Do Bridging Water Molecules Dictate the Structure of a Model Dipeptide in Aqueous Solution?

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Received November 9, 1999
Revised Manuscript Received April 24, 2000

Model dipeptides provide the basic elements of secondary structure (flexible backbone dihedral angles ϕ and ψ) and thus a critical test of peptide model potentials and theories of solvation.[1,2] However, even such simple multi-conformation puzzles have resisted incisive resolution by traditional experimental methods including CD, NMR, and vibrational spectroscopies. In particular, it remains unclear which conformers of alanyl dipeptide (Ac-L-Ala-NHMe, Figure 1) are important in aqueous solution. This report shows that all nine dipolar couplings in the proton nuclear NMR spectrum of AcAlaNHMe dissolved in a water-based liquid crystal can be quantitatively understood in terms of one dominant conformation. Its backbone angles are $\phi \approx -85°$ and $\psi \approx +160°$, similar to the polyproline-II (P_{II}) conformation. P_{II} is one of two equal-energy global minima in a recent electronic structure calculation which placed AcAlaNHMe and four explicit water molecules in an Onsager cavity and optimized the geometry self-consistently using B3LYP/6-31G* density functional theory.[3] The other minimum, α_R ($\phi = -80°$, $\psi = -48°$), is similar to the right-handed α-helix structure. The same two structures have the lowest free energy in a recent molecular dynamics simulation using the CHARMM22 and TIP3P force fields.[4] Based on the calculated P_{II} geometry, we infer that formation of optimal *hydrogen bonding bridges to water* is a key determinant of the structure of AcAlaNHMe in aqueous solution. Since P_{II} is not an energy minimum on the gas-phase potential surface,[3,5,6] we can begin to understand in detail *how the solvent modifies peptide equilibrium structure*.

In liquid crystal NMR (LX-NMR),[7−10] the solvent is a liquid crystalline medium, herein cesium pentadecafluorooctanoate (CsPFO) in D_2O,[11] which subjects the solute to an *orientationally anisotropic mean field*. At 42%/wt, CsPFO forms a lyotropic liquid crystal comprising oblate, disk-shaped *bicelles* about 22 Å thick and 80 Å in diameter (aggregation number ≈ 190) that occupy roughly 24% of the volume of the anisotropic solution.[12]

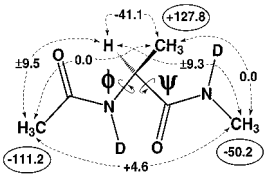

Figure 1. Ac-L-Ala-NHMe structure with best-fit dipolar coupling constants (Hz). Intramethyl couplings in ellipses. Upper and lower set of sign combinations fit spectrum equally well. See Supporting Information Table 1 for details.

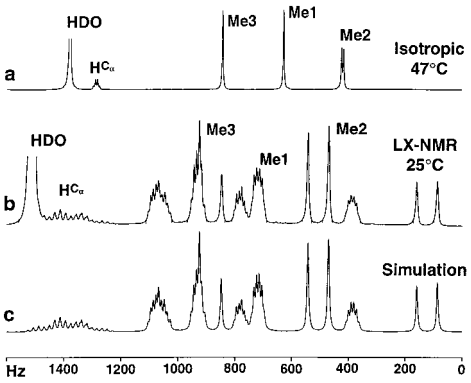

Figure 2. NMR spectra (300 MHz) of AcAlaNHMe in 42%/wt CsPFO in D_2O. Frequency scale with 0 Hz = 0 ppm. (a) Isotropic spectrum at 47 °C. (b) Oriented LX-NMR spectrum at 25 °C. (c) Simulated spectrum using best-fit coupling constants shown in Figure 1.

When heated to the isotropic phase (above 37 °C) and then cooled in the strong magnetic field of an NMR spectrometer, the bicelles form a *macroscopically oriented phase* with its director—the symmetry axis of the mean field—aligned parallel to the magnetic field. Small water-soluble peptides sample primarily an aqueous environment but acquire substantial orientation from transient encounters with the oriented CsPFO bicelles. The resulting NMR spectrum exhibits moderately strong *intramolecular, direct magnetic dipolar couplings* D_{ij} between nuclear spins. To a first approximation, AcAlaNHMe comprises two rigid amide planes attached by tetrahedral hinges to the central C_α. Those D_{ij} with nucleus *i* in one plane and nucleus *j* in the other, or with *i* in one plane and *j* on the central $-CH(CH_3)-$ group, are extremely sensitive to ϕ and ψ and provide the incisive structural handle.

