Structural Transformation of the Peptide Fragments from the Reactive Center Loops of Serpins: Circular Dichroic Studies

HU, Hong-Yu*(胡红雨) CHEN, Yun-Neng(陈韵能) XU, Song-Qin(徐松琴)
XU, Gen-Jun(许根俊)
Institute of Biochemistry and Cell Biology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

Two peptides, derived from the reactive center of ovalbumin (OVARC) and plasminogen activator inhibitor-1 (PAIRC) respectively were chemically synthesized and investigated by circular dichroic spectroscopy. The secondary structural transformation in solution and in solid state was studied. OVARC shows a nascent helical structure in aqueous solution, and its helical content increases under acidic conditions. There is no obvious structural conversion from solution to solid state. PAIRC, however, undergoes a structural transformation from random coil in aqueous solution to a typical β-sheet structure in the solid state. Hexafluoroisopropanol (HFIP) prompts helical structures of the two peptides in solution, but it seems to trigger the structural formation of β-sheets in solid state. The novel structural transformation from random coil or nascent helical structure in aqueous solution to the α-helix in HFIP and to the β-sheet structure in solid state may reflect the conformational polymorphism of the serpin reactive centers and is implicated in the structural features of the amyloid aggregates.

Keywords Serpin reactive center, circular dichroism, structural transformation

Introduction

Serpin (serine proteinase inhibitor) functions as regulating coagulation and fibrinolysis. Dysfunctional serpins are associated with a series of diseases such as thrombosis, emphysema and angioedema.[1] Crystal structures of serpins reveal that they share a common highly ordered tertiary structure with three β-sheets (A—C) and nine α-helices (A—I).[2] A long flexible loop or loop-helix-loop motif constitutes the reactive center whose conformation varies strikingly.[3,4] Folding of the serpin proteins is controlled by a kinetic pathway.[5] Binding to the target proteinase or proteolytic cleavage of serpins can result in a dramatic structural transformation. The cleaved reactive loop may transform into a β-strand being inserted into the preexisting β-sheet structure.[6] It has been proposed that the conformational polymorphism in the reactive center loop is the basis for the unique inhibitor properties. It is also implicated that the flexible reactive center loop is in relation to the aggregation of serpins and the pathogenesis of diverse diseases.[7]

Ovalbumin (OVA) is a non-inhibitory serpin, which was reported to transform into an inhibitory form without cleavage.[8] Study of the heat- or pH-induced structural transformation revealed the conformational polymorphism and cross β-sheet aggregation.[9] The corresponding reactive center is comprised of a short α-helix flanked by two loops.[10] Plasminogen activator inhibitor-1 (PAI) is a typical inhibitory serpin that regulates the activity of plasminogen activator.[11] The reactive center of the active PAI is a long loop, which can insert into the preexisting β-sheet when PAI binds to the plasminogen activator and switches to the latent-form.

To study the structural transformation of the novel reactive center, two peptides that are homologous to the reactive centers of the non-inhibitory OVA and PAI were

* E-mail: hyhu@sunm.shcnc.ac.cn; Tel: 0086-021-64374430; FAX: 0086-021-64338357.
Received January 15, 2001; revised and accepted July 2, 2001.
Project supported by the National Natural Science Foundation of China (No. 39990600).

chemically synthesized. Research on the peptide fragments by circular dichroism (CD) both in solution and in solid state may provide further understanding of the intriguing structural transformation in the intact serpins.

Materials and methods

Peptide synthesis

The peptides were synthesized by the Boc solid-phase method and cleaved from the resins by using hydrogen fluoride. The initial products were purified by preparative HPLC and verified by amino acid analysis.

Materials

Analytical-grade hexafluoroisopropanol (HFIP) was purchased from Fluka. Other reagents were of analytical reagent grade. The peptide was dissolved in a Tris-HCl buffer (25 mmol/L Tris, 50 mmol/L NaCl, pH 7.4) in a stock of 1.0 mg/mL and was diluted to 0.2 mg/mL with buffer or solvent for solution CD measurements. The aqueous solution was adjusted to a proper pH value with dilute NaOH or HCl for pH titration.

