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A study of conformational stability of polypeptide blends by solid state two-dimensional $^{13}C-^{1}H$ heteronuclear correlation NMR spectroscopy

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Abstract

The intermolecular hydrogen-bond interactions in polyglycine (PG)/poly(L-valine) (PLV) blend with a ratio of 1/1 have been studied through high-speed frequency-switched Lee–Goldburg (FSLG) $^{13}C-^{1}H$ heteronuclear correlation (HETCOR) NMR experiments, where the PG/PLV blend sample is prepared by adding trifluoroacetic acid (TFA) solution of their polypeptide mixture containing a 2.0 wt/wt% amount of sulfuric acid (H_2SO_4) to alkaline water. The spectral assignment of the polypeptide blend is made by using multiple proton cross-peaks, which appeared in the HETCOR spectra. Intermolecular correlation peaks between PG(β-sheet) and PLV(β-sheet) appear in the HETCOR spectra with long contact time. From these experimental results, it has been clarified that intermolecular hydrogen-bond interactions (>C=O···H–N<) between the PG(β-sheet) and PLV(β-sheet) are formed and thus the PG/PLV blend with high miscibility is formed.

Keywords: Polypeptide blend; Miscibility; Solid state NMR

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1. Introduction

Synthetic homopolypeptides consist of a repeated sequence of an amino acid, and take some specified conformations such as the α-helix, β-sheet, etc. These individual conformations are transformed into other conformations under certain conditions such as temperature treatment and quenching [1–7]. For example, the main chain of poly(β-benzyl L-aspartate) takes on a right-handed $\alpha(\alpha_R)$-helix form within the temperature range from room temperature to 117 °C and is transformed to the left-handed $\alpha(\alpha_L)$-helix form, the ω-helix form, the β-sheet at temperatures above 117 °C. On the other hand, copolymers of alanine (Ala) and glycine (Gly) [$(\mathrm{Ala, Gly})_n$] take the right-handed α-helix, β-sheet and 3_1-helix form in the solid state as obtained by changing the mixture ratio or by solvent treatment [8–11]. These transformations arise from the energetic stability caused by intramolecular or intermolecular hydrogen-bond (HB) interactions. Thus, by the balance of intramolecular and intermolecular HB interactions in polypeptide blends, it is expected that the strength of intermolecular interaction in the blends is different from those in homopolypeptides and then new conformations can be formed by intermolecular HB interactions, which do not exist originally in homopolypeptides. There are many studies on intermolecular HB interactions in homopolypeptides and copolypeptides in the solid state, but according to our best knowledge there is little study on intermolecular HB interactions in polypeptide blend samples except for our previous works.

Some kinds of polypeptide blend samples [polyglycine/poly(L-alanine), polyglycine/poly(L-valine), poly(L-alanine)/poly(L-valine), poly(D-alanine)/poly(L-valine), and poly(L-alanine)/poly(L-isoleucine)] have been successfully prepared with different blend condition, and their miscibility has been investigated by the observation of ^{13}C NMR chemical shifts and spin-lattice relaxation times in the rotating frame for proton (^{1}H $T_{1\rho}$) [7,12,13]. The conformations of the polypeptide blends are characterized by ^{13}C cross-polarization/magic angle spinning (CP/MAS) NMR method. It has been demonstrated that solid state NMR is a

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very useful means for characterizing conformation and conformational changes of homopolypeptides and copolypeptides. The solid state NMR chemical shift is often characteristic of a specific conformation because of the highly restricted molecular motion in solids. For example, it has been elucidated that the ^{13}C NMR chemical shifts of a number of polypeptides and proteins in the solid state are significantly displaced, depending on their particular conformations such as α-helix, 3_1-helix, ω-helix, β-sheet, etc. [14–19].

On the other hand, ^1H $T_{1\rho}$ is very sensitive to the domain size of individual polypeptides in the blends by spin diffusion process [20]. From these experimental results, it has been found [12,13] that homopolypeptides such as polyglycine (PG: 3_1-helix)/poly(L-valine) (PLV: β-sheet) can blend at the molecular level as prepared by adding a trifluoroacetic acid (TFA) solution of these polymers containing a 2.0 wt/wt% amount of sulfuric acid (H_2SO_4) to alkaline water. Nevertheless, we need more detailed information about the miscibility of their polypeptides to understand the polypeptide blend by using further sophisticated NMR methodology.

