Peptide *de novo* Design: CD Evidences of β-hairpin Formation by Eight-residue Peptide*

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Abstract β-hairpins are popular secondary structural elements in native protein and play important roles either in protein folds or functionalization. Here we describe the design and preliminary structural studies of two peptide sequences LTVd-PGLTV(n7) and LTVGDDTV(n5) synthesized by solid phase peptide synthetic strategy. The circular dichroism (CD)spectra of n5 show a negative minimum near 198 nm, random coil characteristics. On the contrary, the CD spectra of n7 show a minimum at about 218 nm and a maximum at about 196 nm, a typical β-hairpin characteristics, which have been concluded as the common contribution of a β-turn mixed with β-sheets. The results show that β-turn, sequence context and β-sheet forming tendency are determinant of β-hairpin formation and stability.

Keywords: *de novo* protein design, β-hairpin, β-turn, β-sheet, Circular dichroism(CD), HPLC, Peptide synthesis

De novo protein and peptide design, designing peptide sequence with predictable folding pattern, presently a subject of great interest[1], are based on the structural prediction ability of peptide sequences. Helices and β-sheets, the major structural elements of native protein, have been the focus of protein design in many research groups in recent years. Being an aggregation, it is not easy to investigate the folding disciplinary of β-sheet and design β-structure[2]. Alternatively, β-hairpin, two segments of antiparalell β-strands connected with a β-turn and a good template to study the stability and formation of antiparallel β-sheet, has attracted more attention in the past years[3]. In the past decade, β-hairpin studies mainly focused on the conformational stability of fragment from native protein, which supported the rationality of the hierarchical approach of protein folding. Reports on *de novo* design of β-hairpin forming peptide also emerged[4-5]. Studies on β-hairpin show that at least four factors effect β-hairpin formation and stability, such as hydrogen bonding, hydrophobic interaction of side-chains, β-turn type and environment properties[6]. Previously, we described the β-turn structure of a six-residue peptide from BLIP protein, which shows the context of sequence is important in structural stability[7]. Herein, we describe the design, synthesis and circular dichroism (CD) studies of two peptides with different turn sequence.

1 Peptide design

Hydrophobic interaction, one of the most important forces of protein folding, and periodic hydrophobic/hydrophilic residue location of sequence are guidelines of peptide design strategy. To keep the amphiphilic feature of β-hairpin structure, which will conserve structural stability and solubility, the leucine with hydrophobic side chain and valine with best β-sheet forming tendency were selected as building

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block to construct the two strands and enhance the hydrophobic interaction, the thronine with γ-hydroxyl group was used to strengthen the hydrophilic interactions between two strands and increase peptide solubility insolution[5]. Type II′ β-turn GlyAsp, the most abundant β-turn sequence in native protein, and(D-) ProGly reported by Haque[3] were used to investigate the role of β-turn on β-hairpin stability.

H_2N – Leu – Thr – Val – Gly – Asp –
Asp – Thr – Val – COOH n5

H_2N – Leu – Thr – Val – (D-)Pro –
Gly – Leu – Thr – Val – COOH n7

2 Experiment

2.1 Peptide synthesis and purification

The peptides were synthesized on Wang resin (substitution 0.8 mmol·g^{-1}, ACT product, USA) by Fmoc/Bu^t strategy[8]. The first amino acid valine was loaded to the resin by preformed symmetrical anhydride of valine and catalyzed by *N, N*-dimethylaminopyridine. HBTU/HOBT was selected as the coupling reagent. The protected amino acids are FmocLeu, FmocVal, Fmo(D-) Pro, FmocGly, FmocAsp(Bu^t) and FmocThr(Bu^t) (ACT products, USA). The peptides were cleavaged by K reagent[9], and the crude products were purified by RP-HPLC (Gilson Inc., zorbax C_{18} column, 9.4 mm × 250 mm). The peptides were confirmed by RP-HPLC(zorbax C_{18} column, 4.6 mm × 250 mm) and MALDI-TOF mass spectra.

2.2 CD spectra

The far ultraviolet CD spectra were recorded on Jobin Yvon-Spex CD6 at 20 ℃. Samples were prepared using PBS buffer at concentration 1 g·L^{-1} with pH 7.04. Scans were obtained in a range from 184 nm to 260 nm by taking points every 0.5 nm, with an integration time of 1 s and a 2 nm bandwidth. Cell with path length 0.1 mm was used for analysis.