Circular dichroism measurements

All CD measurements were performed on a JASCO-715 spectropolarimeter as previously reported.[12] The spectra were recorded over a wavelength range of 250—190 nm using a cuvette of 1-mm pathlength at a scan speed of 10 nm/min and a time constant of 0.125 s. Data were further processed for noise reduction, baseline subtraction and signal averaging when needed. The data for solution CD were presented as mean residue molar ellipticities ($[\theta]$, deg cm^2/dmol). For the solid-state CD measurements,[13] the peptide solution (~1.0 mg/mL) was cast onto a 2-cm diameter cylindrical quartz glass to evaporate the solvent overnight (12—18 h) until a dry thin film formed on the surface of the glass. The far-UV CD spectra of the solid peptide were directly recorded on the film and the data were presented as ellipticities (θ, mdeg).[14]

Results

Design and secondary structure prediction of the reactive center

Two peptide fragments derived from the reactive center of egg-white ovalbumin (namely OVARC) and plasminogen activator inhibior-1 (PAIRC) were selected as models for the present research. The OVARC sequence forms a loop-helix-loop structure in native OVA while PAIRC sequence appears to be a long loop in native PAI.[6] The sequence alignment and the secondary structure prediction by Chou-Fasman method[15] are displayed in Table 1. The two sequences share homologous residues with almost 50% positives. As expected, P9—P1′ residues of OVARC have a propensity to form an α-helix ($P_\alpha = 1.14$) while P16—P4 of PAIRC tend to a β-sheet ($P_\beta = 1.11$, $P_\alpha = 0.95$). In addition, OVARC shows an amphipathy by helical wheel projection.

Table 1 Amino acid sequence and structural prediction of serpin reactive center loops in this study[6,10]

Peptide	Sequence
	Number (P— P′)
	18 15 10 5 1 1′ 5
	$\lvert P_\alpha = 1.14 \rvert$
OVARC	Ac-NEAGREVVG SAEAGVDAA^SV SEE-NH_2
PAIRC	Ac-NE SG TVAS SSTAV I VSAR^MAPEE-NH_2
	$\lvert P_\beta = 1.11 \rvert$

Ac: acetyl; NH_2: amide; ^: cleavage site. The reactive center, denoted as P15—P5′, extends from 15 residues on the *N*-terminal side of the P1 residue to 5 residues on the *C*-terminal side. Within P1—P1′ is the cleavage site. The underlined sequences represent that they form α-helix in native OVA or β-strand insertion into the preexisting β-sheet of the latent PAI. P_α and P_β are the probabilities of α-helix and β-sheet estimated by the Chou-Fasman method.[15]

Secondary structure in solution: pH effect

The CD spectra of OVARC and PAIRC peptides in aqueous solution are shown in Fig. 1. At neutral pH (pH 7.4), both OVARC and PAIRC show a large negative peak at 198 nm, indicating that the peptides are unstructured and flexible. Nevertheless, the spectrum of OVARC has a shoulder at around 222 nm (Fig. 1A), suggesting the possibility to form a marginally stable structure named nascent α-helix. The shoulder at 222 nm increases under acidic pH conditions, indicative of an α-helical structure (Fig. 1A, inset). PAIRC, however, gives a spectrum without a shoulder at 222 nm. It seems that acid has no effect on the structure (Fig. 1B). In comparison of the two peptides, OVARC contains more negatively charged residues (5 Glu + 1 Asp)

