Available online at www.sciencedirect.com
ScienceDirect
Journal of Magnetic Resonance 190 (2008) 327–332
JMR Journal of Magnetic Resonance
www.elsevier.com/locate/jmr

Communication

High field ^{17}O solid-state NMR study of alanine tripeptides

Kazuo Yamauchi [a], Michi Okonogi [a], Hiromichi Kurosu [b], Masataka Tansho [c], Tadashi Shimizu [c], Terry Gullion [d], Tetsuo Asakura [a,*]

[a] Department of Biotechnology, Tokyo University of Agriculture and Technology, Naka-cho 2-24-16, Koganei-shi, Tokyo 184-8588, Japan
[b] School of Natural Science and Ecological Awareness, Nara Women's University, Kita-Uoya Nishimachi, Nara-shi, Nara 630-8506, Japan
[c] National Institute for Materials Science, 3-13, Sakura, Tsukuba, Ibaraki 305-0003, Japan
[d] Department of Chemistry, West Virginia University, Morgantown, WV 26505, USA

Received 27 March 2007; revised 5 November 2007
Available online 9 November 2007

Abstract

^{17}O chemical shifts of Ala-Ala-Ala, with parallel and anti-parallel β-sheet structures, are observed using a 930-MHz high-resolution solid-state NMR spectrometer. Ala-Ala-Ala serves as a model sheet-forming peptide because it can be easily prepared as either a parallel or an anti-parallel β-sheet structure. Spectral differences between the two samples are observed which arise from molecular packing differences between the two sheet structures. DFT chemical shift calculations are performed for the two samples, and the calculated spectra are in good agreement with the experimental spectra. The DFT calculations provide insight into the nature of the chemical shift differences observed between the two sheet structures.

Keywords: Anti-parallel β-sheet; Parallel β-sheet; ^{17}O chemical shift; Ala-Ala-Ala; DFT

1. Introduction

Solid-state NMR is a useful tool for structural characterization of natural protein fibers with mixed parallel and anti-parallel β-sheet structures. We showed previously [1] that ^{13}C chemical shifts and ^{13}C spin–lattice relaxation times can be used to distinguish between Ala-Ala-Ala peptides prepared as pure parallel or anti-parallel β-sheet structures [2,3]. Both structures have two non-equivalent A and B molecules in the unit cell, and the ^{13}C chemical shifts for ^{13}C nuclei at the same carbonyl positions on the A and B molecules differ considerably. These carbonyl groups are part of the hydrogen bonding network.

Oxygen-17 nuclei (^{17}O) are seldom used in solid-state NMR because of the low sensitivity (natural abundance is 0.037%) and complicated line structure caused by the electric quadrupolar moment. The combination of ^{17}O-labeled samples with higher magnetic fields has minimized these problems, and applications of ^{17}O solid-state NMR of amino acids, peptides and polypeptides with magic angle spinning (MAS) have progressed steadily over the past decade [4–7]. In recent years detailed studies of chemical shifts and quadrupolar tensors have been investigated to understand the NMR parameters of hydrogen bonded structures and how they can be applied to determine hydrogen bond lengths and bond angles [8–11]. In addition, dipolar recoupling experiments have been performed to determine the carbon–oxygen distance of hydrogen bonded systems [12–14]. The unique secondary structures of peptides are stabilized by multiple hydrogen bonds, and oxygen atoms serve as proton acceptors in hydrogen bonds. Therefore, ^{17}O NMR studies are useful and important for characterizing hydrogen bonding and secondary structures of peptides.

An ^{17}O NMR study of Ala-[^{17}O]Ala-Ala having parallel and anti-parallel β-sheet structures is reported here, and it is shown that the ^{17}O NMR parameters can be calculated for both structures using density functional theory (DFT). The use of a 126 MHz ^{17}O NMR (930 MHz for ^{1}H) spectrometer and 90% enriched $H_2{}^{17}O$ as a starting

* Corresponding author. Fax: +81 42 383 7733.
E-mail address: asakura@cc.tuat.ac.jp (T. Asakura).

