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Optimization of the loop length for folding of a helix–loop–helix peptide

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Abstract

We have designed and synthesized compact helix–loop–helix peptides as a scaffold for conformationally defined peptide mimetics. Circular dichroism and sedimentation equilibrium studies suggested that the connection between the two helical segments significantly affected the structural formation. The loop length corresponding to seven glycine residues (approximately 25 Å) was found to be most suitable for the intrachain packing of the α-helices.

Keywords: peptides; polypeptides; structure–activity; circular dichroism; helices.

The helix–loop–helix structure allows polypeptides to form stable α-helices, which often present recognition sequences in biological processes.[1] Hence, the de novo construction of a compact helix–loop–helix peptide, in which the amino acid residues essential for the molecular recognition could be embedded, provides an approach to mimetic and antagonist design. The structural motif can be constructed by the combination of: (1) employing amino acids with high helix-forming propensities; (2) providing a stabilizing hydrophobic interface; and (3) connecting helices. The basic principles for designing α-helices with the desired structural properties have been extensively studied. By contrast, the connecting loop between the two α-helices is less well-defined, because in natural proteins, it has only a minor effect on the global folding and the function.[2] However, in a compact designed peptide, the effect is an important consideration. In this work, we have focused on the role of the connection in the folding of helix–loop–helix peptides, and have optimized the length of the connecting loop.

The helix–loop–helix peptides were designed based on the structural properties of intermolecular antiparallel coiled coils,[3] and were composed of three structural regions, the N-terminal α-helix, the C-terminal α-helix, and the flexible connecting loop, as shown in Fig. 1. In both helical regions, uncharged leucine residues were incorporated into the heptad repeat positions to dimerize the α-helices by hydrophobic interactions.[4] In addition to the hydrophobic interface, charged residues, glutamic acids and lysines, were introduced into the N- and C-terminal α-helices, respectively, to promote intrachain

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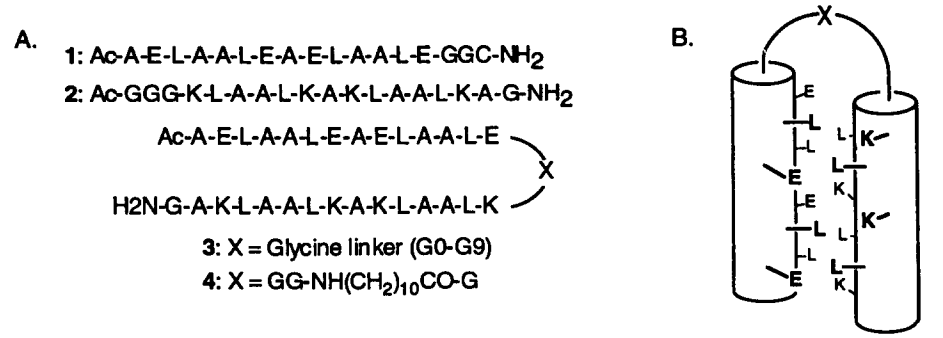

Figure 1. (A) Amino acid sequences of the peptides. (B) A proposed structure of the intramolecular antiparallel helix–loop–helix peptide. The cylinder and the loop represent the α-helices and the linker, respectively. Leucine, glutamic acid, lysine and glycine residues are represented by L, E, K, and G, respectively

salt bridge formation.[5] The N- and C-termini of the peptides were blocked with acetyl and amide groups, respectively. To examine the effect of the connection on the folding, different numbers of glycine residues were used to link the N- and C-terminal α-helical regions. Glycine is a commonly occurring loop residue and a poor α-helix former, and serves as a helix C-cap to prevent further propagation of the helix.[6] The lack of a sidechain maximizes the backbone conformational freedom and minimizes the potential interactions both within the loop and between the loop and the helices, thus simplifying the interpretation of the effect of loop length on the peptide folding. The peptides were prepared by solid phase synthesis using the Fmoc strategy. After purification by HPLC, each peptide was characterized by amino acid analysis and electrospray ionization mass spectrometry.

