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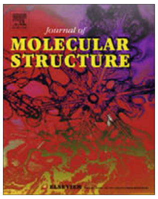

Precise side-chain conformation analysis of L-phenylalanine in α-helical polypeptide by quantum-chemical calculation and ^{13}C CP-MAS NMR measurement

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ABSTRACT

To clarify the positive role of side-chain conformation in the stability of protein secondary structure (main-chain conformation), we successfully calculated the optimization structure of a well-defined α-helical octadecapeptide composed of L-alanine (Ala) and L-phenylalanine (Phe) residues, H–(Ala)$_8$–Phe–(Ala)$_9$–OH, based on the molecular orbital calculation with density functional theory (DFT/B3LYP/6-31G(d)). From the total energy and the precise secondary structural parameters such as main-chain dihedral angles and hydrogen-bond parameters of the optimized structure, we confirmed that the conformational stability of an α-helix is affected dominantly by the side-chain conformation (χ_1) of the Phe residue in this system: model A (*T* form: around 180° of χ_1) is most stable in α-helix and model B (G^+ form: around −60° of χ_1) is next stable, but model C (G^- form: around 60° of χ_1) is less stable. In addition, we demonstrate that the stable conformation of poly(L-phenylalanine) is an α-helix with the side-chain *T* form, by comparison of the carbonyl ^{13}C chemical shift measured by ^{13}C CP-MAS NMR and the calculated one.

1. Introduction

It is a challenge of long standing interest to clarify the correlation between the particular secondary structure (main-chain conformation) and the side-chain conformation in proteins. To accomplish it, the precise quantum-chemical calculation for a well-defined and high molecular weight model peptide is essential. Only a few basic researches on the stability of side-chain conformation have been reported [1–5]. Very recently, we have successfully computed the optimized structure of α-helical octadecapeptides such as H–(Ala)$_{18}$–OH [6], H–(Ala–Gly)$_9$–OH [7], and H–(Ala)$_8$–Pro–(Ala)$_9$–OH [8] (where the abbreviations of Ala, Gly and Pro denote L-alanyl, glycyl and L-prolyl residues, respectively), based on the molecular orbital calculation with DFT (density functional theory)/B3LYP (Beck's three parameter hybrid method using the Lee–Yang–Parr correlation functional) [9–11] at the basis set of extended 6-31G(d). We have also computed the nuclear shielding calculation for the optimized structure, based on the GIAO (Gauge-included atomic orbital) – CHF (Coupled Hartree–Fock) [12,13] approach with B3LYP/6-311G(d,p). As a result, we have found that the hydrogen-bond parameters such as hydrogen-bond distances, hydrogen-bond angles, and hydrogen-bond dihedral angles as well as the conformational parameters of the optimized α-helical conformation are very useful for the conformational analysis of polypeptides. Accordingly, it is possible to clarify the relation between the main-chain conformation and the side-chain conformation of a particular amino-acid residue in polypeptides and proteins.

L-Phenylalanine (Phe) has a hydrophobic and aromatic side chain and is one of the most interesting standard amino acid in considering the structure–function relationship in proteins. It is concerned with an important role in organization of secondary structure such as an enhancement in helicity because of intramolecular aromatic side-chain interaction [14], and biological functions such as amyloid fibril formations of human calcitonin known as a cause of deposits in Alzheimer's disease [15,16] and a bioactive species for the μ opiate receptor found in the mammalian nervous system [17].

From such a standpoint of view, in this paper, we focus on the Phe residue and research the influence of the side-chain conformation on the stability of α-helix conformation of a well-defined model polypeptide containing Phe and Ala residues, H–(Ala)$_8$–Phe–(Ala)$_9$–OH. In particular, we evaluate the side-chain effect of the Phe residue on the stability of α-helix main-chain conformation. Furthermore, we obtain the calculated ^{13}C, ^{1}H, ^{15}N and ^{17}O chemical shifts determined from the nuclear shieldings for each optimized structure, and demonstrate that the stable side-chain

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conformation of the Phe residue in α-helical polypeptide can be identified usefully by comparing the calculated the ^{13}C chemical shifts with those measured by ^{13}C CP-MAS NMR.

2. Experimental

2.1. Quantum-chemical calculation

We have designed, in this study, the following model polypeptides for the quantum-chemical calculation.

