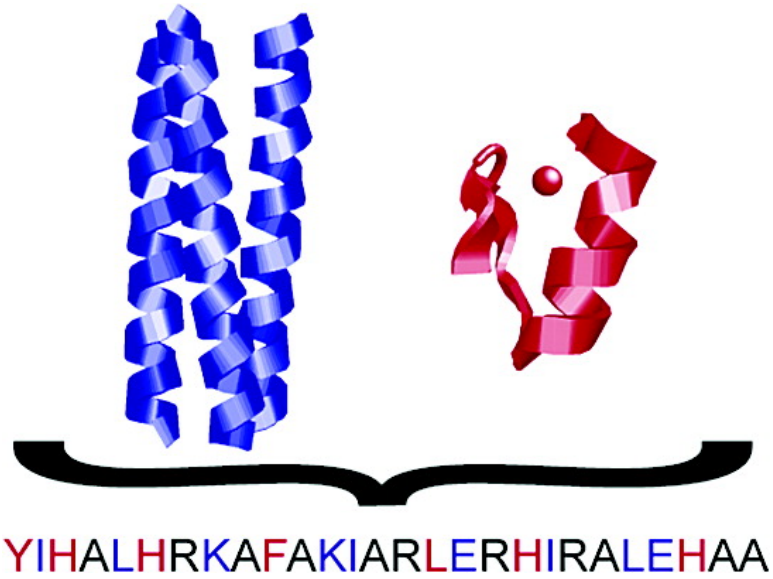
Communication

ZiCo: A Peptide Designed to Switch Folded State upon Binding Zinc

Eleonora Cerasoli, Belinda K. Sharpe, and Derek N. Woolfson

J. Am. Chem. Soc., **2005**, 127 (43), 15008-15009• DOI: 10.1021/ja0543604 • Publication Date (Web): 08 October 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 11 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 10/08/2005

ZiCo: A Peptide Designed to Switch Folded State upon Binding Zinc

Eleonora Cerasoli, Belinda K. Sharpe, and Derek N. Woolfson*,†

Department of Biochemistry, School of Life Sciences, University of Sussex, Falmer BN1 9QG, U.K.

Received July 1, 2005; E-mail: D.N.Woolfson@bristol.ac.uk

A major goal of protein-folding studies is to understand how amino acid sequence determines structure. This is complicated by the growing realization that conformational changes in proteins are key to many biological processes[1] and in disease.[2] Thus, the long-held tenet of protein folding that "one sequence determines one structure" is breaking down to some extent, and some sequences encode two or more folded states.[3] We refer to this as structural duality.[4] Protein design offers a route to test our understanding of sequence-to-structure relationships. Several peptide- and protein-based switches have been designed.[4,5] In some cases, switches between different oligomers or binding states are induced by noncovalent interactions.[5h−j] Other systems introduce structural duality at the sequence level, although only a small number of these have been designed de novo.[4,5a−g] Such systems would have academic and technological value.

We are interested in the de novo design of peptides that encode two distinct sequence patterns for different structures simultaneously within the same sequence, and, thus, may be switched between the different states.[4] Our previously described systems are one-way switches; they are triggered by heat, or the reduction of a disulfide bond, and they are not easily reversible.

Here, we describe the design of a reversible switch between two different folded conformations triggered by a noncovalent interaction, namely, metal binding. Given the roles played by metal ions in biology, the incorporation of a metal-binding site as the trigger may open new routes to the development of peptide-based metal sensors. To date, however, the design of metal-binding sites has primarily been to stabilize a previously unstructured or partially structured peptide, or to incorporate a metal-binding site onto a preformed protein scaffold.[6] To our knowledge, it has not been previously exploited to instigate a conformational switch between two distinct folded states.