As the first step, we attempted to compare the circular dichroism spectra (CD) of the connected peptides with the corresponding non-connected peptides, to characterize the helix–loop–helix structure. In the CD spectra, the N-terminal peptides **1** (10 μM) indicated the characteristics of an unfolded peptide with a minimum ellipticity below 200 nm, and the C-terminal peptide **2** (10 μM) displayed a weak α-helical structure with a small molar ellipticity at 222 nm (Fig. 2). On the other hand, upon mixing **1** with **2** in a 1:1 ratio (10 μM), the intensities of the ellipticity minima at 222 and 208 nm were increased. This suggests that an interchain packing between **1** and **2** with hydrophobic interactions and salt bridges can induce α-helicity into the peptides. Furthermore, a promising result was obtained when peptides **1** and **2** were connected with a flexible linker of oligoglycine (Fig. 2). Peptide $\mathbf{3}_{(G7)}$ exhibited a CD spectrum with higher α-helical content than that of a mixture of **1** and **2**, with large molar ellipticity minimum values at 222 and 208 nm and a maximum ellipticity around 193 nm. These observations show that the N-terminal and C-terminal peptides are associated with each other to form α-helical structures, which are considerably stabilized by the connection with the flexible loop.[7]

As described above, the α-helicity of the connected peptide is sensitive to the folding of the helix–loop–helix structure. Thus, this peptide seems to be a suitable model to detect subtle structural changes by evaluating the α-helicity. Therefore, we systematically examined the effects of the loop length to construct the helix–loop–helix structure, by using a panel of linkers with different numbers of glycine residues. As shown in Fig. 3, a comparison of the molar ellipticity minimum values at 222 nm among the ten peptides $\mathbf{3}_{(G0–G9)}$ shows that peptide $\mathbf{3}_{(G7)}$ exhibits the largest α-helicity, suggesting the formation of a stable helix–loop–helix structure. The peptides $\mathbf{3}_{(G8)}$ and $\mathbf{3}_{(G9)}$, with linkers longer than seven glycines, displayed weak α-helicities as compared with that of $\mathbf{3}_{(G7)}$. On the other hand, as shown in peptides $\mathbf{3}_{(G1–G6)}$, the α-helicity of the peptides decreases as the loop length decreases. Interestingly, peptide $\mathbf{3}_{(G0)}$ without the connecting loop displayed an increased α-helicity. However, peptide $\mathbf{3}_{(G0)}$

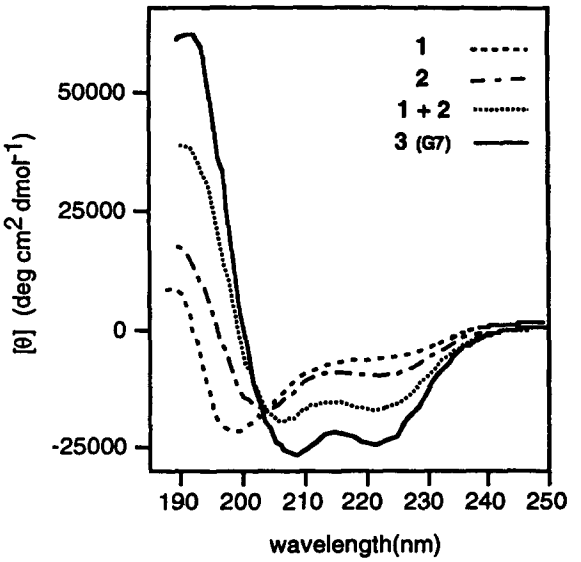

Figure 2. CD spectra of peptides **1**, **2**, $\mathbf{3}_{(G7)}$, and **1+2** (1:1) in 150 mM sodium chloride and 20 mM sodium phosphate, 20°C. The concentration of the peptides was 10 μM

differed from $\mathbf{3}_{(G7)}$ in the oligomeric states. In the CD spectra of various concentrations of the peptides, peptide $\mathbf{3}_{(G0)}$ exhibited a concentration dependence, increasing the α-helicity with oligomerization of the single-stranded peptide (Fig. 4). In contrast, peptide $\mathbf{3}_{(G7)}$ showed no significant change in the CD spectra (Fig. 4). The concentration independence suggests the formation of a monomeric intramolecular helix–loop–helix of $\mathbf{3}_{(G7)}$, which is also supported by sedimentation equilibrium ultracentrifugation experiments.[8] These observations are inconsistent with similar work, which use the four-helix-bundle protein Rop as a model system to explore the role of loop length in protein folding and stability.[2] In the case of Rop, the loop length has no effect on the global folding, because the helices consist of 25 amino acid residues so that the interaction must be quite strong to favor hairpin formation. However, our results reveal that for a compact helix–loop–helix structure, the connection between the two helical segments is one of the predominate factors contributing to the folding and the stability, and the loop length corresponding to seven glycine residues is suitable for the intrachain packing of the α-helices.[9] In the case of the longer linker, from an entropic standpoint, the closure of the linker should require more energy than that for the linker of seven glycines, and therefore could result in structure destabilization. On the other hand, the loop length of up to seven glycines is too short to achieve intrachain packing of the helices.