Recently, two-dimensional (2D) ^{13}C–^1H heteronuclear correlations (HETCOR) NMR method by using frequency-switched Lee–Goldberg (FSLG) ^1H decoupling sequence [21] at high MAS rates has been developed, in order to provide intermolecular and spatial distance information. The HETCOR spectrum often has multiple proton cross-peaks for each carbon, and these cross-peaks can be extremely helpful to assign the spectrum. Thus, this method can be used to characterize the structure of polymers in solid [22–26].

From such background, in this work, we aim to measure 2D FSLG ^{13}C–^1H heteronuclear correlation (HETCOR) NMR spectrum of PG/PLV(50/50) blend sample and to elucidate intermolecular HB interactions between PG and PLV chains and their miscibility.

2. Experimental

2.1. Materials

Poly(L-valine) (molecular weight (M_w) : 1700–1900) in the β-sheet form and polyglycine (M_w : 1000) in the β-sheet form are purchased from Sigma Chemical Co., respectively. Polyglycine in the 3_1-helix (PG-II) form is obtained by precipitation from an aqueous lithium bromide (LiBr) solution of PG in the β-sheet form [8–11]. The conformations of these polypeptides are recognized by ^{13}C CP/MAS NMR. PG/PLV mixture with a ratio of 50/50 (wt/wt%) is dissolved in TFA with a 2.0 wt/wt% amount of H_2SO_4, which is 'random coil' solvent for these polypeptides. Their solutions are added to alkaline water at room temperature and the precipitated blend samples are washed by water and dried under vacuum at temperatures from 308 to 318 K. Homopolypeptides of PG(3_1-helix) and PLV(β-sheet) are prepared using the same condition as PG/PLV(50/50) blend sample.

2.2. NMR measurements

All of NMR experiments are carried out on a Bruker DSX-300 spectrometer operating at 75 MHz for ^{13}C and 300 MHz for ^1H, equipped with a Bruker 4 mm-double tuned MAS probe.

(1) *^{13}C CP/MAS experiments*: In the ^{13}C CP/MAS experiments standard cross-polarization pulse sequence with standard phase cycling is used. The ^1H 90° pulse width is 3.7 μs, contact time is 2 ms, and the pulse delay is 5 s, respectively. The MAS frequency is 7 kHz. Spectra are accumulated 400–512 times to achieve a reasonable signal-to-noise ratio. Each of the samples with about 60–80 μl is used. The observed spectra are recorded at room temperature and the chemical shifts are calibrated by using the upfield peak (29.5 ppm relative to tetramethylsilane) of adamantane.

(2) *2D ^{13}C–^1H HETCOR experiments*: 2D ^{13}C–^1H HETCOR spectra are measured according to the method of van Rossum et al. [21]. The pulse sequence for the 2D ^{13}C–^1H HETCOR experiment is shown in Fig. 1. The sample volume is placed at the middle of an NMR rotor (with volume of about 25 μl) to improve the radio frequency (rf) homogeneity. The MAS rate is set to 13 kHz. Since the Hartmann–Hahn matching and corresponding efficiency of CP magnetization transfer is very sensitive to rf power instabilities at high MAS frequencies, ramped-amplitude CP (RAMP-CP) is used for protons to restore a broader matching profile. The proton rf field strength is set to 81 kHz during the t_1 delay for FSLG decoupling and during acquisition for two pulse phase-modulation (TPPM) decoupling. The phase modulation angle for the TPPM decoupling is set to 15.0°, and the flip-pulse length is optimized to 6.1 μs to yield optimal ^{13}C resolution. Two off-resonance pulses with opposite phases (i.e. $+x$, $-x$ or $+y$, $-y$) during the FSLG decoupling are set to 10.13 μs. The contact time is used to be 0.2, 0.5 and 1.5 ms. The magic angle pulse

