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Communication

Cd²⁺-Induced Conformational Change of a Synthetic Metallopeptide: Slow Metal Binding Followed by a Slower Conformational Change

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A two-stranded α-helical coiled coil was prepared having a Cys_4 metal-binding site within its hydrophobic interior. The addition of Cd^{2+} results in the incorporation of 2 equiv of metal ion, which is accompanied by a conformational change of the peptide, as observed by circular dichroism (CD) spectroscopy. Isothermal titration calorimetry (ITC) shows that the addition of Cd^{2+} is accompanied by two thermodynamic events. A comparison of the time dependence of the ITC behavior with those of the UV absorption and CD behavior allows the assignment of these events to a preliminary endothermic metal-binding step followed by a slower exothermic conformational change.

The three-dimensional structures of proteins are often affected by the binding of transition-metal ions.[1] In some cases, metal-induced protein-folding events are essential for the proper functioning of metalloproteins, as seen for the zinc finger proteins, metallothioneins, and metalloregulatory proteins such as MntR.[2–4] In other cases, the binding of metal ions may cause protein misfolding, which may result in the generation of pathological events. Such may be the case when Cu^{2+}, Fe^{2+}, or Zn^{2+} bind to the amyloid β (Aβ) peptide, resulting in the generation of the amyloid plaques associated with Alzheimer's disease.[5] For these reasons, metal-induced protein-folding processes have been the focus of recent studies through the use of theoretical methods[6] and well-defined model systems.[7–10]

Previous work by our group examined the metal-binding properties of a synthetic 32-residue polypeptide called C16C19-GGY.[11,12] It was found that this peptide undergoes a significant conformational change from a monomeric random coil to a metal-bridged coiled coil upon its binding of various d^{10} metal ions.[13] Interestingly, the oligomerization state of the resulting holoproteins was found to be metal-ion-dependent because the Cd^{2+} adduct was shown to exist as a two-stranded coiled coil containing a single metal ion and the Cu^+ species was found to assemble into a four-stranded coiled coil having a tetranuclear copper center.[11–13] The rich conformational properties of this system must therefore arise from a complicated interplay occurring between the coordination chemistry of the metal ions and the peptide's ability to self-associate into ordered three-dimensional structures. To more easily study the nature of this relationship, we sought to design a related system in which simpler metal-induced conformational behavior can be observed. Here, we report the metal-binding properties of the peptide AQ-C16C19, which exists as a two-stranded α-helical coiled coil in the absence of metal ions and undergoes a small, but noticeable, conformational change when two Cd^{2+} ions bind to a preformed Cys_4 metal-binding site within its hydrophobic interior. In particular, the addition of Cd^{2+} to AQ-C16C19 is seen to produce two stepwise thermodynamic events that are assigned to a preliminary endothermic metal-binding step followed by a slower exothermic conformational change.

The peptide AQ-C16C19 has the sequence Ac-Q(IAA-LEQK)₂(**C**AA**C**EQK)(IAALEQK)GGY-amide, which is similar to that previously used to prepare a stable two-stranded α-helical coiled coil[14] but modified to contain cysteine residues at two of its hydrophobic positions. High-performance size-exclusion chromatography (HPSEC) shows that the purified peptide exists as a dimer (MW = 6.8 kDa), and circular dichroism (CD) spectroscopy shows that it is α-helical in nature, as evidenced by minima at 208 and 222 nm (Figure 1). The observed ellipticity ratio

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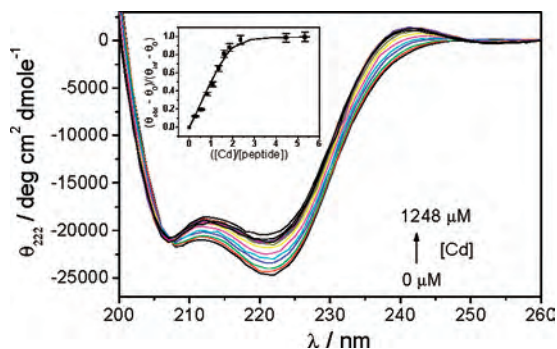(14) Tsurkan, M. V.; Ogawa, M. Y. *Biomacromolecules* **2007**, *8*, 3908.

