Methionine-containing zipper peptides

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SUMMARY

Using circular dichroism, we have examined the effect of single and multiple methionine mutations on the dimerization function of a previously reported engineered leucine zipper peptide. Our results show that the methionine-containing zipper peptides self-associate to form coiled coils that are less stable than that of the reference leucine zipper. The circular dichroism data also indicate that leucine at position d is more tolerant of methionine substitution than isoleucine at position a.

INTRODUCTION

Leucine zipper polypeptides have a characteristic seven-residue repeat, which in the notation of McLachlan and Stewart [1] is termed \( a\textʲb\text^-c\text^-d\text^-e\text^-f\text^-g \). The a and d residues pack in a regular 'knobs and holes' pattern, with the side chains ('knobs') of these amino acids (i.e., hydrophobic \( \beta \)-branched amino acids at the a positions and leucines at the d positions) fitting into the spaces ('holes') between four residues on a neighboring helix. Leucine is by far the most preferred residue at position d, while leucine, isoleucine, methionine, and valine often occur at position a [2]. The hydrophobic interactions established in the dimerization interface are critical for the formation of coiled coils from leucine zipper monomers and can influence the stoichiometry and orientation of the \( \alpha \)-helical bundles [3]. Although several articles have reported the effect of amino acid replacement on the stability [4] and oligomer choice of leucine zipper polypeptides [3], methionine, proposed by Landschulz et al. [5] as the most suitable alternative to leucine, was not included in these studies. Using circular dichroism (CD), we have examined the effect of single (peptides 2 and 3) and multiple methionine mutations (peptides 4 and 5) on the dimerization function of a previously reported engineered leucine zipper peptide (peptide 1) [6]. The work reported here is an extension of a previous publication in which we showed that the dimerization function of a leucine zipper peptide (peptide 3) is abolished upon methionine oxidation [7].

MATERIALS AND METHODS

Peptides 1–5 (Fig. 1) were synthesized on a Milligen 9050 automated peptide synthesizer (continuous flow), employing protocols previously reported from our laboratory [7,8]. After completion of the syntheses, the peptide resins were
simultaneously cleaved and deprotected with trifluoroacetic acid–water–ethanedithiol (76:4:20, v/v/v) for 3 h at room temperature. The filtrate from the cleavage reaction was precipitated in diisopropyl ether–petroleum ether (1:1, v/v) at 0 °C, and the precipitate was collected by filtration. The crude mixtures of peptides 4 and 5 were treated with a 20% solution of ammonium iodide in water for 5 min at room temperature (reduction of the methionine sulfoxide), and after precipitation in diisopropyl ether–petroleum ether (1:1, v/v) the precipitate was dissolved in 1 N AcOH and heated at 60 °C for 30 min (removal of the tert-butyl groups from the sulfonium salts) [9]. The peptides were purified by reversed-phase medium-pressure liquid chromatography on a C_{18} column (0.1% trifluoroacetic acid–acetonitrile gradient). The purity of the final compounds was verified by reversed-phase analytical HPLC (Table 1), and the identity of the final products was assessed by correct mass spectral (matrix-assisted laser-desorption ionization time-of-flight mass spectrometry, MALDI-TOF) and amino acid analyses (Table 1).

CD measurements (Fig. 2) and size-exclusion chromatography experiments (data not shown) were carried out as described previously [8]. Samples were prepared in 100 mM NaCl, 10 mM phosphate buffer (pH = 7.0) and the concentrations were determined by amino acid analyses. Three scans were averaged to improve the signal-to-noise ratio in the CD spectra. The α-helical content was calculated by setting a 100% helix value equal to [θ]_H^{α} = -33.334 deg cm^2 dmol^{-1}. The theoretical molar ellipticity ([θ]_H^{α}) was obtained using the equation [θ]_H^{α} = [θ]_H{α} = x (1 - k/n), where [θ]_H^{α} = -37.400 deg cm^2 dmol^{-1}, n = 23 (number of residues), and k = 2.5 (λ = 222 nm) [10]. The monomer/dimer equilibrium of the peptides is described by the equation (θ - θ_m)/(θ_{max} - θ_m) =

**TABLE 1**

<table>
<thead>
<tr>
<th>Entry</th>
<th>MALDI-TOF* (amu)</th>
<th>HPLCb (min)</th>
<th>Amino acid analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2521.8 (calcd. 2522.0, C_{11}H_{19}N_{2}O_{8}S_{1})</td>
<td>6.7</td>
<td>Lys 4.1 (4), Ala 7.1 (7), Glu 5.9 (6), Ile 2.8 (3), Leu 3.0 (3)</td>
</tr>
<tr>
<td>2</td>
<td>2540.0 (calcd. 2540.0, C_{11}H_{19}N_{2}O_{8}S_{1})</td>
<td>6.6</td>
<td>Lys 4.0 (4), Ala 7.0 (7), Glu 5.6 (6), Ile 3.0 (3), Leu 2.0 (2), Met 1.0 (1)</td>
</tr>
<tr>
<td>3</td>
<td>2539.7 (calcd. 2540.0, C_{11}H_{19}N_{2}O_{8}S_{1})</td>
<td>6.6</td>
<td>Lys 4.0 (4), Ala 7.2 (7), Glu 5.8 (6), Ile 1.9 (2), Leu 3.0 (3), Met 1.0 (1)</td>
</tr>
<tr>
<td>4</td>
<td>2576.5 (calcd. 2576.1, C_{11}H_{19}N_{2}O_{8}S_{1})</td>
<td>6.4</td>
<td>Lys 3.9 (4), Ala 7.0 (7), Glu 5.5 (6), Ile 2.9 (3), Met 3.1 (3)</td>
</tr>
<tr>
<td>5</td>
<td>2575.8 (calcd. 2576.1, C_{11}H_{19}N_{2}O_{8}S_{1})</td>
<td>6.3</td>
<td>Lys 4.1 (4), Ala 7.0 (7), Glu 5.9 (6), Leu 2.9 (3), Met 3.0 (3)</td>
</tr>
</tbody>
</table>

* Negative-ion mode.

b Linear gradient over 10 min of CH_{3}CN–0.09% TFA and H_{2}O–0.1% TFA from 1:49 to 1:0 on a reversed-phase Nucleosil C_{18} column (250 × 4.0 mm; 5 μm, 100 Å), flow rate 2.0 ml min^{-1}, detection at 215 nm, single peaks at the retention time indicated.