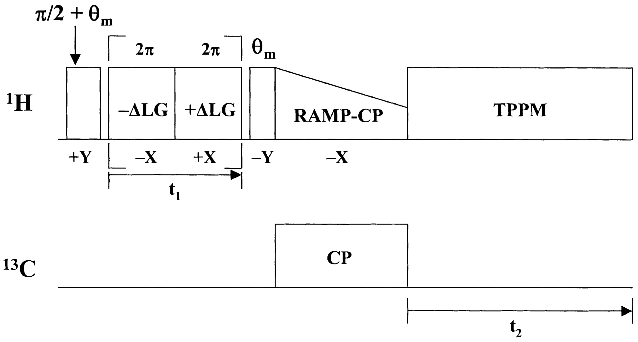

Fig. 1. The pulse sequence for measuring the 2D heteronuclear correlation NMR spectrum with frequency-switched Lee–Goldburg irradiation during the evolution.

length is 3.1 μs. The recycle delay is set to 5 s. Quadrature detection is achieved by using the states-TPPI method. All the data for 128 t_1 increments with 800 scans are collected.

2.3. Structural modeling of PG and PLV with the anti-parallel β-sheet conformation

The structural modeling of PG and PLV with the anti-parallel β-sheet form is carried out by the hybrid distance geometry-dynamical simulated annealing method [27] as contained in the X-PLOR 3.1 program [28]. For structural calculations, the proton–proton distance restrains and the torsion angle restrains ($\phi = -139°$ and $\psi = 135°$) are derived from reference data by Wüthrich et al. [29]. The distance restraints for hydrogen bonding between the amide nitrogen and the carbonyl oxygen atoms in the secondary structure used to be in the range of 2.7–3.3 Å [30–32]. A final set of 10 lowest-energy structures are selected from 30 calculated results, where the structures with distance and dihedral angle >0.5 Å and 5°, respectively, are omitted. The average coordinates of the ensembles of the preferable 10 structure candidates are subjected to 500 cycles of Powell restrained energy minimization to improve non-bonded contacts. The interatomic distances in anti-parallel β-sheet form for PG and PLV are determined by using the Insight II program.

3. Results and discussion

3.1. ^{13}C CP/MAS NMR spectral analysis and conformational characterization

The observed ^{13}C CP/MAS NMR spectra of PG, PLV and PG/PLV(50/50) blend samples are shown in Fig. 2(a)–(c). These spectral assignments in the spectra are made by using reference data as reported previously [33–35]. The obtained ^{13}C chemical shift values of these polypeptide samples are listed in Table 1. The observed spectrum for PG/PLV(50/50) blend sample is decomposed as a sum of Lorentzian signals by computer-fitting and then the fractions for the 3$_1$-helix and β-sheet forms in PGs and PLV are determined. In the ^{13}C CP/MAS spectrum, a new peak for the C=O carbon appears clearly at 169.5 ppm. This peak can be assigned to the C=O carbon of PG in the β-sheet form. Another new peak of the Cα carbon of PG appears at about 44.3 ppm in addition to a small peak assigned to the 3$_1$-helix form (42.6 ppm), and can be assigned to the β-sheet form (44.3 ppm). On the other hand, PG in the 3$_1$-helix form does not change into the β-sheet form by the TFA-alkaline treatment for preparing blend sample (Fig. 2(a)). This shows that the 3$_1$-helix form of PG in the PG/PLV blend is almost transformed to the β-sheet form.

Table 1
Observed solid state ^{13}C chemical shifts of PG, PLV and PG/PLV(50/50) blend sample

Polypeptide sample	^{13}C chemical shift (ppm)				Conformation
	C=O	Cα	Cβ	Cγ	
PG	172.9	42.6			3$_1$-Helix[a]
PG	169.0	44.3			β-Sheet[a]
PLV	172.3	58.6	32.9	19.0	β-Sheet[a]
PG[b]	172.9	42.9			PG 3$_1$-helix
PG/PLV(50/50)	–[c]	42.8			PG 3$_1$-helix
	169.5	44.3			PG β-sheet
	172.2	58.9	32.9	19.0	PLV β-sheet
PLV[b]	172.0	58.6	32.8		PLV β-sheet

[a] Refs. [33–35].

