Peptide Switches

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Reversible Redox Reconfiguration of Secondary Structures in a Designed Peptide**

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α-Helix-to-β-sheet transitions in proteins have been implicated in a number of neurodegenerative diseases.[1] Several models based on switchable peptides to study these fundamental conformational changes have been developed.[2] Switchable peptides are also of interest as new classes of dynamic nanomaterials.[3] From a protein design perspective, stimulus-responsive peptides allow the study of noncovalent forces that control structure and dynamics of proteins and provide a platform for tuning protein function.[2a,4] Herein we describe the design of a redox-triggered peptide that reversibly switches conformations between a predominately α-helical state to a β-sheet aggregate. The peptide adopts a stable conformation in each state. Although several classes of switchable peptides have been described, a vast majority of these are irreversible (helix to sheet) or rely on non-biocompatible triggers, such as pH changes and/or thermal effects.[2a,d,4f,5] Our design utilizes natural amino acid residues and mild triggers in physiological buffers, affording the opportunity to probe conformational changes in model proteins.

The design of the redox-triggered peptide switch is based on the hard/soft acid–base principles that govern ligand reorganization in the tripodal ligand-based chiroptical switches previously described from our groups where the redox state of copper provides efficient control over the nature of the coordinating ligand.[6] We sought to parlay the higher affinity of copper(II) versus copper(I) for carboxylic acids into a redox-sensitive peptide. The designed seventeen residue peptide is α-helical in the absence of metal ions, switches to a β-sheet structure in the presence of Cu^{II}, and reverses to the α-helical state upon electrochemical reduction of the metal to Cu^{I} (Figure 1).

The optimized peptide sequence was modified from a two-stranded coiled-coil peptide described by Kammerer and Steinmetz.[2d,7] Peptide Ac-SIRELEAKIRELELRIG-NH_2 adopts a stable coiled-coil conformation at low temperatures but irreversibly switches to a β-sheet assembly at 70 °C.[7] We reengineered this peptide to convert it into a reversible redox-sensitive switch. The Kammerer–Steinmetz peptide was judiciously designed to harness the polarity of the side-chain groups to stabilize either of the desired conformations. The coiled-coil state is stabilized by the incorporation of the hydrophobic residues at the a and d positions of the heptad and a number of inter- and intrahelical salt bridges. Incorporation of hydrophobic and polar residues at alternating positions drives the β-sheet formation.

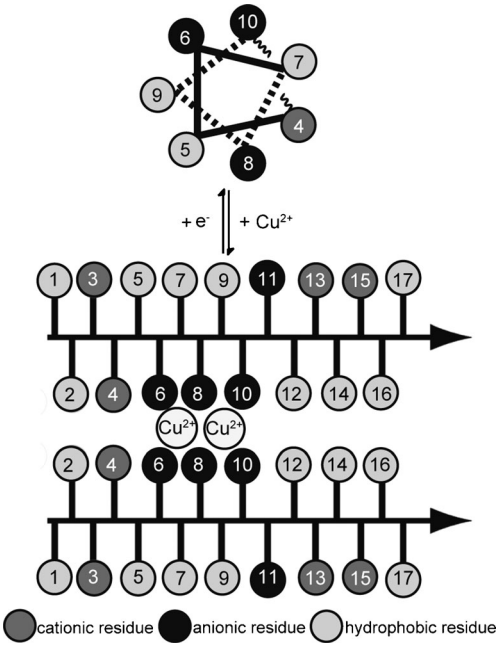

Figure 1. A redox-triggered switchable peptide. A designed α-helical peptide converts into a β-sheet assembly upon addition of Cu^{II}. Reduction of the metal ion causes reversal of the protein conformation.

Table 1: Sequences of the designed peptides. Italicized residues indicate potential Cu^{2+} binding sites.

Peptide	Sequence	Property
1	Ac-SIRKL*EYEIE*ELRLRIG-NH_2	Redox-triggered
2	Ac-SIRKL*EY*AIAELRLRIG-NH_2	Negative control

To convert the peptide from a temperature sensitive irreversible switch into a reversible redox-regulated system, we created a binding site for Cu^{II} on one face of the putative β-sheet (Figure 1). Copper(II) was chosen because it binds to carboxylic acids with relatively high affinity, and more importantly, Cu^{II} can be reduced under mild conditions to the much softer Cu^{I} ion, which associates poorly to hard carboxylate ions compared to Cu^{II}.[8] We began by investigating switchability of analogues of the Kammerer–Steinmetz

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peptide. Evaluation of five designed derivatives (Supporting Information, Table S1) afforded peptide **1** (Table 1), which was found to adopt an α-helical conformation in aqueous buffers while switching to β-sheet assembly upon addition of $CuCl_2$. Other analogues suffered from poor solubility in aqueous solutions.