3 Results and Discussion

3.1 Results

Fig. 1 shows the CD spectra of n5 and n7 in PBS buffer at pH 7.04. The peptide n5 shows a strong negative minimum at about 198 nm, a typical curve of random coil. On the contrary, n7 exhibits a positive maximum at about 196 nm and a broad negative from 210 nm to 225 nm, which have been postulated as what could be the mixed contribution from β-turn and β-sheet[10]. The CD spectra of n7 at different concentration were recorded to investigate the intermolecular interaction. There are no obvious changes in the range from 1.25 mmol·L^{-1} to 12.5 μmol·L^{-1} (Fig. 2). Solvent effects on β-hairpin formation and stability were also investigated. It is very clear from Fig. 3 that both the maximum and minimum of the curve become stronger, the minimum

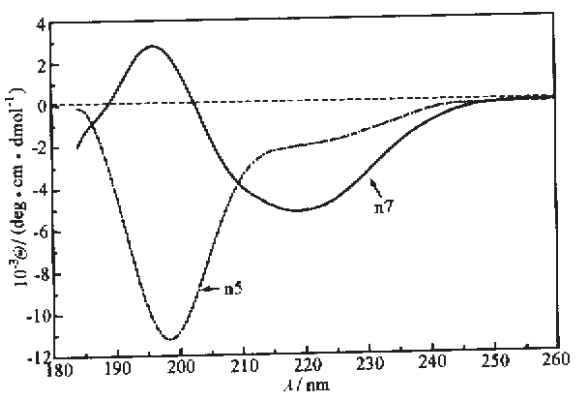

Fig. 1 CD spectra of n7 and n5 in PBS buffer
pH 7.04, concentration 1 g·L^{-1}, 20 ℃

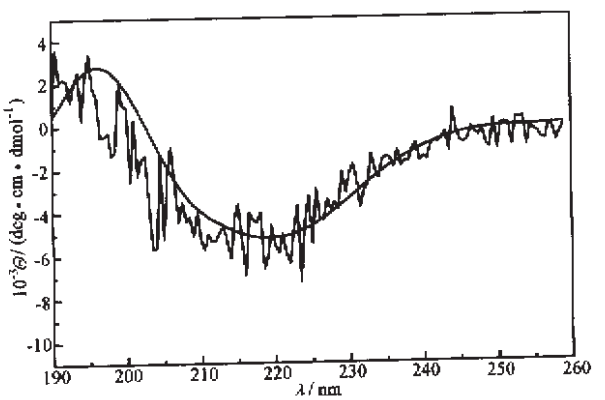

Fig. 2 CD spectra of peptide n7 at different concentration
the smooth line at 1.25 mmol·L^{-1}, the other at 12.5 μmol·L^{-1}

becomes broader and the maximum locates at about 193 nm with TFE(trifluoro ethanol) and SDS(sodium dodecy sulfonate) titration. Obviously, the helix content improved much and the antiparallel sheet disappeared in solution containing SDS or TFE.

3.2 Discussion

The spectra of n7 show a maximum at 196 nm and a broad negative near 218 nm. Similarly, Serrano[5] also reported a β-hairpin peptide BH8 whose CD spectrum in 30% TFE behaves a maximum at 202 nm and a minimum at 216 nm. This kind of spectroscopic characteristics has been postulated as what could be expected for a β-sheet mixed with a β-turn. Differently, n5 with different turn sequence shows random coil feature in the same condition.

Conformational proclivity of the backbone is at least as important as hydrogen bonding and hydrophobic interactions in stabilizing β-hairpin conformations. Evidences have shown that D-ProX(X = Ala, Gly, Ser) turn can increase β-hairpin′s forming ability and conformational stability[3, 11]. In the two sequences described here, n7 with D-ProGly turn behaves as β-hairpin conformation and n5 with GlyAsp turn shows random coil features, this means that β-turn sequence determines the formation and stability of β-hairpin structure. In our previous result, NMR studies show that GD forms type III turn in sequence AAGDYY and no evidence for the existance of hydrogen bond in the structure implicates that proclivity of backbone is determinant of structural formation. We can conclude that sequence context is another important cofactor of β-structure formation.

β-sheet forming potency of amino acid, an essential factor of β-hairpin formation, have been a guideline of designing β-strand. Both leucine and valine are the best β-sheet forming residues, which will enhance the hydrophobic interactions across strands[12]. Thronine with γ-hydroxyl group was also used to construct the two β-strands, which will enforce the cross-strand hydrophilic interactions[10]. Clearly, β-sheet forming potency of amino acid is not determinant of β-hairpin formation comparing with β-turn′s contribution in our results.