[b] Homopolypeptides of PG(3$_1$-helix) and PLV(β-sheet) are prepared using same condition as PG/PLV(50/50) blend sample.

[c] Not determined because of the overlap of the minor C=O (PG: 3$_1$-helix) peak with the major C=O (PLV) peak.

3.2. Two-dimensional ^{13}C–^{1}H HETCOR spectral analysis and structural characterization

2D ^{13}C–^{1}H HETCOR spectrum of PG/PLV(50/50) blend obtained by using the pulse sequence (Fig. 1) with a short contact time (0.2 ms) is shown in Fig. 3(a). The horizontal axis ($F2$) corresponds to the ^{13}C (chemical shift range: 3 to $+182$ ppm), and the vertical axis ($F1$) corresponds to the ^{1}H (chemical shift range: -1 to $+12$ ppm). One-dimensional (1D) ^{13}C CP/MAS spectrum for the horizontal (^{13}C) axis is shown at the top of this figure. In the spectrum, the intense correlations arise from the dipolar coupling between the carbons and their directly bonded protons. The corresponding signals for the Cα and Hα(Cα–Hα) dipolar coupling in PG(β-sheet) and for the Cα–Hα, Cβ–Hβ and Cγ–Hγ dipolar couplings in PLV appear. Further, the other weak correlation peaks are observed for the Cα–Hγ, Cβ–Hγ, Cα–NH and C=O–Hα dipolar couplings in PLV, and for the Cα–NH and C=O–Hα dipolar couplings in PG(β-sheet).

In the ^{1}H spectrum, peaks at 9.3 and 4.7 ppm can be assigned to the NH and Hα protons of PG in the β-sheet form, respectively, and further peaks at 10.3, 5.6, 2.3 and 1.1 ppm to the NH, Hα, Hβ and Hγ protons of PLV in the β-sheet form, respectively. The ^{1}H peaks for PG in the 3$_1$-helix form by the ^{13}C–^{1}H HETCOR method with contact time of 0.2 ms because the 3$_1$-helix form in PG is the minor component. The ^{13}C–^{1}H HETCOR spectrum with contact time of 0.5 ms (Fig. 3(b)) shows the correlation signal between the Cα carbon and the Hα proton of PG in the 3$_1$-helix form, and the ^{1}H chemical shift of the Hα proton is 4.3 ppm. Each of the ^{1}H signals is largely separated in chemical shift enough to analyze corresponding correlation signals.

Further, correlation signals for the C=O–NH, C=O–Hβ, C=O–Hγ and Cγ–Hα dipolar couplings in PLV, and for the C=O–NH dipolar coupling in PG with the β-sheet form