1090-7807/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.jmr.2007.11.006

material for synthesis of Ala-[^{17}O]Ala-Ala provides a way to easily observe differences in the ^{17}O NMR spectra between the two crystalline forms. The good agreement between the DFT calculated and the observed ^{17}O spectra enables us to discuss the origin of the experimental differences in detail.

2. Experimental methods

[^{17}O]Ala was prepared from the alanine methyl ester in Na^{17}OH/methanol solution, where Na^{17}OH was prepared by reacting $H_2{}^{17}O$ (^{17}O, 90%) with Na metal. The [^{17}O]Ala was 9-fluorenylmethyl-oxycarbonyl (Fmoc) protected using Fmoc hydroxyl-succinimide (Fmoc-OSu) in 10% $NaHCO_3$. Ala-[^{17}O]Ala-Ala was manually synthesized starting with Fmoc-Ala-Alko Resin. Fmoc removal was achieved with 20% piperidine solution in dimethyl formamide. *O*-(7-azabenzo-triazolyl)-1,1,3,3-tetramethyluronium hexafluoro-phosphate/diiso-propyl-ethylamine (3 equiv) was used for the coupling reagent. At the end of synthesis the peptide was cleaved from the resin using a solution of 2% triisoproplysilane and 12% thioanisole in trifluoroacetic acid. The product was precipitated from a solution of diethyl ether and purified by reverse-phase HPLC using a water/acetonitrile gradient and a PEGASIL ODS-II C18 column. Samples were prepared as parallel and anti-parallel β-sheets according to previously described recipes [2,3], and the structures of the prepared samples were checked by IR [2,3] and ^{13}C CP/MAS NMR [1]. ^{17}O NMR spectra were acquired with a JEOL ECA930 (21.8 T magnet; ^{1}H frequency is 930 MHz and ^{17}O frequency is 126 MHz) equipped with a 4 mm MAS ^{17}O–^{1}H dual-tuned probe head. A single 10° ^{17}O pulse (relative to the liquid state) and a 50 kHz RF proton decoupling field were used to acquire the data. Approximately 2 million scans were accumulated with a 50-ms repetition time. The MAS spinning speed was 20 kHz and the chemical shifts are reported relative to ^{17}O-labeled water. The spectra were processed with backward linear prediction to recover the first points of the FID; no line broadening factors were applied, but zero filling of the data sets was applied to double the points in the spectra.

2.1. DFT calculation

The Gaussian 03 Rev.D.02 program package was used to perform MO calculations of ^{17}O nuclear shieldings based upon density functional theory (DFT). The B3LYP exchange-correlation functional was employed and the 6-311G(d,p) basis set was used. Atomic coordinates obtained from X-ray diffraction experiments were used as input for the two β-sheet structure calculations [2,3]. Six molecules (3×2) were used for the parallel β-sheet calculation, and eight molecules (4×2) were used for the anti-parallel β-sheet calculation (see Fig. 1). Since the X-ray diffraction data do not give the positions of the hydrogen atoms, their positions were calculated using N–H and C–H distance constraints of 1 and 1.09 Å, respectively. The ^{17}O chemical shifts and calculated results are reasonably interpreted with these positions of hydrogen atoms.

