Characteristic ^{1}H Chemical Shifts of Silk Fibroins Determined by ^{1}H CRAMPS NMR

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ABSTRACT: CRAMPS NMR of ^{1}H was used for the structural analysis of some natural silk fibroins such as *Tussah Antheraea pernyi* and *Bombyx mori* in the solid state. We were able to resolve all expected ^{1}H NMR resonances. When tied to the resolution of ^{13}C NMR via 2D ^{1}H−^{13}C HETCOR experiments, overlapping proton resonance under CRAMPS are able to be further resolved. The simplified ^{1}H signals of these natural proteins could be successfully assigned on the basis of the conformation-dependent ^{1}H chemical shifts of model polypeptides. The ^{1}H chemical shift of the H_α signals of *Tussah A. pernyi* fibroin adopting an α-helix conformation (4.0 ppm) agrees with that of α-helical poly(L-alanine) (3.9 ppm) to within ±0.1 ppm. A well-defined poly(L-alanylglycine), $[Ala\text{-}Gly]_{12}$, was used as a model polypeptide of *B. mori* silk fibroin. The ^{1}H CRAMPS spectra of *B. mori* fibroins adopting the silk I or silk II form were similar to those of $[Ala\text{-}Gly]_{12}$ adopting a corresponding conformation. The H_α chemical shifts of the silk I fibroin were 3.9 ppm (singletlike) whereas those of the silk II fibroin exhibited peaks at 5.0 and 3.9 ppm. Further, we found that the ^{1}H chemical shift of side chains in silk I was downfield by 0.4 ppm compared with that in silk II. Thus, it is possible to assign the ^{1}H CRAMPS NMR spectra of natural proteins such as silk fibroins using model polypeptides of known structure as references.

Introduction

High-resolution and solid-state ^{13}C and ^{15}N NMR spectroscopies have been used successfully for structural analysis of solid proteins such as silk fibroins and collagens.[1−4] The NMR spectra of those solid proteins are rather complicated. However, assignment of the peaks is possible because there exists a database from model polypeptides with known sequences and conformations. It has been shown that high-resolution solid-state NMR spectroscopies for nuclei such as ^{13}C and ^{15}N are very useful for structural analysis of proteins in the solid state. Each individual nucleus reflects unique and different aspects of the structures studied.[5−14] For example, the ^{15}N chemical shift of solid polypeptides is strongly influenced by the main-chain conformation, neighboring amino acid sequence effects, and side-chain effects of the individual amino acid residues.[1,6−11] On the other hand, the ^{13}C chemical shift of main-chain carbonyl carbons depends only on their conformations.[2,3,5,6,12−14] Polypeptides and proteins contain predominantly proton, carbon, nitrogen, and oxygen nuclei. In particular, the information from ^{1}H chemical shifts in the solid state is therefore of great interest.

The ^{1}H CRAMPS (combined rotation and multiple pulse spectroscopy) method is a technique to obtain high-resolution solid-state ^{1}H NMR spectra.[15−25] We have already studied the correlation between the ^{1}H chemical shifts and the conformation of some homopolypeptides such as poly(L-alanine) (PLA), poly(L-leucine), and poly(γ-benzyl-L-glutamate) in the solid state by ^{1}H CRAMPS.[22,24] In these papers, we found that (1) the H_α (α-methine proton) chemical shift of solid polypeptides is conformation-dependent—e.g. right-handed α-helix (α-helix) (3.9−4.0 ppm) and antiparallel β-sheet (β-sheet) forms (5.1−5.5 ppm); (2) on the other hand, H_β (β-methyl or β-methylene protons) is affected strongly by the chemical structure of the individual amino acid residue, but it is relatively insensitive to the main-chain conformation; and (3) the NH (amide proton) chemical shift of fully ^{15}N-labeled polypeptides is conformation-dependent—e.g. α-helix (8.0−8.2 ppm) and β-sheet form (8.6−9.1 ppm), although the NH signal of ^{15}N natural abundant polypeptides is relatively broad due to dipolar coupling to the quadrupolar ^{14}N nucleus. The fact that the conformation of solid polypeptides was able to be determined based on the ^{1}H chemical shift was an important discovery. This implies that the conformation of natural proteins in the solid state may be inferred from ^{1}H chemical shifts. In addition, we compared our results in the solid state to solution NMR measurements of the dependence of α-methine proton chemical shifts on conformation.[22] Therefore, it is important to test systematically the utility of ^{1}H CRAMPS NMR for the structural analysis of proteins in the solid state. However, it was supposed that ^{1}H signals of silk fibroin samples and copolypeptides overlapped due to the resolution limit of ^{1}H CRAMPS. We have improved the resolution limit of ^{1}H CRAMPS by two-dimensional ^{1}H−^{13}C HETCOR (heteronuclear correlation).