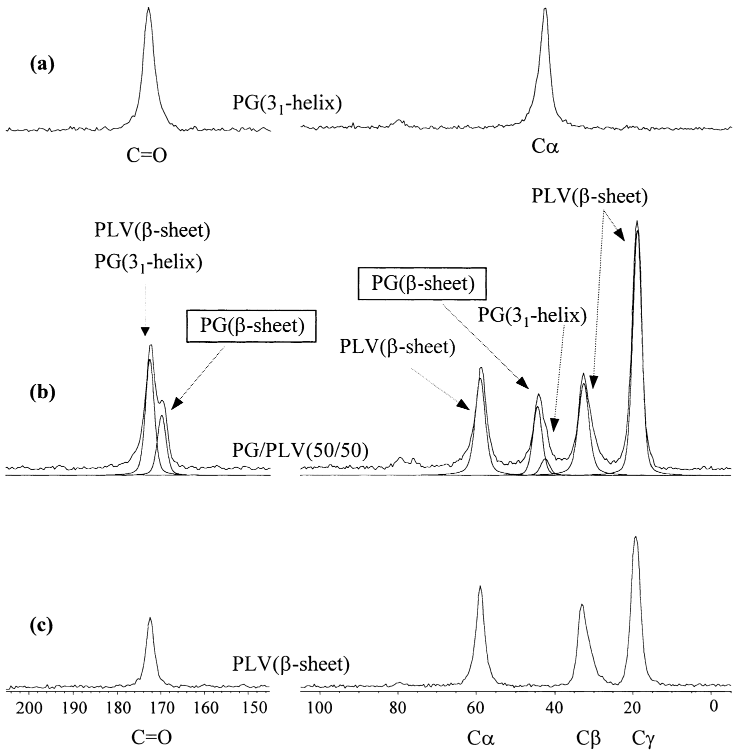

Fig. 2. Expanded ^{13}C CP/MAS NMR spectra for the carbonyl carbon region and for the Cα, Cβ and Cγ carbons region of PG, PLV and PG/PLV(50/50) blend sample. Homopolypeptides of PG(3_1-helix) and PLV(β-sheet) are prepared using same condition as PG/PLV(50/50) blend sample. (a) PG, (b) PG/PLV(50/50) and (c) PLV.

are observed. In addition, it is very significant to show that the intermolecular correlation signals for the PG(β-sheet)Cα–PLV(β-sheet)Hα, PG(β-sheet)Cα–PLV(β-sheet)NH, PG(β-sheet)C=O–PLV(β-sheet)Hα and PG(β-sheet)C=O–PLV(β-sheet)NH dipolar couplings are observed.

On the other hand, ^{13}C–^{1}H HETCOR spectrum with long contact time of 1.5 ms (Fig. 3(c)) shows a significant and new signal. The new correlation signal which appeared between the Cγ carbon of PLV(β-sheet) at 19 ppm and the Hα proton of PG(β-sheet) at 4.7 ppm is shown by an arrow in Fig. 3(c).

In order to get more detailed structural information, ^{1}H slice objections of the Hα (4.7 ppm) and NH (9.3 ppm) protons of PG(β-sheet) and of the Hα (5.7 ppm) and NH (10.3 ppm) protons of PLV(β-sheet) are shown in Fig. 4(a)–(d). From these spectra, intermolecular correlations are more clearly shown. For example, the slice spectra of the Hα proton for PG(β-sheet) show intermolecular correlations with the C=O, Cα and side chain carbons of PLV(β-sheet), and also the sliced spectra of the Hα proton for PLV(β-sheet) show intermolecular correlations with the C=O and Cα carbons of PG(β-sheet). Similarly, the sliced spectra of the NH proton for PG(β-sheet) show intermolecular correlation with the C=O, Cα and side chain carbons of PLV(β-sheet), and those of the NH proton of PLV(β-sheet) show intermolecular correlations with the C=O and Cα carbons of PG(β-sheet).

In order to clarify the relationship between cross-peaks and carbon–proton interatomic distances, molecular model for the anti-parallel β-sheet conformation that the PG and PLV have been calculated using reference data [29] by the X-PLOR 3.1 program, we measured carbon–proton distances from the modeling structure (Table 2). The distances between carbons and their directly bonded proton are ca. 1.1 Å and their signals can be observed in ^{13}C–^{1}H HETCOR spectrum with contact time of 0.2 ms. Further,

Table 2
The calculated carbon–proton distances of anti-parallel β-sheet for the PG and PLV

	Distances (Å)			
	PG–PG	PLV–PLV	PG–PLV	PLV–PG
Cα–Hα	1.08	1.08	3.09–3.30	3.17–3.34
Cα–Hβ	–	2.15	4.30–6.04	–
Cα–Hγ	–	2.53–3.39	3.32–6.65	–
Cα–HN	2.15	2.15	3.93–4.23	4.00–4.23
Cβ–Hα	–	2.15	–	3.90–6.54
Cβ–Hγ	–	2.15	–	–
Cβ–HN	–	2.89	–	4.12–5.04
Cγ–Hα	–	2.68–3.43	–	3.56–6.32
Cγ–Hβ	–	2.15	–	–
Cγ–HN	–	2.67–3.64	–	3.21–6.20
C=O–Hα	2.15	2.15	3.80–4.00	3.72–4.01
C=O–Hβ	–	2.62–2.72	4.34–6.70	–
C=O–Hγ	–	2.39–4.15	3.27–7.10	–
C=O–HN	1.98	1.98	3.17	3.17