Model A H–(Ala)$_8$–Phe(*T*)–(Ala)$_9$–OH (T = *trans* : $\chi_1 = 180°$, $\chi_2 = 90°$).
Model B H–(Ala)$_8$–Phe(G^+)–(Ala)$_9$–OH (G^+ = *gauche*(+): $\chi_1 = -60°$, $\chi_2 = 90°$).
Model C H–(Ala)$_8$–Phe(G^-)–(Ala)$_9$–OH (G^- = *gauche*(−): $\chi_1 = 60°$, $\chi_2 = 90°$).

Here, it should be noted that the different conformations of the side chain as a function of dihedral angles χ_1 (N—C$^\alpha$—C$^\beta$—C^1) and χ_2 (C$^\alpha$—C$^\beta$—C^1—C^2). The χ_1 angle is subject to certain restrictions, which arise from steric hindrance between the side-chain phenyl-1 carbon (C^1) atom and the main-chain atom. In this case, they are referred to as *trans*, *gauche*(+) and *gauche*(−). In the *trans* conformation ($\chi_1 = 180°$), the side chain phenyl C^1 atom is opposite the main chain nitrogen. In the *gauche*(+) ($\chi_1 = -60°$), the C^1 atom is opposite the C═O group when viewed along the C$^\beta$—C$^\alpha$ bond. In the *gauche*(−) ($\chi_1 = 60°$), the side chain is opposite the hydrogen substituent on the C$^\alpha$ atom.

Gaussian 03 Rev. C.02 software program [18] was used for structural optimization based on the quantum-chemical calculation with DFT/6-31G(d). We used the density functional theory (DFT), B3LYP (Beck's three parameter hybrid method using the LYP correlation functional) [9–11] with extended 6-31G(d) basis set for the calculation of optimized structure and the GIAO-CHF approach with DFT/6-311G(d,p) for nuclear shielding calculation. In the calculation, reasonable selection of the initial parameters is quite important, and the initial parameters of an α-helical octadecapeptide were adopted $\phi = -57°$, $\psi = -47°$ and $\omega = 180°$ as main-chain dihedral angles of both the Ala and Phe residues. In addition, three allowed side-chain conformations of the Phe residue, the Phe(*T*) ($\chi_1 = 180°$), Phe(G^+) ($\chi_1 = -60°$), and Phe(G^-) ($\chi_1 = 60°$), were used as initial parameters, which were determined by a single point calculation of *N*-acetyl L-phenylalanine *N*′-methyl amide, CH$_3$CO–Phe–NHCH$_3$.

The initial structural parameters for the Ala residues in α-helical H–(Ala)$_8$–Phe–(Ala)$_9$–OH were used the same values as those reported previously [6–8]. The chief initial structural parameters for the Phe residues are as follows: bond lengths (nm): 0.145 (N—C$^\alpha$), 0.152 (C$^\alpha$—C′), 0.134 (C′—N), 0.154 (C$^\alpha$—C$^\beta$, C$^\beta$—C^1), 0.126 (C′═O), 0.100 (N—H), 0.109 (C$^\alpha$—H$^\alpha$), 0.107 (C$^\beta$—H$^\beta$), 0.154 (C$^\beta$—C^1), 0.140 (C—C: phenyl), 0.110 (C—H: phenyl); bond angles (°): 120.0 (H—N—C$^\alpha$, C$^\alpha$—C′═O, N—C′═O, N—C′—C$^\alpha$, H—N—C′ and C′—N—C$^\alpha$), 109.5 (N—C$^\alpha$—H$^\alpha$, C$^\alpha$—C$^\beta$—H$^\beta$, H$^\beta$—C$^\beta$—C^1 and C$^\alpha$—C$^\beta$—C^1), 109.4 (H$^\alpha$—C$^\alpha$—C$^\beta$ and H$^\beta$—C$^\beta$—H$^\beta$), 120.0 (C—C—C, H—C—C: phenyl).