The solvent effect of n7 has been studied for solution environmental effects on helix and β-hairpin formation, the CD spectra in solution containing SDS or TFE was recorded(Fig. 3). Both the maximum and minimum of the curve are enhanced in PBS solutions containing SDS and TFE, respectively. Shifting of the positive near 193 nm, appearing of a negative shoulder near 208 nm and sharping of the negative near 220 nm mean that α-helical content increases rapidly after adding SDS or TFE to PBS solution. The mechanism of the interaction between peptide and TFE has been reported[13-14]. Comparing to water, TFE almost do not destroy hydrogen bonding, but supply a good hydrophobic surface that will benefit the hydrophobic core formation of the side-chain of peptide and increase conformational stability. Either SDS or TFE with hydrophobic heads tends to aggregate as cluster at a broad concentration range, which will promote the rearrangement of hydrophobic side-chain of the peptide and transfer to a stable conformation. We postulate that the helical conformation of n7, like most of the peptide, could be more stable than β-structure in SDS or TFE solutions.

The conformational stability of peptide n7 in PBS solution was further investigated by thermal denaturation experiment. Fig. 4 shows a cooperative unfolding curve with a midpoint at about 70 ℃. The θ value has almost no change below 60 ℃, it means that the conformation of n7 is thermal stable. Because the minimum of CD spectra near 218 nm, the common contribution from β-turn and β-sheet, exhibits the typical characteristics of β-hairpin, the thermal denaturation was recorded at wavelength 218 nm.

4 Conclusion

Conformation of peptide less than 30 residues is much unstable without disulphide bonding. In this paper, we described two peptides from *de novo* design. The preliminary results of CD study confirmed the β-hairpin formation of n7 and its stability. The absolute different conformations of n7 and n5 show

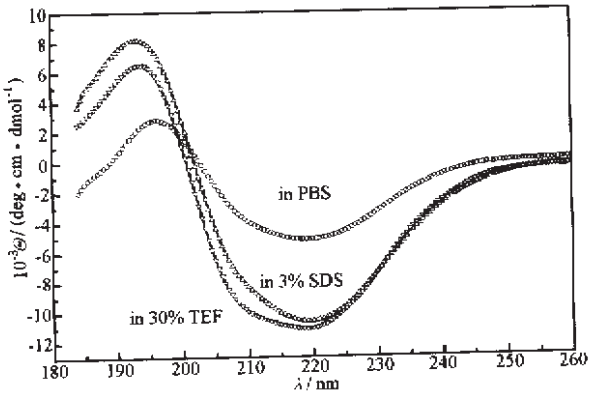

Fig. 3 CD spectra of n7 in PBS buffer, 3% SDS, 30% TFE

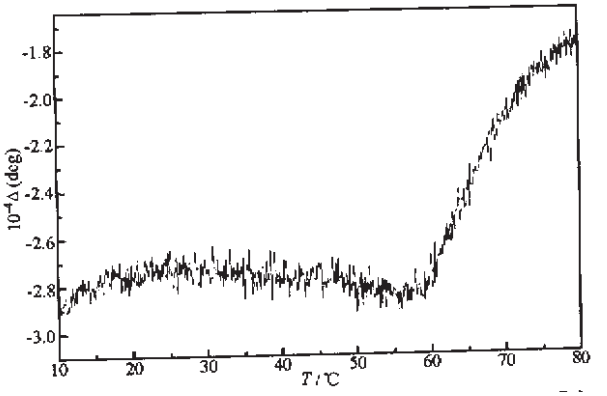

Fig. 4 The thermal denaturation spectra of n7 in PBS solution with a midpoint near 70 ℃ recording at 218 nm

that β-turn, which determines the extend direction of the β-strand and the interaction between two strands, plays a key role in β-hairpin formation, the β-strand forming tendency of amino acid and sequence context are also essential determinants of β-hairpin formation.

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蛋白质全新设计:八残基序列形成发夹结构的圆二色谱*

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摘要 β-发夹是天然蛋白质中丰富的二级结构单元之一,在蛋白折叠和功能方面扮演着重要角色. 文章报导了二条多肽序列(LTVd-PGLTV,n7 和 LTVGDDTV, n5)的设计、合成和园二色谱研究结果. 结果显示, n5 在 198 nm 附近呈现负峰,表现为非规整结构特征;相反,n7 表现为典型的发夹结构特征,在 218 nm 附近呈负峰, 196 nm 附近呈正峰,为 β-转角与 β-折叠的共同贡献. 初步研究表明,β-转角、序列关系和氨基酸形成 β-折叠结构倾向性是 β-发夹结构形成和稳定的决定性因素.

关键词: 蛋白质全新设计, β-发夹, β-转角, β-折叠, 圆二色谱, 高效液相色谱, 多肽合成

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