de novo Design and Conformational Studies of a β-hairpin Forming Peptide*

Sha Yin-Lin Li Yin-Ling[1] Qiu Yang Wang Qi[1] Lai Lu-Hua[1] Tang You-Qi[1]

(*Single Molecule and Nano-biomedicine Laboratory, Department of Biophysics, School of Basic Medical Sciences, Peking University, Beijing 100083; [1]Institute of Physical Chemistry, Peking University, Beijing 100871*)

Abstract A 12-residue peptide ($R^1G^2T^3F^4W^5V^6$ *d*-$P^7S^8V^9N^{10}Y^{11}F^{12}$, β2) with cross-strand amino acid pairs V^6V^9, F^4Y^{11} and Y^3F^{12}, predicted to form β-hairpin, was designed and synthesized by Fmoc/Bu^t strategy. The circular dichroism spectrum of β2 in PBS buffer shows a maximum at about 202 nm and a minimum at about 217.5 nm, a typical β-hairpin characteristics that have been postulated as the common contribution from a β-turn mixed with a β-sheet. The FTIR experiments confirmed the secondary structural contents of β2.

Keywords: *de novo* design, β-hairpin, β-turn, β-sheet, Cross-strand amino acid pairs, CD, FTIR

Designing peptide with a predictable folding pattern is of great interest at present, which is helpful to discover the forces determining protein folds and stability[1-2]. Helices and β-sheet, being suggested as the nucleation sites of protein folding[3-4], are the major elements in native proteins. Because β-sheet tends to aggregate in aqueous solution, it is difficult to discover the folding disciplinary of β-structure. A β-hairpin, constructed by two segments of antiparallel β-strand connected with a β-turn, was a simple template to study the interaction between antiparallel sheets[2]. Several β-hairpin sequences from the fragments of native protein or modified ones were reported in recent years[3, 5-9], and most of them were stabilized using organic reagents, such as TFE and alcohol. According to the reports, factors, like β-turn type, long-range interaction between sheets, hydrophobic interaction are important in β-hairpin formation. Obviously, the solvent effects also play important roles in β-hairpin stability, which were discussed in most of recent reports[3, 7-12]. Here we report a designed 12-residue β-hairpin whose strands were constructed with cross-strand amino acid pairs F^4Y^{11} and V^6V^9 to strengthen the side chain interactions across strands. The peptide was synthesized by solid phase peptide synthesis strategy using Fmoc chemistry, purified by high performance liquid chromatography (HPLC), and investigated by circular dichroism (CD) and FTIR spectra, respectively.

1 Peptide design

A 12-residue peptide with β-hairpin forming propensity was designed (Fig. 1). The strong type II′ β-turn *d*-ProSer, providing a good orientation of the extended structure[10-13], was selected to enhance the structural stability. The best β-sheet forming residues Val, Trp, Phe and Tyr were used to construct the two strands. To enhance the interactions between the two strands, we selected the most favorable β-sheet forming amino acid pairs FY and VV to construct the

Received: February 25, 2002; Revised: April 8, 2002. Correspondent: Sha Yin-Lin (E-mail: shyl@bjmu.edu.cn). *The Project Supported by the National Project of Basic Science Researches (970211006) and NSFC (20103001)

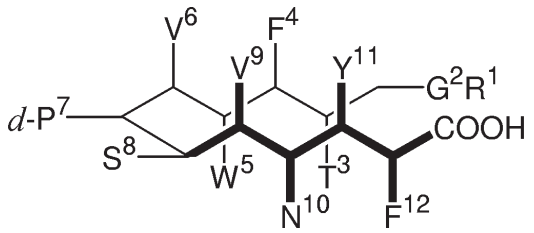

Fig. 1 Molecular model of β2

side chain interactions across strand[14] . In order to increa- sing the peptide solubility in water and destroy the aggregation, the amino acid Arg with a positive charge in neutral or acidic solution was spaced by Gly to the *N*-terminal of the peptide[11].

