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Precise structural analysis of α-helical copolypeptide H-(Ala-Gly)$_9$-OH by quantum chemical calculation and high-resolution solid-state NMR measurement

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ABSTRACT

We computed the optimized structure of sequential 18-mer copolypeptide H-(Ala-Gly)$_9$-OH ($C_{45}H_{74}N_{18}O_{19}$) adopting an right-handed α-helix (α_R-helix) conformation with the basis set of DFT/6-31G(d), and then calculated the nuclear shieldings of the optimized structure with the basis set of DFT/6-311G(d,p). As a result, we confirmed highly accurate conformational parameters characteristic to the α_R-helical H-(Ala-Gly)$_9$-OH, which were identical with those of the individual Ala and Gly residues. Most of these parameters were fundamentally the same as those obtained for the optimized α_R-helical H-(Ala)$_{18}$-OH. Furthermore, it was found that the calculated isotropic ^{13}C and ^{15}N chemical shifts were dependent on the nature of individual amino acid residues, which were greatly in good agreement with those of α_R-helical model copolypeptides consisting of L-alanine and glycine residues measured by high-resolution solid-state NMR.

1. Introduction

It is very useful to analyze the secondary structure of polypeptides and proteins by a precise structural calculation. Very recently, noticeable progress in computer science has made possible to realize a precise structural optimization of polypeptide molecule to characterize the secondary structure [1,2]. To achieve the goal of this study successfully, it is necessary, as the minimum requirement, to complete the optimized structural calculation of sequential copolypeptide chain by using the density functional theory [3] (DFT: B3LYP (Becke's three parameter hybrid method using the Lee, Yang and Parr (LYP) correlation functional) [4,5]) with extended 6-31G(d) basis set level, which has been accepted as trustworthy for a credible molecular orbital theory at present. In addition, it is necessary to calculate the nuclear shieldings of the optimized structure by using the gauge-included atomic orbital (GIAO)-Coupled Hartree–Fock (CHF) approach [6,7] with B3LYP/6-311G(d,p) basis set level, which has been also authorized in nuclear shielding calculation.

In our previous paper [1], it has been reported that the precise structural optimization calculation and its nuclear shieldings of the most acceptable α_R-helical poly(L-alanine) (H-(Ala)$_{18}$-OH) have been successfully accomplished using Gaussian03 program with B3LYP/6-31G(d) and B3LYP/6-311G(d,p) basis sets, respectively. As a result, we have proved the highly precise structural geometry and conformational parameters characteristic to the most acceptable α_R-helical H-(Ala)$_{18}$-OH: dihedral angles $(\phi,\psi,\omega) = (-62°,-43°,178°)$, hydrogen-bond distances $\mathbf{R}_{O\ldots H} = 0.205$ nm and $\mathbf{R}_{O\ldots N} = 0.303$ nm, and hydrogen-bond angles C′═O…H = 149° and N—H…O = 160°. These parameters have been in good agreement with those of X-ray and neutron diffraction data in protein databank (PDB). Furthermore, the calculated isotropic ^1H and ^{13}C chemical shifts have been identical with those of α_R-helical poly(L-alanine) measured by the high-resolution solid-state NMR (^1H CRAMPS and ^{13}C CP-MAS NMR). These conformational parameters and chemical shifts are very useful to elucidate the nature of the electronic structure and to determine the precise secondary structure of proteins theoretically [1,2]. This implies that such a precise structural calculation has a very high capacity of prediction of a secondary structure. However, for this purpose, we must solve the problem whether the optimization calculation in such precise level is reliable for copolypeptides as models of natural proteins, as a continuation of our previous work [1].