Three silk fibroin samples with known sequences and conformations were prepared: (1) *Tussah Antheraea pernyi* (α-helix form), (2) *Bombyx mori* I (silk I form), (3) *B. mori* II (silk II form).[1] Opinions are divided on

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Table 1. Synthetic Conditions and Conformational Characteristics of Silk Fibroin and Polypeptide Samples

sample[a]	synthetic method[b] or origin; treatment	conformation[c]	H_α	H_β	C=O	C_α[d]	C_β[e]
Tussah A. pernyi	silk gland; air-drying at 20 °C	α-helix	4.0	1.5	176.9, 173.5	53.2, 43.4	16.8
B. mori I	silk gland; air-drying at 20 °C	silk I	3.9	1.6	177.2, 171.2	51.7, 44.1	17.3
B. mori II	silk gland; immersion in methanol	silk II	5.0, 3.9	1.2	172.6, 170.2	49.2, 42.9	20.2
PG I	Sigma (without further treatment)	polyglycine I (β-sheet)	4.3		169.2	44.3	
PG II	Sigma (cast from HCOOH/$CaCl_2$)	polyglycine II (3_1-helix)	3.7		172.7	42.5	
$[Ala\text{-}Gly]_{12}$ I	Fmoc; dialysis from LiBr solution	silk I	3.6	1.5	177.4, 170.6	51.3, 43.9	17.2
$[Ala\text{-}Gly]_{12}$ II	Fmoc; as synthesized	silk II	5.0, 3.6	1.2	173.2, 169.8	49.6, 43.5	21.5

[a] Abbreviations: PG, poly(glycine); $[Ala\text{-}Gly]_{12}$, poly(L-alanylglycine); *Tussah A. pernyi*, *Tussah A. pernyi* silk fibroin; *B. mori*, *B. mori* silk fibroin. [b] Sigma, purchased from Sigma Co.; Fmoc, 9-fluorenylmethyl methoxycarbonyl (method). [c] Conformations of these samples were determined by the ^{13}C and/or ^{15}N CP-MAS NMR, and IR spectroscopic methods. [d] ^{13}C chemical shifts of α-methine (or α-methylene) carbons of L-alanine and glycine residues. [e] ^{13}C chemical shift of β-methyl carbon of L-alanine residue.

the secondary structure of the silk I form, though the existence of the silk I form has long been known. In general, *A. pernyi* consists mainly of 51% L-alanine, 24% glycine, and 11% L-serine residues, and *B. mori* contains approximately 43% glycine, 32% L-alanine, and 15% L-serine residues.[26,27] Therefore, ^{1}H chemical shifts of model polypeptides such as PLA, poly(glycine) (PG), and poly(L-alanylglycine) $[Ala\text{-}Gly]_{12}$, which show characteristic differences in conformation will be a guide for the assignment of the spectra of these silk fibroins in the solid state. Since the ^{1}H chemical shifts of solid PLA have been previously obtained,[22,24] in the present work, those of PG and $[Ala\text{-}Gly]_{12}$ were at first clarified in comparison with their conformations. Recently, we have found that the model polypeptide $[Ala\text{-}Gly]_{12}$ takes a stable silk I form in the solid state by dissolution in aqueous LiBr solution (9 M) and slow dialysis against distilled water.[28]

In this work, we pose three questions: (1) Can we obtain highly resolved ^{1}H NMR spectra of the natural silk fibroins in the solid state? (2) Are the conformations of the three silk fibroins distinguishable by ^{1}H CRAMPS spectra? (3) Can we apply the ^{1}H chemical shifts of model polypeptides to characterize the solid conformation of natural proteins?

