Pergamon

*Bioorganic & Medicinal Chemistry Letters*, Vol. 6, No. 3, pp. 229-232, 1996

0960-894X/96 $15.00 + 0.00

S0960-894X(96)00009-1

# DISRUPTION OF COILED COIL FORMATION BY METHIONINE OXIDATION

Carlos García-Echeverría

*Pharmaceuticals Division, Ciba-Geigy Ltd., CH-4002 Basel, Switzerland*

**Abstract:** On the basis of previous data, we have designed a leucine-zipper peptide whose folding preferences are controlled by the oxidation state of a single methionine residue. The peptide Ac-Lys-Ala-Glu-Ile-Glu-Ala-Leu-Lys-Ala-Glu-Met-Glu-Ala-Leu-Lys-Ala-Glu-Ile-Glu-Ala-Leu-Lys-Ala-$NH_2$ self-associates in aqueous media to form a parallel coiled coil, but the dimerization function of the peptide is abolished upon conversion of the methionine side chain to its sulfoxide form.

The leucine-zipper coiled coil is an important structural motif in gene regulation[1] and a frequent molecular scaffold in the *de novo* protein design.[2] Leucine-zipper polypeptides have a characteristic seven-residue repeat, conventionally termed **a•b•c•d•e•f•g**, with a preponderance of hydrophobic β-branched amino acids at the **a** position and leucines at the **d** position.[3] We have recently shown by circular dichroism that a hydrophobic core mutation in the central region, but not at the *N*-terminus, of a wild-type leucine zipper has a dramatic effect on the folding properties of the peptide.[4] On the basis of this result and using a reported peptide sequence,[5] we have designed a leucine zipper peptide (peptide **1**) whose folding preferences are controlled by the oxidation state of a single methionine residue.[6] Thus, the dimerization function of the leucine zipper peptide is abolished upon conversion of methionine to its sulfoxide form (peptide **2**).

Ac-Lys-Ala-Glu-Ile-Glu-Ala-Leu-Lys-Ala-Glu-Xxx-Glu-Ala-Leu-Lys-Ala-Glu-Ile-Glu-Ala-Leu-Lys-Ala-$NH_2$

(Xxx= Met, peptide **1**; Xxx= Met(O), peptide **2**)

N-1-pyrenebutyrolyl-Gly-Gly-Lys-Ala-Glu-Ile-Glu-Ala-Leu-Lys-Ala-Glu-Met-Glu-Ala-Leu-Lys-Ala-Glu-Ile-Glu-Ala-Leu-Lys-Ala-$NH_2$ (peptide **3**)

(Xxx= Met, peptide **3**; Xxx= Met(O), peptide **4**)

Peptides **1** and **3** were synthesized on a Milligen 9050 automated peptide synthesizer (continuous flow), employing the fluorenylmethoxycarbonyl (Fmoc) strategy.[7] The required Fmoc-amino acids (3 equiv.; single coupling) were incorporated using their 2,4,5-trichlorophenyl esters with minimum reaction times of 30 min. Side chains were protected with the following groups: *tert*-butyl for glutamic acid; and *tert*-butyloxycarbonyl for lysine. Incorporation of 1-pyrenebutyric acid was carried out as previously described.[8] After completion of the syntheses, the peptide resins were simultaneously cleaved/deprotected with trifluoroacetic acid/water/ethanedithiol (76:4:20, v/v/v) for 3 h at room temperature, and the crude compounds were purified by reversed-phase medium-pressure liquid chromatography on a $C_{18}$-column (0.1 % trifluoroacetic acid-acetonitrile gradient). Peptides **2** and **4** were obtained by oxidation of peptides **1** and **3**, respectively, with 5 % hydrogen peroxide (90 min at room temperature). The purity of these peptides was verified by reversed-phase

analytical HPLC,[9] and the identity of the final products assessed by correct amino acid and mass spectral (matrix-assisted laser-desorption ionization time-of-flight mass spectrometry, MALDI-TOF) analyses.[10]

