ORGANIC LETTERS

2002
Vol. 4, No. 26
4655−4657

Conformational Stability of Helical Peptides Containing a Thioamide Linkage

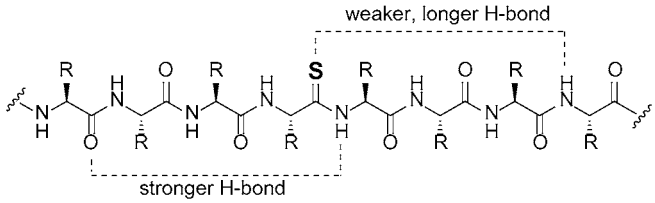
Julia H. Miwa,* Letha Pallivathucal, Shyla Gowda, and Katherine E. Lee

Department of Chemistry, Wellesley College, Wellesley, Massachusetts 02481

jmiwa@wellesley.edu

Received October 8, 2002

ABSTRACT



Thioxo peptide analogues of the α-helical peptide GCN4-p1 were synthesized and evaluated for helicity and oligomeric state. Sedimentation equilibrium and CD measurements indicate that the thioxo peptides fold into parallel α-helical coiled coil structures essentially identical to the native structure. This work marks the first incorporation of a thioamide linkage into the backbone of an α-helix and demonstrates that a thioamide linkage is compatible with positions within the helix as well as near the C-terminus.

In efforts to understand and control peptide and protein conformation, amino acids with unnatural side chains (side chains other than the 20 standard coded amino acids) and altered backbone structures have proven to be useful tools.[1] Thioxylated amino acids, in which the carbonyl oxygen is replaced with a sulfur atom, have been used in studies of biologically active peptides[1c,2] but have not been exploited as conformational constraints in peptide and protein design. The thioamide is a nearly isosteric replacement for the amide.[3] The thioamide NH is a stronger hydrogen bond donor than the amide NH, while the sulfur is a weaker hydrogen bond acceptor than the amide oxygen.[4] Recent computational studies suggest that although the bulkier thioamide restricts the available ϕ and ψ angles of the amino acids flanking the thioamide group, backbone conformations compatible with the three major types of regular secondary structures (α-helix, β-sheet, and β-turn) are accessible to thioxylated amino acids.[5] A thioamide has been incorporated into a peptide that adopts a β-hairpin conformation,[6] but thioamides have not been observed in the backbones of α-helical peptides. Thioxylated amino acids are expected to be compatible with the last four positions (at the C-terminus) of a helix, because at these sites the thioamide NH would be hydrogen bonded but the sulfur would not. At other positions in the helix, the thioamide is postulated to produce an interruption in the helical conformation because of the increase of approximately 0.9 Å in the C−S···H−N distance.[7]

Insertion of a thioamide linkage into a peptide with a high propensity to adopt an α-helical conformation could have one of several outcomes: the thioamide might be incorporated into the hydrogen bonding network of the helix, the thioamide might serve as a helix breaker and prevent folding

(1) (a) Karle, I. L.; Das, C.; Balaram, P. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3034. (b) Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 13701. (c) Spatola, A. F. *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*; Weinstein, B., Ed.; Dekker: New York, 1983; p 267.

(2) Clausen, K.; Spatola, A. F.; Lemieux, C.; Schiller, P. W.; Lawesson, S.-O. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 305.

(3) Bardi, R.; Piazzesi, A. M.; Toniolo, C.; Jensen, O. E.; Omar, R. S.; Senning, A. *Biopolymers* **1988**, *27*, 747.

(4) (a) Dudek, E. P.; Dudek, G. J. *J. Org. Chem.* **1967**, *32*, 823. (b) Lee, H. J.; Choi, Y.-S.; Lee, K.-B.; Park, J.; Yoon, C.-J. *J. Phys. Chem. A* **2002**, *106*, 7010.

(5) Tran, T. T.; Zeng, J., Treutlein, H.; Burgess, A. W. *J. Am. Chem. Soc.* **2002**, *124*, 5222.

(6) Miwa, J. H.; Patel, A. K.; Vivatrat, N.; Popek, S. M.; Meyer, A. M. *Org. Lett.* **2001**, *3*, 3373.

(7) La Cour, T. F. M. *Int. J. Peptide Protein Res.* **1987**, *30*, 564.