Figure 1. CD spectrum of the AQ-C16C19 peptide (89.8 μM) with successive additions (anaerobic) of Cd^{II} in 20 mM acetate buffer (pH 5.4; temperature 303 K). Inset: Plot of fractional saturation of sites vs mole ratio ([Cd]/[peptide]). The solid line shows the best fit of data points to eq 2.

of $\theta_{222}/\theta_{208} \approx 1.1$ is greater than that seen for monomeric helices for which $\theta_{222}/\theta_{208} = 0.86$ and is consistent with the assignment of a coiled-coil structure.[15] The CD spectrum of the peptide is concentration-dependent, and the change in θ_{222} was analyzed in terms of a two-state monomer−dimer equilibrium[16] (data not shown) to yield $K_d = 6.5 \pm 0.6$ μM and a maximum ellipticity of $\theta_{max} = -33\,400 \pm 200$. Together, these results show that the apopeptide exists as a two-stranded coiled coil and can become 97% helical at high concentrations.[16]

The metal-binding properties of AQ-C16C19 were studied by UV and CD spectroscopy as well as ITC. Figure 2 (inset) shows that the addition of Cd^{2+} to the peptide solution results in the appearance of a new absorption band at 240 nm, which is assigned to a Cys-SH to Cd^{2+} ligand-to-metal charge-transfer transition. The increase in absorbance with Cd^{2+} addition was analyzed in terms of the equilibrium

$$\frac{1}{n}[Cd_n \cdot Peptide] \rightleftharpoons \frac{1}{n}[Peptide] + Cd$$
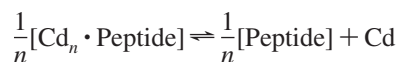
for which

$$K_d = \frac{[Peptide]_{free}^{1/n}[Cd]_{free}}{[Cd_nPeptide]^{1/n}} \quad (1)$$

$$R = \frac{K_d}{[Peptide]_{total}}\left\{\frac{S}{1-S}\right\}^{1/n} + n(S) \quad (2)$$

where $R = [Cd]_{total}/[Peptide]_{total}$, $S = (A - A_0)/(A_\infty - A_0)$ or $(\theta - \theta_0)/(\theta_\infty - \theta_0)$ and [Peptide] refers to the molar concentration of the peptide dimer (Figure 2). The absorption titration data can be accurately described by the binding isotherm (eq 2) to yield values of $n = 1.6 \pm 0.02$ and $K_d = 18 \pm 0.4$ μM for the metal-binding process.[17] The AQ-C16C19 peptide thus binds two Cd^{2+} ions to cysteine residues located within its hydrophobic interior. Interestingly, the ^{113}Cd NMR spectrum of the holopeptide consists of a single resonance at 459 ppm, indicating that the two Cd^{2+} ions either are in magnetically equivalent sites or are in rapid exchange. This chemical shift is similar to that reported for
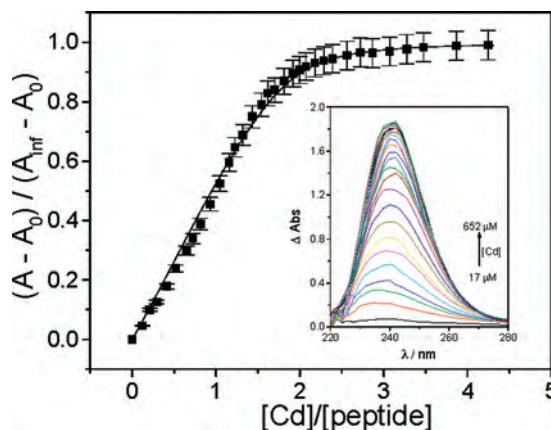
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Figure 2. Plot of fractional saturation of sites vs mole ratio [Cd]/[peptide] obtained from the spectrophotometric titration of AQ-C16C19 peptide with successive additions (anaerobic) of Cd^{II}. The data points are the average of two titrations with 128 and 119 μM peptide solutions in a 50 mM acetate buffer (pH 5.4; temperature 303 K). The solid line shows the best fit of data points to eq 2. Inset: Appearance of a new absorption band upon Cd^{2+} addition.