Our design process involved the merger of sequence motifs for two different target folded conformations. For the metal-free structure, we chose the coiled coil. Sequence-to-structure relationships for this fold are well understood.[7a] For instance, hydrophobic residues alternate three and four residues apart along the sequence—or with patterns closely similar to this—and the precise nature of these residues largely determines coiled-coil stability and 3D structure. The second, metal-bound structure was based on the classical zinc-finger motif[8] and designed helix-loop-helix structures that bind zinc.[9] Figure 1 shows the hydrophobic-repeat and consensus sequences for these targets and the designed sequence based on the superimposition of these two motifs. The aim was that the peptide would display structural duality, forming an amphipathic helix for coiled-coil oligomerization in the absence of Zn^{2+} and a metal-bound monomer with buried hydrophobic residues in the presence of Zn^{2+}. In the final design, the two zinc-ligating Cys were replaced with His, to avoid problems with Cys oxidation;[10] and a noncanonical coiled-coil pattern—namely, a 3−4−4−3−4−3−4 hydrophobic pattern as seen in the structure of the hemagglutinin trimer[7b]—was employed to give flexibility between the β-hairpin and α-helix of the alternate zinc-binding target structure.[11] We dubbed the resulting peptide ZiCo for Zinc-binding/Coiled coil.

```
hpphpppphpppphpphpppphpphpppph  (A)
YxCxxCxxxFxxxxxLxxHxxxxxHxx  (B)
YIHALHRKAFAKIARLERHIRALEHAA  (C)
```

Figure 1. (A) Noncanonical coiled-coil pattern of hydrophobic (h) and polar (p) residues; (B) zinc-finger consensus sequence; (C) peptide ZiCo.

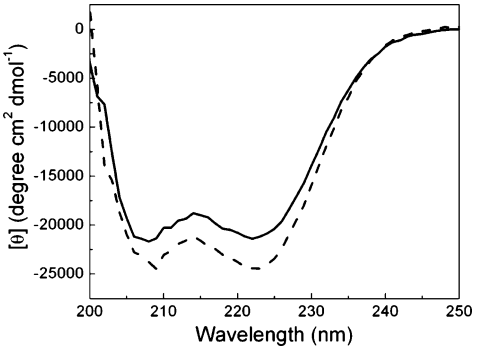


Figure 2. CD spectra for ZiCo without (—) and with (- - -) zinc. Conditions: 100 μM peptide, 5 °C, 50 mM sodium phosphate, pH 7.5, 50 mM NaCl; and with 100 μM $ZnCl_2$ (dashed spectra).

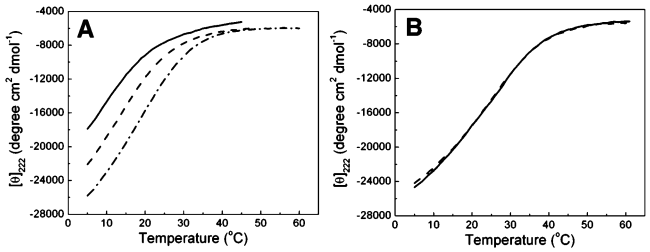


Figure 3. Thermal unfolding curves for ZiCo: (A) without zinc at 50 μM (—), 100 μM (- - -), and 200 μM (- • - • -) peptide; (B) with equimolar zinc at 50 μM (—) and 100 μM (- - -) peptide.

ZiCo was synthesized by Fmoc-based solid-phase methods, purified by RP-HPLC, and confirmed by MALDI-TOF mass spectrometry (Supporting Information).