Experimental Section

Materials. Silk fibroin samples adopting different kinds of conformations were prepared as follows.[1,29] *Tussah A. pernyi* (α-helix form) was prepared by air-drying from the posterior division of the silk gland at 20 °C. *B. mori* I (silk I form) was prepared by air-drying from the silk gland for 1 day at 20 °C. *B. mori* II (silk II = antiparallel β-sheet form) was prepared by immersion in methanol.

Poly(glycine) (MW = 5200) was purchased from Sigma Chemical Co. All the other polypeptide samples were synthesized in our laboratory. A monodispersed alternating sequential copolypeptide $[Ala\text{-}Gly]_{12}$ was synthesized as a model of *B. mori* silk fibroins by a solid-phase peptide synthetic method using Fmoc [9-fluorenylmethoxycarbonyl] and HATU [*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] as the N-protecting group and C-active esters of amino acid, respectively. The solid-phase synthesis was performed on a PerSeptive Biosystems 9050 Plus PepSynthesizer.

Conformational characterization of these samples was made on the basis of conformation-dependent ^{13}C, ^{15}N chemical shifts determined using CP−MAS NMR and also from the characteristic bands in the IR spectra.[30,31]

Synthetic conditions and characteristics of these samples are summarized in Table 1.

^{1}H CRAMPS NMR Measurements. The solid-state ^{1}H CRAMPS NMR measurements were performed on a Chemagnetics CMX 300 spectrometer operating at 300 MHz, equipped with a 5 mm CRAMPS probe. The BR-24 pulse sequence[32] was used for homonuclear decoupling of ^{1}H. The $\pi/2$ pulse width was 1.3 μs. The rotational frequency was exactly controlled at 2.0 kHz, and the cycle time of BR-24 was 108 μs. Silicone rubber (δ 0.12) relative to tetramethylsilane $(CH_3)_4Si$ (δ 0) was used as an internal standard. The ^{1}H chemical shift was calculated with a scaling factor of 0.40 (theoretical value: 0.385) for all samples, which was determined experimentally. Experimental errors of the ^{1}H chemical shifts are estimated to be less than ±0.1 ppm in the range of 0−15 ppm. The total measurement time for one sample was 5−10 min.

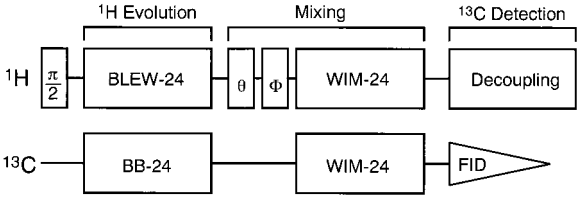

Figure 1. Pulse diagram of 2D heteronuclear-correlation experiment. The technique was proposed by Burum and Bielecki.[33] For all scans, $\theta = X$, $\phi = 63°$. All pulses expect ϕ are 90° pulses.

2D ^{1}H−^{13}C HETCOR NMR Measurements. The solid-state 2D ^{1}H−^{13}C HETCOR (heteronuclear correlation) NMR spectra were obtained on a Bruker DSX-300 using a standard Bruker 4 mm CP−MAS probe. The pulse diagram of the 2D HETCOR, proposed by Burum and Bielecki,[33] is illustrated in Figure 1. The BLEW-24 pulse sequence[33] was used for homonuclear decoupling of ^{1}H. The $\pi/2$ pulse width was 2.8 μs for both ^{13}C and ^{1}H under CP conditions. The spinning speed was set to 5.0 kHz. The ^{1}H chemical shift under the BLEW-24 was calculated with an experimentally determined scaling factor 0.29 (theoretical value: 0.212) for all samples. The total measurement time for one sample ranged from 15 to 35 h.