3. Results and discussion

Fig. 2 shows the ^{17}O NMR spectra of Ala-[^{17}O]Ala-Ala with the parallel and anti-parallel β-sheet structures. The ^{17}O spectra are clearly different for the two samples. Since

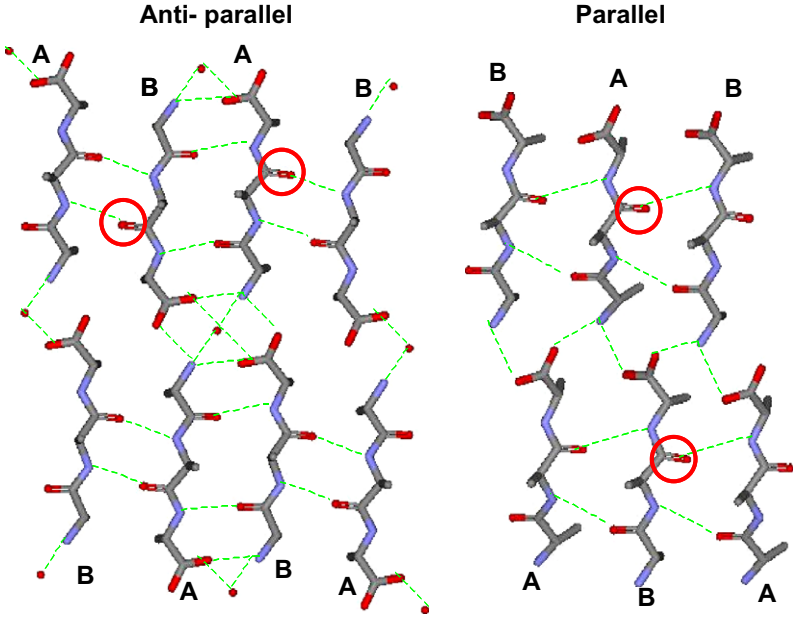

Fig. 1. Molecular structures of the parallel β-sheet and anti-parallel β-sheet samples. Each crystal has two unique molecules, A and B, in the unit cell. The chemical shift DFT calculations are performed with these sets of molecules, and the parameters provided in Table 2 are for the molecules with circles in the figure.