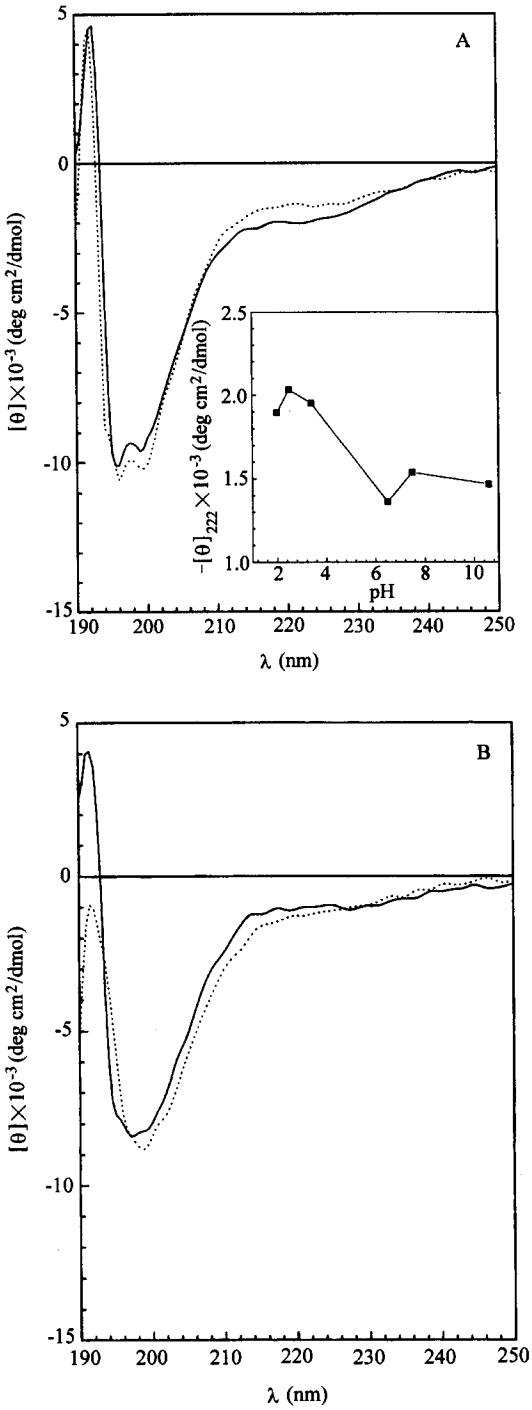

Fig. 1 pH effects on the CD spectra of OVARC peptide (A) and PAIRC peptide (B). The pH titration was performed in a peptide solution (25 mmol/L Tris, 50 mmol/L NaCl) and the pH values were recorded with a pH meter (Orion 710A). The figures show the spectra recorded at pH 7.4 (dotted) and 2.1 (solid). The inset is the plot of $[\theta]_{222}$ of OVARC versus pH values.

than PAIRC (3 Glu). The protonation of the negatively charged carboxylic groups under acidic condition may reduce the charge repulsion that is usually unfavorable to structural formation.

HFIP *induced secondary structural formation*

TFE and HFIP are the structure inducers that are commonly used to mimic hydrophobic conditions for structural formation of amphipathic peptide.[16] Fig. 2A shows the HFIP induced structural formation of OVARC peptide. In a 28.6% HFIP solution, the negative peak shifts to 205 nm from 198 nm in aqueous solution. It is also a negative peak around 222 nm, which gives an increase of $[\theta]_{222}$ from -1340 to -5680 deg cm^2/dmol. The characteristic spectra suggest that OVARC transforms into a structured α-helical conformation with the increase of HFIP concentration. Similarly, PAIRC peptide can also switch to an α-helical secondary structure (Fig. 2B) but with lower helical content (compared with OVARC) as estimated from the ellipticity values at 222 nm. Fig. 3 illustrates the plot of $[\theta]_{222}$ fraction versus the HFIP concentration. Both OVARC and PAIRC show sigmoidal curves with midpoints (C_m) of 16.3% and 13.2%, respectively. C_m is the transition midpoint that reflects effect of HFIP on the secondary structural formation of a peptide. The results indicate that PAIRC is more sensitive to HFIP induction than OVARC.

