Polymer Journal

SHORT COMMUNICATION

Structural Study of Silk-like Peptides Modified by the Addition of the Cell Adhesive Sequence, RGD, Using ^{13}C CP/MAS NMR

By Chikako TANAKA,[1,2] *Atsushi* ASANO,[2] *Takuzo* KUROTSU,[2] *and Tetsuo* ASAKURA[1,*]

KEY WORDS: Silk-like Peptide / *Anaphe* Silk Fibroin / Cell Adhesive Sequence RGD / ^{13}C CP/MAS NMR /

Silk fibroins from different species have original primary structure and the fibers show different mechanical properties. *Bombyx mori* silk fibroin fiber is a well-known natural fiber which has good tensile properties including high strength and Young's modulus. In the past decade, *B. mori* silk has come to the forefront as a biomaterial with high environmental stability, good biocompatibility and workability.

In our previous paper,[1] we reported detailed structural characteristics of the silk fibroin from *Anaphe reticulata*, one of the wild silkworms which is highly abundant in equatorial and southern Africa. The stress-strain curve of the *Anaphe* silk fiber is similar to that of *B. mori* silk fiber, but the amino acid composition is simpler; the sum of Ala and Gly content accounts for more than 91 mol %, which is in contrast to the case of *B. mori* silk fibroin (72.9 mol %). The main sequence of *Anaphe* silk fibroin was only mixture of $(AAG)_{n1}$ and $(AG)_{n2}$. Thus, these simple sequences detected in *Anaphe* silk fibroin will be candidates of fundamental sequences in molecular design of silk-like materials for tissue engineering.

The short sequence, Arg-Gly-Asp (RGD) has been used as an effective cell adhesion sequence for development of biomaterials and we reported several cell adhesive silk-like proteins by introducing the sequence TGRGDSPA from fibronectin into several fundamental sequences from silk fibroins.[2,3] This RGD unit has been reported to lie on the conformationally mobile loop in fibronectin.[4] Therefore, it seems important to examine whether the flexibility of the RGD unit still remain after introduction it into the silk fibroin sequence and reversely, influence of the presence of RGD unit on the local structure of the silk fibroin sequence.

In this paper, we synthesized the peptides where the sequences $(AAG)_{n1}$ or $(AG)_{n2}$ present in both N- and C-terminal parts and the cell-adhesive sequence, ASTGRGD-SPAAS in the central part.[2,3] The ^{13}C CP/MAS NMR was used to characterize these structures in the solid state. Especially, the conformation-dependent ^{13}C chemical shift coupled with ^{13}C selective labeling of the peptides is used here to obtain structural information in detail.

EXPERIMENTAL

Sample Preparations

The peptide 1: $(AG)_7$ASTGRGDSPAAS$(AG)_7$ and peptide 2: $(AG)_3$[3-^{13}C]AG$(AG)_3$AST[2-^{13}C]GR[1-^{13}C]GDSPAAS-$(AG)_7$ were synthesized by the F-moc solid-phase method. Similarly, peptide 3: $(AAG)_5$ASTGRGDSPAAS$(AAG)_5$ and peptide 4: $(AAG)_2$A[3-^{13}C]AG$(AAG)_2$AST[2-^{13}C]GR-[1-^{13}C]GAS$(AAG)_5$ were synthesized. The peptides were then dissolved in 55 w/w % lithium thiocyanate aqueous solution and dialyzed against distilled water for 4 d at 4 °C using cellulose tubes (MWCO;1000), and freeze-dried (Treatment: Dialysis). The lyophilized peptides were dissolved in trifluoroacetic acid (TFA) and precipitated by addition of diethyl ether (Treatment: TFA). The peptides were also dissolved in formic acid (FA) and then air-dried (Treatment: FA).

Solid State ^{13}C CP/MAS NMR Observation

The solid state ^{13}C CP/MAS NMR spectra were observed on a Chemagnetics CMX-400 spectrometer operating at 100.04 MHz for the ^{13}C nucleus, with TPPM decoupling, a CP contact time of 1 ms, a recycle delay of 4 s, and magic angle spinning rate of 7 kHz. The chemical shifts were calibrated indirectly through the adamantine peak relative to TMS.