In this study, we aimed to test whether the precise calculation method is applicable to the secondary structure optimization of a simple copolypeptide as a model of protein structural analysis or not. For this, we applied to calculate the optimized structure of a well-defined α_R-helical sequential 18-mer copolypeptide consists of L-alanine (Ala) and glycine (Gly) residues, H-(Ala-Gly)$_9$-OH ($C_{45}H_{74}N_{18}O_{19}$). Here, the Gly is the smallest α_R-helix breaking amino acid residue [8], and the Ala is the very strong α_R-helix

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supporting residue. Therefore, it is meaningful to confirm theoretically whether the alternate copolypeptide of repeating Ala-Gly sequences at counterbalance stabilizes an α_R-helix conformation or not, in relation to the secondary structural analysis of silk fibroins such as *Bombix mori* [9]. Furthermore, we compared the calculated isotropic ^{13}C and ^{15}N chemical shifts of the Ala and Gly residues with those of the individual Ala and Gly residues of the α_R-helical model copolypeptides, respectively, by ^{13}C and ^{15}N NMR measurements. From this study, it would be expected that the optimization of a hypothetical precise secondary structure (main-chain conformation) of copolypeptides would give valuable information in structural analysis of polypeptides and proteins.

2. Experimental

2.1. Quantum chemical calculation

We calculated with the Gaussian03 D.01 program [10] for structural optimization with the basis set of B3LYP/6-31G(d), and used with the B3LYP/6-311G(d,p) basis set for nuclear shielding calculation. The geometrical parameters of the model compound were optimized for bond lengths, bond angles, and dihedral angles. The main initial parameters of α_R-helical H-(Ala-Gly)$_9$-OH used in this study were as follows; (1) Ala residues – bond lengths (nm): 0.145 (N—C$^\alpha$), 0.152 (C$^\alpha$—C′), 0.134 (C′—N), 0.153 (C$^\alpha$—C$^\beta$), 0.126 (C′=O), 0.100 (N—H), 0.109 (C$^\alpha$—H$^\alpha$ and C$^\beta$—H$^\beta$), bond angles (degree): 120.0 (H—N—C$^\alpha$, C$^\alpha$—C′=O, N—C′=O, N—C′—C$^\alpha$, H—N—C′, C′—N—C$^\alpha$ and H—N—H), 111.1 (N—C$^\alpha$—C$^\beta$ and N—C$^\alpha$—C′), 109.5 (N—C$^\alpha$—H$^\alpha$), 108.6 (H$^\alpha$—C$^\alpha$—C′ and H$^\alpha$—C$^\alpha$—C$^\beta$), 107.8 (C′—C$^\alpha$—C$^\beta$), dihedral angles (degree): −57.0 (ϕ: C′—N—C$^\alpha$—C′), −47.0 (ψ: N—C$^\alpha$—C′—N) and 180.0 (ω: C$^\alpha$—C′—N—C$^\alpha$); (2) Gly residue – bond lengths (nm): 0.145 (N—C$^\alpha$), 0.152 (C$^\alpha$—C′), 0.134 (C′—N), 0.126 (C′=O), 0.100 (N—H), 0.109 (C$^\alpha$—H$^\alpha$ and C$^\alpha$—H$^{\alpha'}$), 0.096 (O—H), 0.143 (C′—O), bond angles (degree): 120.0 (H—N—C$^\alpha$, C$^\alpha$—C′=O, N—C′=O, N—C′—C$^\alpha$, H—N—C′ and C′—N—C$^\alpha$), 110.4 (N—C$^\alpha$—C′), 109.5 (N—C$^\alpha$—H$^\alpha$ and N—C$^\alpha$—H$^{\alpha'}$), 109.4 (H$^\alpha$—C$^\alpha$—H$^{\alpha'}$), 109.0 (H$^\alpha$—C$^\alpha$—C′ and C′—C$^\alpha$—H$^{\alpha'}$), dihedral angles (degree): −57.0 (ϕ: C′—N—C$^\alpha$—C′), −47.0 (ψ: N—C$^\alpha$—C′—N), and 180.0 (ω: C$^\alpha$—C′—N—C$^\alpha$).

