Polymer Journal (2010) 42, 354–356

www.nature.com/pj

RAPID COMMUNICATION

Local conformation of serine residues in a silk model peptide, (Ala–Gly–Ser–Gly–Ala–Gly)$_5$, studied with solid-state NMR:REDOR

Polymer Journal (2010) **42,** 354–356; doi:10.1038/pj.2010.2; published online 27 January 2010

INTRODUCTION

The primary structure of the heavy chain of *Bombyx mori* silk fibroin contains multiple repetitions of $(GAGAGS)_n$, which make up 49% of the total silk fibroin.[1] The native silk fibroin fiber and various model peptides have been studied for decades to analyze the conformation of silk fibroin molecules. The role of Ser residue in the unique repetitive sequence is of particular interest, as it carries a hydroxyl group that contributes to intra- and intermolecular hydrogen bonding.

The dynamics and structure of the Ser residue in *B. mori* silk fibroin, including the role of Ser residue in the conformation of the silk fibroin, have been studied with stable isotope-labeled silk fibroin and a variety of sequential model peptides using ^{2}H solid-state nuclear magnetic resonance (NMR), ^{13}C CP/MAS NMR and molecular dynamics simulations.[2–7] However, it is still important to clarify the local structure of the Ser residue depending on its position in the repeated AGSGAG sequences because the Ser residue controls the silk fibroin structure through intramolecular hydrogen bonding or intermolecular hydrogen bonding formations of the side chain OH group.

In this study, to investigate changes in the local conformation of Ser residues in the silk fibroin model peptide $(AGSGAG)_5$ with the Silk II form (the structure of silk fibroin after spinning), a kind of solid-state NMR experiment, rotational-echo double-resonance NMR (REDOR),[8] was applied to five kinds of [1-^{13}C]Gly-Ser-[^{15}N]Gly-labeled $(AGSGAG)_5$ peptides with different labeled positions. Through the determination of internuclear atomic distances between [1-^{13}C]Gly and [^{15}N]Gly nuclei in sequence [1-^{13}C]Gly-Ser-[^{15}N]Gly, the local conformation of the Ser residue was determined. It is difficult to obtain single crystals of the peptide and, therefore, solid-state NMR is more effective than X-ray diffraction analysis at obtaining the exact local conformation.

EXPERIMENTAL PROCEDURE

Sample preparations

Five kinds of $(Ala–Gly–Ser–Gly–Ala–Gly)_5$ peptides with different [1-^{13}C]Gly-Ser-[^{15}N]Gly positions were synthesized by the Fmoc solid-phase method. The sequences of peptides with their respective isotopic labels are listed in Table 1. The crude peptides were dissolved in 9 M LiBr and then dialyzed (molecular weight cutoff=1 kDa) against distilled water for 3 days at 4 °C. The naturally precipitated peptides were dried after dialysis.

REDOR Measurements

^{13}C-detected ^{13}C-^{15}N REDOR experiments were performed on a Bruker DSX-400 AVANCE spectrometer (Bruker, Rheinstetten, Germany) operating at 400.1 MHz for ^{1}H, 100.6 MHz for ^{13}C and 40.5 MHz for ^{15}N using a BL 4 mm Bruker MAS probe. The MAS spinning speed was 7 kHz and the recycle delay was set to 5 s. π pulses for ^{13}C and ^{15}N channels were 16.0 and 12.8 μs, respectively, and a total of 512 acquisitions were collected. REDOR evolution times ranged up to 24.3 ms. The values of $\Delta S/S_0 = 1 - S/S_0$, where S_0 and S represent the ^{13}C signals obtained in the absence of dipolar dephasing and in the presence of dipolar dephasing, respectively, were computed as the ratios of peak intensities in the REDOR spectra. REDOR results have been corrected by calculation for the natural abundance background.

Protein Data Bank analysis

X-ray crystallographic data from the Protein Data Bank at the Research Collaboratory for Structural Bioinfomatics (RCSB) were used to search the torsion angles of Ser residues in the Gly–Ser–Gly sequences of natural proteins.[9] The structures of proteins determined at 2.0 Å resolution or better and with an *R* factor $\leqslant$20% were used. A subset of 310 occurrences were obtained from the database after excluding multiple entries of proteins with a similarity greater than 50%. Thereafter, the atomic distance between the carbonyl carbon of the first Gly and the amide nitrogen of the third Gly residues in the Gly–Ser–Gly sequence was calculated and a histogram was prepared, as shown in Figure 1.