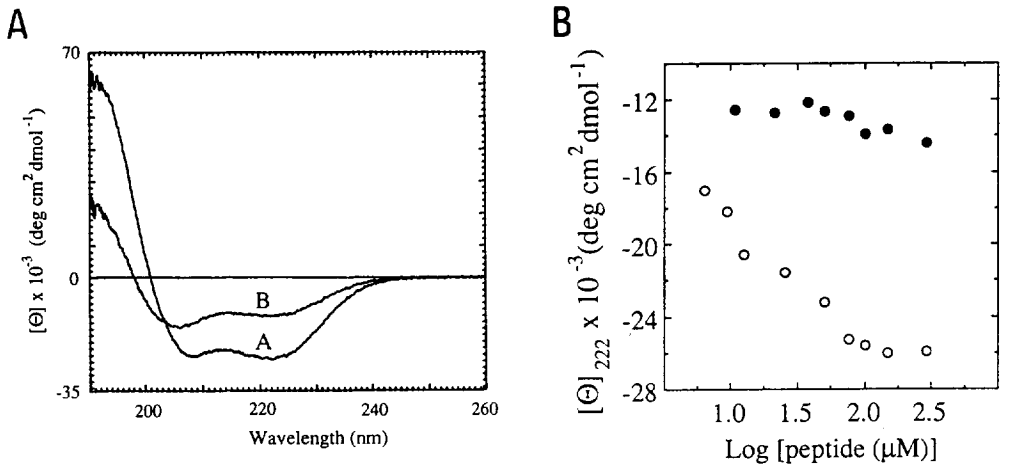


*Figure 1.* **A)** Circular dichroism of peptide **1** (c=150 μM; T= 20 °C). (A) 100 mM NaCl, 10 mM phosphate buffer, pH= 7.0. The CD spectrum of peptide **2** is also shown (c= 150 μM; T= 20 °C). (B) 100 mM NaCl, 10 mM phosphate buffer, pH= 7.0. **B)** Effect of peptide concentration on the mean residue ellipticity [Θ] at 222 nm for peptide **1** (○) and peptide **2** (●) in 100 mM NaCl, 10 mM phosphate buffer, pH= 7.0, T= 20 °C.

Although methionine is not a common residue in the hydrophobic **a** or **d** positions of wild-type leucine zippers,[11] we posited that this amino acid would be suitable at the **a** position of an engineered leucine-zipper peptide. To test this idea, the isoleucine residue in the central position of a recently published leucine zipper sequence[5] was replaced by methionine, which acts as a "switchable" amino acid.[12] The benignancy of this replacement was studied by circular dichroism and fluorescence spectroscopy. The CD-spectrum of peptide **1** in an aqueous buffer (100 mM NaCl, 10 mM phosphate buffer, pH= 7.0) shows the characteristic minima of an α-helix at 208 nm and 222 nm (Figure 1A).[13] The variation of the molar ellipticity minima at 222 nm with the peptide concentration (Figure 1B) is attributed to the existence of an equilibrium between monomeric peptide and multimeric species ($K_d$= 4.8 ± 1.0 μM);[14] a decrease in the peptide concentration shifts the equilibrium toward the monomeric peptide, decreasing the α-helical content (78 % at c=150 μM *versus* 51 % at c= 4.7 μM).[15] CD-studies on model coiled coil peptides have reported a $[\Theta]_{222}/[\Theta]_{208} \approx 1.03$ for coiled coil conformations in benign medium[16] and a $[\Theta]_{222}/[\Theta]_{208} \approx 0.86$ for single-stranded α-helix polypeptides when dissolved in different amounts of TFE.[2e,16d,17] The value of $[\Theta]_{222}/[\Theta]_{208}$= 1.04 (c= 150 μM, 20 °C) obtained for peptide **1** in aqueous buffer supports a coiled coil conformation, which is disrupted in 90 % TFE ($[\Theta]_{222}/[\Theta]_{208}$= 0.81; c= 150 μM, 20 °C; data not shown). The relative orientation of the polypeptide chains in the coiled coil conformation was determined by fluorescence spectroscopy. Peptide **1** was appended at the *N*-terminus with 1-pyrenebutyric acid, using a glycine-glycine dipeptide as a spacer (peptide **3**). It has been shown that a pyrene-labeled leucine zipper polypeptide displays excimer fluorescence (maximum at 480 nm) when the polypeptide self-associates to form a parallel coiled coil and monomer fluorescence (maxima at 380, 400 nm) when the peptide adopts a single-stranded conformation.[8] Representative fluorescence-emission

spectra of peptide **3** are shown in Figure 2A. An excimer fluorescence band with maxima at 480 nm was observed for this peptide in aqueous buffer, but not in 2,2,2-trifluoroethanol. These results, which are in agreement with the solvent-dependent conformation determined by circular dichroism, confirmed that peptide **1** self-associates in benign medium to form a parallel coiled coil.