Secondary structure in dry films

Protein or peptide samples can form dry films on the surface of quartz glass, which allowed the secondary structure of solid-state samples to be studied.[14] Fig. 4 is the CD spectra of OVARC peptide in the solid state. Compared with the solution spectrum (dotted) that shows a small shoulder at 222 nm and a negative peak at 198 nm, the solid-state spectrum exhibits a larger shoulder at around 222 nm and a shift of the negative peak to 202 nm (solid), suggesting that OVARC peptide becomes less flexible in the solid state. As for the solid-state PAIRC sample (Fig. 5), the spectrum shows a strong negative peak at 218 nm and a positive peak at 196 nm, which designates a typical β-sheet dominant secondary structure. Intriguingly, when the solid sample was prepared by evaporation of the OVARC peptide in 25%

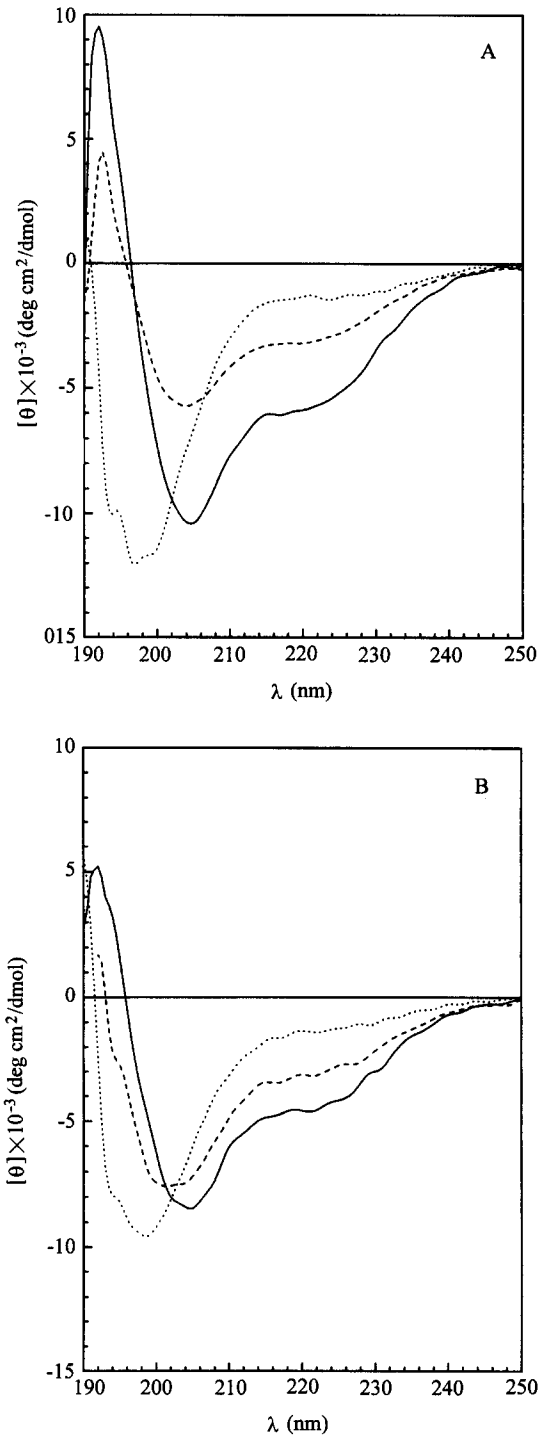

Fig. 2 HFIP effects on the CD spectra of OVARC (A) and PAIRC (B). The peptide solution (25 mmol/L Tris, 50 mmol/L NaCl, pH 7.4) was mixed with different concentration (V/V) of HFIP. The HFIP concentrations are 0% (dotted), 16.7% (A, dashed), 13.8% (B, dashed) and 28.6% (solid), respectively.

