Polymer Journal, Vol. 35, No. 3, pp 293—296 (2003)

SHORT COMMUNICATIONS

Conformational Characterization of (Val-Pro-Gly-Val-Gly)$_6$ with ^{13}C Solid State NMR

Tetsuo ASAKURA,[*,†] Jun ASHIDA,[*,**] and Kosuke OHGO[*]

[*]*Department of Biotechnology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184–8588, Japan*
[**]*Varian Technologies Japan Ltd., Minato, Tokyo 108–0023, Japan*

(Received October 22, 2002; Accepted January 9, 2003)

KEY WORDS Elastin / (VPGVG)$_6$ / 2D Spin-Diffusion Nuclear Magnetic Resonance(NMR) under off Magic Angle Spinning /

The repeated pentapeptide sequence, (Val-Pro-Gly-Val-Gly)$_n$, has been considered to be an essential repeated sequence for producing the elastic character of elastin.[1] The previous structural studies have been concentrated to clarify whether the unit, Pro-Gly, in the sequence takes β-turn structure.[2–4] However, there are very limited structural information on the other parts of the pentapeptide sequence. By employing the mathematical methods of deriving a linear helical correlation structures from a conformation of *cyclo*-(Val^1-Pro^2-Gly^3-Val^4-Gly^5)$_3$ obtained by X-Ray diffraction analysis,[3] Urry's group reported the torsion angles as Val^1(ϕ, ψ) = (−120°, 100°), Val^4(ϕ, ψ) = (−120°, 80°) and Gly^5(ϕ, ψ) = (−150°, −130°) together with the Pro-Gly unit; (Pro^2(ϕ, ψ) = (−60°, 120°) and Gly^3(ϕ, ψ) = (120°, −40°)). However, in general, *cyclo*-peptides take limited conformations because of the presence of many constraints such as steric constraints and hydrogen bonding formation as well as "cyclic" without the end groups. The extension of the information on the structures of *cyclo*-peptides to linear peptide with similar sequence should be performed very carefully. Thus, it is better to obtain the information on the torsion angles directly for the linear pentapeptide sequence in the solid state if it is possible.

In our previous papers,[5–7] ^{13}C solid state NMR such as especially 2D spin-diffusion solid state NMR under off magic angle spinning (OMAS) coupled with ^{13}C isotope double labeling of specific residues has been successfully used to determine the torsion angles of the backbone amino acid residues for the silk model peptides, (Ala-Gly)$_{15}$ and (Ala-Gly-Gly)$_{10}$ in the several solid state forms. Especially, a new structural model of (Ala-Gly)$_{15}$ as a model of *Bombyx mori* silk fibroin before spinning could be proposed.[5]

In this paper, we synthesized four kinds of the repeated pentapeptide sequences, (VPGVG)$_6$, (VPGVG)$_2$VP[1-^{13}C]G$_{13}$[1-^{13}C]V$_{14}$G(VPGVG)$_3$, (VPGVG)$_2$VPG[1-^{13}C]V$_{14}$[1-^{13}C]G$_{15}$(VPGVG)$_3$, (VPGVG)$_2$VPGV[1-^{13}C]G$_{15}$[1-^{13}C]V$_{16}$PGVG(VPGVG)$_2$ in order to obtain the information on the torsion angles of the backbone of two Val residues (Val$_{14}$ and Val$_{16}$) and one Gly residue (Gly$_{15}$) using both 2D spin-diffusion NMR under OMAS. Especially, the local conformation of the Gly$_{15}$ residue was analyzed in detail from the comparison of the calculated and observed 2D spin-diffusion NMR spectra.

EXPERIMENTAL

The ^{13}C-labeled and un-labeled peptides mentioned above were synthesized by the solid-phase method. The samples are purified by HPLC with water and are freeze-dried. The ^{13}C CP/MAS NMR measurements were performed on a Chemagnetics Infinity 400 MHz spectrometer. A ^{1}H $\pi/2$ pulse of duration 3.0–6.0 μs and a contact time of 1 ms was used. The 2D spin-diffusion NMR spectra were obtained with Varian ^{Unity}*INOVA* 400 NMR spectrometer and 7 mm Jakobsen-type double-tuned MAS probe at off magic angle condition (θ_m + 9°) and sample spinning of 6 kHz at room temperature. The scaling factor of the 2D spin-diffusion spectra were $1/2\,(3\cos^2(\theta_m + 9°) - 1) = -0.206$. The mixing time of 2 s was optimized for spin diffusion between intramolecular specific carbon atoms of selectively isotope-labeled Val and Gly residues; however, for no spin-diffusion between intermolecular carbon atoms.[5–7] The contact time was set to 2 ms using the Variable-Amplitude CP technique.[8] About 400 scans with a number of t_1 points of 150 and a recy-