cadmium-substituted horse liver alcohol dehydrogenase in which the catalytic Cd^{2+} ion was thought to have a Cys$_2$-His ligand set also containing a labile water molecule.[18]

Figure 1 shows that the addition of Cd^{2+} to the peptide produces a noticeable conformational change as evidenced by CD spectroscopy. Increasingly more positive values of θ_{222} are observed that saturate at higher concentrations of metal ion, indicating a change in helicity from 72% to 59%. The spectra further show isodichroic points at 207 and 249 nm, which indicates the existence of a two-state conformational change upon the addition of Cd^{2+}. Interestingly, the HPSEC behavior of the metallopeptide remains unchanged from that of the apopeptide, which shows that it remains a dimer. The inset to Figure 1 shows that the CD titration behavior can be accurately described by eq 2, which yields values of $n = 1.7 \pm 0.1$ and $K_d = 15 \pm 2$ μM for the metal-binding process. These results are in agreement with those observed from the absorption data discussed above.

Isothermal titration calorimetry (ITC) was used to study the thermodynamic events associated with the binding of Cd^{2+} to the AQ-C16C19 dimer. In these experiments, care was taken to account for the effects of the buffer on the observed enthalpy changes by use of an acetate buffer (pH 5.5)[19] and dilution experiments, which showed that these effects were negligible. Sufficient time (600 s) between successive injections ensured completion of the reaction in each step. Figure 3A shows that each addition of metal ion is accompanied by an initial endothermic response followed by a smaller exothermic one. This behavior is reminiscent of that previously reported for the addition of Cu^{2+} to apoazurin in which metal binding was followed by a conformational change of the protein.[20] However, in that system, it was found that the initial binding event was exothermic followed by an endothermic conformational change.

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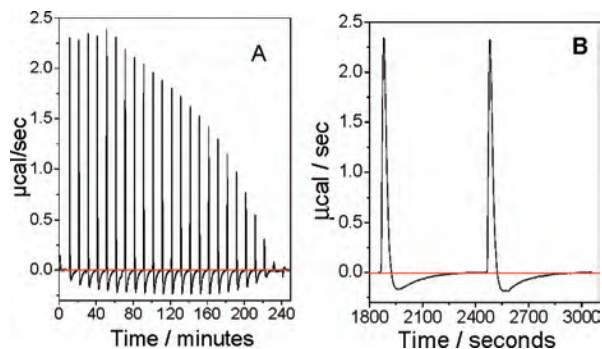(20) Blasie, C. A.; Berg, J. M. *J. Am. Chem. Soc.* **2003**, *125*, 6866.

Figure 3. ITC titration of 218.5 μM peptide with 3.7 mM Cd^{II} in a 200 mM acetate buffer, pH 5.4, 303 K: (A) raw titration data; (B) magnified raw data showing two stepwise events upon addition of Cd^{II} to the peptide.
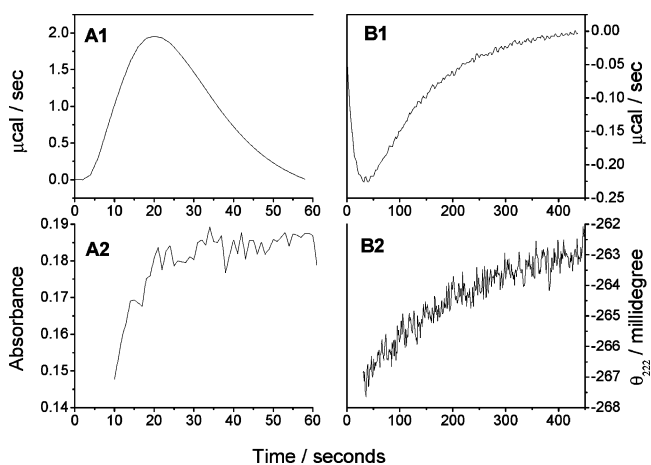