Circular dichroism (CD) spectra of ZiCo without Zn^{2+} showed minima at 208 and 222 nm indicative of α-helix (Figure 2). Consistent with this, the FT-IR spectrum had a single band at 1651 ± 2 cm^{-1} (Supporting Information).[12] The intensity of the CD signal at 222 nm suggested ∼50% helix.[13] This structure unfolded with a sigmoidal curve upon heating (Figure 3A), indicative of a cooperatively folded structure, albeit of low thermal stability: the midpoint of the unfolding from the first derivative of the thermal unfolding curves was 14 ± 1 °C at 100 μM peptide. As expected for a coiled-coil oligomer, the T_M was concentration dependent: at 50 μM peptide the T_M was 10 ± 1 °C, and 18 ± 1 °C at 200 μM peptide (Figure 3A). Analytical ultracentrifugation (AUC) data gave a molecular weight of 8510 ± 461 Da when fitted to a single-species model, and a K_D of 10−100 μM (Figure 4A) with a monomer−trimer equilibrium model. Together, these data are

† Present address: School of Chemistry, University of Bristol, Bristol, BS8 1TS, UK.

10.1021/ja0543604 CCC: $30.25

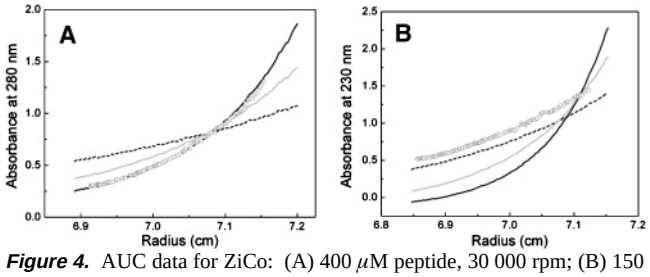


Figure 4. AUC data for ZiCo: (A) 400 μM peptide, 30 000 rpm; (B) 150 μM peptide with zinc, 36 000 rpm. Experimental data are shown as open circles, and theoretical curves for monomers, dimers, and trimers as broken, gray, and black lines, respectively. Conditions: 5 °C, 50 mM sodium phosphate, pH 7.5, 50 mM NaCl. (See Supporting Information.)

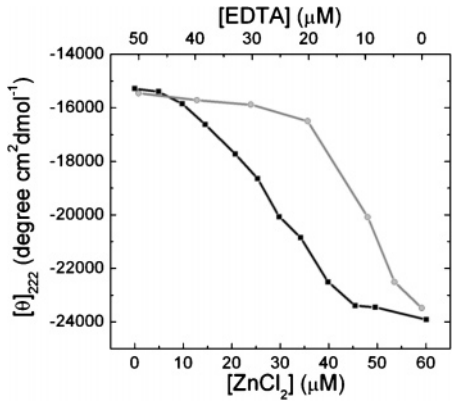


Figure 5. Zinc binding (black squares) and EDTA-induced release (gray circles) by ZiCo monitored by CD spectroscopy. Conditions: 5 °C, 50 mM sodium phosphate, pH 7.5, 50 μM peptide (for both titrations).

consistent with ZiCo forming an α-helical coiled-coil oligomer in the absence of zinc as designed.

Upon the addition of zinc, both the CD (Figure 2) and FT-IR spectra changed; the intensity of the CD signal increased indicative of ∼15% more helix, and the FT-IR spectra had an additional component at 1633 ± 2 cm^{-1} indicative of β-sheet. The thermal unfolding behavior also changed. First, zinc stabilized the structure. Second, with zinc, the T_M of ZiCo was concentration independent at 23 ± 1 °C, consistent with the peptide folding as a monomer. This was confirmed by AUC, which fitted best to a single-species model with a molecular weight of 3142 ± 328 Da (Figure 4B), in reasonable agreement with the predicted monomer molecular weight with zinc of 3296 Da.

To investigate zinc binding further, and to check for reversibility, CD spectroscopy was used to follow zinc titrations at 50 and 100 μM peptide concentrations. In both cases, saturation was achieved at equimolar concentrations of peptide:Zn^{2+} (Figure 5), and no further changes were observed upon excess Zn^{2+} addition.[14] Furthermore, the switches were reversible as the signal returned to that of the peptide alone upon addition of EDTA. The noncoincidence of the binding and release curves indicated the expected higher affinity of EDTA for zinc, which is reported as 10^{-14} M.[10a] The binding affinity of ZiCo was measured directly by isothermal titration calorimetry (ITC); the titration curves fitted to single-site binding models and returned K_D values in the range of 3−5 μM (Supporting Information).