2 Materials and methods

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[15] . The first amino acid Phe was loaded to the resin by preformed symmetrical anhydride method and catalyzed by *N*, *N*-dimethylaminopyridine. 2-(1H-benzotriazol-1-yl)-1, 1, 3, 3-tetramethyl uranium hexafluorophosphate (HBTU) and 1-hydroxybenzotrizole (HOBT) were selected as the coupling reagents. The protected amino acids are FmocLeu, Fmoc(*d*-)Pro, FmocVal, FmocGly, FmocPhe, FmocTrp, FmocAsn, FmocAsp (Bu^t), FmocTyr (Bu^t), FmocArg(Mtr) and FmocThr (Bu^t) (ACT products, USA). The peptide resin was cleavaged by K reagent, and the crude products were purified by RP-HPLC (Gilson Inc. France, 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 spectroscopy

The CD spectra were recorded on Jobin Yvon-Spex CD6 at 20 ℃. Samples were prepared using PBS buffer at about 0.68 mmol·L^{-1} with pH 7.04. Scans were obtained in a range between 184 nm and 260 nm by taking points every 0.5 nm, with an integration time of 1 s and a 2 nm bandwidth. Cells with path length of 0.1 mm were used.

2.3 Furier transferred infared spectroscopy

The IR spectra were obtained on Bio-Rad FTS 165 FTIR spectrometer equipped with a DTGS detector at room temperature. The solution of β2 was prepared in D_2O at 6.8 mmol·L^{-1} and the path length was 25 μm for the measurement. The spectrum was obtained by averaging 400 scans with a nominal resolution of 4 cm^{-1}, and then Fourier transforming with a triangular apodization function. Curve fitting was performed with the Bio-Rad Win-IR CURVEFIT. AB program. Trifluoroacetic acid used both in peptide synthesis and in the HPLC mobile phase was removed after several lyophilization-solubilization cycles in 10 mmol·L^{-1} HCl solution.

3 Results and discussion

In order to destroy the peptide aggregation and increase the solubility in water, the residue Arg was

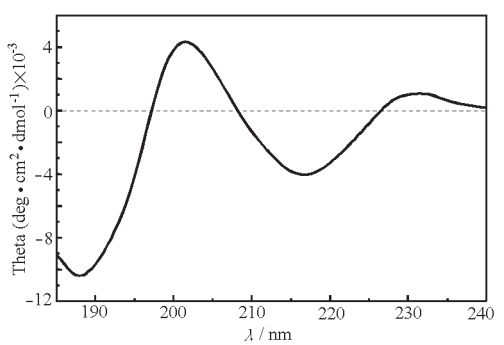

Fig. 2 The CD spectrum of β2 in PBS buffer
pH 7.04, 0.68 mmol·L^{-1} at 20 ℃

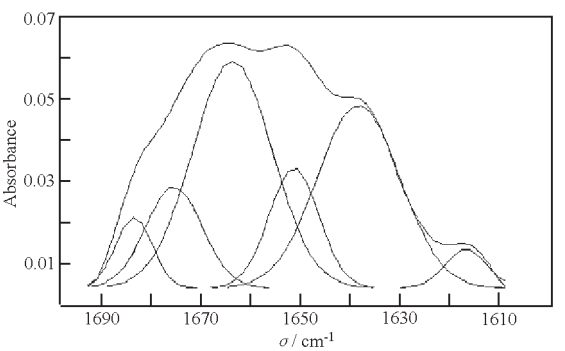

Fig. 3 Decomposition of the amide I band of β2 in PBS buffer (D_2O) at 20 ℃

Table 1 The secondary structural contents, percentage area and assignment of β2

Band position(cm^{-1})	Area(%)	Assignment
1684	5.3	β-turn
1676	11.7	β-sheet
1663	36.7	β-turn
1650	12.2	helix
1636	30.9	β-sheet
1613	3.2	side chain

linked on the *N*-terminal of the peptide whose positive charge repulsion in acidic or neutral condition will prevent aggregation[11]. As a result, β2 exhibited excellent solubility in water, and the CD spectra of β2 did not show concentration dependence at a range 0.68 mmol·L^{-1} ~ 68 μmol·L^{-1} in PBS(data not shown).