Finally, as an approach to developing peptide mimetics, we examined whether the glycine linker used in peptide $\mathbf{3}_{(G7)}$ could be replaced with a simplified methylene compound of the same molecular length. Peptide **4**, which possesses a linker, Gly-Gly-$NH(CH_2)_{10}CO$-Gly, exhibited a CD spectrum almost identical to that of peptide $\mathbf{3}_{(G7)}$. This is beneficial for peptide-mimetic design; the replacement will allow one to minimize the molecular size and reduce the number of synthetic steps. In this work, we have demonstrated optimization of the loop length for folding of a helix–loop–helix peptide. Although our study focused on a single peptide, our findings should have general applicability in a system with a helix–loop–helix structure. We are now using the helix–loop–helix as a scaffold for conformationally defined peptide mimetics.

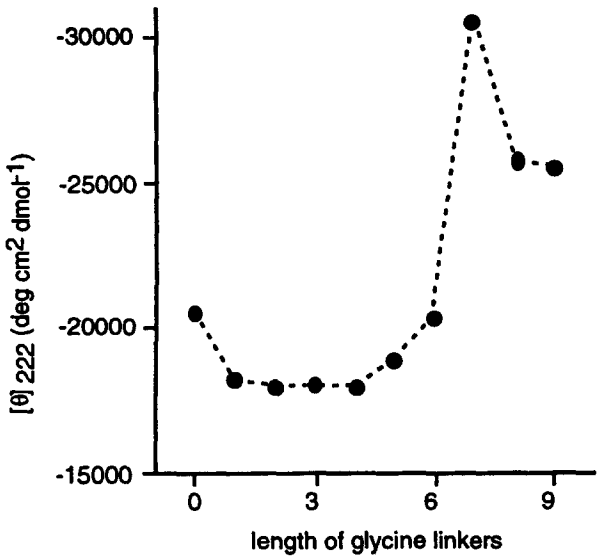

Figure 3. Effect of the length of the glycine linker on the α-helical content of the peptides. The CD spectra of peptides $3_{(G0\text{-}G9)}$ were measured in 150 mM sodium chloride and 20 mM sodium phosphate at 20°C. The concentration of the peptide was 10 μM. The mean molar residue ellipticity at 222 nm was determined as a 28-residue peptide

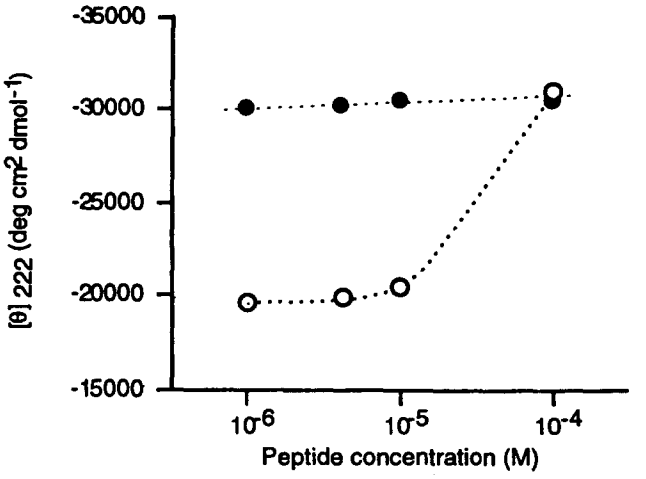

Figure 4. Peptide concentration dependence of $3_{(G0)}$ (white) and $3_{(G7)}$ (black) in 150 mM sodium chloride and 20 mM sodium phosphate at 20°C. The mean molar residue ellipticity was determined as a 28-residue peptide

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7. The structure of $\mathbf{3}_{(G7)}$ was also supported by the 2D ^{1}H NOESY spectra (mixing time: 50 msec, Bruker DMX 600) in ^2H_2O (pH 5.0) at 10°C. The large number of dNN NOE connectivities and the fine dispersion of the chemical shifts in the amide–amide region (7.4–8.8 ppm) suggest the helical conformation.
8. The sedimentation equilibrium study on $\mathbf{3}_{(G7)}$ (500 μM) at a rotor speed of 53000 rpm suggests that the peptide is monomeric in solution; observed molecular mass 3190 Da, expected mass for monomer 3302 Da.
9. The length of seven glycine residues in an extended form is approximately 25 Å.