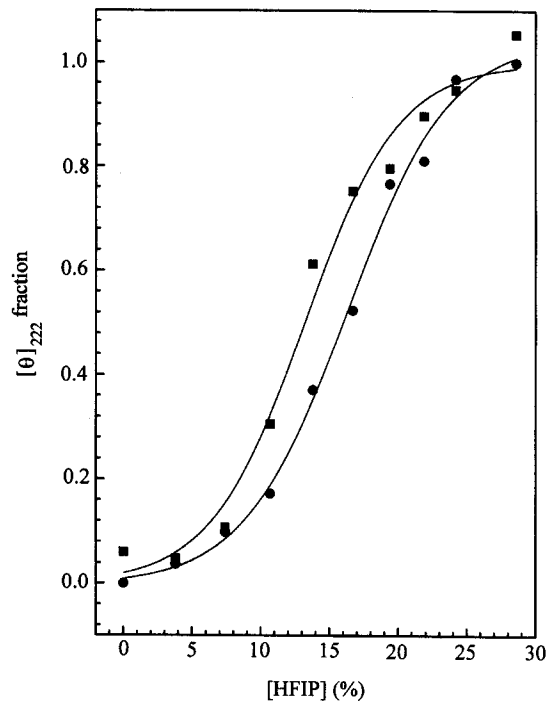

Fig. 3 HFIP induced structural formation of OVARC (circle) and PAIRC (square) peptide. The midpoints (C_m) of the transitions are 16.3% HFIP for OVARC and 13.2% HFIP for PAIRC.

HFIP solution, its spectrum becomes a broad negative peak at around 218 nm (Fig. 4, dashed), manifesting that it has transformed into a β-sheet-like structure from the α-helix in HFIP solution (see Fig. 2). Analogously, when the dry film was prepared from a 25% HFIP sample, PAIRC also forms a β-sheet structure in solid state. It seems that both OVARC and PAIRC undergo structural transformation from α-helical structures in HFIP solutions to the β-sheet structures in dry films.

Discussion

The secondary structure prediction suggests that OVARC possesses an α-helix probability whereas PAIRC has a potential of β-sheet. The present CD experiments show that OVARC peptide forms a small percentage of α-helix in aqueous solution, and the helical content increases under acidic conditions, while PAIRC presents a random coil structure as the reactive loop in the active serpin. The flexible peptide fragments that correspond to the serpin reactive centers have the potential to form

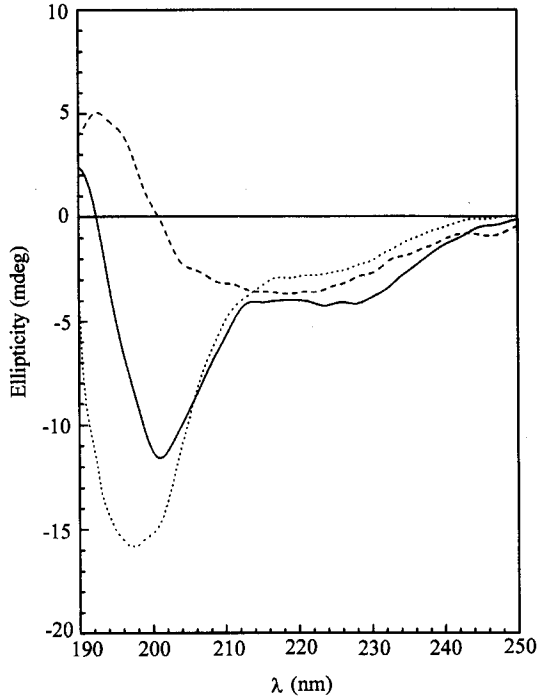

Fig. 4 CD spectra of OVARC peptide in dry films. The peptide solution sample was cast onto the surface of a cylindrical quartz glass with 2 cm in diameter for evaporating overnight (12—18 h) for the dry thin film. (1) The dry film was made from 0.15 mg OVARC peptide (1.0 mg/mL, 150 μL) in aqueous solution (pH 7.4) (solid); (2) from 0.15 mg OVARC (0.75 mg/mL, 200 μL) in 25 % HFIP (dashed); (3) The solution spectrum (dotted) was carried out in a Tris-HCl buffer (25 mmol/L Tris, 50 mmol/L NaCl, pH 7.4) and the peptide concentration was 0.2 mg/mL.