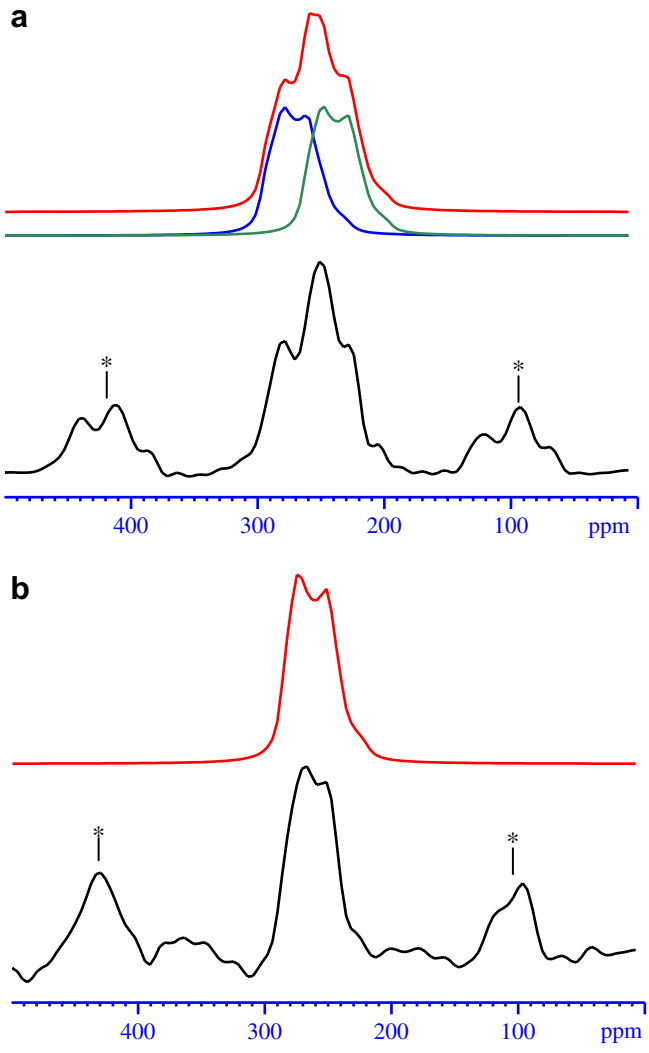

Fig. 2. ^{17}O MAS NMR spectra of Ala-[^{17}O]Ala-Ala using a 21.8-T magnet. (a) anti-parallel β-sheet structure and (b) parallel β-sheet structure. An '*' marks a spinning sideband caused by the sample rotation (20 kHz spinning rate). The black lines are the observed spectra and the red lines are fits to the spectra obtained with the dmFIT program. The spectrum for the parallel β-sheet can be fitted by assuming a single ^{17}O chemical environment; whereas, the spectrum for the anti-parallel β-sheet requires two ^{17}O different chemical environments (blue and green spectra). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

the 21.8 T spectrometer provides excellent spectral sensitivity and resolution, it is possible to discuss the observed differences between the ^{17}O NMR spectra for the two sheet structures. Fig. 2 also shows fits to the spectra generated with the dmFIT line-simulation program [15]. The fitting parameters are listed in Table 1. As shown in Fig. 1 and summarized in Table 1, the fit to the spectrum of the anti-parallel structure requires two chemically distinct ^{17}O sites while the fit to the spectrum of the parallel structure requires a single ^{17}O environment. X-ray crystallography data shows there are two non-equivalent coordination molecules, A and B, in both anti-parallel and parallel Ala-Ala-Ala. Consequently, both samples could have ^{17}O spectra

Table 1
Observed ^{17}O chemical shift (ppm), quadrupolar coupling constant (MHz) and asymmetry parameters of the Ala-[^{17}O]Ala-Ala

	σ_{iso}	χ_Q	η
Anti-parallel	302 ± 5	8.7 ± 0.5	0.40 ± 0.1
	270 ± 5	8.7 ± 0.5	0.35 ± 0.1
Parallel	293 ± 5	8.7 ± 0.5	0.30 ± 0.1
	293 ± 5	8.7 ± 0.5	0.30 ± 0.1

with contributions from two magnetically distinct ^{17}O sites. The observed differences between the two experimental ^{17}O NMR spectra were examined with the assistance of DFT calculations.

Calculated spectra were obtained with the Gaussian 03 program using the GIAO-CHF method and B3LYP(DFT)/6-311G(d,p) basis set [16]. The calculated ^{17}O nuclear shielding tensor components, quadrupolar coupling constants, χ_Q, electric field gradient (EFG) tensor components, and quadrupolar asymmetry parameters, η, are summarized in Table 2. The DFT calculated ^{17}O spectra (not shown) reproduce the features of the experimental spectra. The assignment of the observed ^{17}O resonances to the A and B molecules can be easily done using results from the DFT calculations. The high-field and low-field peaks for the anti-parallel β-sheet sample are assigned to the ^{17}O nuclei on the A and B molecules, respectively. Both the DFT calculation and experimental results show the same tendency; that is, the ^{17}O chemical shift difference between ^{17}O labels on the A and B molecules is larger for the anti-parallel β-sheet sample than for the parallel β-sheet sample.

The geometric parameters [2,3] of the oxygen atoms in the A and B molecules for the two tri-peptides are summarized in Fig. 3. Previous workers [17] suggested a good correlation between the direct hydrogen bond length and isotropic chemical shift of ^{17}O nuclei. The direct hydrogen bond lengths $R_{N\cdots O}$ for the ^{17}O labels (the ^{17}O labels on the central Ala residues) are $R_{N(B)\cdots O(A)} = 2.915$ Å and $R_{N(A)\cdots O(B)} = 2.919$ Å for the anti-parallel β-sheet sample, and $R_{N(B)\cdots O(A)} = 3.350$ Å and $R_{N(A)\cdots O(B)} = 3.201$ Å for the parallel β-sheet sample [2,3]. The difference in the direct hydrogen bond length, $\Delta_R = |R_{N(B)\cdots O(A)} - R_{N(A)\cdots O(B)}|$, is very small for the anti-parallel sample ($\Delta_R = 0.004$ Å) and quite large for the parallel sample ($\Delta_R = 0.149$ Å), but the