[†]To whom correspondence should be addressed (Tel & Fax: +81–42–383–7733, E-mail: asakura@cc.tuat.ac.jp).

cle delay of 2 s were accumulated for every 2D experiment. Here, the repetition time of 2 s was carefully determined from the obtained 1D OMAS spectra under several repetition times. The intensity of OMAS spectra of repetition time of 2 s was the same as those of 4 s and of 8 s; however; that of 1 s was a little smaller. The principal values of the chemical shift tensors for the carbonyl carbon atoms of the isotope-labeled Val and Gly residues were determined with the 1D OMAS spectra.[5] The square deviation, χ^2, was introduced to demonstrate the difference between the observed and calculated spectra quantitatively.[7] The definition of the χ^2 was

$$\chi^2(\phi, \psi) = \frac{1}{\sigma^2} \sum_{i=1}^{N} [E_i - S_i(\phi, \psi)]^2$$

where σ is the root-mean-squared noise in the experimental spectrum, N is the number of intensities analyzed, E_i are the observed intensities and $S_i(\phi,\psi)$ are the calculated intensities.

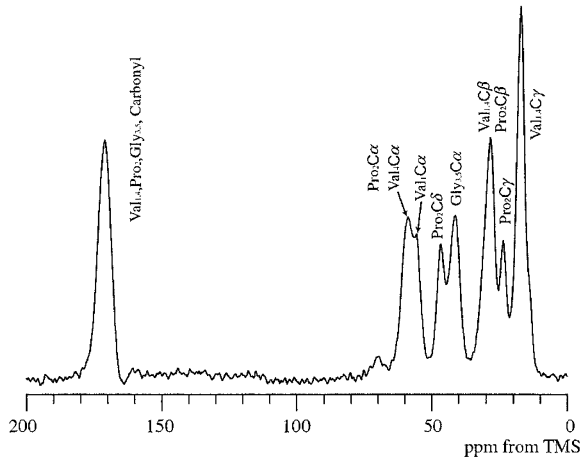

Figure 1. ^{13}C CP/MAS NMR spectrum of $(VPGVG)_6$ together with the assignment.

RESULTS AND DISCUSSION

Figure 1 shows the ^{13}C CP/MAS NMR spectrum of $(V^1P^2G^3V^4G^5)_6$ after lyophilization together with the assignment. This spectrum is essentially the same as that of longer polypeptide, $[(VPGVG)_4(VPGKG)]_{39}$.[9] Thus, the number of the repeated pentapeptide sequence, 6, of $(VPGVG)_6$ seems enough in analyzing the local structure of elastin.

The observed 2D spin-diffusion NMR spectra under OMAS of $(VPGVG)_2VP[1\text{-}^{13}C]G_{13}[1\text{-}^{13}C]V_{14}G(VPGVG)_3$, $(VPGVG)_2VPG[1\text{-}^{13}C]V_{14}[1\text{-}^{13}C]G_{15}(VPGVG)_3$, $(VPGVG)_2VPGV[1\text{-}^{13}C]G_{15}[1\text{-}^{13}C]V_{16}PGVG(VPGVG)_2$ are shown in Figure 2a, 2b, and 2c, respectively. Here, these spectra contain information on the torsion angles, ϕ and ψ or Val_{14}, Gly_{15}, and Val_{16}