RESULTS AND DISCUSSION

The ^{13}C CP/MAS NMR spectra (upper spectra; a, c and e) of the peptide 2 (solid line) and peptide 1 (broken line) after several sample treatments, Dialysis, TFA and FA were shown together with the difference spectra (peptide 2 − peptide 1: lower spectra, b, d and f) in Figure 1. The difference spectra give clearly the information on the local structure of the 7th Ala residue in $(AG)_n$ sequence part by the ^{13}C-labeled Ala Cβ peak. The spectra are quite different among Dialysis, TFA and FA treatments, which is a similar tendency reported for the ^{13}C CP/MAS NMR spectra of $(AG)_{15}$ without RGD sequence previously (Figure 2). The sharp peak at 16.7 ppm in Figures 1b and 2a indicates that the structure is silk I (type II β-turn structure).[5] However, additional broad peak was observed at lower field in Figure 1b, which means the presence of β-sheet structure.[6] Thus, the cell-adhesive sequence, ASTGRGDSPAAS in the central part disturbs

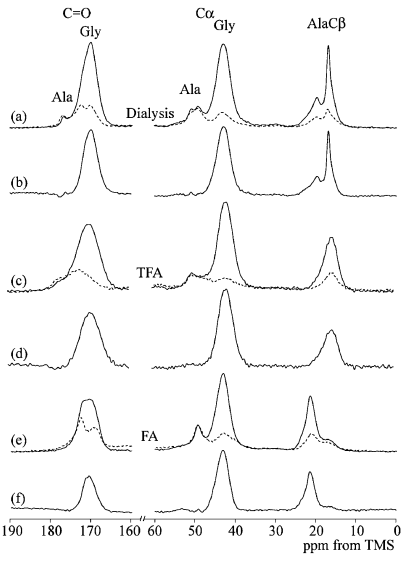

Figure 1. ^{13}C CP/MAS NMR spectra of peptide 1 (broken lines), peptide 2 (solid lines of a, c and e) and the difference spectra (b, d and f) after Dialysis (a and b), TFA (c and d) and FA treatments (e and f).

[1]Department of Biotechnology, Tokyo University of Agriculture and Technology, Koganei 184-8588, Japan
[2]Department of Applied Chemistry, National Defense Academy, Yokosuka 239-8686, Japan
*To whom correspondence should be addressed (Tel: +81-42-388-7025, Fax: +81-42-383-7733, E-mail: asakura@cc.tuat.ac.jp).

 doi:10.1295/polymj.PJ2008218

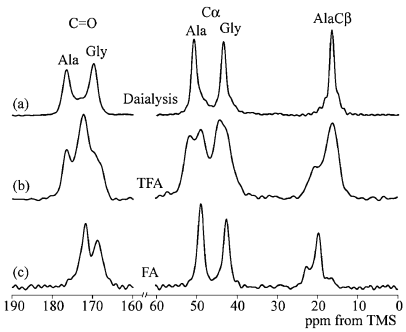

Figure 2. ^{13}C CP/MAS NMR spectra of $(AG)_{15}$ without RGD unit after Dialysis (a), TFA (b) and FA treatments (c).

partially the local conformation of the 7th Ala residue and promotes the structural change from silk I to β-sheet structure.

As shown in Figure 1d, the structural change to random coil occurs by TFA treatment, which can be noted by the broad Ala $^{13}C\beta$ peak with the same chemical shift as the silk I.[7] The small peak assigned to β-sheet was observed together with the broad random coil peak for $(AG)_{15}$ (Figure 2b). Thus, contrary to the case of Dialysis treatment, the presence of the cell-adhesive sequence in the peptides 1 and 2, prevents the structural change from random coil to β-sheet at the 7th Ala residue. Figure 1e and 1f show that the Ala^{7th} residue took mostly β-sheet structure by FA treatment. However, the main peak in Figure 1f is slightly broader than the main peak of $(AG)_{15}$ after FA treatment in Figure 2c. The smaller peak at the lower field of Ala Cβ in Figure 2c could not be observed in Figure 1f and the chemical shifts of the main peaks are slightly different between two Figures. Thus, distorted β-sheet structure can be proposed for the $(AG)_n$ part of the peptides 1 and 2 as discussed previously.[6] Thus, the structure of $(AG)_n$ part changes largely by several solvent treatments although the presence of the cell-adhesive sequence, ASTGRGDSPAAS in the central part disturbs slightly the regular structure of $(AG)_n$. This several solvent treatments can be used for change the structure and property depending on the use of biomaterials.

