on both Co(II) complexes confirms not only the proposed primary coordination spheres but also shows six Co(II)–C$_p$ methyl group distances in Co(II)-IGA-Pen. These results demonstrate that ligand stericities in simple peptides can be designed to provide asymmetric coordination spheres such as those commonly observed in natural metalloproteins.

Metalloprotein design is an active field of bioinorganic chemistry aimed at delineating the structure–function relationships of natural metalloproteins. De novo metalloprotein design implemented using either rational or combinatorial methodologies provides a constructive approach to the design of novel metalloproteins. These studies have provided detailed insight into the role of transition metal ions in protein folding, oligomerization and stability as well as revealing the engineering specifications of their design and the factors that modulate metalloprotein chemical properties and reactivities.

We are utilizing peptide-based coordination complexes, protein maquettes, as aqueous soluble and stable synthetic analogues of natural metalloproteins to explore the chemical consequences of using noncoded amino acid ligands. By studying natural to non-natural ligand amino acid substitutions in otherwise invariant peptide sequences, we are expanding the repertoire of ligands available for metalloprotein design. Our approach is to use solid-phase peptide synthesis methods coupled with de novo design to evaluate potential ligands prior to their incorporation into natural metalloprotein scaffolds via expressed protein ligation or sophisticated molecular biological methods.

In this contribution, we introduce the cysteine analogue L-penicillamine (Pen, Figure 1) as a noncoded amino acid ligand for metalloprotein design. L-Penicillamine, whose enantiomer is a systemic treatment for copper overload in Wilson’s disease, was chosen for its similar basicity (pK$_a$ of 7.9 vs 8.3 for Cys) and yet greater steric bulk at the C$_p$ methyl group in comparison to cysteine. Pen was incorporated at the cysteine positions of the IGA ferredoxin maquette sequence to evaluate the effect on Co(II) metal ion affinity and spectroscopy. The designed primary structure of each ligand of the penicillamine containing peptide ligand (IGA-Pen),

(15) The peptide ligands were synthesized using standard Fmoc/tBu solid-phase peptide synthesis methodologies. For IGA-Pen, double coupling was employed for the Pen amino acids followed by resin capping.
NH₂-KLI(Pen)EGG⁺(Pen)IG(Pen)GA(Pen)+GGW−CONH₂, is that of the IGA ferredoxin maquette which binds a [4Fe-4S]²⁺ cluster. The data demonstrate that IGA-Pen binds Co(II) with high affinity using only three of the four potential Pen ligands whereas the IGA maquette binds Co(II) avidly via four cysteine thiolytles. The Co(II) affinities of IGA and IGA-Pen are compared with IAA, recently shown to bind Co(II) with a Kₐ of 5 μM at pH 6.5, to determine the effect of the Ala⁹Gly sequence modification and the use of Pen ligands on Co(II) affinity, respectively. Furthermore, the spectroscopic effects of Pen ligation are evaluated in comparison to the Cys analogue to assess the role of steric in modulating the metal coordination environment.

Figure 2A shows that the IGA ligand containing four Cys residues binds 1 equiv of Co(II) as evidenced by UV−vis spectroscopy. Titration of Co(II) into IGA (75 μM) at pH 7.5 (20 mM HEPES, 100 mM KCl) results in increasing absorbance at 304 nm [ε = 3800 M⁻¹cm⁻¹] with a shoulder at 340 nm [ε = 3200 M⁻¹cm⁻¹] consistent with S→Co(II) charge-transfer bands. The extinction coefficient of the highest energy CT transition suggests four Cys thiolate ligands (ε = 950 M⁻¹cm⁻¹ per Co−S bond). The appearance of ligand field bands at 630 nm [ε = 400 M⁻¹cm⁻¹], 686 nm [ε = 570 M⁻¹cm⁻¹], and 728 nm [ε = 540 M⁻¹cm⁻¹] consistent with A₄ → T₁(P) transitions demonstrates that the Co(II) is in a tetrahedral coordination environment. Indeed, the UV−vis spectrum of Co(II)-IGA is comparable to Co(II)-substituted rubredoxin and other designed tetrahedral proteins.

Figure 2B shows a sharp break in the titration curve due to the tight formation of a 1:1 metal/ligand complex between the IGA and Co(II) at pH 7.5 whose dissociation constant, Kₐ(Co(II)) value, is tighter than 500 nM.

