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Conformational analysis of α-helical polypeptide included L-proline residue by high-resolution solid-state NMR measurement and quantum chemical calculation

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Abstract

We challenged the problem about the stabilization mechanism of an α-helix formation for polypeptides containing L-proline (Pro) residue. We computed the optimized structure of α-helical poly(L-alanine) molecules including a Pro residue, H-$(Ala)_8$-Pro-$(Ala)_9$-OH, based on the molecular orbital calculation with density functional theory, B3LYP/6-31G(d) and the ^{13}C and ^{15}N chemical shift values based on the GIAO-CHF method with B3LYP/6-311G(d,p), respectively. It was found that two kinds of optimized structures, 'Bent structure' and 'Included α-helix structure', were preferred structures in H-$(Ala)_8$-Pro-$(Ala)_9$-OH. In addition, based on the precise ^{13}C and ^{15}N chemical shift data of the simple model, we successfully analyzed the secondary structure of well-defined synthetic polypeptide H-$(Phe\text{-}Leu\text{-}Ala)_3$-$Phe^C$-Pro-$Ala^N$-$(Phe\text{-}Leu\text{-}Ala)_2$-OH (FLA-11P), the secondary structure of which was proven to the 'Included α-helix structure'.

Keywords: α-Helix; L-Proline; Included structure; Bent structure; CP-MAS NMR; Quantum chemical calculation

As an α-helix or β-sheet conformation is unique and beautiful secondary structure (main-chain conformation) of polypeptides and proteins, elucidation of the formation mechanism and the stability of secondary structure are especially important subjects. Above all, it is quite valuable to demonstrate the stability of an α-helix conformation of the polypeptide included L-proline (Pro) residue, since only the Pro residue does not have an amide proton and strongly destabilize an α-helix structure [1,2]. Whether the Pro residue is practically included in a stable α-helix structure or it breaks an α-helix structure? Which structural parameter of the Pro residue is more effective in conformational stability, main-chain conformation or side-chain conformation?

Up to now, the crystal structural analysis by X-ray [3,4] or neutron [5] diffraction and the three dimensional (3D) structural analysis by solution NMR [6] have been applied mainly to determine the protein structure. In addition, high-resolution solid-state NMR methods [7–17] have advanced in recent years, which have unlimited possibilities for secondary structural analysis of solid proteins. Since each method has many advantages and limitations for protein structural analysis, the establishment of the reliable molecular structure optimization based on calculation chemistry is essential. The quantum chemical calculation has been increasingly applied for this system very recently, but it is considerably limited case using semi-empirical calculation method because of the calculation costs in the hugeness (very large molecular size) of protein molecule. On the other hand, recent tremendous developments of computer technology made possible to calculate at the non-empirical calculation level for small model peptides [18–20].

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In our previous paper [21], we have successfully calculated the optimization structure of the α-helical H-(Ala)$_{18}$-OH based on the molecular orbital theory by using non-empirical calculation alone. As a result, we have confirmed highly accurate conformational parameters characteristic to the typical α-helical poly(L-alanine) (PLA) as follows; intrinsic 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; hydrogen-bond angles $\angle C'{=}O\ldots H = 149°$ and $\angle N{-}H\ldots O = 160°$. Furthermore, the calculated ^{13}C and ^{15}N chemical shift values of α-helical H-(Ala)$_{18}$-OH were 176.15 (C′=O), 53.29 (C^{α}) and 12.14 (C^{β}), and 98.80 ppm (N).

These results indicated that the calculated secondary structural parameters and the ^{13}C and ^{15}N chemical shift values have been useful for the conformational analysis of other common amino acid residues except for glycine (Gly) and L-proline (Pro) residues, as the basic information for the standard α-helix.