Table 2
Calculated ^{17}O nuclear shielding (ppm), their tensor components (ppm), quadrupolar coupling constants (MHz), and asymmetry parameters of the Ala-[^{17}O]Ala-Ala

Molecule	σ_{iso}	σ_{11}	σ_{22}	σ_{33}	χ_Q	η
Anti-parallel (A)	33.6	−168.5	−68.8	338.2	8.53	0.476
Anti-parallel (B)	2.3	−229.5	−102.8	339.3	8.84	0.388
Δ(A–B)	31.3	61.0	33.9	−1.1	−0.31	0.088
Parallel (A)	−9.8	−266.7	−105.3	342.7	8.58	0.301
Parallel (B)	−13.5	−265.4	−122.3	347.0	8.91	0.304
Δ(A–B)	3.8	−1.4	17.0	−4.4	−0.33	−0.003

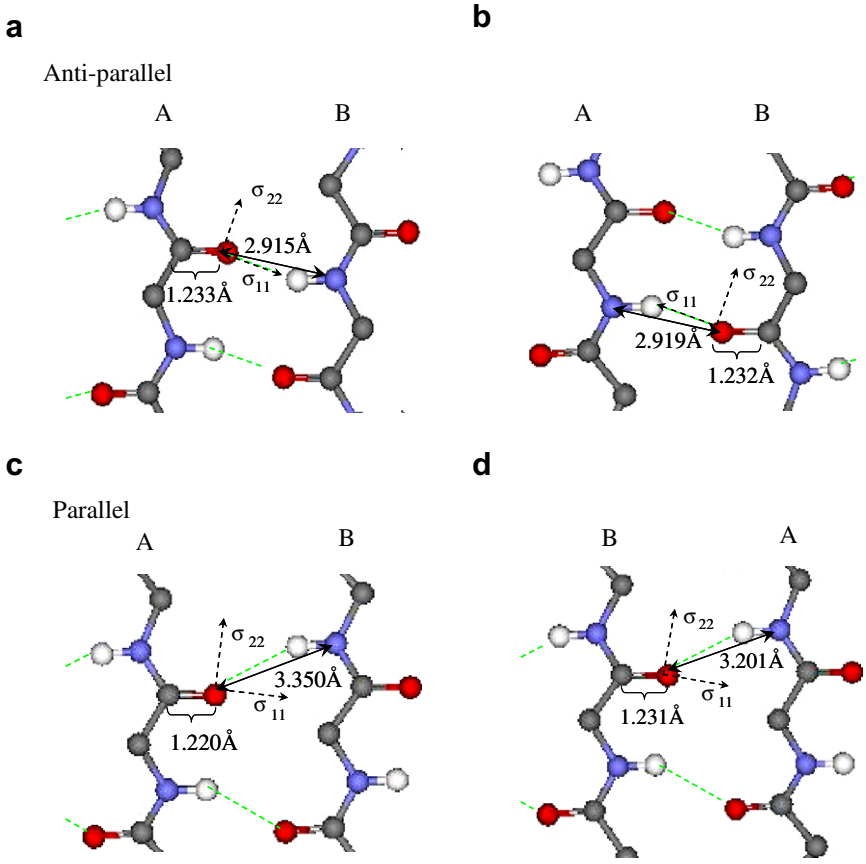

Fig. 3. Direct hydrogen bond lengths, $R_{N\cdots O}$, and C=O bond lengths determined with X-ray crystallography and the directions of DFT-calculated chemical shift tensors of the ^{17}O atoms for Ala-[^{17}O]Ala-Ala with anti-parallel β-sheet and parallel β-sheet structures. The lengths were obtained from X-ray crystallographic data [2,3] and the error scales of length (R factors) are 0.031 and 0.057.