A difference in structure of the two forms of ZiCo was further supported by 1D ^{1}H NMR spectra of ZiCo without and with Zn^{2+} (Supporting Information). Spectra recorded without Zn^{2+} showed little dispersion, consistent with an all α-helical fold without buried aromatic residues. However, those with zinc present showed greater chemical shift dispersion and sharper peaks consistent with a smaller alternately folded species. We are pursuing high-resolution structural studies of both forms.

In summary, we describe the design and solution-phase characterization of a peptide that reversibly switches between a trimeric α-helical coiled coil and a zinc-bound folded monomer. Understanding how to switch the folded and oligomerization states of such peptide (and protein) systems by small-molecule binding could lead to the development of new sensing devices.[15]

Acknowledgment. We thank David Papapostolou and Max Ryadnov for many valuable discussions and peptide synthesis, Geoff Kelly and Tom Frenkiel for access to and assistance with NMR spectroscopy at NIMR, Mill Hill, and the BBSRC and MRC of the U.K. for financial support (B17958 and G0300539).

Supporting Information Available: Materials and methods, and HPLC, MALDI-TOF, FT-IR, ITC, and NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Lee, K. N.; Park, S. D.; Yu, M. H. *Nat. Struct. Biol.* **1996**, *3*, 497−500. (b) Whisstock, J.; Skinner, R.; Lesk, A. M. *Trends Biochem. Sci.* **1998**, *23*, 63−67. (c) Skehel, J. J.; Wiley, D. C. *Annu. Rev. Biochem.* **2000**, *69*, 531−569.
(2) (a) Rochet, J. C.; Lansbury, P. T. *Curr. Opin. Struct. Biol.* **2000**, *10*, 60−68. (b) Cohen, F. E. *J. Mol. Biol.* **1999**, *293*, 313−320. (c) Carrell, R. W.; Gooptu, B. *Curr. Opin. Struct. Biol.* **1998**, *8*, 799−809.
(3) (a) Minor, D. L.; Kim, P. S. *Nature* **1996**, *380*, 730−734. (b) Kuznetsov, I. B.; Rackovsky, S. *Protein Sci.* **2003**, *12*, 2420−2433.
(4) (a) Pandya, M. J.; Cerasoli, E.; Joseph, A.; Stoneman, R. G.; Waite, E.; Woolfson, D. N. *J. Am. Chem. Soc.* **2004**, *126*, 17016−17024. (b) Ciani, B.; Hutchinson, E. G.; Sessions, R. B.; Woolfson, D. N. *J. Biol. Chem.* **2002**, *277*, 10150−10155.
(5) (a) Dalal, S.; Balasubramanian, S.; Regan, L. *Nat. Struct. Biol.* **1997**, *4*, 548−552. (b) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 12609−12610. (c) Mutter, M.; Gassmann, R.; Buttkus, U.; Altmann, K. H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1514−1516. (d) Fezoui, Y.; Hartley, D. M.; Walsh, D. M.; Selkoe, D. J.; Osterhout, J. J.; Teplow, D. B. *Nat. Struct. Biol.* **2000**, *7*, 1095−1099. (e) Takahashi, Y.; Yamashita, T.; Ueno, A.; Mihara, H. *Tetrahedron* **2000**, *56*, 7011−7018. (f) Cerpa, R.; Cohen, F. E.; Kuntz, I. D. *Fold. Des.* **1996**, *1*, 91−101. (g) Zhang, S.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 23−28. (h) Gonzalez, L.; Plecs, J. J.; Alber, T. *Nat. Struct. Biol.* **1996**, *3*, 510−515. (i) Gonzalez, L.; Brown, R. A.; Richardson, D.; Alber, T. *Nat. Struct. Biol.* **1996**, *3*, 1002−1009. (j) Lin, Q.; Barbas, C. F.; Schultz, P. G. *J. Am. Chem. Soc.* **2003**, *125*, 612−613.