Next, we calculated the structural optimization for adamantane ($C_{10}H_{16}$) as a reference material of ^1H and ^{13}C chemical shift using the same basis set as B3LYP/6-31G(d) in structural optimization and B3LYP/6-311G(d,p) for nuclear shielding calculation. We used the ^1H and ^{13}C chemical shift values of adamantane methine proton and methine carbon as 1.87 ppm (CRAMPS) [1,2,11], and 29.47 ppm (CP-MAS) [1,2,12] from tetramethylsilane (TMS; δ 0), respectively. Here, the ^1H and ^{13}C nuclear shieldings of TMS were estimated as 32.03 and 177.77 ppm, respectively, as reported previously [1,2]. In addition, we used the ^{15}N chemical shift value as 98.80 ppm from $^{15}NH_4NO_3$ (δ 0), where the ^{15}N nuclear shieldings of $^{15}NH_4NO_3$ was estimated as 213.21 ppm.

2.2. Sample

A well-defined copolypeptide sample containing glycine residue, FLA-11G, H-(Phe-Leu-Ala)$_2$-Phe-Leu^C-Ala-Phe-Gly-Ala^N-(Phe-Leu-Ala)$_2$-OH (where Phe = L-phenyl alanine, Leu = L-leucine, Ala = L-alanine, Leu^C = 1-^{13}C labelled L-leucine, and Ala^N = 2-^{15}N labelled L-alanine residues), was synthesized by solid-phase peptide synthesis using 9050 PLUS Pepsynthesizer (PerSeptive Biosystems) [2,9]. In this experiment, we used Fmoc-Ala-PEG-PS resin (Fmoc = 9-fluorenylmethoxycarbonyl) as a solid supporting resin. The Fmoc group was eliminated from Fmoc-Ala-PEG-PS resin with piperidine, and HATU (*O*-(7-azabenzotriazol-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) was used for activation reaction. After the final step of synthesis, the polypeptide as synthesized was cleaved from the solid support by using 95% trifluoroacetic acid, then treated with diethyl ether to precipitate.

2.3. NMR measurement

The solid-state ^{15}N CP-MAS NMR measurement was performed using a Bruker DSX300 spectrometer operating at 30.42 MHz, equipped with a CP-MAS probe. The ^{15}N chemical shift was calibrated indirectly by external $^{15}NH_4Cl$ (18.0 ppm relative to $^{15}NH_4NO_3$). The experimental error in the isotropic ^{15}N chemical shifts was estimated to be less than ±0.5 ppm [9].

3. Results and discussion

3.1. Precise characteristic parameters of α_R-helical structure

We computed a precise structural optimization and determined its characteristic parameters of the α_R-helical structure of H-(Ala-Gly)$_9$-OH. Fig. 1 shows the optimized molecular structure of α_R-helical H-(Ala-Gly)$_9$-OH (the dotted line represents the hydrogen-bonding formation).