Results and Discussion

^{1}H CRAMPS Spectra of Silk Fibroins. Figure 2 shows the 300 MHz ^{1}H CRAMPS NMR spectra of three silk fibroins: (A) *Tussah A. pernyi* fibroin (α-helix), (B) *B. mori* I (silk I form), and (C) *B. mori* II (silk II form). We succeeded in obtaining highly resolved ^{1}H NMR spectra of these silk fibroins in the solid state. These represent the first high-resolution solid-state ^{1}H NMR spectra of such natural proteins. The spectra of these fibroins are separated into four regions (NH, side chain phenyl, H_α, and side chain protons). The ^{1}H NMR spectrum of *Tussah A. pernyi* fibroin is roughly similar to that of α-helical PLA[22] except for the side chain phenyl proton signal. This result correlates with the fact that the *Tussah A. pernyi* fibroin contains approximately 51% L-alanine residues and takes the form of an α-helix. It is worth noting that the H_α chemical shift of α-helical *Tussah A. pernyi* fibroin (4.0 ppm) agrees very closely with that of α-helical PLA (3.9 ppm). In addition, the ^{1}H chemical shifts of the side chain H_β protons of the L-alanine residue in the *Tussah A.* pernyi fibroin (δ 1.5) agree roughly with the H_β chemical shift

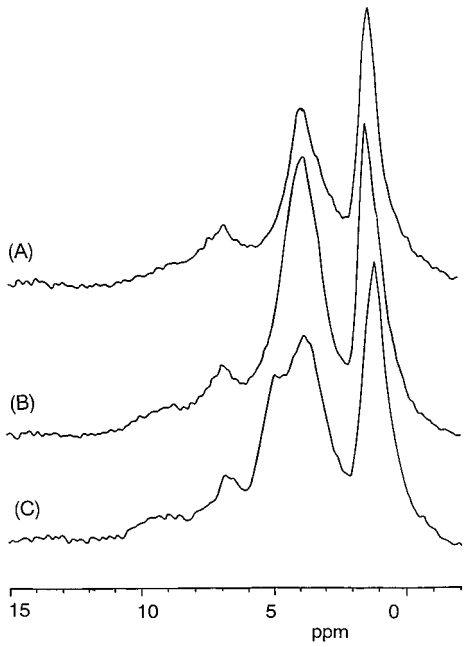

Figure 2. 300 MHz ^{1}H CRAMPS NMR spectra of silk fibroins: (A) *Tussah A. pernyi* fibroin (α-helix), (B) *B. mori* I (silk I), and (C) *B. mori* II (silk II) in the solid state (64 scans). Peak assignment: NH, 10−8 ppm; side chain phenyl, 6.9 ppm; H_α, 3.9−5.0 ppm; side chain, 1.6−1.2 ppm.

of α-helical PLA (δ 1.4). This result indicates that the conformational aspects of *Tussah A. pernyi* fibroin may be inferred on the basis of the conformation-dependent ^{1}H chemical shifts of the model polypeptides (PLA). We conclude that ^{1}H CRAMPS NMR can be diagnostic for the conformational analysis of silk fibroins in the solid state.

Next, we discuss ^{1}H NMR spectra of the two *B. mori* fibroins, silk I form and silk II form (β-sheet),[1,4] as shown in Figure 2, parts B and C. It is noted that the amino acid sequence (primary structure) of these two samples is identical, but their conformations are different. The ^{1}H CRAMPS NMR spectra were exactly diagnostic of these two conformations. We found that the H_α signals of *B. mori* I (silk I form) and *B. mori* II (silk II form) showed one (δ 3.9) and two resonances (δ 5.0 and 3.9), respectively. In addition, the ^{1}H chemical shifts of side chain H_β protons (mainly from the L-alanine residue) of *B. mori* I (δ 1.6) were different from those of *B. mori* II (δ 1.2). The ^{1}H chemical shifts of the side chain protons also depend on the conformation such as silk I and silk II clearly. As reported in our previous work,[22] the side chain proton chemical shifts of polypeptides showed only a small amount of conformation dependency between the α-helix and β-sheet conformations. These results are interpreted as meaning that the ^{1}H chemical shifts of the side chain protons are fundamentally conformation-dependent but the ^{1}H shift of the side chain in α-helical polypeptides is similar to that in the β-sheet.