RESULTS AND DISCUSSION

The Ala Cβ peak of the ^{13}C CP/MAS NMR spectrum for nonlabeled $(AGSGAG)_5$ consists of three peaks at 16.7, 19.9 and 22.1 p.p.m., with relative fractions of 40, 28 and 32%,

Table 1 The samples for REDOR measurements and fraction of random coil for given Ser residue

Sample no.	*Sample*	*Random coil (%)*
Ser-3	A[1-^{13}C]GS[^{15}N]GAG AGSGAG AGSGAG AGSGAG AGSGAG	20 ± 10
Ser-9	AGSGAG A[1-^{13}C]GS[^{15}N]GAG AGSGAG AGSGAG AGSGAG	30 ± 8
Ser15	AGSGAG AGSGAG A[1-^{13}C]GS[^{15}N]GAG AGSGAG AGSGAG	30 ± 5
Ser-21	AGSGAG AGSGAG AGSGAG A[1-^{13}C]GS[^{15}N]GAG AGSGAG	40 ± 5
Ser-27	AGSGAG AGSGAG AGSGAG AGSGAG A[1-^{13}C]GS[^{15}N]GAG	30 ± 5

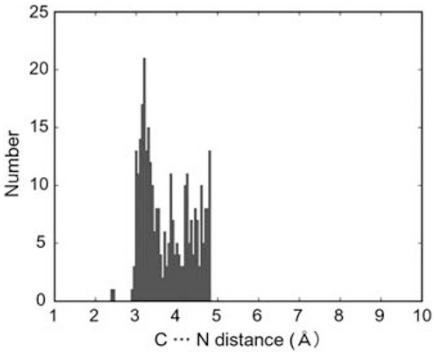

Figure 1 The distribution of internuclear atomic distances between the carbonyl carbon of the first Gly and the amide nitrogen of the third Gly in the sequence, Gly–Ser–Gly.

respectively.[10] The latter two peaks observed at 19.9 and 22.1 p.p.m. were already assigned to an antiparallel β-sheet conformation with different intermolecular arrangements. The peak at 16.7 p.p.m. was assigned to a random coil. Thus, sample $(AGSGAG)_5$ is a mixture of antiparallel β-sheet and random coil structures.

As an example, the ^{13}C-observed REDOR spectra of peptide **Ser-15** are shown in Figure 2, illustrating the full-echo (S_0), rotational spin-echo with ^{15}N π pulse-applied (S) and substantial-echo (ΔS) amplitudes, retained at 20.9 ms of evolution time and a MAS frequency of 7 kHz. The intense peak at 169.5 p.p.m. is the center band of the ^{13}C label placed in the peptide, and the resonances at 100 and 240 p.p.m. are spinning sidebands. All other resonances are from the natural abundance ^{13}C contributions. The experimental $\Delta S/S_0$ values for five labeled peptides are plotted against dipolar evolution time in Figure 3. The theoretical REDOR curve for 4.75 Å (the broken line in the plot of **Ser-15**), which corresponds to the β-sheet distance, is not fitted to the observed REDOR curve, indicating that the conformation of the Ser residue is a mixture of random coil and antiparallel β-sheet structures, as expected. To determine the fraction of antiparallel β-sheets and random coil from the REDOR distance information, it is necessary to know the corresponding internuclear atomic distance for the random coil of the Ser residue. By averaging the distances shown in Figure 1, 3.80 Å was obtained as the random coil distance of the Ser residue in Gly–Ser–Gly.

The theoretical REDOR curves were calculated by changing the fraction of the distance that corresponds to the β-sheet distance, 4.75 Å, and that which corresponds to the random coil distance, 3.80 Å, as shown in Figure 3 (solid line). The theoretical REDOR curve of the fraction, 30% random coil and 70% β-sheet, is the best-fitted one for Ser-15. The same fitting process was performed for the remaining four peptides, and the fraction of random coil was determined as listed in Table 1. The fractions of Ser residues in $(AGSGAG)_5$ with random coil conformation were determined to be from 20 to 40% and the rest was β-sheet.

