Redox Control of Secondary Structure in a Designed Peptide

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Received September 16, 1993

The development of strategies for modulating the conformation (and thereby the function) of a polypeptide chain is a fundamental challenge in the emerging field of protein design.[1] Many natural proteins are regulated by posttranslational modification of amino acid residue side chains, processes that are often enzymatically facilitated and reversible.[2] We report the use of a chemically reversible side chain modification, methionine oxidation to the sulfoxide form,[3,4] to control the secondary structural preference (α-helix vs β-strand) of an 18-residue peptide. These results have important implications for the design of proteins that can be induced to switch between two very different folding patterns.[5,6]

Our approach is based on the tendency for peptides capable of adopting amphipathic secondary structures (i.e., conformations displaying distinct polar and nonpolar surfaces) to be stabilized in those secondary structures by aggregation in aqueous solution. Thus, peptides containing a strict alternation of polar and nonpolar residues form intermolecular β-sheets,[7,8] because the side chains of adjacent residues in a β-strand project from opposite sides of the extended backbone. Peptides containing a polar/nonpolar pattern that matches the α-helical periodicity of 3.6 residues/turn form helical bundles.[8,9] Our experiments proceeded from the hypothesis that peptides of variable and controllable conformational preference could be generated through the use of three types of residues: permanently polar, permanently nonpolar, and "switchable". Methionine is well suited as the switchable residue because the side chain ($CH_2CH_2SCH_3$) is hydrophobic in its usual thioether form but is expected to become hydrophilic upon conversion to the sulfoxide form.[3,4]

Our design strategy is illustrated for peptide **1**, Ac-YLKAMLEAMAKLMAKLMA-NH_2, in Figure 1. Leucine (L) is the permanently nonpolar residue, and lysine (K) and glutamic acid (E) are the permanently polar residues; alanine (A) serves as a "neutral" residue. In the reduced (thioether) form, **1** can adopt an α-helical folding pattern in which the methionine residues form part of the hydrophobic helical face. Oxidation of these residues destroys the amphipathic nature of the α-helix; however, sulfoxide form **1°** displays the polar/nonpolar alternation required for an amphipathic β-strand, with the methionine sulfoxide residues forming part of the strand's hydrophilic face. In practice, interconversion between **1** and **1°** can be achieved by standard methods,[10] and the thioether and sulfoxide forms are readily distinguishable by HPLC and mass spectrometry.

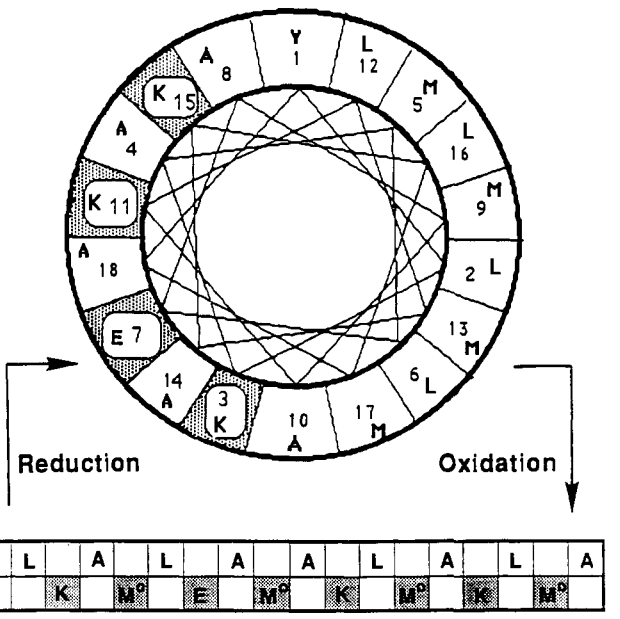

Figure 1. Schematic depiction of peptide **1** in the α-helical state (upper) and peptide **1°** in the β-strand state (lower). These depictions highlight the well-defined polar and nonpolar surfaces displayed by each redox state in the secondary structure illustrated (polar residues are stippled). A = alanine, E = glutamic acid, K = lysine, L = leucine, M = methionine, M° = methionine sulfoxide, and Y = tyrosine.