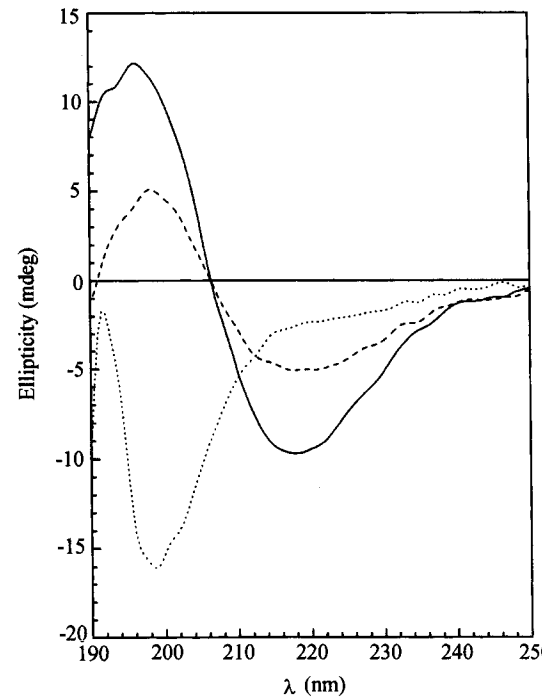

Fig. 5 CD spectra of PAIRC peptide in dry thin films. (1) The dry film was made from 0.15 mg PAIRC peptide (1.0 mg/mL, 150 μL) in aqueous solution (pH 7.4) (solid); (2) from 0.15 mg PAIRC (0.75 mg/mL, 200 μL) in 25% HFIP (dashed); (3) The solution spectrum (dotted) was obtained in a Tris-HCl buffer (25 mmol/L Tris, 50 mmol/L NaCl, pH 7.4) and the peptide concentration was 0.2 mg/mL.

compact structures under specific conditions. The present results by peptide research demonstrate that the model peptides form α-helices in HFIP solution or β-sheet-like structures in the solid state. It could be concluded that the synthetic peptides in different solvents or states may reflect the structural transformation occurring in the native serpin, which could mimic the conformational polymorphism of segments in native proteins (especially in the serpin family).

Some peptides in solution are unstructured due to their flexibility, but it is likely to form ordered structures in the anomalous solid-state form.[17] Structural study of the solid-state peptide and protein is still in its infancy. CD spectroscopy as well as FT-IR in dry films may provide valuable information on secondary structural formation. Indeed, HFIP is a helix enhancer, and OVARC has a propensity to α-helix formation. The β-sheet-like structure of the peptides associated in the dry films from HFIP solution remains problematic when elucidating the structural formation in the solid state. HFIP and TFE are Bronsted acids that provide proton to form hydrogen bonds with the carboxylic groups of the peptide chain, which are conducive to structural formation in solution especially to α-helix.[18] On the other hand, hydration effects contribute equally to the structural formation and polymerization. Slow evaporation of the cosolvents reduces hydration effects and breaks down the hydrogen bonds between peptide and HFIP molecules through excluding HFIP from the peptide sample, and subsequently leads to an increase of the chain-chain interactions that favor the cross β-sheet structure in the solid-state form. The intriguing α-helix to β-sheet transformation by evaporating solvent may resemble the amyloidogenic pathway

of amyloid β-peptide (Aβ) accelerated by organic osmolytes such as glycerol.[19]