calculated chemical shift differences between ^{17}O nuclei on A and B molecules is 31.3 ppm for the anti-parallel β-sheet sample and 3.8 ppm for the parallel β-sheet sample (Table 2). Therefore, the small ^{17}O chemical shift differences for ^{17}O labels on the A and B molecules in the anti-parallel β-sheet sample and the large chemical shift differences between the ^{17}O labels in the parallel β-sheet sample cannot be explained just by differences in hydrogen bond lengths. Recently, Pike et al. [18] reported a strong linear correlation for the isotropic ^{17}O chemical shifts of carbonyl oxygens with C=O bond lengths for ^{17}O-labeled sites. The C=O bond lengths, $R_{C=O}$, of the ^{17}O nuclei of the central Ala residue for our samples are $R_{C=O}(A) = 1.233$ Å and $R_{C=O}(B) = 1.232$ Å for the anti-parallel β-sheet sample and $R_{C=O}(A) = 1.220$ Å and $R_{C=O}(B) = 1.231$ Å for the parallel β-sheet sample. The larger chemical shift difference observed between ^{17}O nuclei on the A and B molecules is for the anti-parallel β-sheet (which has nearly identical $R_{C=O}$ values for the A and B molecules) and the smaller chemical shift difference is found for the parallel β-sheet structure. Hence, the observed chemical shift differences for the Ala-Ala-Ala samples cannot be attributed to differences in C=O bond lengths.

The calculated chemical shift principal values σ_{11}, σ_{22} and σ_{33} provide insight into the nature of the experimentally observed differences in ^{17}O chemical shifts. The results tabulated in Table 2 show a large difference in σ_{11} ($\Delta\sigma_{11} = 61.0$ ppm) and σ_{22} ($\Delta\sigma_{22} = 33.9$ ppm) between ^{17}O nuclei on the A and B molecules and a very small difference in σ_{33} ($\Delta\sigma_{33} = -1.1$ ppm) for the anti-parallel β-sheet sample. The differences in the calculated chemical shifts between A and B molecules are small for the parallel β-sheet sample: $\Delta\sigma_{11} = -1.4$ ppm, $\Delta\sigma_{22} = 17.0$ ppm and $\Delta\sigma_{33} = -4.4$ ppm. Thus, the origin of the large difference in isotropic chemical shifts for ^{17}O nuclei on the A and B molecules in the anti-parallel β-sheet comes primarily from the large difference in σ_{11} (and to a lesser degree from σ_{22}). Calculations show little difference in chemical shifts for the ^{17}O nuclei on the A and B molecules in the parallel β-sheet, which is consistent with the observed NMR spectra. The direction of σ_{11} (see Fig. 3) is slightly deviated from the C=O bond direction, and the angle, θ, between the C=O bond and the σ_{11} direction is $\theta = 14°$ for the A molecule and $\theta = 18°$ for the B molecule in the parallel β-sheet structure. For the anti-parallel β-sheet sample, the angles are $\theta = 9°$ for the A molecule and $\theta = 14°$ for the B molecule. The orientation of σ_{22} is approximately perpendicular to the C=O bond and in the nodal plane of the amide bond for both samples.

There is an orientational property between chemical units on neighboring molecules that may be responsible for the observed chemical shifts. The angle, α, between lines

