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Metal-Mediated Peptide Assembly: Use of Metal Coordination to Change the Oligomerization State of an α -Helical Coiled-Coil

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Metal coordination is used to alter the oligomerization state of a designed peptide structure. The 30-residue polypeptide AQ-Pal14Pal21 contains two metal-binding 4-pyridylalanine (Pal) residues on its solvent-exposed surface and exists as a very stable two-stranded α -helical coiled-coil. Upon the addition of Pt(en)-(NO₃)₂, a significant conformational change to a metal-bridged, four-helix bundle is seen.

The biological activity of peptides and proteins often depends on their abilities to form specific three-dimensional structures. As such, the structural diversity of biomolecules makes their supramolecular assembly an attractive target for developing new nanoscale materials having useful new properties. Significant effort is therefore being devoted to both understand and control the various factors which can be used to regulate the assembly of biomolecules into new three-dimensional structures.^{1–5}

The self-assembly of α -helical coiled-coils is a well-studied process that can serve as a convenient starting point for creating new types of biological supramolecules. These structures exist as an intertwining of two or more α -helices driven by the specific interchain packing of hydrophobic side chains from complementary peptide chains.⁶ It has been demonstrated that synthetic coiled-coils can be constructed from amphipathic peptides whose sequences are based on a seven-residue repeat (abcdefg) in which positions “a” and “d” are occupied by hydrophobic amino acids, and positions “b”, “c”, and “f” are occupied by hydrophilic residues (Figure 1). An interesting feature of coiled-coils is that their oligomerization states can be varied from peptide dimers to

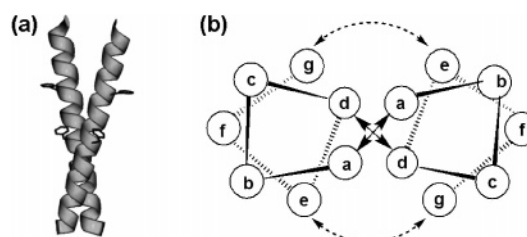


Figure 1. (a) Two-stranded α -helical coiled-coil of AQ-Pal14Pal21. (b) Wheel diagram in which the positions marked a–g indicate the amino acid sequence of one heptad repeat looking down the helical axis. The solid arrows indicate interchain hydrophobic interactions, and the hatched arrows indicate electrostatic interactions. The 4-pyridylalanine residues occupy the solvent-exposed “f” positions of the second and third heptad repeats, respectively.

tetramers through subtle alteration of the packing interactions occurring within their hydrophobic cores, even through single amino acid substitutions.⁷

Our group has recently shown that the binding of different metal ions within the hydrophobic core of synthetic coiled-coils can also produce significant changes to the oligomerization state of the resulting metal–peptide assemblies.^{8–10} Thus, the binding of Cd²⁺ to the C16C19-GGY peptide, which contains two cysteine residues at its hydrophobic heptad “a” and “d” positions of the third heptad repeat, produces a conformational change from a disordered random coil of the apo-peptide to a two-stranded coiled-coil in which a single Cd²⁺ ion bridges two peptide chains.¹⁰ It was later observed that the binding of Cu¹⁺ to this same peptide produces a four-stranded coiled-coil which contains a luminescent tetranuclear copper cluster⁹ that exhibits interesting photoinduced electron-transfer properties.¹¹

The work described here explores a different use of metal coordination to alter the oligomerization state of coiled-coils

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and shows how the coordination of metal ions to the hydrophilic *exterior* of a stable two-stranded coiled-coil can result in the formation of a tetrameric metal–peptide assembly.

Solid-phase peptide synthesis was used to prepare AQ-Pal14Pal21, which is a 30-residue polypeptide having the sequence:



in which X is the non-natural amino acid, 4-pyridylalanine (Pal). After cleavage from the resin, the crude peptide was purified by reverse-phase C₁₈ HPLC and analyzed by MALDI-MS (calcd, 3299.90; obsd, 3300.15). The sequence of AQ-Pal14Pal21 closely follows the design of Hodges and co-workers^{12,13} for the construction of very stable, two-stranded α -helical coiled-coils in which the hydrophobic core of the coiled-coil was formed by the placement of isoleucine and leucine residues at the heptad “a” and “d” positions respectively, and the helical nature of the peptide was reinforced by placing a weakly hydrophobic, yet helix-stabilizing alanine residue at the heptad “b” and “c” positions. Chemical denaturation studies discussed below show that AQ-Pal14Pal21 does indeed form a stable two-stranded coiled-coil. The most important feature of AQ-Pal14Pal21 is that 4-pyridylalanine residues were placed at positions 14 and 21 of the sequence which occupy the most highly solvent-exposed “f” positions of the second and third heptad repeats. This peptide thus contains two metal-binding sites on its hydrophilic surface. Separation of the Pal residues two helical turns away from one another was done in order to discourage the formation of intrachain chelates, as this would require severe bending of the peptide. The AQ-Pal14Pal21 peptide was thus designed to bind two separate metal centers along the length of its sequence.