The CD curve of β2 presented in Fig. 2, exhibits a maximum near 202 nm and minimum near 217.5 nm, the typical characteristics of β-hairpin, and which have been postulated as the mixed contribution from β-turn to β-sheet[16]. The CD curve of the β-hairpin peptide BH8 in 30% TFE shows similar characteristics of a maximum at 202 nm and a similar minimum at 216 nm[11], which supports our postulation.

Obviously, β-turn plays important roles in designed β-hairpin formation and stability. It determines not only the turn conformation, but also the pattern of interstrand residue pairing and the backbone hydrogen bonding register between the β-strands [10, 13]. We reported that the turn sequence *d*-ProSer behaves well than GlySer[17], and Haque and his colleagues[1] also reported that the β-hairpin could be stabilized using the enforced turns like *d*-Pro(*d*-)Ala, *d*-ProGly or *d*-ProAla. They also pointed out that the conformational proclivity of the backbone is at least as important as hydrogen bonding and hydrophobic interactions in stabilizing β-hairpin conformation. To test the roles of the turn sequence played in the β-hairpin formation of β2, we synthesized a B1 mutant ($R^1G^2T^3F^4Y^5V^6G^7D^8V^9N^{10}Y^{11}F^{12}$), characterized its structure by CD spectroscopy. The CD curve of a negative near 193 nm and a broad weak negative between 205 nm and 218 nm imply that the β2 mutant lost β-hairpin conformation (data not shown) and confirmed the β-turn contribution to β-hairpin formation of β2.

The conformation of β2 in PBS (D_2O) was also investigated by FTIR, and Fig. 3 shows the decomposed band together with the deconvoluted amide I infrared spectra. Six bands located at 1683, 1674, 1662, 1646, 1633 and 1612 cm^{-1} were observed and the bond position, percentage area, and assignment of the components are shown in Table 1.

Theoretical calculations of antiparallel β-sheet predict a high frequency infrared component near 1676 cm^{-1}[18], and many previous studies took this peak as diagnostic for antiparallel β-structures[19-20]. So we assigned the maximum band at 1676 cm^{-1} to the high frequency component of β-sheet. Consequently, the band at 1636 cm^{-1} is assigned to the low frequency component of β-sheet[21]. Two bands located at 1684 and 1663 cm^{-1} are assigned to β-turn[22]. Unfortunately, it is not clear why one type of turn gives rise to more than one band, but this assignment is consistent with the studies of β-turn structure and β-hairpin structure[18-22]. The band at 1650 cm^{-1} is always assigned to helix in D_2O[19, 24-26]. It is clear that the band at 1613 cm^{-1} is considered as from absorbency of aromatic side chains, like Tyr, Trp and Phe[27-29].

4 Conclusion

CD and FTIR experiments revealed that β2 forms β-hairpin structure in solution. The β-turn is key factor of the β-hairpin formation, and it determines

not only the turn conformation but also the orientation of the strands and the interactions between the two strands of β-hairpin of β2. Usually, it spends much effort to design a peptide sequence with special folds in *de novo* protein design due to the complex interactions and the unpredictable factors of protein. The success of designing the simple template of β-hairpin by cross-strand amino acid pair strategy would supply an economic way to design β-sheet related structures.