The conformation of peptide **1** was probed with circular dichroism (CD) spectroscopy (Figure 2A). When the peptide was in the metal-free state, the CD spectrum of 25 μM peptide solution (in 10 mM MOPS buffer, pH 7, with 15% acetonitrile, MOPS/MeCN) showed two minima near 205 nm and 220 nm, and a maximum at 190 nm, which is consistent with those observed for canonical α-helices. The peptide was estimated to be about 40% helical based on maximal helicity observed in a trifluoroethanol titration experiment (Supporting Information, Figure S3).[2c,9] Analytical ultracentrifugation (AUC) analysis of 25 μM peptide **1** in MOPS/MeCN at 60000 rpm (Figure 2C) suggested a molecular weight of 2050 Da when fit to a non-interacting discrete species model, consistent with the peptide folding as a monomer (2158 Da).

Upon addition of $CuCl_2$ to the peptide solution, the CD spectra changed significantly. Both minima at 205 nm and 220 nm disappeared and a new minimum formed around 215 nm, indicating β-sheet conformation. Titration of peptide solution with $CuCl_2$ showed that excess of Cu^{II} was needed to completely drive the peptide to adopt the β-sheet structure (Figure 2B). The titration curve fit to the "one site-specific binding" equation provides a dissociation constant (K_d) of 75 ± 2 μM and a Hill coefficient of $+3.4$, which is indicative of positive cooperativity.[10] The β-sheet conformation was further supported by IR spectroscopy on the peptide film (Supporting Information, Figure S4). In contrast, the negative control peptide **2**, in which two glutamic acid residues designed for Cu^{II} binding were mutated to alanines, did not respond to Cu^{II}, as expected, even at much higher $CuCl_2$ concentration (Supporting Information, Figure S6).

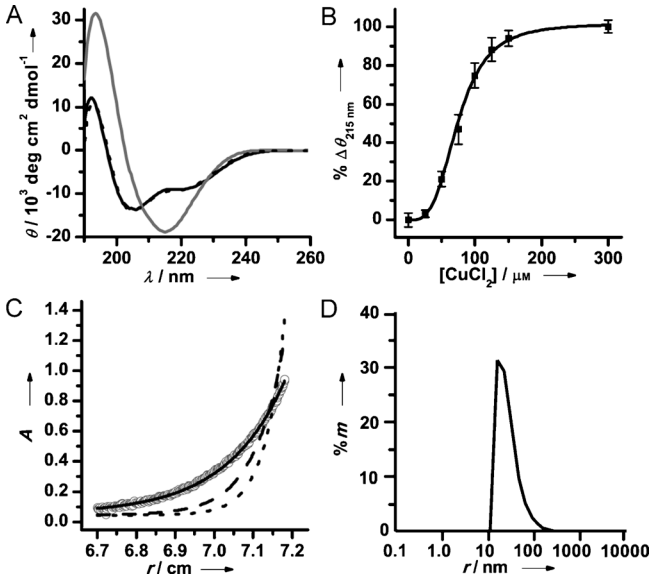

Figure 2. Characterization of peptide **1** in the absence or presence of Cu^{II}. A) CD spectra of peptide **1** (25 μM) in MOPS/MeCN (black ——), in MOPS/MeCN with $CuCl_2$ (0.3 mM; gray ——), and in MOPS/MeCN with $CuCl_2$ (0.3 mM) and hydrazine (0.35 mM, black -----). B) Cu^{II} binding by 25 μM peptide **1** monitored by CD spectroscopy. Experimental data (■), fit curve (——). C) AUC data for peptide **1** (25 μM) without $CuCl_2$ at 60000 rpm. Experimental data (○) and theoretical curves for monomers (——), dimers (-----), and trimers (•••••) are shown. D) DLS spectra of peptide **1** (25 μM) with $CuCl_2$ (0.3 mM).

Dynamic light scattering (DLS) data (Figure 2D) suggests that **1** forms large aggregates with radii ranging from 10 nm to 100 nm. These aggregates were so large that instead of distributing through the entire cell, they fully sedimented in the AUC test even at low rpm of 8000 (Supporting Information, Figure S7). Metal-ion screening showed that **1** selectively responds to Cu^{II} to form β-sheet aggregates; zinc(II) chloride did not induce the conformational transition at a tenfold higher concentration than $CuCl_2$. Other ions tested did not cause the α–β conformational change (Supporting Information, Table S2 and Figure S8).

One-electron redox switching was achieved by reducing Cu^{II} to Cu^{I} with hydrazine followed by reoxidation back to Cu^{II} with bleach. It was discovered by serendipity and then confirmed that small amounts of boron and aluminum salts, such as those that may leach from bleach solution stored in glass, gave the most reversible oxidation results (see Supporting Information for detailed conditions). Upon reduction of Cu^{II} to Cu^{I}, the peptide is fully released from the peptide-Cu β-sheet complex back to the α-helix state. CD spectra for the peptide after the redox cycle are identical to those of the original peptide. Sequential addition of bleach (3 equivalents to hydrazine) oxidizes Cu^{I} back to Cu^{II}, which again interacts with the peptide to form the β-sheet assembly (Figure 3A).