The 300 MHz proton NMR spectrum of Ac-L-Ala-NHMe (used as purchased from Bachem) dissolved at 0.3%/wt in 42%/wt CsPFO in D_2O (prepared as in ref 11) is shown in Figure 2. The LX-NMR dipolar-coupled spectrum of the partially oriented AcAlaNHMe at 25 °C is obtained with deuterium decoupling to remove dipolar coupling between $H^{C\alpha}$ and the two D^N deuterons. The simulation of the dipolar-coupled spectrum was carried out using the program XSIM.[13] Input includes four adjustable chemical shifts, the scalar coupling $J(H^{C\alpha},H^{C\beta})$ fixed at +7.3 Hz by the isotropic spectrum, and nine adjustable D_{ij}. The nuances of the intensity pattern in the region 750−1500 Hz are exquisitely sensitive to the *relative signs* of the D_{ij}. Exhaustive exploration shows that precisely *two* sign combinations (Figure 1) fit the spectrum qualitatively better than all others. The best-fit D_{ij} are accurate to ±0.2 to 0.4 Hz. The mean distance between methyl protons at either end of AcAlaNHMe(P_{II}) is 7.3 Å, yet we readily observe a 4.6 Hz dipolar coupling.

The dipolar coupling between nuclei *i* and *j* depends on the internuclear distance r_{ij} and on the angular distribution of the vector $\boldsymbol{r}_{ij}$ in space:[14]

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10.1021/ja993953+ CCC: $19.00
Published on Web 05/27/2000

$$D_{ij} = K_{ij}\langle r_{ij}^{-3} P_2(\cos\theta_{ij})\rangle = \frac{2}{3}\sum_{n=1}^{N} f_n Tr(\mathbf{S}_n \mathbf{G}_n^{ij}) \qquad (1)$$

Here K_{ij} contains fundamental constants, θ_{ij} is the angle between $\boldsymbol{r}_{ij}$ and the *z*-axis (magnetic field axis), and P_2 is the second Legendre polynomial, $P_2(x) = (3x^2 - 1)/2$. The brackets indicate an average over the motion of the molecule, including both isomerization and reorientation in space. At the right, D_{ij} is rewritten as a discrete sum over N distinct conformers, each representing a free energy minimum in the conformational space of the dipeptide, with f_n the fractional population of conformer n.[15] This assumes that barriers to isomerization are large enough ($2k_BT$ or larger) that the dipeptide spends much more time in local free-energy minima than in the act of isomerization. $\mathbf{S}_n$ is the symmetric, traceless 3×3 orientation tensor for conformer n, describing the angular distribution of a set of Cartesian axes fixed in the conformer. $\mathbf{G}_n^{ij}$ is a 3×3 "geometry matrix" consisting of products of projection cosines of the ij internuclear vector onto the same conformer-fixed axes.

Our eventual strategy will use N candidate geometries from theory to fit more than $5N$ experimental coupling constants to eq 1 in a least-squares sense, with the $5N$ independent elements of the $f_n\mathbf{S}_n$ as fitting parameters. At present, limited to nine observed couplings for AcAlaNHMe, we make the bold assumption that a *single conformer* dominates. In attempting to fit the data to a one-conformer model, we used different detailed local geometries from the energy minima found in the recent density functional calculations.[3] For each choice of frozen local geometry, we varied ϕ and ψ on a 10° grid of 1296 conformer geometries and solved the least-squares problem at each (ϕ,ψ). Methyl rotors sample a uniform distribution of internal rotation angles.