The circular dichroism (CD) spectrum of the AQ-Pal14Pal21 apopeptide consists of minima at 208 and 222 nm, demonstrating that the peptide exists within an α -helical conformation. The observed value of $\theta_{222} = -26\,500 \text{ deg}\cdot\text{cm}^2/\text{dmol}$ indicates that the apopeptide is 76% helical by comparison to that predicted for a pure 30-residue α -helix.¹⁴ The observed value of $\theta_{222}/\theta_{208} = 1.04$ is within the generally accepted range indicating the formation of an α -helical coiled-coil.⁷ In contrast, monomeric α -helices are expected to display values of $\theta_{222}/\theta_{208} \approx 0.86$, as the magnitude of the CD band at 208 nm is sensitive to the presence of interacting α -helices, as it is polarized parallel to the helical axis.¹⁵

The molecular weight of the AQ-Pal14Pal21 apopeptide was determined to be 7 kDa by static light scattering methods (data not shown) which provide a direct means of determining molar masses by analyzing the angular dependence of

scattered light intensity.¹⁶ These results show that the peptide exists as a coiled-coil dimer in solution. This conclusion is consistent with the original peptide design and is confirmed by high performance size exclusion chromatography (HPSEC) which has been shown to be an effective method of determining the molecular weight of small α -helical coiled-coils.¹⁷

The conformational stability of the AQ-Pal14Pal21 coiled-coil was studied by guanidinium chloride (Gdn·HCl) denaturation studies. The denaturation curve (Supporting Information) shows that the intensity of the CD band at 222 nm decreases with increasing concentrations of Gdn·HCl, having a midpoint concentration of $[\text{Gdn}\cdot\text{HCl}]_{1/2} \approx 6$. This behavior can be accurately fit to a two-state dimer/monomer unfolding model^{18,19} to yield an unfolding free energy of $\Delta G^{\text{H}_2\text{O}} = 49.8 \pm 1.3 \text{ kJ/mol}$. For comparison, the previously studied H21-(30-mer), which has been shown to exist as a two-stranded coiled-coil by analytical ultracentrifugation and EPR line-broadening experiments,²⁰ has an unfolding free energy of $\Delta G^{\text{H}_2\text{O}} = 32.3 \pm 1.2 \text{ kJ/mol}$.

The AQ-Pal14Pal21 peptide was reacted with Pt(en)(NO₃)₂ in water at 60 °C for several days. The presence of two labile NO₃ ligands in the Pt complex allows each metal center to bind two separate peptide chains. Evidence for pyridylalanine coordination to the Pt center was obtained by comparing changes seen in the UV spectrum of the reaction mixture with those observed upon the reaction of Pt(en)(NO₃)₂ with pyridine. In both cases, the characteristic 256 nm band of the pyridine ligands grew in intensity and blue-shifted to 250 nm upon reaction with the Pt center.

Analysis of the crude reaction mixture by SDS PAGE (Figure 2) shows the presence of two species having different molecular weights. The one at ca. 3 kDa is assigned to the unreacted apopeptide which travels as a peptide monomer under the denaturing gel conditions. Significantly, the major species appearing at ca. 14 kDa indicates the presence of a peptide tetramer. No other products were observed. As this species exists only after Pt(en)(NO₃)₂ has been added to the peptide solution and is stable in the presence of sodium dodecyl sulfate, it is assigned to the formation of a new Pt-bound tetrameric peptide assembly, (Pt-peptide)₄. No such reaction was observed when an analogous coiled-coil peptide that lacked the Pal residues was treated with the Pt complex.

Figure 2 shows the reverse-phase C₁₈ HPLC results of the crude reaction mixture. In contrast to the SDS-PAGE results, which showed only the presence of a tetrameric Pt–peptide product, the reverse-phase chromatogram shows that at least four different species actually exist in the reaction mixture. The major species (peak 2) is assigned to the unreacted apopeptide and the remaining species are ascribed to new Pt–peptide products (peaks 1, 3, and 4). Of these, peaks 1

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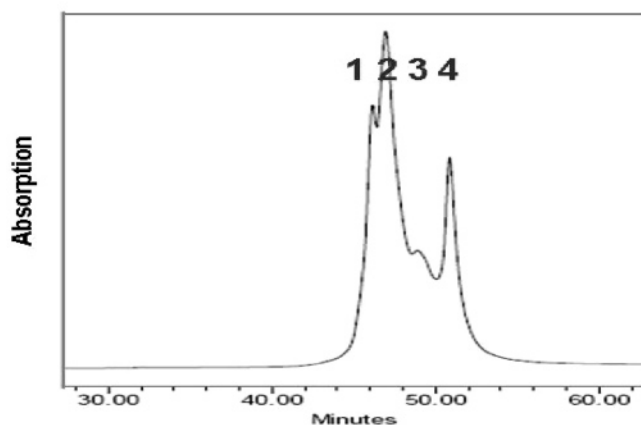
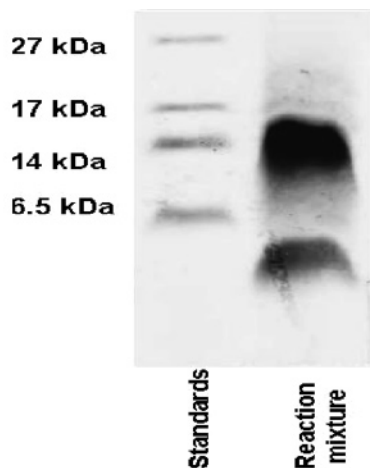


Figure 2. SDS-PAGE (top) and reverse-phase HPLC analysis (bottom) of the crude reaction mixture (30 mM AQ-Pal14Pal21 + 30 mM Pt(en)-(NO₃)₂).