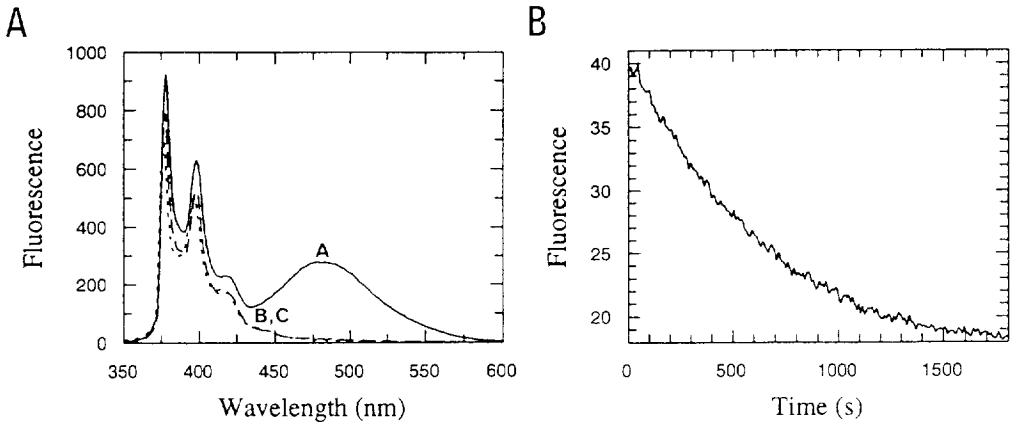


*Figure 2.* **A)** Fluorescence emission spectra ($\lambda_{ex}$ = 308 nm) of peptide **3** (c= 5 μM; T= 20 °C). (A) 100 mM NaCl, 10 mM phosphate buffer, pH= 7. (B) 2,2,2-Trifluoroethanol. The fluorescence emission spectra of peptide **4** is also shown (c= 5 μM; T= 20 °C). (C) 100 mM NaCl, 10 mM phosphate buffer, pH= 7.0. **B)** Time-line of the decrease in the excimer fluorescence at $\lambda_{em}$= 480 nm ($\lambda_{ex}$ = 308 nm) upon addition of hydrogen peroxide (150 μl of 30 % $H_2O_2$) to a solution of peptide **3** (c= 5 μM; phosphate buffer; T= 20 °C; V= 2.0 ml).

In accordance with our expectations, oxidation of the methionine side chain has a dramatic effect on the conformation of the 23-mer peptide. Peptide **2** exhibits a circular dichroism spectrum with molar ellipticity minima at 206 nm and 220 nm (Figure 1A). The CD spectrum profile is a population-weighted superimposition of α-helix, β-sheet, and random coil reference CD spectra. The CD pattern, the value of the ratio $[\Theta]_{222}/[\Theta]_{208}$= 0.85, and the low α-helical content (41 %, c= 150 μM)[15] provide evidence that peptide **2** is not stabilized in a coiled coil conformation. The default folding autonomy of peptide **2** was also confirmed by fluorescence spectroscopy. Thus, the pyrene-labeled peptide (peptide **4**) shows a monomer fluorescence emission spectrum in aqueous buffer (Figure 2A) identical to the fluorescence emission profile of peptide **3** in 2,2,2-trifluoroethanol, a known disrupter of the coiled-coil structure. Furthermore, a time-line decrease in the excimer fluorescence maximum at 480 nm was observed by fluorescence monitoring of the oxidation reaction of peptide **3** with hydrogen peroxide (Figure 2B). The lack of dimerization function of peptide **2** is ascribed to the hydrophilic character of the methionine sulfoxide side chain. Its polar nature disrupts the otherwise conserved hydrophobic dimerization interface, destabilizing the coiled coil and impeding the formation of a cooperatively folded structure.