Consequently, the ^{1}H chemical shifts of the H_α signal and side chain proton signals are useful for conformational analysis of *B. mori* silk fibroins in the solid state. It is also worthy noting that the ^{1}H chemical shifts of *B. mori* I were near to those of *Tussah A. pernyi* fibroin (α-helix). The chemical shift difference between the H_α and H_β protons can only be regarded as significant (*Tussah A. pernyi*, 4.0−1.5 = 2.5 ppm; *B. mori* I, 3.9−1.6 = 2.3 ppm). Thus, it is apparent that all the ^{1}H chemical shift differences between the *Tussah A. pernyi* (α-helix) and *B. mori* I (silk I) is distinguishable, although these chemical shift differences are smaller than the ^{15}N chemical shift differences (L-alanine: α-helix, δ 97.7; silk I, δ 102.0. L-serine: α-helix, δ 97.7; silk I, δ 95.4. Glycine: α-helix, δ 84.7; silk I, δ 86.9).[1] However, the peak intensity of the side chain peak of *Tussah A. pernyi* fibroin was higher than that of *B. mori*. The reason for the different peak heights is perhaps explained with the idea that the L-alanine content in the *Tussah A. pernyi* fibroin is much higher than that in *B. mori* silk fibroin. The side chain proton signals of the silk fibroins should be mainly associated with the methyl protons of the L-alanine residue. Therefore, we can easily distinguish between the *Tussah A. pernyi* fibroin and *B. mori* I from the peak intensity ratio of H_α to H_β in the ^{1}H CRAMPS NMR spectra.

In conclusion, ^{1}H CRAMPS NMR is a useful tool for conformational analysis of silk fibroins in the solid state, but a problem remains. Why does the shape of the H_α signals of the *B. mori* depend on the conformation? To investigate this question, we examined the 2D ^{1}H−^{13}C HETCOR NMR spectra of these silk fibroins. This technique can provide well-resolved proton chemical shift information for the silk fibroins where it is impossible to resolve the proton resonances with ^{1}H CRAMPS alone.

2D ^{1}H−^{13}C HETCOR NMR Spectra of Silk Fibroins. Figure 3 shows the 2D ^{1}H−^{13}C HETCOR NMR spectra of silk fibroins: (A) *Tussah A. pernyi* fibroin (α-helix), (B) *B. mori* I (silk I), and (C) *B. mori* II (silk II) in the solid state. We can assign the ^{1}H peaks of these silk fibroins on the basis of the ^{13}C signals, which are known,[2] because the correlation is clearly resolved in the 2D spectra for proton-carbon pairs. Therefore, the assignment of the ^{1}H peaks is readily made. From Figure 3, we see that (1) the H_α proton chemical shifts of L-alanine residue were almost the same as for the glycine residue and can be seen as overlapped in *Tussah A. pernyi* fibroin and *B. mori* I, (2) in contrast, the H_α chemical shift of the L-alanine residue is downfield (1.1 ppm) from that of the glycine residue in *B. mori* II, and (3) the H_α and H_β peaks of L-serine residue appear at around 1.5−2.5 ppm and are conformation-dependent. However, the signal of the L-serine residue did not produce a strong effect on the ^{1}H NMR spectra because *B. mori* silk fibroins have a low L-serine content compared to L-alanine or glycine content. Consequently, it was clear that the H_α signals of the glycine and L-alanine residues overlapped in *Tussah A. pernyi* fibroin and *B. mori* I and that the H_α signal of the glycine residue was separated from that of the L-alanine residue in *B. mori* II.

In conclusion, solid-state 2D HETCOR spectra of these silk fibroins were successfully obtained, and the correlation was clear. Therefore, we can assign ^{1}H peaks of the silk fibroins from 2D HETCOR spectra on the basis of ^{13}C signals. The 2D HETCOR technique allows the resolution of ^{1}H CRAMPS to be tied to the higher resolution associated with ^{13}C chemical shifts. However, these 2D HETCOR spectra have the disadvantage of having a smaller scaling factor and, therefore, lead to a larger error for ^{1}H chemical shifts. Consequently, we require ^{1}H NMR spectra of some silklike model polypeptides such as PG and $[Ala\text{-}Gly]_{12}$ to clarify their assignment in the ^{1}H NMR spectra in *B. mori* silk fibroins.