The CD spectrum of PAIRC peptide in dry film demonstrates evidently that a cross β-sheet structure has been formed as revealed in Aβ peptide by solid-state NMR studies.[20] From aqueous solution to a dry film, PAIRC undergoes a structural transformation as the reactive loop segments in latent PAI. It is proposed that the solid-state and the aggregated forms of the peptide share physical and morphological similarities. The β-sheet structure of PAIRC in dry film might be analogous to the cross β-sheet of amyloidogenic peptide in the aggregated form. A pair of or numerous peptide chains associate or get tangled into fibrils with β-strand structure in the solid state[21] as described explicitly in the amyloid β-peptide aggregate.[20,22] Attentions to the cross β-sheet structure in solid state will provide insight into the mechanism of structural transformation in peptide and protein aggregates.[23] It is likely that the synthetic peptide fragments and the segments of the serpin reactive center may resemble the amyloidogenic peptides, both of which are prone to form cross β-sheet structure and aggregate. The amyloid-like aggregates are the principal components of pathological deposits implicated in pathogenesis of some amyloid diseases.[24,25]

References

1 Stein, P. E.; Carrell, R. W. *Nat. Struct. Biol.* **1995**, *2*, 96.

2 Lesk, A. M. *Trends Biochem. Sci.* **1998**, *23*, 63.

3 Stein, P. E.; Leslie, A. G.; Finch, J. T.; Carrell, R. W. *J. Mol. Biol.* **1991**, *221*, 941.

4 Stein, P. E.; Chothia, C. *J. Mol. Biol.* **1991**, *221*, 615.

5 Wang, Z.; Mottonen, J.; Goldsmith, E. J. *Biochemistry* **1996**, *35*, 16443.

6 Wright, H. T. *BioEssays* **1996**, *18*, 453.

7 Sifers, R. N. *Nat. Struct. Biol.* **1995**, *2*, 355.

8 Mellet, P.; Michels, B.; Bieth, J. G. *J. Biol. Chem.* **1996**, *271*, 30311.

9 Hu, H. Y.; Du, H. N. *J. Prot. Chem.* **2000**, *19*, 177.

10 Stein, P. E.; Leslei, A. G. W.; Finch, J. T. *Nature* **1990**, *347*, 99.2

11 Mottonen, J. *Nature* **1992**, *355*, 270.

12 Hu, H. Y.; Lu, Z. X.; Du, Y. C. *J. Pept. Res.* **1997**, *49*, 113.

13 Hu, H. Y.; Li, Q.; Cheng, H. Q.; Du, H. N. *Biopolymers* **2001**, *62*, 15.

14 Safar, J.; Roller, P. P.; Ruben, G. C.; Gajdusek, D. C.; Gibbs, C. Jr., J. *Biopolymers* **1993**, *33*, 1461.

15 Chou, P. Y.; Fasman, G. D. *Biochemistry* **1974**, *13*, 222.

16 Stoss, R. W.; Truckes, D.; Wemmer, D. E. *Biopolymers* **1992**, *32*, 1695.

17 Gasset, M. A.; Baldwin, M. A.; Lloyd, D. H. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 10940.

18 Hu, H. Y.; Lu, Z. X.; Du, Y. C. *Chin. J. Chem.* **1998**, *16*, 153.

19 Yang, D. S.; Yip, C. M.; Huang, T. H. J.; Chakrabartty, A.; Fraser, P.E. *J. Biol. Chem.* **1999**, *46*, 32970.

20 Lansbury, P. T.; Costa, P. R.; Grifiths, J. M. *Nat. Struct. Biol.* **1995**, *2*, 990.

21 Wood, S. J.; Maleeff, B.; Hart, T.; Wetzel, R. *J. Mol. Biol.* **1996**, *256*, 870.

22 Zagerski, M. G.; Barrow, C. J. *Biochemistry* **1992**, *31*, 5621.

23 Hu, H. Y.; Xu, G. J. *Prog. Biochem. Biophys.* **1999**, *26*, 9 (in Chinese).

24 Koo, E. H.; Lansbury, P. T.; Kelly, J. W. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 9989.

25 Tran, P. B.; Miller, R. J. *Trends Neurosci.* **1999**, *22*, 194.

(E0101152 SONG, J.P.; DONG, L.J.)