defined by the C=O and N–H bond directions are significantly different for the two samples. The angle, $\alpha_{(A)}$, between the C=O bond direction of the A molecule and N–H bond direction of the B molecule is −136°, and the angle, $\alpha_{(B)}$, between the C=O bond direction of the B molecule and N–H bond direction of the A molecule is −155.17° for the anti-parallel β-sheet structure. The corresponding angles are −136° and −139° for the parallel β-sheet structure. Thus, the differences in the angles, $\Delta\alpha = \alpha_{(A)} - \alpha_{(B)}$, are considerably different for the two samples, with $\Delta\alpha = 19°$ for the anti-parallel β-sheet sample and $\Delta\alpha = 3°$ for the parallel β-sheet sample. Since the orientation of the calculated σ_{11} is approximately parallel to the C=O bond (and σ_{22} is oriented nearly perpendicular to the C=O bond), we propose that the relative orientation between the C=O and N–H bond directions has the most influence on the observed differences in chemical shifts. Hence, the primary difference in the oxygen electronic environment occurs within the nodal plane of the amide bond, especially in the C=O bond direction, and not in the direction perpendicular to the amide plane. The DFT results leads us to the conclusion that the relative orientation between the C=O bond and N–H bond of the hydrogen-bonded pair most affects the ^{17}O chemical shift and is the cause of the observed chemical shift differences between the two peptide samples.

The quadrupole coupling asymmetry parameters, η, were also examined by DFT calculations; the calculated η values and quadrupolar coupling constants are summarized in Table 2. The difference in η values between the A and B molecules is similar to the behavior of the respective isotropic chemical shifts described above. The difference in asymmetry parameter, $\Delta\eta = \eta_{O(A)} - \eta_{O(B)}$, is large for the anti-parallel β-sheet ($\Delta\eta = 0.088$, with $\eta_{O(A)} = 0.476$ and $\eta_{O(B)} = 0.388$) but is very small for the parallel β-sheet ($\Delta\eta = -0.003$, with $\eta_{O(A)} = 0.301$ and $\eta_{O(B)} = 0.304$). As with the previous discussion of chemical shifts, the difference in η values between ^{17}O nuclei on A and B molecules cannot be explained either by the differences in hydrogen bond lengths or C=O bond lengths found between A and B molecules. Instead, the difference in the angles between the C=O and N–H bond orientations account for the differences in asymmetry parameter.

The quadrupole coupling constants χ_Q (in MHz) for the two samples were also examined by DFT calculations. Takahashi et al. and Yamada et al. reported that the χ_Q values increase with $R_{N\cdots O}$ [17,11]. For example, χ_Q changes from 8.18 MHz at $R_{N\cdots O} = 2.90$ Å to 8.27 MHz at $R_{N\cdots O} = 3.20$ Å for their *N*-methylacetamide/formamide model [17]. The calculated χ_Q values are 8.53 MHz and 8.58 MHz for ^{17}O nuclei on the A molecules of the anti-parallel β-sheet sample (with $R_{N(B)\cdots O(A)} = 2.915$ Å) and parallel β-sheet sample (with $R_{N(B)\cdots O(A)} = 3.350$ Å), respectively. Hence, the substantial difference (0.44 Å) in $R_{N(B)\cdots O(A)}$ between the samples and the miniscule difference in the corresponding χ_Q values for the A molecules makes the direct hydrogen bonding length $R_{N\cdots O}$ an unlikely factor alone for interpreting differences in χ_Q.

In conclusion, the observed differences in the ^{17}O NMR spectra between the anti-parallel and parallel β-sheet samples arise primarily from the differences in the angle between the C=O bond and N–H bond orientations.

Acknowledgements

Financial support was provided by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan (18105007), SENTAN, JST (JAPAN) and NSF Grant CHE-0603697.

References

[1] T. Asakura, M. Okonogi, Y. Nakazawa, K. Yamauchi, Structural analysis of alanine tripeptide with antiparallel and parallel β-sheet structures in relation to the analysis of mixed β-sheet structures in *Samia cynthia ricini* silk protein fiber using solid-state NMR spectroscopy, J. Am. Chem. Soc. 128 (2006) 6231–6238.

[2] J. Fawcett, N. Camerman, A. Camerman, The structure of the tripeptide L-alanyl-L-alanyl-L-alanine, Acta Cryst. B31 (1975) 658–665.

[3] A. Hempel, N. Camerman, A. Camerman, L-Alanyl-L-alanyl-L-alanine: parallel pleated sheet arrangement in unhydrated crystal structure, and comparisons with the antiparallel sheet structure, Biopolymers 31 (1991) 187–192.