Figure 2A also shows the UV−vis spectrum of Co(II)-complex of IGA-Pen (bold) (A). Evaluation of the metal−ligand stoichiometry of the Co(II)-IGA (○) and Co(II)-IGA-Pen (●) complexes. All experiments were performed at 75 μM protein concentration in 20 mM HEPES, 100 mM KCl at pH 7.5. A binding site is defined in IGA as four S-donors and three in IGA-Pen.

References:
Accurate Co(II) dissociation constants were obtained for Co(II)-IGA and Co(II)-IGA-Pen at pH 6.6 where proton competition for metal ion binding is more pronounced. Figure 3 shows the anaerobic titration of Co(II) into 5 μM peptide ligand at pH 6.6 as followed by UV–vis spectroscopy in a 10 cm path length quartz cuvette. The Co(II)-IGA titration curve (C) was fit to a 1:1 metal/peptide binding model with a $K_d^{\text{Co(II)}}$ value of 2 μM at pH 6.6. This value is slightly tighter than the 5 μM value measured at pH 6.5 for IAA suggesting that the Ala9Gly sequence change results in a minimal change in Co(II) affinity. In contrast, the binding curve of Co(II)-IGA-Pen (■) shows significantly weaker Co(II) affinity than observed for Co(II)-IGA, consistent with loss of one S ligand. Fitting to a 1:1 binding model in which each binding site is defined as three penicillamine ligands resulted in a $K_d^{\text{Co(II)}}$ value of 98 μM at pH 6.6, some 49-fold weaker (2.2 kcal/mol) than Co(II)-IGA. The expected $[\text{H}^+]^3$ dependence of the Co(II)-IGA-Pen $K_d^{\text{Co(II)}}$ value suggests this value is 78 nM at pH 7.5.

Further evidence for the proposed primary coordination spheres of Co(II)-IGA and Co(II)-IGA-Pen is provided by their EXAFS spectra at the Co K-edge as shown in Figure 4. The FT for Co(II)-IGA (dashed line) shows a single peak at $R + \alpha$ of 1.9 Å, corresponding to 4 S donors at 2.31 Å (Table S1, Figure S4). In contrast, the FT for Co(II)-IGA-Pen shows a first shell scattering peak that is substantially lower in magnitude with its center of gravity shifted, owing to the appearance of a shoulder to low $R$. These data are best modeled with 3 S donors at 2.27 Å and one N/O donor at 2.13 Å. Interestingly, the substitution in the Pen side-chains is also apparent in the EXAFS. A new peak, absent in the FT for Co(II)-IGA, appears in the FT for Co(II)-IGA-Pen (indicated by the arrow). Single scattering fits are consistent with a shell of 6 C at 3.45 Å. This distance is too short to be the $\beta$-carbons of Pen, and smaller coordination numbers led to unreasonably small Debye–Waller factors, supporting its assignment as scattering from the $\beta$-CH$_3$ carbons consistent with the model in Figure 1.

These simple Co(II)-peptide complexes serve as water soluble synthetic analogues of Co(II)-substituted Zn(II) proteins. The pseudotetrahedral (S-Cys)$_4$ coordination motif observed in Co(II)-IGA is a model for both structural (S-Cys)$_4$ coordinated Zn(II) sites, e.g., DNA polymerase III$^{24a}$ and alcohol dehydrogenase,$^{24b}$ as well as for the reactive Zn(II) site in the DNA repair enzyme Ada.$^{24c}$ The (S-Cys)$_3$-(H$_2$O)$_1$ site in Co(II)-IGA-Pen mimics the structural site in TRAIL$^{25a}$ and the catalytic Zn(II) site observed in 5-aminolevulinate dehydratase.$^{25b}$

In conclusion, we have shown the feasibility of using the steric of a non-natural amino acid ligand to produce an asymmetric primary coordination sphere at a mononuclear metal ion. Our efforts are now focused on detailed spectroscopic and electronic structure studies of these Co(II) complexes, evaluating the reactivity of the corresponding Zn(II) maquettes and introducing further asymmetry into the metal primary coordination sphere.

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Supporting Information Available: Mass spectrum of IGA-Pen, derivation of $K_d$ fitting equation, sedimentation equilibrium analytical ultracentrifugation data, and fits to Fourier transformed EXAFS data for Co(II)-IGA and Co(II)-IGA-Pen. This material is available free of charge via the Internet at http://pubs.acs.org. IC0497679