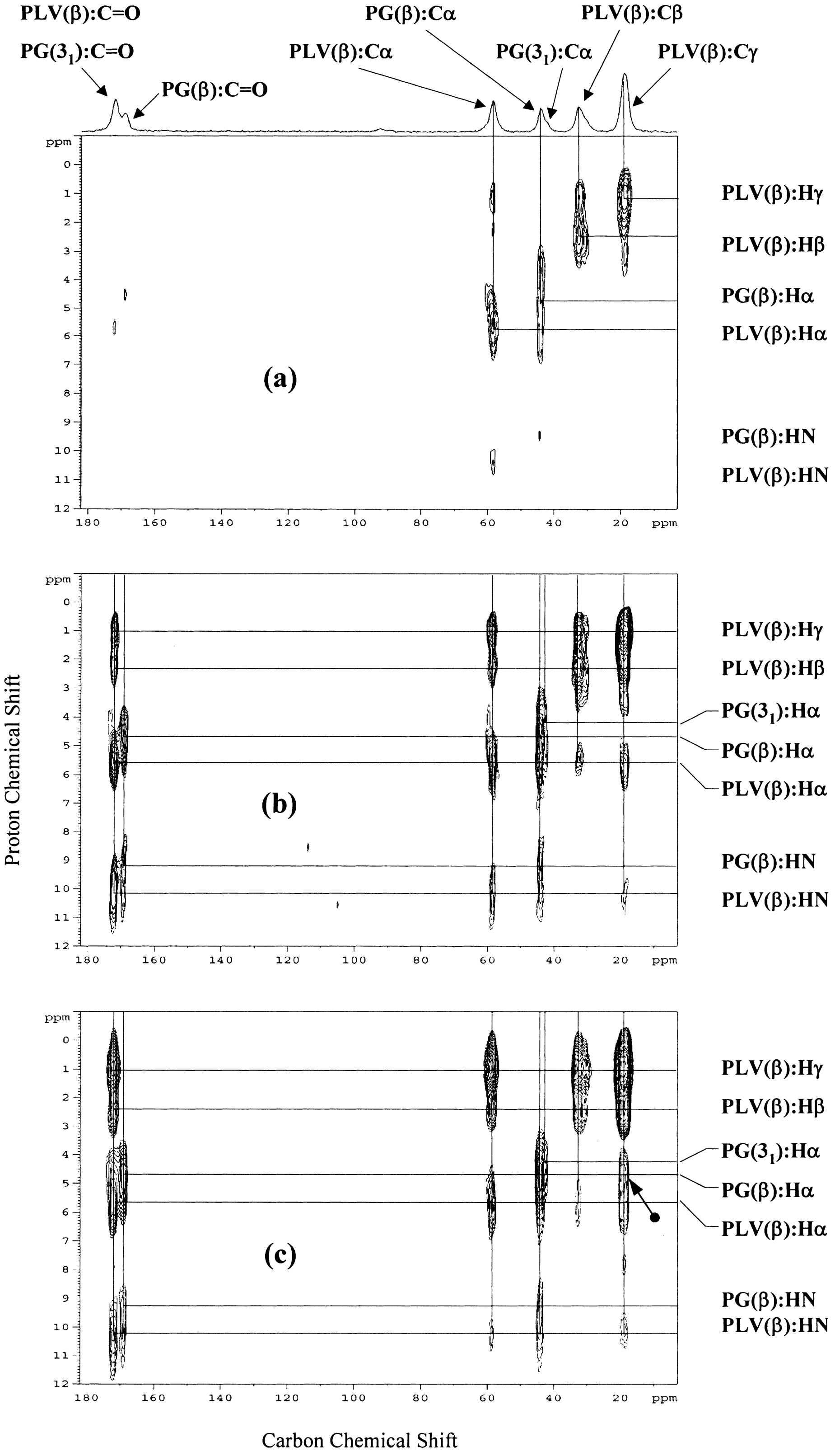

Fig. 3. The 2D FSLG $^{13}C-^{1}H$ HETCOR spectrum of PG/PLV(50/50) blend sample with contact time of (a) 0.2 ms, (b) 0.5 ms and (c) 1.5 ms.