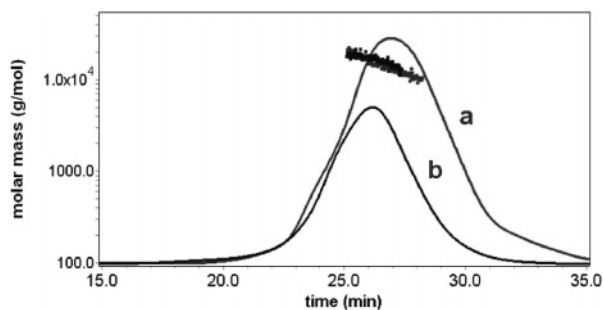


Figure 3. Molar mass distribution plot by multangle light scattering of purified samples of fractions 1 (a) and 4 (b). The solid lines indicate the HPSEC traces using absorption detection at 250 nm. The hashed lines are the weight-averaged molecular masses for the two compounds. Elution conditions: 6 M GdnHCl, 50 mM KH₂PO₄, 100 mM KCl, pH 7.

and 4 were successfully isolated from the reaction mixture by preparative HPLC. SDS-PAGE further showed that these two species were indeed peptide tetramers, and static light scattering measurements (Figure 3) determined that the molecular weights of fractions 1 and 4 were 12.7 ± 0.9 and 14.3 ± 0.6 kDa, respectively, which are within experimental error to one another. The CD spectra of fractions 1 and 4 were taken separately in H₂O, and both were found to display negative signals at 208 and 222 nm with ellipticity ratios of $\theta_{222}/\theta_{208} \approx 1.04$ and 1.02, respectively. The results indicate that the new metal-peptides are coiled-coils, as was the case

for the AQ-Pal14Pal21 apo-peptide. However, θ_{222} for the two metal-peptide samples were only $-16\,600$ and $-15\,400$ deg·cm²/dmol, respectively to show that metal coordination distorts the peptides and reduces their helical content from that of the apo-peptide.

The above data show that Pt(en) coordination to the pyridylalanyl residues of AQ-Pal14Pal21 alters the peptide conformation from that of a two-stranded coiled-coil to a metal-stabilized, four-stranded coiled-coil. Under denaturing conditions, SDS-PAGE, size exclusion chromatography, and static light scattering experiments all showed no evidence for the presence of other metal-peptide oligomers. However, different forms of the metal-stabilized peptide tetramer can be resolved by reverse-phase HPLC. Such differences in the hydrophobic properties of these assemblies suggests that they might have different peptide-peptide packing interactions. These may include the presence of parallel vs antiparallel coiled-coils, and/or differences in the way the Pt centers cross-link adjacent peptide chains.

The observation that only tetrameric Pt-peptide products are formed when AQ-Pal14Pal21 reacts with Pt(en)(NO₃)₂ is in marked contrast to the previously reported behavior for the reaction of Pal14C19_{ox} with *fac*-Re(CO)₃Br₃.²¹ In that earlier work, coordination of Pal14C19_{ox} to the *cis* positions of the Re(CO)₃Br core produced a continuous series of metal-peptide products in which Re complexes bridged from 2 to >5 coiled-coil units in linear chains. It is speculated that two significant differences in the design of the AQ-Pal14Pal21 peptide might account for its different behavior. First, whereas the dimeric coiled-coil conformation of the Pal14C19_{ox} peptide was rigorously enforced by the presence of an interchain disulfide cross-link, the forces controlling the oligomerization state of AQ-Pal14Pal21 remain noncovalent in nature. This feature might impart greater plasticity to the conformational properties of the peptide, allowing it to form a different oligomerization state upon the addition of Pt(en)(NO₃)₂. Second, the presence of only one metal-binding Pal residue on the surface of each peptide chain of Pal14C19_{ox} suggests that the resulting metal-peptide synthons might experience an enhanced degree of rotational freedom about their Pt-Pal bonds to favor formation of linear chains. In contrast, the presence of two Pt-Pal bonds in each AQ-Pal14Pal21 chain might reduce the rotational disorder between adjacent metal-peptide units and favor formation of the cyclic (Pt-peptide)₄ species as an enthalpic product. It is anticipated that such insight into the formation of discrete metal-peptide assemblies may lead to the generation of new bioinspired molecular devices.

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Supporting Information Available: Denaturation curve. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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