The rms error contour plot using frozen local geometry elements from Suhai's C_7^{ax} minimum[3] is provided in Supplementary Figure 1. Remarkably, a *single conformer* with $\phi_0 = -90°$ and $\psi_0 = +140°$ fits all nine data extremely well when the +9.5, +9.3 Hz sign combination of Figure 1 is chosen. The rms error is 0.14 Hz, and no single error exceeds the error estimate from the spectral fits. The range of angles over which the fit is good (rms error < 0.4 Hz) is quite narrow ($\Delta\phi = \pm 8°$ and $\Delta\psi = \pm 16°$). The fit is unacceptable (4−5 Hz rms error) near the α_R conformation. A second local minimum with rms error of 1.0 Hz (2.5 times experimental uncertainties) occurs at the less physically interesting geometry $\phi = +80°$, $\psi = +130°$. When the alternative −9.4, −9.2 Hz sign combination is chosen, the rms error at the P_{II} conformation soars to 4.5 Hz and no (ϕ,ψ) fits the data at all well. These results depend weakly on the local geometry chosen. For the +9.5, +9.3 Hz sign choice, the rms error at the P_{II} minimum among eight choices of local geometry varied from 0.14 to 0.9 Hz, with four geometries giving rms errors below 0.45 Hz. In all cases the best fit has ϕ_0 in the range $-85 \pm 5°$ and ψ_0 in the range $+160 \pm 20°$, clearly P_{II}-like.

Additional dipolar couplings can test for the presence of a minority conformation such as the α_R conformer also favored by theory. ^{15}N and ^{13}C labels will provide these. Perhaps α_R lies 1 kcal/mol or more higher than P_{II} in free energy, but it is also possible that α_R has a substantial population but orients much more weakly than P_{II}. Measurements on AcAlaNHMe in different liquid crystalline media may provide new insight into the orientation mechanism. For now, we infer that P_{II} is a free energy minimum in our solution of AcAlaNHMe in CsPFO/D_2O. In early interpretations of CD and traditional NMR data for Ac-L-AlaNHMe, the P_{II} structure was considered likely to be important in aqueous solution, but C_7^{eq}, C_5^{ext}, and α_R were frequently mentioned as well.[16−18]

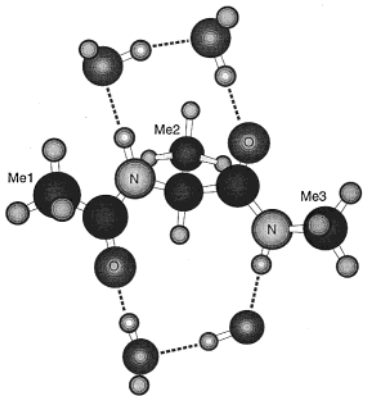

Figure 3. Calculated polyproline II (P_{II}) geometry from Suhai and coworkers (ref 1) optimized using density functional electronic structure theory plus four explicit water molecules in an Onsager solvent cavity.

Since both electronic structure theory[3] and the latest MD simulations[4] agree with experiment that P_{II} is an energy minimum, the theoretical results can then provide a more detailed understanding of how the geometry is determined. In Suhai's optimized structure of AcAlaNHMe + 4 H_2O in a spherical Onsager cavity (Figure 3), P_{II} forms two *double-water bridges*. Evidently the gas-phase intrachain potential (which favors the C_5^{ext} and C_7^{eq} structures) combines with the effects of explicit hydrogen bonding to determine the overall energy minimum. P_{II} is seemingly better configured than C_5^{ext} or C_7^{eq} to make strong water bridges. P_{II} is unusual in that both of the O−C···N−H units to which water dimers bind are coplanar within 25°, permitting nearly linear hydrogen bond angles throughout both bridges. The two bridges differ substantially in overall size. Such a bridging structure suggests that effects of cooperative hydrogen bonding may be quite important in a priori prediction of peptide structure and energetics.[19] The most recent MD simulations using the CHARMM22 potential[20] for AcAlaNHMe and the TIP3P potential[21] for water *also* find P_{II} and α_R as the lowest free-energy structures.[4] Examination of the MD configurations for evidence of single-, double-, and triple-water bridges becomes very interesting. On the electronic structure side, it is important to test the sensitivity of Suhai's results to the number of explicit water molecules in the cavity.

With help from theoretical geometries, LX-NMR seems poised to unravel the conformational preferences of a variety of small peptides. The resulting insight into equilibrium conformational preferences in turn can guide development of better model peptide potentials and more accurate theories of solvation.

Acknowledgment. We thank Professor S. Suhai for sending details of his calculated geometries. J.C.W. thanks the UW-Madison Graduate School and Chemistry Department for sabbatical support, C.-D.P and E.T.S. for their kind hospitality during that leave. E.T.S. acknowledges support from the National Science Foundation (DMR-9412701).

Supporting Information Available: Table of best-fit chemical shifts and dipolar couplings and figure of contour plot (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA993953+

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