[4] G. Wu, Recent developments in solid-state nuclear magnetic resonance of quadrupolar nuclei and applications to biological systems, Biochem. Cell. Biol. 76 (1998) 429–442.

[5] K. Yamauchi, S. Kuroki, I. Ando, T. Ozaki, A. Shoji, ^{17}O NMR chemical shifts and quadrupole coupling constants in solid poly(L-alanine)s determined using a high-speed MAS technique, Chem. Phys. Lett. 302 (1999) 331–336.

[6] K. Yamauchi, S. Kuroki, I. Ando, High-resolution solid-state ^{17}O NMR studies of polyglycines and their hydrogen-bonded structures, J. Mol. Struct. 602/603 (2002) 171–175.

[7] V. Lemaître, M.E. Smith, A. Watts, A review of oxygen-17 solid-state NMR of organic materials – towards biological applications, Solid State Nucl. Magn. Reson. 26 (2004) 215–235.

[8] E.Y. Chekmenev, K.W. Waddell, J. Hu, Z. Gan, R.J. Wittebort, T.A. Cross, Ion-binding study by ^{17}O solid-state NMR spectroscopy in the model peptide Gly-Gly-Gly at 19.6 T, J. Am. Chem. Soc. 128 (2006) 9849–9855.

[9] K.W. Waddell, E.Y. Chekmenev, R.J. Wittebort, Peptide ^{17}O chemical shielding and electric field gradient tensors, J. Phys. Chem. B 110 (2006) 22935–22941.

[10] G. Wu, S. Dong, R. Ida, N. Reen, A solid-state ^{17}O nuclear magnetic resonance study of nucleic acid bases, J. Am. Chem. Soc. 124 (2002) 1768–1777.

[11] K. Yamada, S. Dong, G. Wu, Solid-state ^{17}O NMR investigation of the carbonyl oxygen electric-field-gradient tensor and chemical shielding tensor in amides, J. Am. Chem. Soc. 122 (2000) 11602–11609.

[12] A. Brinkmann, A.P.M. Kentgens, Sensitivity enhancement and heteronuclear distance measurements in biological ^{17}O solid-state NMR, J. Phys. Chem. B 110 (2006) 16089–16101.

[13] A. Brinkmann, A.P.M. Kentgens, Proton-selective ^{17}O–^{1}H distance measurements in fast magic-angle-spinning solid-state NMR spectroscopy for the determination of hydrogen bond lengths, J. Am. Chem. Soc. 128 (2006) 14758–14759.

[14] T. Gullion, K. Yamauchi, M. Okonogi, T. Asakura, ^{13}C–^{17}O REAPDOR NMR as a tool for determining secondary structure in polyamides, Macromolecules 40 (2007) 1363–1365.

[15] D. Massiot, F. Fayon, M. Capron, I. King, S. Le Calvé, B. Alonso, J.O. Durand, B. Bujoli, Z. Gan, G. Hoatson, Modelling one- and two-dimensional solid-state NMR spectra, Magn. Reson. Chem. 40 (2001) 70–76.

[16] D. Sitkoff, D.A. Case, Theories of chemical shift anisotropies in proteins and nucleic acids, Prog. Nucl. Magn. Reson. Spec. 32 (1998) 165–190.

[17] A. Takahashi, S. Kuroki, I. Ando, T. Ozaki, A. Shoji, Hydrogen-bonded structure and NMR parameters of oxygen-17 labeled poly(L-alanine)s as studied by solid state oxygen-17 NMR spectroscopy, J. Mol. Struct. 442 (1998) 195–199.

[18] K.J. Pike, V. Lemaitre, A. Kukol, T. Anupõld, A. Samoson, A. P Howes, A. Watts, M.E. Smith, R. Dupree, Solid state ^{17}O NMR of amino acids, J. Phys. Chem. B 108 (2004) 9256–9263.