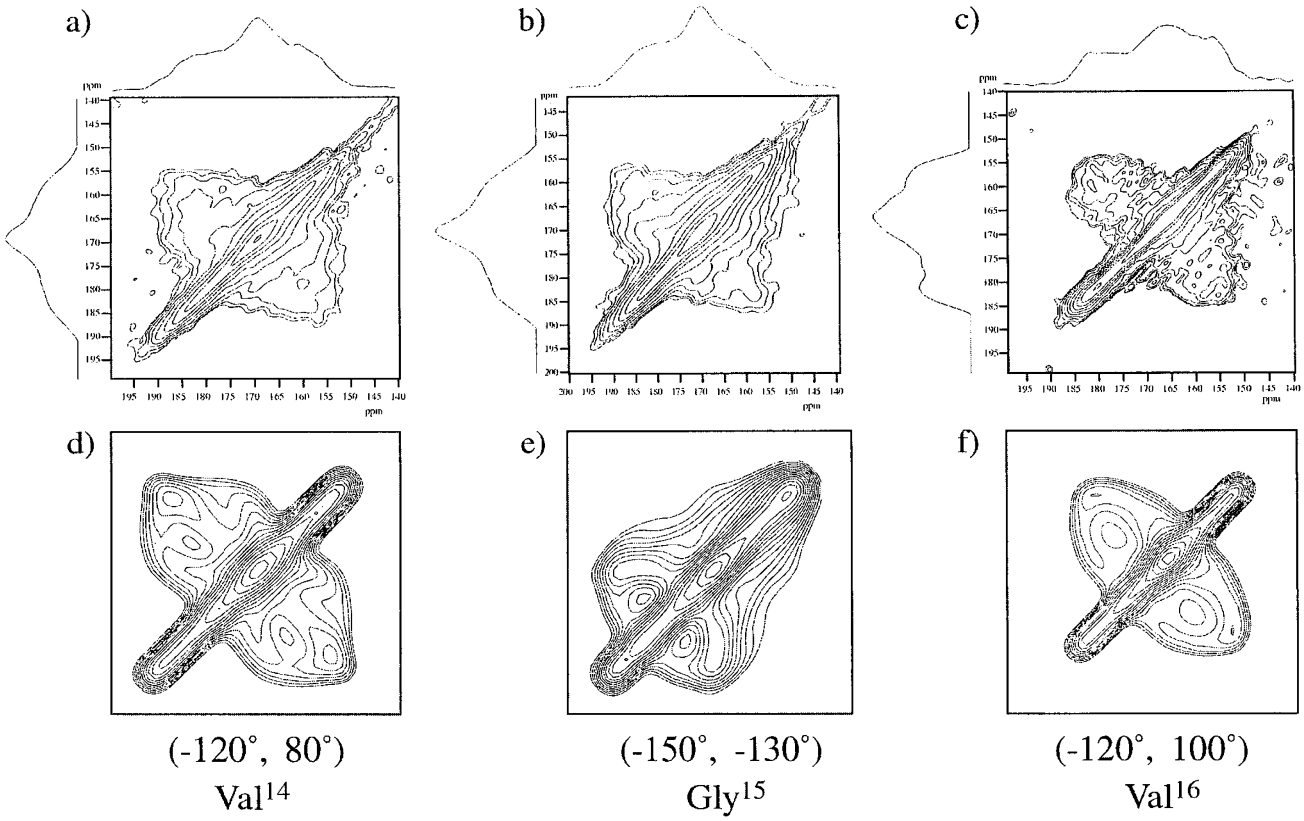

Figure 2. The observed 2D spin-diffusion NMR spectra of a) $(VPGVG)_2VP[1\text{-}^{13}C]G_{13}[1\text{-}^{13}C]V_{14}G(VPGVG)_3$, b) $(VPGVG)_2VPG[1\text{-}^{13}C]V_{14}[1\text{-}^{13}C]G_{15}(VPGVG)_3$, and c) $(VPGVG)_2VPGV[1\text{-}^{13}C]G_{16}[1\text{-}^{13}C]V_{17}PGVG(VPGVG)_2$. The calculated spectra are also shown by assuming d) $Val_{14}(\phi, \psi) = (-120°, 80°)$, e) $Gly_{15}(\phi, \psi) = (-150°, -130°)$, and f) $Val_{16}(\phi, \psi) = (-120°, 100°)$. The agreement is poor.