In this communication, it has been shown that a specifically designed leucine zipper peptide containing a single methionine residue in an **a** position self-associates to form a parallel coiled-coil.[18] Oxidation of this amino acid abrogates the dimerization function of the peptide. The possibility of controlling the conformational preferences of engineered leucine-zipper polypeptides by the above method adds an extra feature to the use of coiled-coil motifs in the *de novo* protein design and in protein-folding studies.

## Acknowledgements

I thank R. Wille for his technical assistance, Dr. E. John for the use of the CD instrument and the Friedrich Miescher-Institut for allowing me to use their fluorescence spectrometer.

## References and Notes

1. (a) Kerppola, T.; Curran, T. *Nature* **1995**, 373, 199-200. (b) Cohen, C.; Parry, D.A.D. *Science* **1994**, 263, 488-489. (c) Pathak, D.; Sigler, P.B. *Current Biology* **1992**, 2, 116-123. (d) Abel, T.; Maniatis, T. *Nature* **1989**, 341, 24-25.
2. (a) Slate, C.A.; Weninger, S.C.; Church, F.C.; Erickson, B.W. *Int. J. Peptide Protein Res.* **1995**, 45, 290-298. (b) Graddis, T.J.; Myszka, D.G.; Chaiken, I.M. *Biochemistry* **1993**, 32, 12664-12671. (c) O'Shea, E.K.; Lumb, K.J.; Kim, P.S. *Current Biology* **1993**, 3, 658-667. (d) Moser, R. *Protein Eng.* **1992**, 5, 323-331. (e) Engel, M.; Willians, R.W.; Erickson, B.W. *Biochemistry* **1991**, 30, 3161-3169.
3. (a) Hurst, H.C. *Protein Profile* **1994**, 1, 125-134. (b) Landschulz, W.H.; Johnson, P.F.; McKnight, S.L. *Science* **1988**, 240, 1759-1764.
4. García-Echeverría, C. *Lett. Pept. Sci.* **1994**, 1, 255-262.
5. The peptide Ac-Lys-Ala-Glu-Ile-Glu-Ala-Leu-Lys-Ala-Glu-Ile-Glu-Ala-Leu-Lys-Ala-Glu-Ile-Glu-Ala-Leu-Lys-Ala-$NH_2$ was reported by Su, J.Y.; Hodges, R.S.; Kay, C.M. *Biochemistry* **1994**, 33, 15501-15510. Kay and co-workers found that a minimum of three heptad repeats was required to form a stable two-stranded α-helical coiled-coil.
6. This work expands the "switchable residue" concept of Dado and Gellman: (a) Dado, G.P.; Gellman, S.H. *J. Am. Chem. Soc.* **1993**, 115, 12609-12610. Gellman used methionine residues to control the secondary structure preference, α-helix *versus* β-strand, of an 18-mer peptide. For a discussion on the conformational properties of methionine, see: (b) Gellman, S.H., *Biochemistry* **1991**, 30, 6633-6635.
7. Atherton, E.; Sheppard, R.C. In *The Peptides*; Gross, E.; Meinhofer, J., Eds.; Academic Press: New York, 1987; Vol. 9, pp 1-38.
8. García-Echeverría, C. *J. Am. Chem. Soc.* **1994**, 116, 6031-6032.
9. Linear gradient over 20 min of $CH_3CN$-0.09% TFA and $H_2O$-0.1% TFA from 1:19 to 19:1 on a $C_{18}$-column (250 x 4.0 mm; 5 μm, 100 Å), flow rate 1.5 ml/min, detection at 215 nm; single peak at $t_R$= 11.73 (peptide **1**); $t_R$=10.68 (peptide **2**); $t_R$= 13.08 (peptide **3**); and $t_R$=12.52 (peptide **4**).
10. Quantitative amino acid analyses of the peptides revealed amino acid compositions within 5% of the expected values. MALDI-TOF mass spectra (negative or positive-ion mode): calc. 2540.0 [M-H], found 2539.7 (peptide **1**); calc. 2556.0 [M-H], found 2555.4 (peptide **2**); calc. 2884.4 [M+H], found 2884.8 (peptide **3**); calc. 2900.4 [M+H], found 2900.9 (peptide **4**).