Next, 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 [19] (for ^{13}C) and 1.87 ppm [20] (for ^{1}H) from tetramethylsilane (TMS: δ 0). The ^{15}N and ^{17}O chemical shift references were defined as 98.80 ppm from ammonium nitrate (^{15}NH$_4$NO$_3$, δ 0) [21], and 303.0 ppm from external liquid water (δ 0) for α-helical poly(L-alanine) [22], respectively. The calculated chemical shifts (δ) were determined from the nuclear shieldings (σ) using the following equations: $\delta_C = 177.77 - \sigma_C$ (for ^{13}C), $\delta_H = 32.03 - \sigma_H$ (for ^{1}H), $\delta_N = 213.21 - \sigma_N$ (for ^{15}N), and $\delta_O = 237.33 - \sigma_O$ (for ^{17}O), respectively [6–8].

2.2. Samples

Poly(L-phenylalanine) (MW = 5000–15,000; Lot. No. 044H5530) was purchased from Sigma–Aldrich Inc.

2.3. NMR measurements

The ^{13}C CP-MAS NMR measurements were performed using a Bruker DSX300 spectrometer operating at 75.48 MHz equipped with a CP-MAS probe. The isotropic ^{13}C chemical shifts were calibrated indirectly by external adamantane (29.5 ppm relative to TMS) [19], and the experimental error in the isotropic ^{13}C chemical shifts was estimated to be less than ±0.3 ppm.

3. Results and discussion

3.1. Determination of stable side-chain conformation (χ_1 and χ_2) of CH$_3$CO–Phe–NHCH$_3$

Prior to put the calculation of the optimized structure of H–(Ala)$_8$–Phe–(Ala)$_9$–OH throughout carefully, we searched locally allowed stable side-chain conformation (χ_1 and χ_2) of the Phe residue in order to determine the sets of minimum side-chain conformation. At a first step, we calculated single-point energy of the simplest model peptide such as CH$_3$CO–Phe–NHCH$_3$ by changing a side-chain dihedral angle χ_1 under given condition. Fig. 1 shows the plots of the calculated single-point energy against χ_1 of CH$_3$CO–Phe–NHCH$_3$ under the given dihedral angles of an α-helix ($\phi = -62°$, $\psi = -42°$, $\omega = 178°$) and another side-chain dihedral angle χ_2 (=90° [5]). As seen in Fig. 1, we confirmed three χ_1 values corresponding to the energy minimum point at −59.3°, 49.7° and −173.3°. At a second step, similarly as mentioned above, we calculated a single-point energy of CH$_3$CO–Phe–NHCH$_3$, by changing another dihedral angle (χ_2) under the given condition (χ_1) (not shown). Thus, we roughly determined three sets of energy minimum side-chain conformations as follows: (χ_1, χ_2) = (−59.3°, 106.4°) (total energy = −727.095562931 a.u.: minimum), (49.7°, 85.4°) (−727.093121891 a.u.: +6.40 kJ/mol), and (−173.3°, 86.4°) (−727.090860330 a.u.: +12.33 kJ/mol). Accordingly, it was confirmed that the three dihedral angles χ_1 (−59.3°, 49.7°, and −173.3°) are almost the same as the Phe(G^+) ($\chi_1 = -60°$), Phe(G^-) ($\chi_1 = 60°$), and Phe(*T*) ($\chi_1 = 180°$), respectively, and that the calculated χ_2 (85.4°, 106.4°, and 86.4°) are around 90°. Based on these results, we adopted the locally allowed three sets of dihedral angles (χ_1, χ_2) of the Phe residue as G^+ (−60°, 90°), G^- (60°, 90°), and *T* (180°, 90°), respectively, as the initial parameters in the structural optimization calculation of α-helical H–(Ala)$_8$–Phe–(Ala)$_9$–OH.

3.2. Main-chain conformation (secondary structure) of the optimized α-helical H–(Ala)$_8$–Phe–(Ala)$_9$–OH

First of all, we calculated the optimized structure of α-helical octadecapeptide containing Phe residue, H–(Ala)$_8$–Phe–(Ala)$_9$–OH, by using three sets of locally allowed stable side-chain conformations of the Phe residue as initial parameters as follows: the Phe(*T*), Phe(G^+) and Phe(G^-) conformations.

Fig. 2 shows the optimized α-helical structures of H–(Ala)$_8$–Phe–(Ala)$_9$–OH, with a full DFT/6-31G(d) calculation. The dashed line represents the hydrogen-bonding formation. From the total energy of the optimized structure for the models A: Phe(*T*), B: Phe(G^+) and C: Phe(G^-), it was estimated that the most stable structure was model