Figure 4. Comparison of the kinetics of Cd^{II} addition to peptide by UV, CD, and ITC. Layers A1 and A2: Comparison of the endothermic trace of ITC ([peptide] = 218.5 μM; [Cd] = 28 μM) with UV titration ([peptide] = 220 μM; [Cd] = 26 μM. Layers B1 and B2: Comparison of the exothermic trace of ITC ([peptide] = 217 μM; [Cd] = 260 μM) with CD titration in a 200 mM acetate buffer, pH 5.4. The CD titration is the average of four titrations ([peptide] = 205.5 μM; [Cd] = 245 μM, [peptide] = 120 μM; [Cd] = 183 μM, [peptide] = 133 μM; [Cd] = 157 μM. ITC traces were translated to begin at time equals zero.

Examination of Figure 3B shows that the two thermodynamic events observed upon the addition of Cd^{2+} to AQ-C16C19 occur on very different timescales. Thus, to test the hypothesis that fast metal binding is accompanied by a slower conformational change, the time profiles of the ITC signals were compared with those of the UV absorption and CD spectra. Figure 4 (layers A1 and A2) shows that both the initial endothermic event and the UV absorption changes occur in about 1 min, and layers B1 and B2 show that that the changes in the CD intensity and the secondary exothermic event both occur on a ca. 50-fold longer timescale. In comparison, the binding of Hg^{2+} to a weakly associating peptide was earlier shown to occur on the millisecond timescale when performed under conditions similar to those used in Figure 4.[7] The endothermic process seen in the ITC data is thus assigned to a metal-binding event. This assignment is consistent with the observation of entropy-controlled Zn^{2+} binding to a tetracysteine-containing peptide at low pH, where the cysteine residues are protonated.[10] Endothermic heat changes accompanying metal−protein interactions have
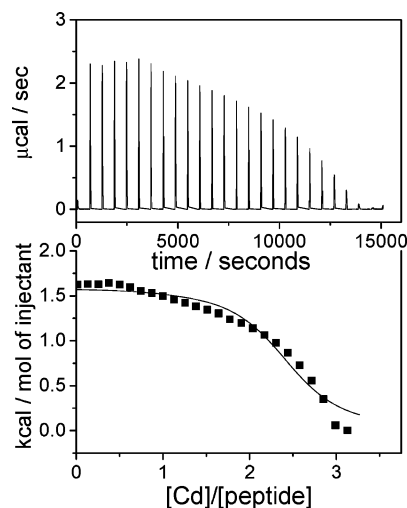

Figure 5. Fits of the endothermic heat changes to a one set of sites model for the ITC titration of 218.5 μM peptide with 3.7 mM Cd^{II} in a 200 mM acetate buffer, pH 5.4, 303 K. The solid line shows the best fit.

also been reported for Cu^{2+} binding to the Cys site in bovine serum albumin,[21] and in a control experiment, we observe only endothermic changes when Cd^{2+} was added to a solution of L-cysteine at pH 5.4. As a further test of this assignment, the endothermic peaks in the ITC titration data were separately analyzed according to one set of sites binding model. As shown in Figure 5, a reasonable fit to the data was obtained in which $n = 2.4 \pm 0.04$ and $K_d = 10 \pm 2$ μM, which are in agreement with the results of the UV and CD titrations discussed above. The analysis also yields values of $\Delta G = -6.9$ kcal/mol and $\Delta H = 1.6$ kcal/mol to give $\Delta S = +28.1$ cal/mol/K. This shows that the metal-binding process is entropy-controlled, which is likely due to disruption of the metal ion hydration sphere and structured water surrounding the nonpolar residues of the peptide scaffold upon metal binding. It is noted that the nature of conformational change giving rise to the exothermic event remains unclear at this time.

In summary, the above study demonstrates the complementary use of UV, CD, and ITC to elucidate metal binding to peptides and proteins. The Cys-containing de novo designed peptide undergoes a metal-induced helical transition after two Cd ions bind to the peptide dimer. The data illustrate that these changes are entropically driven, with dehydration of both the peptide scaffold and metal ion exerting considerable influence on the reaction enthalpies, entropies, and free energy.

Supporting Information Available: ^{113}Cd NMR protocol. This material is available free of charge via the Internet at http://pubs.acs.org.

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