residues, respectively. The spectra calculated with previous torsion angles reported from X-Ray diffraction analysis of *cyclo*-(V^{1}P^{2}G^{3}V^{4}G^5)$_3$;[3] (Val^4(ϕ, ψ) = (−120°, 80°), Gly^5(ϕ, ψ) = (−150°, −130°) and Val^1(ϕ, ψ) = (−120°, 100°) in V^{1}P^{2}G^{3}V^{4}G^5 unit are also shown in Figure 2d, 2e, and 2f. For the calculations of the 2D spin-diffusion spectra, the chemical shift tensor values of Val and Gly carbonyl carbon atoms obtained from the 1D OMAS powder patterns were used. The agreement between the observed and calculated spectra is poor, indicating that the torsion angles of these three residues, Val$_{14}$, Gly$_{15}$, and Val$_{16}$, especially the Gly residue, are different from the reported corresponding angles for *cyclo*-(V^{1}P^{2}G^{3}V^{4}G^5)$_3$.

In order to examine the torsion angles of the Gly residue, the 2D spin diffusion spectra are calculated as a function of ϕ and ψ for each 20°. The results are shown in Figure 3. The patterns are the same between (ϕ, ψ) and (−ϕ, −ψ) and therefore only the area, −180° < ϕ < 0° and −180° < ψ < 180°, are shown. For further analysis, Ramachandran map of χ^2 defined in Experimental section was prepared and shown in Figure 4. The χ^2 deviation between the observed and calculated spectra was more than 25% for the torsion angles (ϕ,ψ) = (−150°, −130°) reported previously[3] when the smallest χ^2 deviation at (ϕ, ψ) = (−80°, −20°) was assumed to be 0%. Thus, the Gly^5 residue does not adopt the torsion angle (ϕ,ψ) = (−150°, −130°).

The value of χ^2 deviation is mostly the same among (ϕ, ψ) = (−80°, −20°), (−20°, −80°) (−80°, 120°), and (−120°, 80°). However, the agreement between the observed and calculated spectra seems still poor even for these four cases. Thus, further calculation is required. The sample, (VPGVG)$_6$ is soluble in water and purified from the aqueous solution. We reported recently the conformational distribution map of Ac-Gly-NHMe molecule in aqueous solution calculated with molecular dynamics method as shown in Figure 5.[10] The

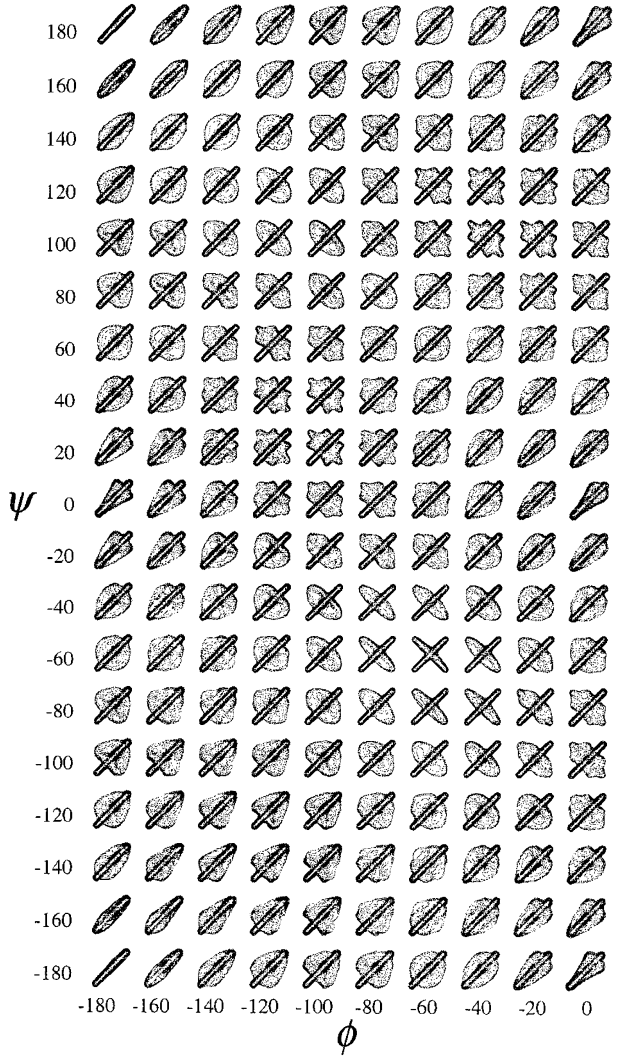

Figure 3. The 2D spin-diffusion NMR spectra calculated for Gly residue as a function of (ϕ, ψ) for each 20°. The patterns are the same between (ϕ, ψ) and (−ϕ, −ψ) and therefore only the area, −180° < ϕ < 0° and −180° < ψ < 180°, are shown.