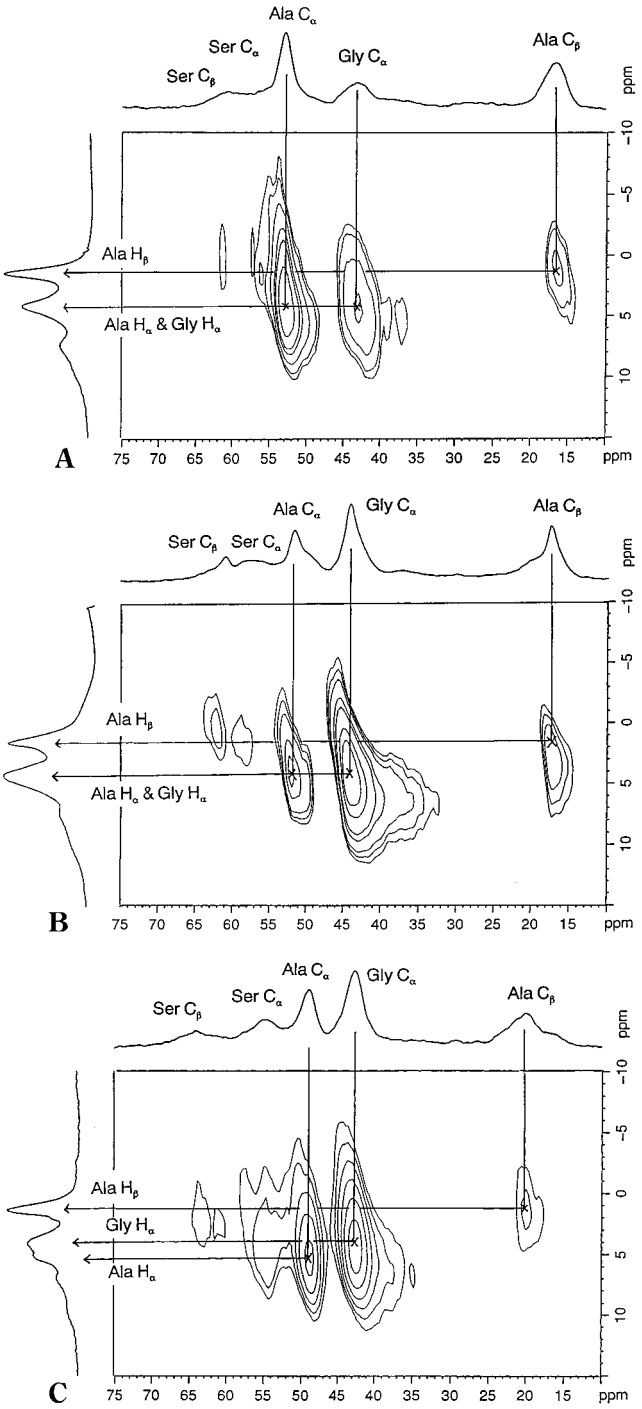

Figure 3. 2D ^{1}H−^{13}C HETCOR spectra of silk fibroins: (A) *Tussah A. pernyi* fibroin (α-helix), (B) *B. mori* I (silk I), and (C) *B. mori* II (silk II) in the solid state. Peak assignments are also shown.

^{1}H CRAMPS Spectra of Model Polypeptides of Silk Fibroins. Figure 4 shows the 300 MHz ^{1}H CRAMPS NMR spectra of poly(glycines): (A) PG I (polyglycine I form) and (B) PG II (polyglycine II form). In PG, polyglycine I (antiparallel β-sheet) and polyglycine II (intermolecular hydrogen-bonded 3_1-helix) are the stable forms in the solid state. The ^{1}H CRAMPS NMR spectra showed high-resolution proton signals separated into two regions (NH and H_α) for PG. We observed that (1) the ^{1}H chemical shift of the H_α methylene protons was conformation-dependent (δ 4.3, PG I; δ 3.7, PG II) and (2) the NH signal was relatively broad due to residual dipolar coupling between the

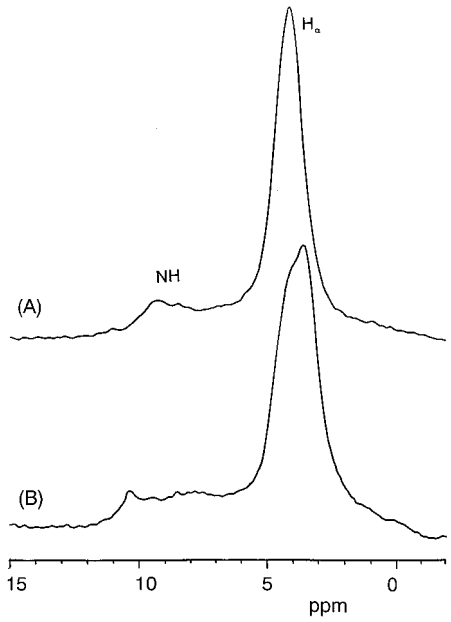

Figure 4. 300 MHz ^{1}H CRAMPS NMR spectra of poly(glycine): (A) PG I (polyglycine I) and (B) PG II (polyglycine II) in the solid state (32 scans). Peak assignment: NH, 10−8 ppm; H_α, 4.3−3.7 ppm.