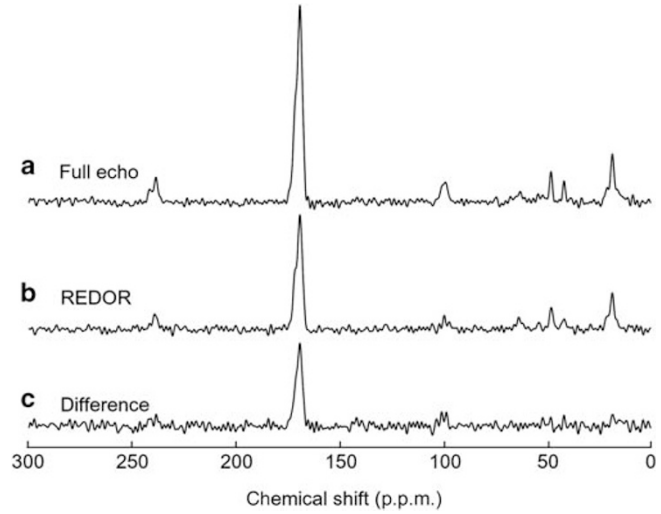

Figure 2 A series of REDOR spectra of peptide Ser-15, AGSGAGAGSGAGA[1-^{13}C]GS[^{15}N]GAGAGSGAGAGSGAG. The full-echo spectrum (**a**), REDOR spectrum (**b**) and the difference spectrum (**c**) (**a** and **b**), retained at 20.9 ms of evolution time and a MAS frequency at 7 kHz.

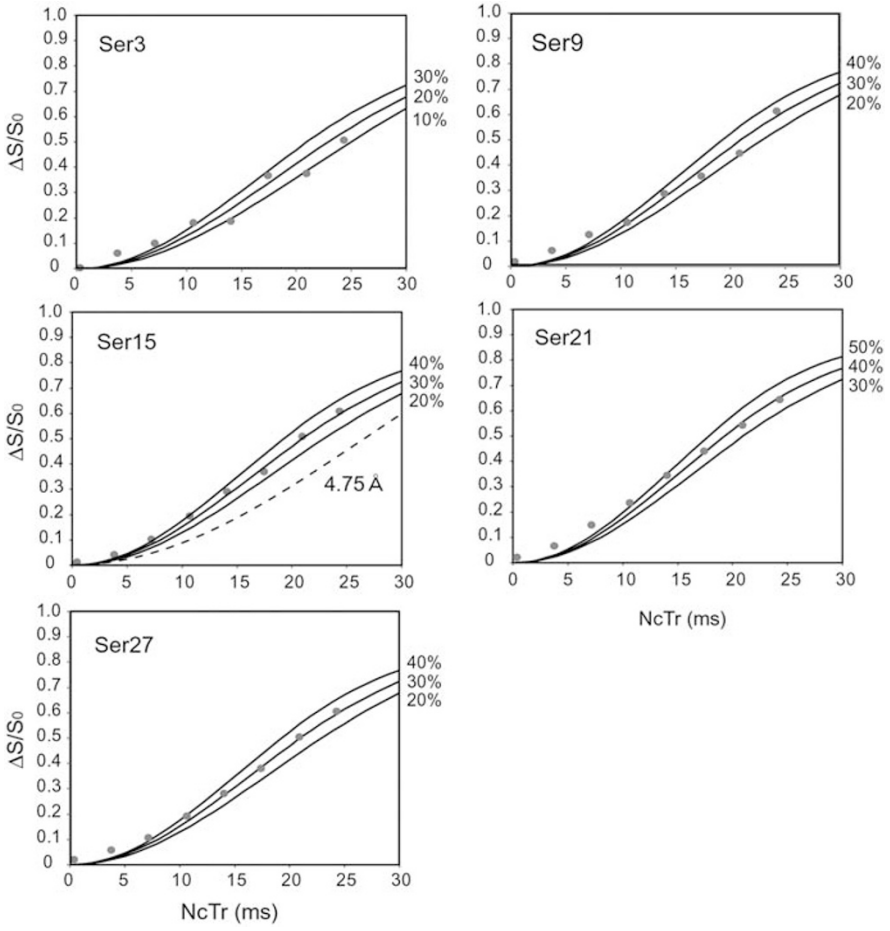

Figure 3 Observed and calculated REDOR plots for the five [1-^{13}C]Gly-Ser-[^{15}N]Gly-labeled $(AGSGAG)_5$ peptides with different labeled positions. Solid lines represent the REDOR curves calculated by changing the fractions of the random coil (3.80 Å) and β-sheet (4.75 Å) forms. The broken line in the plot of Ser-15 corresponds to the β-sheet (4.75 Å).