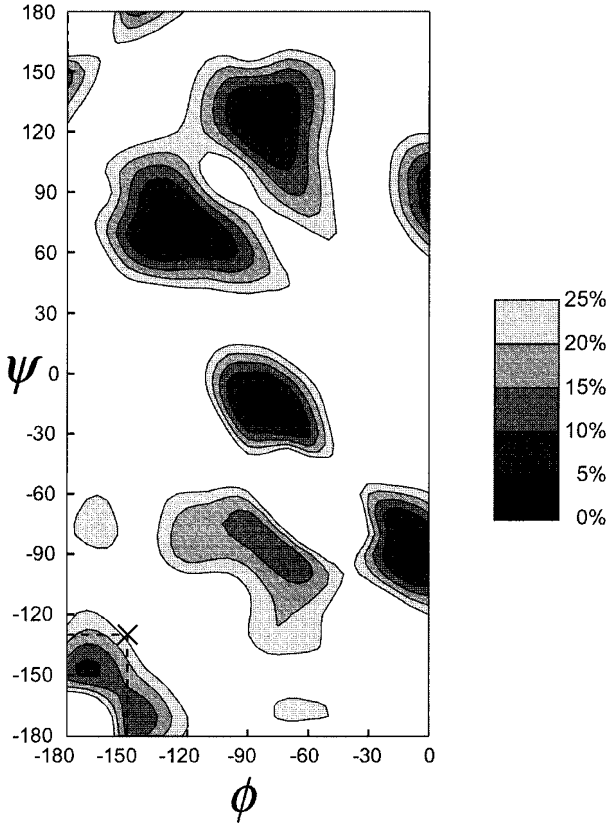

Figure 4. Contour plot of χ^2 deviation for 2D spin diffusion spectrum of (VPGVG)$_2$VPG[1-^{13}C]V$_{14}$[1-^{13}C]G$_{15}$(VPGVG)$_3$. Here $\chi^2(\phi, \psi) = \frac{1}{\sigma^2}\sum_{i=1}^{N}[E_i - S_i(\phi, \psi)]^2$where σ is the root-mean-squared noise in the experimental spectrum, N is the number of intensities analyzed, E_i are the experimental intensities, $S_i(\phi,\psi)$ are the calculated intensities. The smallest χ^2 deviation at (ϕ, ψ) = (−80°, −20°) was assumed to be 0%. X indicates the torsion angles (ϕ,ψ = −150°, −130°) reported previously from the X-Ray diffraction analysis of *cyclo*-(V^{1}P^{2}G^{3}V^{4}G^5)$_3$.[3]

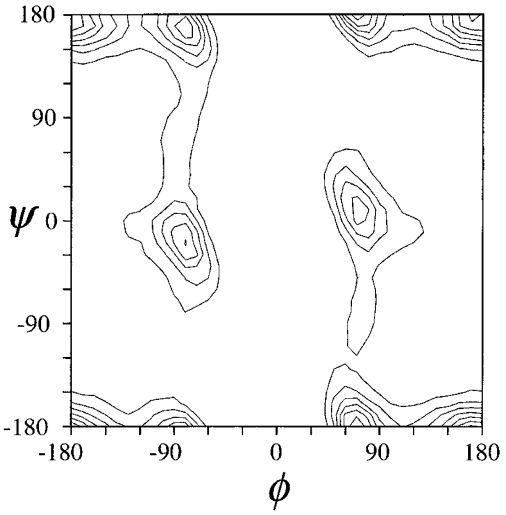

Figure 5. The conformational distribution map of Ac-Gly-NHMe as a function of the torsion angles of ϕ and ψ in aqueous solution reported previously.[10]