To solve the problem whether the Pro residue is practically included in a stable α-helix structure, in this study, we designed and synthesized a model polypeptide included the Pro residue in the center of an α-helix, then carried out the high-resolution ^{13}C and ^{15}N solid-state NMR measurements and quantum chemical calculation. However, since it was extremely difficult to prepare the same model polypeptide in calculation and experiment, from the limitations of the molecular size in structural calculation and the restriction in reaction condition (mainly solubility) in peptide synthesis, we arranged respectively the well-defined best model peptide. The sample, H-(Phe-Leu-Ala)$_3$-Phe^{C}-Pro-Ala^{N}-(Phe-Leu-Ala)$_2$-OH (FLA-P11) was synthesized for NMR measurements, where Phe = L-phenylalanine, Leu = L-leucine, Phe^{C} = (1-^{13}C)-labeled L-phenylalanine, and Ala^{N} = (2-^{15}N)-labeled L-alanine residues. On the other hand, H-Ala$_8$-Pro-Ala$_9$-OH was designed as a simplest model for the quantum chemical calculation. Gaussian03 software program [22] was used for structural optimization based on the molecular orbital calculation with DFT/6-31G(d). We have used the density functional theory [23–25] (DFT: B3LYP (Becke's three parameter hybrid method using the LYP correlation functional)) with extended 6-31G(d) basis set for the calculation of optimized structure and the gauge-included atomic orbital (GIAO)-Coupled Hartree–Fock (CHF) approach [26,27] with B3LYP/6-311G(d,p) basis set for the calculation of nuclear shieldings. The selection of the initial parameters is quite important. In our calculation, for the first step, we searched some stable dihedral angles (ϕ,ψ) of the Pro residue of Ac-Ala-Pro-NHMe and Ac-Pro-Ala-NHMe, and we obtained two sets of locally allowed stable main-chain conformation ($\phi{-}61°, \psi = 150°; -30°$) as the initial parameters for the next step. The initial parameters used for the Ala and Pro residues in α-helical H-Ala$_8$-Pro-Ala$_9$-OH were as follows; Ala dihedral angles $(\phi,\psi) = (-57°, -47°)$ [28], Pro dihedral angles: $(\phi,\psi) = (-61°, -30°)$ or $(\phi,\psi) = (-61°, -150°)$, ring conformation: Up and Down. We used the ^{13}C nuclear shieldings of TMS (δ 0) as 177.77 ppm [21] and the ^{15}N nuclear shielding of $^{15}NH_4NO_3$ (δ 0) as 213.21 ppm, as described previously [21].

Well-defined model polypeptide, FLA-11P, were synthesized by solid-phase peptide synthesis using 9050 PLUS Pepsynthesizer (PerSeptive Biosystems) [15]. 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% TFA, then it was treated with diethyl ether to precipitate.

The solid-state ^{13}C CP-MAS NMR measurement was performed using a Bruker DSX300 spectrometer operating at 75.48 MHz, equipped with a CP-MAS probe. The ^{13}C chemical shifts were calibrated indirectly by external adamantane (29.5 ppm relative to TMS) [15]. The experimental error in the isotropic ^{13}C chemical shifts was estimated to be less than ±0.3 ppm [15]. 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 [15].

Fig. 1 shows the ^{13}C (A) and ^{15}N (B) CP-MAS NMR spectra of FLA-11P, H-(Phe-Leu-Ala)$_3$-Phe^{C}-Pro-Ala^{N}-(Phe-Leu-Ala)$_2$-OH, respectively. The maximum peak appeared at 171.4 ppm was assigned to C′=O of the ^{10}Phe^{C} residue bonded to the ^{11}Pro residue. In general, it is evident that the ^{13}C chemical shift value of α-helical poly(L-phenylalanine) is 175.1 ppm, which is about 1–2 ppm smaller than that of α-helical poly(L-alanine) (176–177 ppm) [7,15,16,21]. From the ^{13}C chemical shift value of the ^{10}Phe^{C} residue alone, it is concluded that the secondary structure of the ^{10}Phe^{C} residue in FLA-11P may not be an α-helix.

Next, the single peak appeared at 99.1 ppm was assigned to the ^{12}Ala^{N} residue bonded to the ^{11}Pro residue. The ^{15}N chemical shift value of the ^{12}Ala^{N} residue was 99.1 ppm, which was equal to that of α-helical poly(L-alanine) (98.8 ppm) [10,11]. Taking into consideration that the ^{15}N chemical shift value was greatly dependent upon the nearest neighboring amino acid residue (amino acid sequence) as well as secondary structure, it is favorable to conclude that the secondary structure of the ^{12}Ala^{N} residue in FLA-11P was an α-helix in this case. However, it seemed that both results obtained from the ^{13}C and ^{15}N chemical shifts were apparently inconsistent.

Therefore in this study, we attempted to analyze the precise secondary structure using the most reliable molecular orbital calculation for the polypeptide at the present. We