10.1021/ol027056d CCC: $22.00
Published on Web 12/05/2002

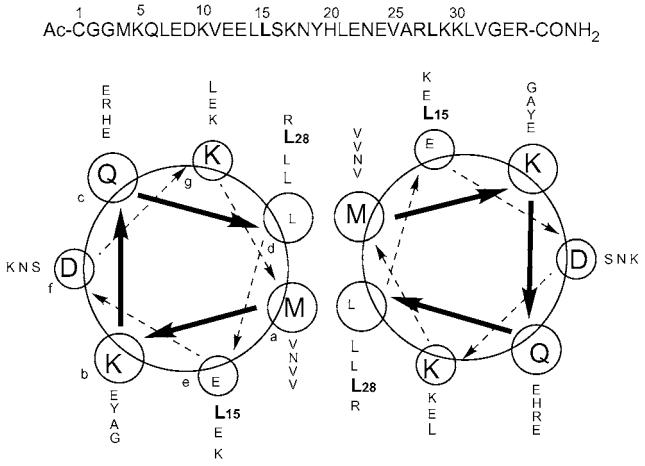


Figure 1. Sequence and helical wheel diagram of thioxo peptides T15 and T28. The carbonyl oxygen of Leu-15 is replaced with sulfur in peptide T15; the carbonyl oxygen of Leu-28 is replaced with sulfur in peptide T28.

of the helix, or the helix might fold with a distortion at the thioamide region, with the thioamide sulfur excluded from the hydrogen bonding network. We have investigated these possibilities by inserting thioamide linkages at two selected sites in a peptide that forms a two-stranded coiled coil in aqueous solution.

We chose a sequence corresponding to the leucine zipper of the yeast bZIP transcription factor GCN4 as the model α-helix for this work. O'Shea et al. have demonstrated that a synthetic peptide based on this sequence, GCN4-p1, folds into a stable α-helix that dimerizes to form a parallel, two stranded coiled coil.[8] The effect of modifications on the conformation of the peptide can be assessed by observing the helical structure using circular dichroism spectroscopy and by measuring the oligomerization state using analytical ultracentrifugation. Goodman and Kim have reported the amide proton exchange rates and predicted hydrogen-bond lengths for the synthetic peptide GCN4-p2N, which contains the same leucine zipper sequence.[9] Because each thioamide is expected to perturb the length and strength of two hydrogen-bonds in the helix, these data were helpful in selecting sites for thioamide incorporation.

We selected two sites in the GCN4-p1 sequence for thioxylation (Figure 1) and prepared two thioxo peptides (T*xx*, where *xx* = position in sequence of thioxylated amino acid), each with a single thioamide linkage. The two thioxo peptides were designed to allow an examination of the thioamide linkage in different local environments within the same helix. A comparison of the locations of the thioamide linkages in the two peptides is shown in Figure 1.

Peptide T15 has a thioxylated leucine residue near the exposed exterior of the coiled coil (position e of the helical wheel). Incorporation of a thioamide at this position would be expected to strengthen the hydrogen-bond between the carbonyl of Glu-12 and the Ser-16 NH (position f), and to lengthen and weaken the hydrogen bond between the Leu-15 carbonyl (thiocarbonyl) and the Tyr-19 NH (position b). Amide protons at position f in the native peptide exhibit high rates of exchange and form unusually long hydrogen bonds, while those at position b exhibit low rates of exchange and are of moderate length. Thus, a thioamide at this position of the helix might stabilize the helix by strengthening what appears to be a relatively weak hydrogen bond. Peptide T28 has the thioxylated leucine at the hydrophobic interface between the coils (position d) and is expected to strengthen the hydrogen bond between the Val-25 carbonyl and the Lys-29 NH (position e), while weakening and lengthening the hydrogen-bond between the Leu-28 carbonyl (thiocarbonyl) and the Val-32 NH (position a). Amide protons at positions e and a in the native peptide exhibit low rates of hydrogen−deuterium exchange and form unusually short hydrogen bonds.[9] Because peptide T28 has its thioamide located near the C-terminus of the helix, it was postulated that the thioxo peptide might retain some of its helical structure even if the thioamide caused unwinding at the C-terminus of the peptide.

Thioxo peptides T15 and T28 were synthesized by automated peptide synthesis using Fmoc/*tert*-butyl protection and the Rink amide resin. Thioxylation was achieved using a thionitrobenzotriazolide prepared from Fmoc-leucine.[10] Thioxo peptides were cleaved from the resin with a low concentration of trifluoroacetic acid (TFA) to prevent acidolytic cleavage at the amide immediately following the thioamide linkage (TFA/thioanisole/H_2O/ethanedithiol/dimethyl sulfide (82.5:5:5:2.5:5)),[11] purified by RP-HPLC, and characterized by mass spectrometry. Analytical HPLC was used to verify the purity of the thioxo peptides prior to each experiment.