11. Methionine residues have been found in the **a** positions of CREBA (CRE-binding protein), ATF6 (activating transcription factor), and YAP1 (yeast AP1-binding factor), and in the **d** positions of TGA1b (TGACG-sequence-specific binding proteins, plants) and BZLF1 (EBV immediate early protein; ref. 3(a)). It has also been found in an **a** position of GCN4 (general control protein 4, yeast): (a) O'Shea, E.K.; Klemm, J.D.; Kim, P.S.; Alber, T. *Science* **1991**, 254, 539-544; and in a **d** position of the l-Myc gene product: Prendergast, G.C.; Lawe, D.; Ziff, E.B. *Cell* **1991**, 65, 395-407.
12. The term *switchable* is taken from reference 6(a).
13. Woody, R.W. In *The Peptides: Analysis, Synthesis, and Biology*, Hruby, V.J., Ed.; Academic Press: New York, Vol. 8, 1985, pp 15-114.
14. Size-exclusion chromatography experiments indicated that peptide **1** has essentially the same molecular weight as that of Ac-Lys-Ala-Glu-Ile-Glu-Ala-Leu-Lys-Ala-Glu-Ile-Glu-Ala-Leu-Lys-Ala-Glu-Ile-Glu-Ala-Leu-Lys-Ala-$NH_2$, which was used as standard and has been reported to adopt a two-stranded α-helical coiled coil conformation in aqueous media (ref. 5). In accordance with this result, the monomer/dimer equilibrium of peptide **1** is described by the equation $(\Theta - \Theta_m) / (\Theta_{max} - \Theta_m) = 2\, c_o\, [1 - (\Theta - \Theta_m) / (\Theta_{max} - \Theta_m)]^2 / K_d$, where $\Theta$ is the molar ellipcity at 222 nm, $c_o$ is the peptide concentration, $\Theta_{max}$ is the molar ellipticity of the folded dimer, and $\Theta_m$ is the molar ellipticity of the unfolded monomer. $\Theta_m$ was taken to be 2500 deg $cm^2$ $dmol^{-1}$ (Scholz, J.M.; Qian, H.; York, E.J.; Stewart, J.M.; Baldwin, R.L. *Biopolymers* **1991**, 31, 1463-1470. The above equation was fitted (GraFit 3.0, Erithacus Software Limited) to $\Theta$ (Figure 1B, peptide **1**) to obtain $K_d$= 4.8 ± 1.0 μM and $\Theta_{max}$= 29 247 ± 537 deg $cm^2$ $dmol^{-1}$.
15. The α-helical content was calculated by setting a 100% helix value equal to $[\Theta]_H^n$= -33 334 deg $cm^2$ $dmol^{-1}$. The theoretical molar ellipticity ($[\Theta]_H^n$) was obtained using the equation $[\Theta]_H^n = [\Theta]_H^\infty \times (1 - k/n)$, where $[\Theta]_H^\infty$= -37 400 deg $cm^2$ $dmol^{-1}$, n= 23 (number of residues), and k= 2.5 (λ= 222 nm). Chen, Y.-H; Yang; J.T.; Chau, K.H. *Biochemistry* **1974**, 13, 3350-3359.
16. (a) Zhou, N.E.; Kay, C.M.; Hodges, R.S. *Biochemistry* **1992**, 31, 5739-5746. (b) Hodges, R.S.; Zhou, N.E.; Kay, C.M.; Semchuk, P.D. *Pept. Res.* **1990**, 3, 123-137. (c) Hodges, R.S.; Semchuk, P.D.; Taneja, A.K.; Kay, C.M.; Parker, J.M.R.; Mant, C.T. *Pept. Res.* **1988**, 1, 19-30. (d) Lau, S.Y.M.; Taneja, A.K.; Hodges, R.S. *J. Biol. Chem.* **1984**, 259, 13253-13261.
17. Zhou, N.E.; Kay, C.M.; Hodges, R.S. *J. Biol. Chem.* **1992**, 267, 2664-2670.
18. It has been shown by genetic analysis of the GCN4 leucine zipper that single mutagenesis in the **a** or **d** positions by methionine was completly benign. Hu, J.C.; O'Shea, E.K.; Kim, P.S.; Sauer, R.T. *Science* **1990**, 250, 1400-1403.

(*Received in Belgium* 2 *October* 1995; *accepted* 13 *December* 1995)