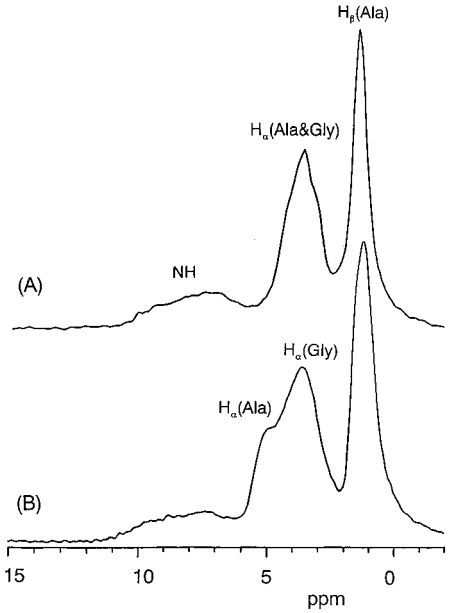

Figure 5. 300 MHz ^{1}H CRAMPS NMR spectra of poly(L-alanylglycines): (A) $[Ala\text{-}Gly]_{12}$ I (silk I) and (B) $[Ala\text{-}Gly]_{12}$ II (silk II) in the solid state (32 scans). Peak assignment: NH, 10−8 ppm; H_α, 5.0−3.6 ppm; H_β, 1.2−1.5 ppm.

quadrupolar ^{14}N nuclei and the amide protons.[24] This result shows that the ^{1}H chemical shift of the H_α signal is useful for conformational analysis of solid PG, although the difference in the H_α chemical shift is relatively small. These ^{1}H chemical shift values of PG support the assignment of the H_α signal of glycine residue in ^{1}H NMR spectra of *B. mori* silk fibroins.

Figure 5 shows the 300 MHz ^{1}H CRAMPS NMR spectra of solid poly(L-alanylglycines): (A) $[Ala\text{-}Gly]_{12}$ I (silk I form)[28] and (B) $[Ala\text{-}Gly]_{12}$ II (silk II form). Poly(L-alanylglycines) were used as a model polypeptide of *B. mori* silk fibroin because it contains predominantly L-alanine and glycine residues, and because the X-ray diffraction data of *B. mori* fibroin are similar to that of

poly(L-alanylglycines).[27] As shown in Figure 5, the ^{1}H CRAMPS NMR spectra showed highly resolved proton signals separated into three regions (NH, H_α, and H_β) for [Ala-Gly]$_{12}$. It was deduced from the spectra that: (1) the H_α signal of glycine and L-alanine residues in [Ala-Gly]$_{12}$ I overlapped (δ 3.6) and gave a single peak, whereas that of [Ala-Gly]$_{12}$ II separated into two peaks (δ 5.0, 3.6), (2) the ^{1}H chemical shift of the H_β of L-alanine residue in the silk I form (δ 1.5) appears downfield by 0.3 ppm compared with that in the silk II form (δ 1.2), and (3) the NH signal is relatively broad. As expected, the ^{1}H CRAMPS spectra of [Ala-Gly]$_{12}$ I and [Ala-Gly]$_{12}$ II were similar to that of *B. mori* I and *B. mori* II forms, respectively.