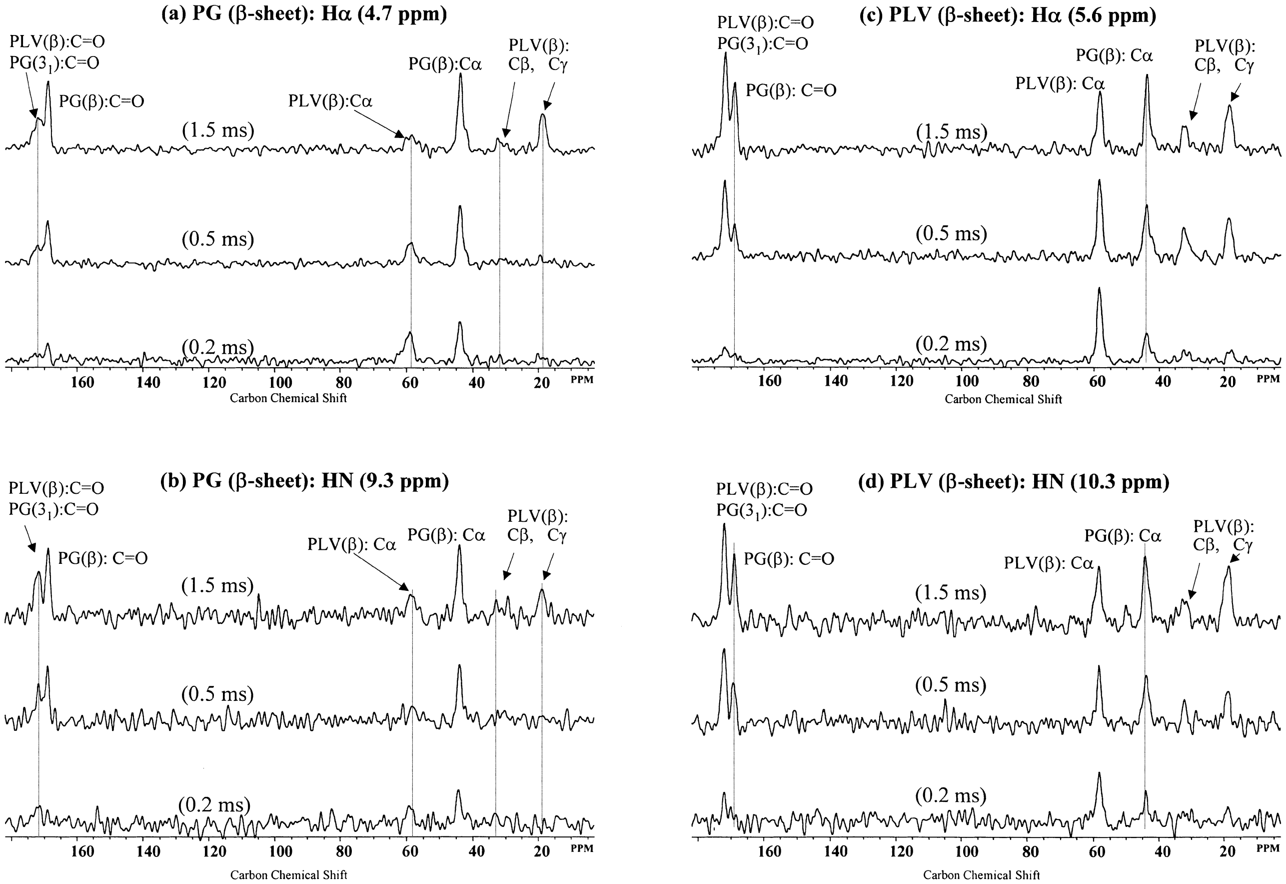

Fig. 4. 1D slices along the horizontal axis of the FSLG $^{13}C-^{1}H$ HETCOR spectrum taken at (a) 4.7 ppm (PG(β):Hα), (b) 9.3 ppm (PG(β):NH), (c) 5.6 ppm (PLV(β):Hα) and (d) 10.3 ppm (PLV(β):NH) on the ^{1}H chemical shift axis. The contact time used in this experiment: 1.5 ms (top), 0.5 ms (middle) and 0.2 ms (bottom).

in the $^{13}C-^{1}H$ HETCOR spectrum with a contact time of 0.5 ms the signals corresponding to another intramolecular carbon–proton distance less than 3 Å are observed.

