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Precise structural analysis of α-helical poly(L-alanine) by quantum chemical calculation

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Abstract

It is extremely important to elucidate the formation mechanism of the secondary structure in polypeptides and proteins. This enables the stability of the three-dimensional structure of proteins to be predicted theoretically. For this purpose, the development of a precise structural calculation is indispensable. We computed the optimized structure of a poly(L-alanine) (PLA) molecule, H—Ala$_{18}$—OH ($C_{54}H_{92}N_{18}O_{19}$), adopting a right-handed α-helix (α_R-helix) conformation based on the molecular orbital calculation with density functional theory (DFT/6-31G(d)). As a result, we confirmed highly accurate conformational parameters characteristic to the "most acceptable α_R-helix PLA" as follows; intrinsic dihedral angles $(\phi, \psi, \omega) = (-62°, -43°, 178°)$; hydrogen-bond distances $\mathbf{R}_{O...H} = 0.205$ nm and $\mathbf{R}_{O...N} = 0.303$ nm; hydrogen-bond angles $\angle C{=}O...H = 149°$and $\angle N{-}H...O = 160°$. Furthermore, we calculated the ^{1}H, ^{13}C, ^{15}N and ^{17}O nuclear shieldings of the optimized α_R-helical H—Ala$_{18}$—OH with the base set of DFT/6-311G(d,p), and demonstrated that the calculated isotropic ^{13}C and ^{1}H chemical shifts were identical with those measured by high-resolution solid-state NMR.

Keywords: Poly(L-alanine); α-Helix; Quantum chemical calculation; Chemical shift tensors; Conformational parameter; Hydrogen bond

1. Introduction

The right-handed α-helix (α_R-helix) conformation found by Pauling et al. [1] in 1951 is a very important secondary structure (main-chain conformation), and the characteristic parameters of the "classical α_R-helix [2,3]" have been widely used in the study of protein structure. Recently, structural analytical data of natural proteins by neutron diffraction technique came to be accumulated, and structure calculation of a large molecule became possible because the performance of the personal computer has been remarkably progressed. Thus, the decision of accurate structural parameters has been required.

It has been already demonstrated that the isotropic chemical shifts and the principal values of the chemical shift tensors of the ^{1}H, ^{13}C, ^{15}N and ^{17}O nuclei were dependent mainly on the local conformations of synthetic polypeptides and natural proteins in the solid state by high-resolution solid-state NMR measurements [4–20]. These chemical shift parameters are very important to elucidate the relation between the nature of protein structure and the electronic structure, and to predict a precise structure of proteins theoretically.

In this paper, we calculated a precise molecular structure of "most acceptable α_R-helix" poly(L-alanine), H—Ala$_{18}$—OH ($C_{54}H_{92}N_{18}O_{19}$), molecular size of which is very large, based on the molecular orbital theory (DFT/6-31G(d)), and obtained the structure geometry and characteristic parameters of the α_R-helix conformation. In addition, the nuclear

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shieldings calculated for the optimized "most acceptable α_R-helix" H—Ala$_{18}$—OH were compared to the NMR chemical shifts measured, and we verified the reliability of this structural calculation and the effectiveness of the computational approaches. To make this study succeed, we especially considered the following points.

First, we chose the α_R-helical H—Ala$_{18}$—OH as a model of the calculation. The α_R-helix is stabilized by the "intra-molecular hydrogen bonds" between ith C=O and $(i+4)$th N—H atoms, and therefore the inter-molecular interactions in the main-chain atoms should become very small in this system. The L-alanine (Ala) residue is very strong α_R-helix supporting and the smallest L-amino acid residue, which is absolutely necessary condition for the highly precise structural calculation of a single α_R-helical molecule at present. Since naturally occurring polypeptides and proteins are full of varieties, these simple systems are highly useful in such a powerful study. Interestingly, PLA is also used to measure several other parameters including the stability of the α-helical or β-sheet conformation [4–20]. Therefore, we adopted here the well-defined α_R-helical poly(L-alanine), H—Ala$_{18}$—OH (octadeca peptide), as a longer model peptide.