References

1 Haque T S, Little J C. *J. Am. Chem. Soc.,* **1994, 116:** 4105
2 Arnold P A, Shelton W R. *J. Am. Chem. Soc.,* **1997, 119:** 3181
3 Blanco F J, Jimenez M A. *Biochemistry,* **1994, 33:** 6004
4 Alba E, Blanco F J. *Eur. J. Biochem.,* **1995, 233:** 283
5 Sha YL, Huang YL. *Acta Phys. -Chim. Sin.,* **2002, 18**(6): 504 [沙印林,黄永亮. 物理化学学报 (*Wuli Huaxue Xuebao*), **2002, 18**(6): 504]
6 Blanco F J, Jimenez M A. *Eur. J. Biochem.,* **1991, 200:** 345
7 Cox J P L, Evans P A. *J. Mol. Biol.,* **1993, 234:** 483
8 Viguera A R, Jimenez M A. *J. Mol. Biol.,* **1996, 255:** 507
9 Searle M S, Zerella R. *Protein Engineering,* **1996, 7:** 559
10 Marina R A, Blanco F J. *J. Mol. Biol.,* **1997, 273:** 898
11 Marina R A, Blanco F J. *Nature Structural Biology,* **1996, 3:** 604
12 Sha Y, Huang Y. *Protein and Peptide Lett.,* **1998, 5:** 259
13 Alba E D, Rico M. *Protein Science,* **1997, 6:** 2548
14 Smith C K, Regan L. *Science*, **1995, 270:** 980
15 Fields G B, Noble R L. *Int. J. Peptide Protein Res.,* **1990, 35:** 161
16 Johnson Jr. W J. *Ann. Rev. Biophys. Biophys. Chem.,* **1988, 17:** 145
17 Cao A, Sha Y. *Protein and Peptide Lett.,* **1998, 5:** 53
18 Krimm S, Bendekar J. *Adv. Protein Chem.,* **1986, 38:** 181
19 Byler D M, Susi H. *Biopolymers,* **1986, 25:** 469
20 Holloway P W, Mantsch H H. *Biochemistry,* **1989, 28:** 931
21 Jiang H, Song Z. *Biochimica Biophysica Acta,* **1996, 1294:** 121
22 Arrondo J L R, Blanco F J. *FEBS Lett.,* **1996, 384:** 37
23 Arrondo J L R. *Biochimica Biophysica Acta,* **1988, 952:** 261
24 Casal H L, Kohler U. *Biochimica Biophysica Acta,* **1988, 957:** 11
25 Venyaminov S Y, Kalnin N N. *Biopolymers,* **1990, 30:** 1259
26 Kalnin N N. *Biopolymers,* **1990, 30:** 1273
27 Venyaminov S Y, Kalnin N N. *Biopolymers,* **1990, 30:** 1243
28 Chirgaze Y N, Fedorov O V. *Biopolymers,* **1975, 14:** 679
29 Fabian H, Nauman D. *Biochemistry,* **1992, 31:** 6532

β- 发夹多肽的全新设计和构象研究*

沙印林 李银玲[1] 邱 阳 王 琦[1] 来鲁华[1] 唐有祺[1]

(北京大学基础医学院生物物理学系,单分子与纳米生物医学实验室,北京 100083;
[1] 北京大学物理化学研究所,北京 100871)

摘要 引入跨股氨基酸队的方法进行 β- 发夹结构的设计,序列[$R^1G^2T^3F^4W^5V^6$ *d*-$P^7S^8V^9N^{10}Y^{11}F^{12}$, β2] 中包含二个氨基酸对 V^6V^9 和 F^4Y^{11}, 并以 *d*-P^7S^8 作转角来稳定结构. 多肽合成采用 Fmoc/Bu^t 固相合成方法. 圆二色谱研究显示, β2 在 202 nm 呈现正峰,在 217.5 nm 处呈负峰,为 β 转角和 β 折叠共同贡献的叠加,是典型的 β- 发夹结构圆二色谱特征. 红外光谱研究进一步验证了圆二色谱的结果, 表明 β2 在溶液中主要以 β- 发夹结构存在.

关键词: 全新设计, β- 发夹, β- 转角, β- 折叠片, 跨股氨基酸对, 圆二色谱, 红外光谱
中图分类号: O641

2002-02-25 收到初稿, 2002-04-08 收到修改稿. 联系人:沙印林(E-mail: shyl@bjmu.edu.cn). *国家攀登计划(970211006) 和国家自然科学基金(20103001)资助项目