Both signals of H_α in [Ala-Gly]$_{12}$ I and *B. mori* I are almost the same singlet. This result indicates that the H_α chemical shift values of L-alanine and glycine residues were almost the same in the silk I form. This trend agrees with the result from the 2D HETCOR spectrum of *B. mori* I. The H_α chemical shift of [Ala-Gly]$_{12}$ I (δ 3.6) is close to that of *B. mori* I (δ 3.9). Furthermore, it is interesting that the H_α chemical shifts of the glycine residue of [Ala-Gly]$_{12}$ I and *B. mori* I are close to that of PG II (δ 3.7). On the other hand, the H_β chemical shift of the L-alanine residue in [Ala-Gly]$_{12}$ I (δ 1.5) agrees with the ^{1}H chemical shifts of the side chain signals of *B. mori* I (δ 1.6). We, thus confirmed the assignment of the ^{1}H CRAMPS NMR spectra of *B. mori* I (δ 10−8, NH of all amino acid residues; δ 6.9, phenyl proton of mainly L-tyrosine residue; δ 3.9, H_α of all amino acid residues (mainly H_α of Ala and Gly); δ 1.6, side chain of all amino acid residues (mainly H_β of Ala)).

By contrast, H_α signals of both [Ala-Gly]$_{12}$ II and *B. mori* II were doublets. Also, the H_α chemical shifts of [Ala-Gly]$_{12}$ II (δ 5.0 and 3.6) are close to those of *B. mori* II (δ 5.0 and 3.9). Both H_α chemical shifts of [Ala-Gly]$_{12}$ II (δ 5.0 and 3.6) correspond to that of PLA (δ 5.1)[22] and PG (δ 4.3) adopting the β-sheet form, respectively. The most interesting result here is that the H_α chemical shift of the glycine residues is the same between silk I and silk II forms (in both *B. mori* silk fibroins and [Ala-Gly]$_{12}$) and is not very conformation-dependent in this system, whereas that of the L-alanine residue is apparently conformation-dependent. Both chemical shift displacements may offer a key to clarify the silk I structure. On the other hand, the H_β chemical shift of [Ala-Gly]$_{12}$ II (δ 1.2) agrees completely with that of PLA adopting a β-sheet form. These results show that ^{1}H chemical shifts of the model polypeptide are useful for the conformational analysis of silk fibroins in the solid state. Thus, we can assign the ^{1}H NMR spectrum of *B. mori* II as below: NH of all amino acid residues (δ 10−8), phenyl proton of mainly L-tyrosine residues (δ 6.9), H_α of all amino acid residues except for glycine (mainly H_α of Ala)(δ 5.0), H_α of glycine residue (δ 3.9), and side chain of all amino acid residues (mainly H_β of Ala) (δ 1.2)).

In conclusion, we were able to show that ^{1}H chemical shifts reflect the conformations of model polypeptides such as PG and [Ala-Gly]$_{12}$ and of natural silk fibroins. Further, we confirmed that the well-defined poly(L-alanylglycines) [Ala-Gly]$_{12}$ (silk I and silk II forms) were suitable models for the structural study of natural silk fibroins using high-resolution solid-state NMR. We confirmed the ^{1}H peak assignment of the silk fibroins on the basis of the conformation-dependent ^{1}H chemical shifts of model peptides, utilizing ^{1}H CRAMPS NMR.

Chemical shifts of our model polypeptide [Ala-Gly]$_{12}$ may play an important role for testing new structures of silk I and silk II forms proposed very recently by Lazo and Downing.[34]

Conclusion

We have successfully measured the ^{1}H CRAMPS NMR spectra of some silk fibroins and their model polypeptides in the solid state. We found that (1) the ^{1}H peaks of natural silk fibroins were able to be assigned on the basis of the ^{1}H chemical shifts of their model polypeptides and of the 2D ^{1}H−^{13}C HETCOR NMR spectra, (2) the conformation of silk fibroins were distinguishable by the ^{1}H chemical shifts of the H_α and H_β protons, (3) the peak height and the ^{1}H chemical shifts of side chain protons are as follows. (A) *Tussah A. pernyi* fibroin (α-helix): δ 4.0 (H_α), δ 1.5 (side chain, L-alanine H_β main: relatively high peak). (B) *B. mori* I (silk I): δ 3.9 (H_α), δ 1.6 (side chain, L-alanine H_β main: relatively low peak). (C) *B. mori* II (silk II): δ 5.0 (H_α, L-alanine main), δ 3.9 (H_α, glycine), δ 1.2 (side chain, L-alanine H_β main). Thus, ^{1}H CRAMPS NMR spectra is a useful tool for the conformational analysis of silk fibroins in the solid state. Also, the information from ^{1}H CRAMPS is considerably augmented by the addition of ^{1}H−^{13}C HETCOR NMR.

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