Next, we computed the optimized structure of high molecular weight single polypeptide chain stabilized by intra-molecular hydrogen bonds, by using the density functional DFT(B3LYP [21–23]) in structural optimization, with extended DFT/6-31G(d) basis set, and DFT/6-311G(d,p) GIAO-CHF method in nuclear shielding calculation. The DFT/6-31G(d) basis set calculation is accepted as trustworthy for a credible molecular orbital theory, and the DFT/6-311G(d,p) GIAO-CHF method is also authorized in nuclear shielding calculation. In principle, it is possible to evaluate the calculated ^{1}H, ^{13}C, ^{15}N and ^{17}O isotropic chemical shifts (and the principal values of the chemical shift tensors) of polypeptides and proteins with those observed directly by high-resolution solid-state NMR measurement, because the molecular fluctuation in solid-state NMR was negligibly small compared to that in solution NMR. Therefore, we can anticipate that the chemical shift data measured by high-resolution solid-state NMR become a good control for the theoretical results. Recently, tremendous progress in computer has made possible to materialize a precise calculation of the ^{13}C or ^{15}N chemical shift anisotropy tensors in small model peptides with non-empirical calculations [9,10,17,24,25]. As yet these methods have never been applied for the precise structural optimization of the huge polypeptide molecule.

We succeeded, for the first time, in calculating the precise structural geometry and conformational parameters of the "most acceptable α_R-helix" of H—Ala$_{18}$—OH using Gaussian03 quantum chemical calculation, and obtained a lot of valuable and important information characteristics to the "most acceptable α_R-helix", which were identical with those from X-ray and neutron diffraction data-base in proteins (PDB) and with observed NMR chemical shift data.

2. Experimental

2.1. Quantum chemical calculation

We calculated with the Gaussian03 program Revision C.02 [26] for structural optimization at the base set of DFT/6-31G(d) and we used the gauge-included atomic orbital (GIAO)-Coupled Hartree–Fock (CHF) approach with the density functional theory (Becke's three parameter hybrid method [21–23] using the LYP correlation functional) for the calculation of nuclear shieldings. The basis set we used was DFT/6-311G(d,p) 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$_{18}$—OH used in this study were as follows; 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$^\beta$), 0.096 (O—H), 0.143 (C′—O); bond angles (degree): 120 (H—N—C$^\alpha$, C$^\alpha$—C′=O, N—C′=O, N—C′—C$^\alpha$, H—N—C′, and C′—N—C$^\alpha$), 111 (N—C$^\alpha$—C$^\beta$ and N—C$^\alpha$—C′), 110 (N—C$^\alpha$—H$^\alpha$), 109 (H$^\alpha$—C$^\alpha$—C′ and H$^\alpha$—C$^\alpha$—C$^\beta$); dihedral angles (degree): −57.0 (ϕ: C′—N—C$^\alpha$—C′), −47.0 (ψ: N—C$^\alpha$—C′—N), 180.0 (ω: C$^\alpha$—C′—N—C$^\alpha$) and 180.0 (C-terminal group O=C′—O—H: *trans*).

Next, we also calculated the structural optimization for adamantane ($C_{10}H_{16}$) as a reference material of ^{1}H and ^{13}C chemical shifts using the same basis set as DFT/6-31G(d) in structural optimization and DFT/6-311G(d,p) for nuclear shielding calculation. We evaluated the ^{13}C and ^{1}H chemical shift values from the ^{13}C and ^{1}H nuclear shieldings of adamantane methine carbon and methine proton, whose chemical shifts are 29.47 ppm (CP-MAS) [27] and 1.87 ppm (CRAMPS) [28] from tetramethylsilane (TMS; δ 0), respectively. From this, we confirmed the ^{13}C and ^{1}H nuclear shieldings of TMS as 177.77 ppm and 32.03 ppm, respectively.

2.2. Samples

The α_R-helical (1-^{13}C)-labeled poly(L-alanine), [Ala (1-^{13}C)]$_n$-100 (20 atom% of ^{13}C), was synthesized by heterogeneous polymerization of the mixture of *N*-carboxy L-alanine-anhydride (Ala-NCA) and Ala(1-^{13}C)-NCA in acetonitrile using *n*-butylamine as an initiator [4–6]. The sample was washed with dry acetonitrile using Soxhlet extraction apparatus.

2.3. NMR measurements

The solid-state ^{13}C CP-MAS measurement was performed using a Bruker DRX600 spectrometer operating at 150.93 MHz, equipped with a CP-MAS probe. The ^{13}C chemical shift tensor components (δ_{11}, δ_{22}, and δ_{33} from the downfield side) were obtained from the side-band analysis obtained at slow spinning speed. The ^{13}C chemical shifts were calibrated indirectly by external adamantane