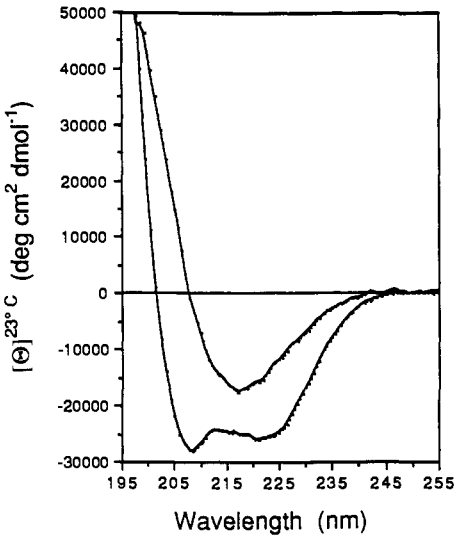

Figure 2. Circular dichroism of peptides **1** (882 μM; lower curve) and **1°** (402 μM) in H_2O at room temperature. Data were obtained on an OLIS-modified Cary 60 instrument and are expressed in terms of mean residue ellipticity. Peptide concentration was determined from tyrosine absorbance at 276 nm in 6 M guanidinium hydrochloride.[16]

Circular dichroism (CD) data (Figure 2) indicate that each redox state displays the expected secondary structure in aqueous solution. For **1**, the presence of helix is indicated by the minima at 208 and 222 nm.[11] CD data at 23 °C indicate **1** to be roughly 45% helical at 4 μM and roughly 100% helical at 441 μM (percent

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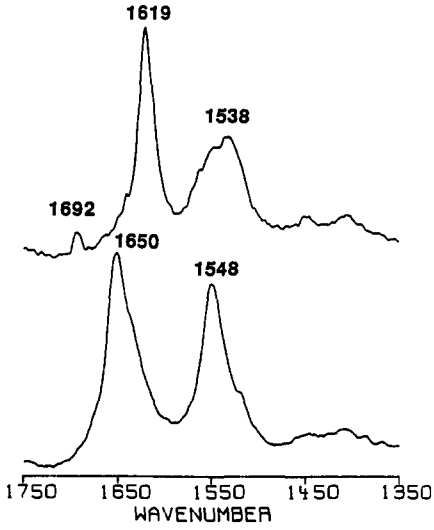

Figure 3. Amide I/II region IR data for peptides **1** (1760 μM; lower curve) and **1°** (1610 μM; upper curve) in H_2O at room temperature. Data were obtained on a Nicolet 740 FT-IR spectrometer, using a circular internal reflectance cell with a ZnSe crystal.

helicity was calculated on the basis of the limiting Θ_{222} observed for **1** in trifluoroethanol (TFE) titration experiments[12]). The promotion of helix formation by increasing peptide concentration is consistent with the expectation that the helical form of **1** is stabilized by aggregation. For **1°**, the minimum at 216 nm and zero ellipticity at 208 nm indicate β-sheet.[11] The population of β-sheet did not change between 4 and 1600 μM, with a consistent Θ_{216} of −17 900 deg·cm^2·dmol^{-1}, which is very similar to the ellipticity of polylysine in the β-sheet form.[13] This concentration independence implies that the β-sheet aggregates are extremely stable.[14]

Solution infrared (IR) spectroscopic data (Figure 3) support the secondary structural assignments discussed above. For **1** in H_2O, the maximum at 1650 cm^{-1} is consistent with the presence of α-helical conformations, although amide I bands in the region 1650–1660 cm^{-1} in H_2O have been attributed also to 3_{10}-helix and "random coil" conformations.[15] For **1°**, the combination of a strong absorbance at 1619 cm^{-1} and a weaker band at 1692 cm^{-1} is very characteristic of antiparallel β-sheet formation.[15a] The lack of absorbance in the range 1640–1670 cm^{-1} indicates that helical and random coil states are not significantly populated.

We have shown that selective and reversible side chain oxidation provides a powerful tool for specifying the secondary structural preferences of methionine-rich sequences. Methionine oxidation is generally considered to be an undesirable adventitious process,[3] but our work hints that this side chain modification might not always be deleterious: since we can put methionine oxidation to good use in the test tube, biological systems may also have harnessed this reversible modification for structural and/or functional purposes. Our results also raise the intriguing possibility that longer synthetic peptides, in which methionine-rich segments are connected by short loop-forming segments, will be subject to redox control of tertiary structure, which could lead to corresponding functional control.

Acknowledgment. We thank Professor C. Sih and Mr. G. Girdaukas for access to and assistance with the CD spectropolarimeter. G.P.D. is the recipient of a National Research Service Award (T32 GM08932) from the National Institute of General Medical Sciences. S.H.G. thanks the Office of Naval Research Young Investigator Program, the National Science Foundation Presidential Young Investigator Program, Eastman-Kodak, and Procter & Gamble for support.

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