Contrary to these Ala Cβ peak behavior, both the chemical shifts and line widths of the Gly $^{13}C\alpha$ peak of the Gly^{18th} residue and Gly ^{13}CO peak of the Gly^{20th} residue do not change by these treatments, Dialysis, TFA and FA (Figure 1b, d and f). And these peaks are broader compared with the case of Figure 2. This means the cell adhesive sequence took random coil structure, that is, in conformationally mobile state, independent on the solvent treatment and the presence of $(AG)_n$ sequence at both terminal sides in the peptide 1 and 2. This is suitable for maintaining the cell-adhesive character.

The ^{13}C CP/MAS NMR spectra of the peptide 4 (solid line) and peptide 3 (broken line) after several sample treatments, Dialysis, TFA and FA were shown together with the difference spectra (peptide 4 − peptide 3) as shown in Figure 3.

The local structure of the 8th Ala residue in $(AAG)_n$ sequence part can be discussed by the chemical shift and line width of the Ala $^{13}C\beta$ peak in the difference spectra. The peak shapes are similar between Dialysis and FA treatments (Figures 3b and 3f). Namely, the Ala residue took mostly distorted β-sheet structure. On the other hand, the structure is random coil after TFA treatment (Figure 3d). In addition, as shown in Figure 3, both the

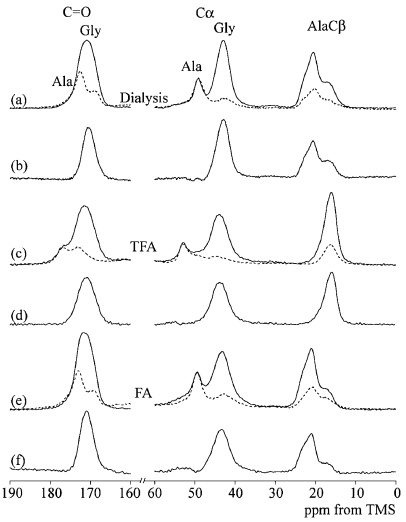

Figure 3. ^{13}C CP/MAS NMR spectra of peptide 3 (broken lines), peptide 4 (solid lines of a, c and e) and the difference spectra (b, d and f) after Dialysis (a and b), TFA (c and d) and FA treatments (e and f).

chemical shifts and wide line widths of the Gly $^{13}C\alpha$ peak of the Gly^{19th} residue and Gly ^{13}CO peak of the Gly^{21th} residue does not change by solvent treatments. Similar to peptides 1 and 2, the cell adhesive sequences in peptides 3 and 4 took random coil structure independent on the solvent treatment, which is also suitable for biomaterials with high cell-adhesive character.

Acknowledgment. TA acknowledges the support of Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan (18105007).

Received: September 14, 2008
Accepted: October 18, 2008
Published: November 27, 2008

REFERENCES

1. C. Tanaka, R. Takahashi, A. Asano, T. Kurotsu, H. Akai, K. Sato, P. D. Knight, and T. Asakura, *Macromolecules*, **41**, 796 (2008).
2. T. Asakura, C. Tanaka, M. Yang, J. Yao, and M. Kurokawa, *Biomaterials*, **25**, 617 (2004).
3. M. Yang, C. Tanaka, K. Yamauchi, K. Ohgo, M. Kurokawa, and T. Asakura, *J. Biomed. Mater. Res. A*, **84**, 353 (2008).
4. L. A. Main, S. T. Harvey, M. Baron, J. Boyd, and D. I. Campbell, *Cell*, **71**, 671 (1992).
5. T. Asakura, J. Ashida, T. Yamane, T. Kameda, Y. Nakazawa, K. Ohgo, and K. Komatsu, *J. Mol. Biol.*, **306**, 291 (2001).
6. T. Asakura, J. Yao, T. Yamane, K. Umemura, and S. A. Ulrich, *J. Am. Chem. Soc.*, **124**, 8794 (2002).
7. T. Asakura, A. Kuzuhara, R. Tabeta, and H. Saito, *Macromolecules*, **18**, 1841 (1985).