The correlation signals between PG and PLV corresponding to intermolecular distance less than 4 Å are observed in the $^{13}C-^{1}H$ HETCOR spectra with a contact time of 0.5 ms. Further, the correlation signal between PG(Hα) and side chain of PLV(Cγ) are observed only in the $^{13}C-^{1}H$ HETCOR spectra with a long contact time of 1.5 ms. For the modeling structure, the intermolecular distance between PG(Hα) and side chain of PLV(Cγ) are obtained to be about 3.56–6.32 Å range.

These intermolecular correlation peaks mean that intermolecular CP occurs between the carbon and proton of intermolecular-interacting polypeptides in blend. There may be two pathways of the observed intermolecular CP. One is that a direct transfer from proton to carbon exists, and another is that a change in the magnetization by spin diffusion (homonuclear Hartmann–Hahn transfer) exists. It is thought that the former is much more efficient than the latter because the former comes from only one magnetization transfer process, but the latter comes from two magnetization transfer processes. Further, it can be said that as $^{13}C-^{1}H$ HETCOR pulse program is used in this experiment, the intermolecular correlations through strong heteronuclear dipolar couplings are mainly observed and thus the appearance of cross-peaks due to spin diffusion in the proton reservoir is avoided. From the above experimental results, it can be concluded that the backbones of the two kinds of PG(β-sheet) and PLV(β-sheet) are very close to each other and thus they are highly miscible at the molecular level. On the other hand, side chain ^{1}H protons of PLV(β-sheet), especially Hγ proton, have very weak intermolecular correlation with the carbons of PG(β-sheet). The molecular modeling shows that side chain protons are not so far from each other, as the distances of PLV(β-sheet)Hγ–PG(β-sheet)Cα and PLV(β-sheet)Hγ–PG(β-sheet)C=O are in 3.32–6.65 and 3.27–7.10 Å range, respectively. It is caused by rapid rotational motion of the methyl group of PLV.

The CP experiments on polymer blend systems, to be a mixture of two polymers in which one is deuterated and another is protonated, have been carried out in order to elucidate their miscibility by information whether protons in protonated polymer are cross-polarized to deuterons of another deuterated polymer or not [36–41]. These studies shows that effective $^{1}H-^{13}C$ CP transfer may be limited to about 10 Å. In the present FSLG $^{13}C-^{1}H$ HETCOR experiments, spin diffusion in protons can be avoided to a certain extent. From the above experimental results, it is found that PG with the β-sheet form and PLV with the β-sheet form are blended with each other on the scale below 10 Å.

In our previous work, the miscibility scale of PG/PLV has been shown to be about 3 nm by the ^{1}H $T_{1\rho}$ experiments [12,13]. These values are consistent with each other because the value of 2–3 nm is upper-limit of the domain size [20]. Thus, it can be said that the ^{13}C–^{1}H HETCOR experiments do not conflict with the ^{1}H $T_{1\rho}$ experiments reported previously. These results show that PG and PLV chains in the blend are very close to each other enough to make intermolecular hydrogen bonds.

Finally, from these experimental results, it is concluded that in the polypeptide blend as prepared by the TFA-alkaline treatment PG chains by changing from the 3_1-helix form to the β-sheet form are incorporated into PLV chains with the β-sheet form by forming hydrogen bonds with PLV. This means that PG chains in the β-sheet form hydrogen-bonded with PLV chains in the β-sheet form are energetically more stable than the 3_1-helix form of the PG chains hydrogen-bonded by themselves.

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