The aim of this work was to reveal the local conformation of Ser residues of the *B. mori* silk model peptide, $(AGSGAG)_5$. The ratio of two components, antiparallel β-sheet and random coil, at each Ser residue in $(AGSGAG)_5$ was obtained by comparison of the observed REDOR plots with calculated ones.[11] The results reveal that the conformational distribution is different among Ser residues and is within 20–40%, depending on the position of the repeated sequence, Ala–Gly–Ser–Gly–Ala–Gly.

ACKNOWLEDGEMENTS

TA acknowledges support from the Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan (18105007) and grants from the Bio-oriented Technology Research Advancement Institution (BRAIN), Japan.

Yu Suzuki and Tetsuo Asakura

Department of Biotechnology, Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan
E-mail: asakura@cc.tuat.ac.jp

1 Zhou, C., Confalonieri, F., Jacquet, M., Perasso, R., Li, Z. & Janin, J. Silk fibroin: structural implications of a remarkable amino acid sequence. *PROTEINS: Structure, Function, and Genetics* **44,** 119–122 (2001).
2 Yamane, T., Umemura, K. & Asakura, T. The structural characteristics of *Bombyx mori* silk fibroin before spinning as studied with molecular dynamics simulation. *Macromolecules* **35,** 8831–8838 (2002).
3 Asakura, T., Ohgo, K., Ishida, T., Taddei, P., Monti, P. & Kishore, R. Possible implications of serine and tyrosine residues and intermolecular interactions on the appearance of silk I structure of *Bombyx mori* silk fibroin-derived synthetic peptides: high-resolution C-13 cross-polarization/magic-angle spinning NMR study. *Biomacromolecules* **6,** 468–474 (2005).
4 Sato, H., Kizuka, M., Nakazawa, Y. & Asakura, T. The influence of ser and tyr residues on the structure of *Bombyx mori* silk fibroin studied using high-resolution solid-state C-13 NMR spectroscopy and C-13 selectively labeled model peptides. *Polymer J.* **40,** 184–185 (2008).
5 Saito, H., Tabeta, R., Kuzuhara, A. & Asakura, T. A H-2 Nmr-Study of [Ser-3,3-2H2]-Silk and [Ala-3, 3,3-2H3]-silk fibroins in the solid-state - role of side-chain moiety in stabilization of secondary structure. *Bulletin of the Chem. Soc. Jpn.* **59,** 3383–3387 (1986).
6 Saito, H., Ishida, M., Yokoi, M. & Asakura, T. Dynamic features of side-chains in tyrosine and serine residues of some polypeptides and fibroins in the solid as studied by high-resolution solid-state C-13 NMR-spectroscopy. *Macromolecules* **23,** 83–88 (1990).
7 Kameda, T., Ohkawa, Y., Yoshizawa, K., Naito, J., Ulrich, A. S. & Asakura, T. Hydrogen-bonding structure of serine side chains in *Bombyx mori* and *Samia cynthia ricini* silk fibroin determined by solid-state H-2 NMR. *Macromolecules* **32,** 7166–7171 (1999).
8 Gullion, T. & Schaefer, J. Rotational-echo double-resonance NMR. *J. Mag. Resonance* **81,** 196–200 (1989).
9 Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N. & Bourne, P. E. The protein data bank. *Nucleic Acids Res.* **28,** 235–242 (2000).
10 Asakura, T. & Yao, J. 13C CP/MAS NMR study on structural heterogeneity in *Bombyx mori* silk fiber and their generation by stretching. *Protein Sci.* **11,** 2706–2713 (2002).
11 Ohgo, K., Ashida, J., Kumashiro, K. K. & Asakura, T. Structural determination of an elastin-mimetic model peptide, $(Val\text{-}Pro\text{-}Gly\text{-}Val\text{-}Gly)_6$, studied by C-13 CP/MAS NMR chemical shifts, two-dimensional off magic angle spinning spin-diffusion NMR, rotational echo double resonance, and statistical distribution of torsion angles from protein data bank. *Macromolecules* **38,** 6038–6047 (2005).