conformational character of the Gly residues in $(Ala\text{-}Gly)_{15}$ with silk I form (The silk structure before spinning in the solid state) could be interpreted well with this map. Therefore the spectrum was calculated again with both Figures 3 and 5 by considering the weight averaged distribution of each conformation. The calculated spectrum is shown in Figure 6b together with the observed spectrum Figure 6a. Although there is still small discrepancy, the agreement seems much improved. Actually, the χ^2 deviation between the experimental and simulated spectra decreased by 6.7% than the case of $(\phi, \psi) = (-80°, -20°)$. Thus, the averaged conformation (Figure 5) will be possible conformation of the Gly^5 residue. We are now doing a similar simulation for other residues, two Val residues. Thus, it seems incorrect to apply the torsion angles obtained from the cyclic elastin model peptides to those for the linear peptides. Rodriguez-Cabello *et al*. studied the structure of poly(GVGVP) with thermal, wide-angle X-Ray diffraction and vibrational Raman analysis.[11] The structure was amorphous with no definite conformation.

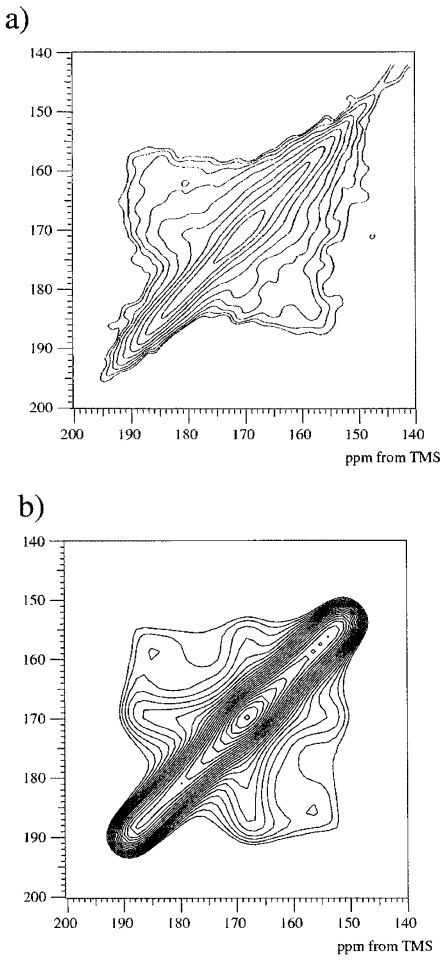

Figure 6. The observed a) and calculated b) spin-diffusion NMR spectra of Gly residue where the calculation was performed with both Figures 3 and 5. The observed 2D spin-diffusion NMR spectrum is for the Gly_{15} residue of $(VPGVG)_2VPG[1\text{-}^{13}C]V_{14}[1\text{-}^{13}C]G_{15}(VPGVG)_3$.

REFERENCES

1. D. W. Urry, *J. Phys. Chem., B Mater surf. interfaces biophys*, **101**, 11007 (1997).
2. M. Hong, R. A. McMillan, and V. P. Conticello, *J. Biomol. NMR*, **22**, 175 (2002).
3. C. M. Venkatachalam and D. W. Urry, *Macromolecules*, **14**, 1225 (1981).
4. H. Reiersen, A. R. Clarke, and A. R. Rees, *J. Mol. Biol.*, **283**, 255 (1998).
5. T. Asakura, J. Ashida, T. Yamane, T. Kameda, Y. Nakazawa, K. Ohgo, and K. Komatsu, *J. Mol. Biol.*, **306**, 291 (2001).
6. J. Ashida, K. Ohgo, and T. Asakura, *J. Phys. Chem. B Mater surf. interfaces biophys*, **106**, 9434 (2002).
7. J. Ashida, K. Ohgo, K. Komatsu, A. Kubota, and T. Asakura, *J. Biomol. NMR*, **25**, 91 (2003).
8. O. Peersen, X. Wu, I. Kustanovich, and S. O. Smith, *J. Magn. Reson. Ser. A*, **104**, 334 (1993).
9. R. A. McMillan and V. P. Conticello, *Macromolecules*, **33**, 4809 (2000).
10. T. Yamane, K. Umemura, and T. Asakura, *Macromolecules*, **35**, 8831 (2002).
11. J. C. Rodriguez-Cabello, M. Alonso, M. I. Diez, M. I. Caballero, and M. M. Herguedas, *Macromol. Chem. Phys.*, **200**, 1831 (1999).