(6) (a) Ghosh, D.; Pecoraro, V. L. *Inorg. Chem.* **2004**, *43*, 7902−7915. (b) Ghosh, D.; Pecoraro, V. L. *Curr. Opin. Chem. Biol.* **2005**, *9*, 97−103. (c) Nitz, M.; Sherawat, M.; Franz, K. J.; Peisach, E.; Allen, K. N.; Imperiali, B. *Angew Chem., Int. Ed.* **2004**, *43*, 3682−3685. (d) Tanaka, T.; Mizuno, T.; Fukui, S.; Hiroaki, H.; Oku, J.; Kanaori, K.; Tajima, K.; Shirakawa, M. *J. Am. Chem. Soc.* **2004**, *126*, 14023−14028.
(7) (a) Woolfson, D. N. *Adv. Protein Chem.* **2005**, *70*, 79−112. (b) Bullough, P. A.; Hughson, F. M.; Skehel, J. J.; Wiley, D. C. *Nature* **1994**, *371*, 37−43.
(8) (a) Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1996**, *118*, 3053−3054. (b) Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1997**, *119*, 3443−3450. (c) Godwin, H. A.; Berg, J. M. *J. Am. Chem. Soc.* **1996**, *118*, 6514−6515. (d) Berg, J. M.; Godwin, H. A. *Annu. Rev. Biophys. Biomol. Struct.* **1997**, *26*, 357−371. (e) Michael, S. F.; Kilfoil, V. J.; Schmidt, M. H.; Amann B. T.; Berg J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 4796−4800. (f) Krishna, S. S.; Majumdar, I.; Grishin, N. V. *Nucleic Acids Res.* **2003**, *31*, 532−550.
(9) (a) Handel, T.; DeGrado, W. F. *J. Am. Chem. Soc.* **1990**, *112*, 6710−6711. (b) Regan, L.; Clarke, N. D. *Biochemistry* **1990**, *29*, 10878−10883.
(10) (a) Kimura, E.; Aoki, S. *BioMetals* **2001**, *14*, 191−204. (b) Walkup G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1997**, *119*, 3443−3450.
(11) Hicks, M. R.; Walshaw, J.; Woolfson, D. N. *J. Struct. Biol.* **2002**, *137*, 73−81.
(12) (a) Dong, A.; Huang, P.; Caughey, W. S. *Biochemistry* **1990**, *29*, 3303−3308. (b) Haris, P. I.; Severcan, F. *J. Mol. Catal. B: Enzym.* **1999**, *7*, 207−221. (c) Surewicz, W. K.; Mantsch, H. H.; Chapman, D. *Biochemistry* **1993**, *32*, 389−394.
(13) Myers, J. K.; Pace, N. C.; Scholtz, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2833−2837. The amount of helix was calculated in the following way: $F_{helix} = ([\theta]_{obs} - [\theta]_{coil}) / ([\theta]_{helix} - [\theta]_{coil})$, where $[\theta]_{coil} = +640$ degree cm^2 $dmol^{-1}$ and $[\theta]_{helix} = [-42500\ (1 - 3/n)]$ (n = number of residues).
(14) Most experiments were carried out in phosphate buffer (pH 7.5) as this proved to be more suitable for the study of both folded conformations. However, given the low solubility of zinc phosphate, higher concentrations of zinc were tested in Tris-HCl, pH 7.5.
(15) (a) Kimura, E.; Aoki, S. *BioMetals* **2001**, *14*, 191−204. (b) Dwyer, M. A.; Looger, L. L.; Hellinga, H. W. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 11255−11260. (c) Ostermeier, M. *Protein Eng. Des. Select.* **2005**, *18*, 359−364.

JA0543604