The optimized structure of H-(Ala-Gly)$_9$-OH was maintained typical α_R-helix conformation, which was quite similar to the α_R-helical H-(Ala)$_{18}$-OH as reported previously [1]. Therefore, it was assumed that the optimized α_R-helical structure of H-(Ala-Gly)$_9$-OH was one of the stable secondary structure. The secondary structure of inner Ala and Gly residues (residual nos. 6–13) were assumed to be typical α_R-helix, whereas both end portions (the residual nos. 1–5 and 14–18) of the H-(Ala-Gly)$_9$-OH were formed only one hydrogen-bond per each residue except for residual numbers 4, 14 and 15. However, it is meaningful that the C′=O of the N-terminal residue (1st Ala residue: ^{1}Ala) displayed a hydrogen-bond with the N—H of 4th Gly residue (^{4}Gly) (which is not an α_R-helix but a 3_{10}-helix) and the N—H of 5th Ala residue (^{5}Ala) was free (no hydrogen-bond). On the other hand, the hydrogen-bond was maintained between the C-terminal N—H of the ^{18}Gly residue and the C′=O of ^{14}Gly residue, and between the C-terminal O—H of the ^{18}Gly residue and the C′=O of ^{15}Ala residue, respectively, which were not the typical α_R-helix. Therefore, we calculated the mean values of the inner 6 residues (^{7}Ala to ^{12}Gly residue) for the safety to determine the characteristic conformational parameters and chemical shift values of the most acceptable α_R-helical H-(Ala-Gly)$_9$-OH. The calculated structural geometry and conformational parameters of the α_R-helical H-(Ala-Gly)$_9$-OH were as below; (1) Ala residues – bond lengths (nm): 0.146 (N—C$^\alpha$), 0.154 (C$^\alpha$—C′), 0.135 (C′—N), 0.153 (C$^\alpha$—C$^\beta$), 0.123 (C′=O), 0.102 (N—H), 0.109 (C$^\alpha$—H$^\alpha$ and C$^\beta$—H$^\beta$); bond angles (degree): 118.9 (H—N—C$^\alpha$), 109.2 (N—C$^\alpha$—H$^\alpha$), 110.1 (N—C$^\alpha$—C$^\beta$), 110.9 (C′—C$^\alpha$—C$^\beta$), 111.9 (N—C$^\alpha$—C′), 104.5 (H$^\alpha$—C$^\alpha$—C′), 110.1 (H$^\alpha$—C$^\alpha$—C$^\beta$), 120.3 (C$^\alpha$—C′=O), 123.0 (N—C′=O), 116.7 (N—C′—C$^\alpha$), 120.1 (H—N—C′), 121.0 (C′—N—C$^\alpha$); dihedral angles (°): −61.8 (ϕ: C′—N—C$^\alpha$—C′), −42.6 (ψ: N—C$^\alpha$—C′—N), 177.9 (ω: C$^\alpha$—C′—N—C$^\alpha$): hydrogen-bond distances (nm): 0.200 ($R_{Oi\ldots Hi+4}$), 0.299 ($R_{Oi\ldots Ni+4}$), 0.314 ($R_{C'i\ldots Hi+4}$), 0.415 ($R_{C'i\ldots N_i+4}$); hydrogen-bond angles (degree): 151.0 (C'_i=O…H_{i+4}), 163.6 (N_{i+4}—H…O_i); hydrogen-bond dihedral angle (degree): 150.1 (C'_i=O…H—N_{i+4}); (2) Gly residues – bond lengths (nm): 0.145 (N—C$^\alpha$), 0.154 (C$^\alpha$—C′), 0.135 (C′—N), 0.123 (C′=O), 0.102 (N—H), 0.109 (C$^\alpha$—H$^\alpha$ and C$^\alpha$—H$^{\alpha'}$) (where H$^{\alpha'}$ denotes the other proton bonded to the C$^\alpha$ of the Gly residue); bond angles (degree): 119.0 (H—N—C$^\alpha$), 111.2 (N—C$^\alpha$—H$^\alpha$), 107.7 (N—C$^\alpha$—H$^{\alpha'}$), 113.3 (N—C$^\alpha$—C′), 106.2 (H$^\alpha$—C$^\alpha$—C′), 109.6 (H$^{\alpha'}$—C$^\alpha$—C′), 108.7 (H$^\alpha$—C$^\alpha$—H$^{\alpha'}$), 120.5 (C$^\alpha$—C′=O), 123.3 (N—C′=O), 116.2 (N—C′—C$^\alpha$), 120.5 (H—N—C′), 120.4 (C′—N—C$^\alpha$); dihedral angles (°): −61.7 (ϕ: C′—N—C$^\alpha$—C′), −43.6 (ψ: N—C$^\alpha$—C′—N), 176.5 (ω: C$^\alpha$—C′—N—C$^\alpha$):