Previous studies of GCN4-p1 analogues have shown that analogues that lack helical structure are monomeric in solution, while in analogues that retain helical structure, disruption of key side chain interactions produces alterations in oligomerization state.[12] Sedimentation equilibrium experiments indicate that both T15 and T28 are dimeric at 4 °C. The apparent molecular mass measured for T15 was 7790, and the apparent molecular mass for T28 was 7800. The molecular weight of the monomeric peptide is 4115. These results suggest that both thioxo peptides adopt parallel coiled coil structures similar to that of the native peptide.

Circular dichroism (CD) spectroscopy further confirms the helical conformation of the thioxo peptides. CD spectra for both T15 and T28 show the signature features of α-helical structure, with strong negative bands at 208 and 222 nm (Figure 2). The $\pi\pi^*$ band of the thioamide appears as a negative band at 270 nm.[13] Helix content can be estimated

(8) (a) O'Shea, E. K.; Rutkowski, R.; Kim, P. S. *Science* **1989**, *243*, 538. (b) O'Shea, E. K.; Klemm, J. D.; Kim, P. S.; Alber, T. A. *Science* **1991**, *254*, 539.

(9) Goodman, E. M.; Kim, P. S. *Biochemistry* **1991**, *30*, 11615.

(10) Shalaby, M. A.; Grote, C. W.; Rapoport, H. *J. Org. Chem.* **1996**, *61*, 9045.

(11) Miwa, J. H.; Margarida, L. A.; Meyer, A. E. *Tetrahedron Lett.* **2001**, *42*, 7189.

(12) (a) Akey, D. L.; Malashkevich, V. N.; Kim, P. S. *Biochemistry* **2001**, *40*, 6352. (b) Lumb, K. J.; Carr, C. M.; Kim, P. S. *Biochemistry* **1994**, *33*, 7361.

(13) Sandstrom, J. *Chirality* **2000**, *12*, 162.

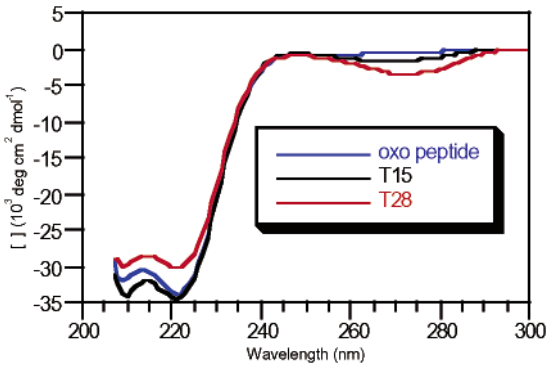


Figure 2. Circular dichroism spectra for 30 μM T15 (black) and 30 μM T28 (red), pH 7, 25 °C indicate that both peptides are helical. The CD spectrum of the native (oxo) peptide (blue) is provided for reference. Samples were prepared in 50 mM sodium phosphate and 150 mM NaCl. Spectra were acquired on an Aviv 62DS spectrometer.

from $[\theta]_{222}$ by assuming that a value of -35×10^{-3} deg cm^2 dmol^{-1} corresponds to a helix content of 100% for a 35 residue coiled coil.[8a,14] The spectra indicate that T15 is fully helical while T28 is approximately 85% helical. The thermal stability of the helical structure was determined at peptide concentrations of 30 μM by monitoring the change in $[\theta]_{222}$ as a function of temperature. The melting temperature (T_M) was taken as the minimum of the first derivative of $[\theta]_{222}$ vs T^{-1} (K^{-1}). Both peptides showed reversible melting behavior. T28 had a T_M of 70 °C, which is comparable to the melting temperature for the native peptide,[12b] while T15 had a T_M of 80 °C, indicating that the thioamide confers increased thermal stability to the helical structure.

We have demonstrated that a thioamide linkage can be incorporated into the backbone of an α-helix. Thioxo peptide analogues of GCN4-p2N fold into α-helical parallel coiled coils with no evidence of local unfolding in the region surrounding the thioamide. Contrary to predictions based on computational studies, the thioamide can be incorporated in the middle of the α-helix as well as at a site near the C-terminus of the helix. The results suggest that the increase in hydrogen-bond strength conferred by the thioamide may increase the thermal stability of an α-helix, particularly when the stronger hydrogen-bond replaces a relatively weak hydrogen-bond in the native peptide. These results have implications for the use of thioxylated amino acids in peptide and protein design.

Acknowledgment. We thank Peter S. Kim and the Whitehead Institute for Biomedical Research for laboratory space, technical assistance, and supplies. Funding was provided by the Wellesley College Brachman Hoffman Fund (J.H.M.).

OL027056D

(14) Chen, Y.-H.; Yang, J. T.; Chau, K. H. *Biochemistry* **1974**, *13*, 3350.