The kinetics of the Cu^{I}/Cu^{II}-induced structural change was monitored by CD intensity change at 215 nm (Figure 3B and C). With 25 μM peptide and 0.3 mM $CuCl_2$, the pseudo first-order rate was determined to be $(1.1 \pm 0.2) \times 10^{-3}\ s^{-1}$. The rate of the conformational change increases in a linear manner with increasing concentration of either peptide or $CuCl_2$, indicating that the rate-determining step involves bimolecular interaction between the peptide and the Cu^{II} ion (Supporting Information, Figure S9).[5d] The reverse process triggered by hydrazine reduction showed faster kinetics as monitored by CD, giving an apparent first-order rate of $(7.2 \pm 0.9) \times 10^{-3}\ s^{-1}$.

The β-sheet aggregated state was further studied with atomic force microscopy (AFM) (Figure 4). When tapping in the buffer, peptide **1** itself gave a blank surface with no detectable species. Addition of $CuCl_2$ to the peptide **1** solution led to random-shaped large aggregates. The radius of the aggregates was on the order of tens of nanometers, which is consistent with the DLS results. After reduction of Cu^{II} by hydrazine, the aggregates disappeared as expected, which was consistent with the reversal of the CD signal and was further confirmed by DLS measurements (Supporting Information, Figure S10).

In conclusion, we have described the design and characterization of a peptide that switches reversibly between an α-helical monomer and a Cu^{II}-bound β-sheet aggregate. Interconversion between the two states was achieved by reduction and oxidation of copper. Typically, generation of β-sheet assemblies from α-helices is considered to be an irreversible process owing to the higher stability of the aggregate over

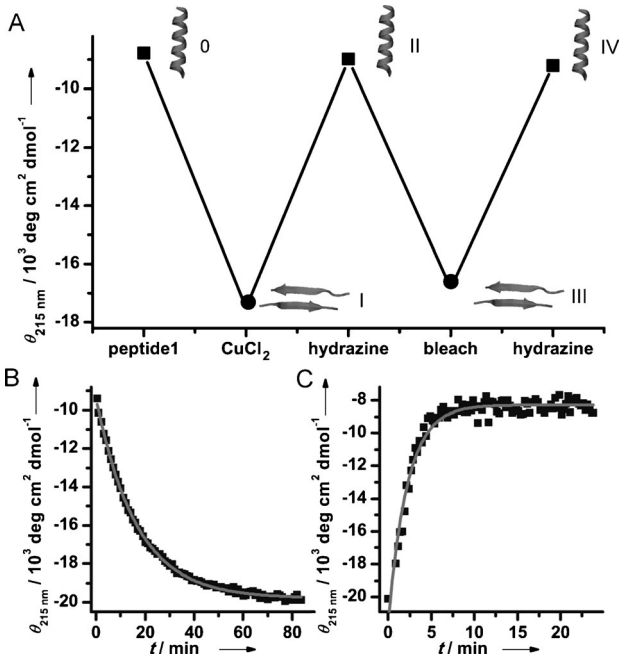

Figure 3. Peptide **1** reversibly responds to the oxidation state of Cu. A) Conformation of peptide **1** (50 μM) in MOPS/MeCN was triggered by addition of $CuCl_2$ (0.3 mM, I), hydrazine (0.25 mM, II), bleach (0.75 mM, III), or hydrazine (0.35 mM, IV). B) α-to-β transition of peptide **1** trigged by $CuCl_2$ and monitored by CD at 215 nm. Experimental data is shown in black squares and fit curve is shown as gray line. C) β-to-α transition of peptide **1**–Cu^{II} complex trigged by hydrazine and monitored by CD at 215 nm. Experimental data is shown in black squares and fit curve is shown as gray line.

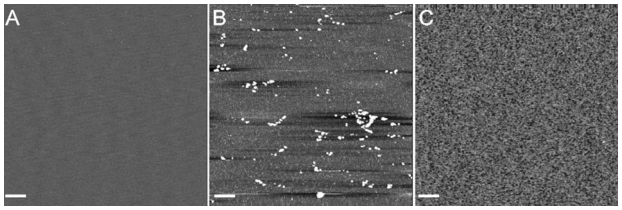

Figure 4. AFM image of A) peptide **1** in solution; B) peptide **1** with $CuCl_2$ in solution; and C) peptide **1** with $CuCl_2$ after reduction. Scale bars: 200 nm.

individual helices. However, peptide **1** is a rare example of a β-sheet aggregate switching back to the α-helical state and may offer a model for conformational changes associated with disease states.[11] Our model provides a basis for relating the size of the Cu aggregate to its irreversibility. Cu^{II} has been implicated in the formation of prion assemblies by coordinating to histidine sites. Based on the hypothesis that numerous proteins can form amyloid fibrils,[12] we conjecture that the interaction of carboxylate groups to Cu^{II} under physiological conditions may play an important, and yet unappreciated, role in such processes. In ongoing experiments, we are examining conserved protein domains that may selectively respond to the presence of Cu^{II}.

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