Communication

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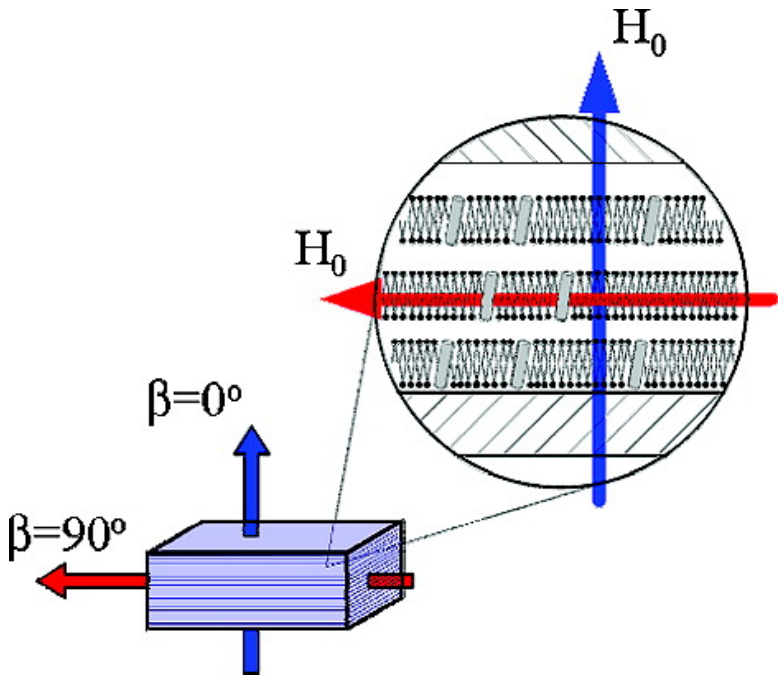
J. Am. Chem. Soc., **2008**, 130 (38), 12584-12585 • DOI: 10.1021/ja803734k • Publication Date (Web): 03 September 2008



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Published on Web 09/03/2008

Comparison of "Polarization Inversion with Spin Exchange at Magic Angle" and "Geometric Analysis of Labeled Alanines" Methods for Transmembrane Helix Alignment

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Transmembrane-anchored WALP peptides of sequence acetyl−GWW(LA)$_n$LWWA−[ethanol]amide—and the corresponding KALP peptides having KK instead of WW anchors—have been used extensively to characterize fundamental aspects of lipid/protein interactions.[1−4] Of particular interest has been the intrinsic tilt of these single-span α-helical peptides in hydrated lipid bilayer membranes. Consistently nonzero tilt angles (with respect to the bilayer normal) have been observed using solid-state ^{2}H NMR and the geometric analysis of labeled alanines (GALA) method.[5−7] The GALA analyses of the 19-residue WALP19 ($n = 6$) as well as WALP23 ($n = 8$), and KALP23, have indicated quite small average tilt angles τ in the range of about 4°−12° in several lipids. These experimental results have differed from predictions of much larger average tilt angles based upon molecular dynamics simulations.[8−10] A possible resolution to the discrepancy has entailed suggestions of averaging effects due to large-scale rotational motions of the peptide helices.[11,12]

In contrast to GALA, the polarization inversion with spin exchange at magic angle (PISEMA) approach to transmembrane helix orientation[13,14] uses ^{15}N−^{1}H dipolar coupling. Because of the relative orientations of Ala-CD$_3$ side chains and ^{15}N-amide planes in an α-helix, the GALA and PISEMA methods are expected to have different sensitivities to molecular motion. For this reason, we sought to analyze a new peptide/lipid system using both methods. For this direct comparison, we introduce acetyl−GGALW(LA)$_6$LWLAGA−ethanolamide, which we have prepared with ^{15}N and with ^{2}H labels. This peptide, designated GWALP23, features a single Trp (W) anchor near each end.

GWALP23 was synthesized with 65−100% ^{2}H-Ala at positions 7 and 17, 9 and 15, or 11 and 13; or with ^{15}N labels on residues 7−17 or 13−17. Peptides were cleaved from Wang resin using ethanolamine, thus avoiding any use of (potentially damaging) trifluoroacetic acid. Mechanically aligned samples of GWALP23 in dilauroylphosphatidylcholine (DLPC, 1/20; 45% hydration, w/w) were prepared,[5] and solid-state NMR spectra were recorded (40 °C, $\beta = 0°$)[4] using Bruker Avance-300 (^{2}H) and Varian Inova 500 (^{15}N−^{1}H) spectrometers and established pulse sequences.[4,5,13]

Figure 1 illustrates ^{2}H NMR spectra for labeled samples of GWALP23 in DLPC, with peak assignments based upon ^{2}H abundance at the different Ala positions.[15] From the magnitudes of the ^{2}H quadrupolar splittings, it is apparent that the peptide is significantly tilted with respect to the DLPC bilayer normal.[5]

We also labeled GWALP23 with ^{15}N in the peptide planes of residues 7−17, or the subset of residues 13−17. The two-dimensional PISEMA spectra for these samples (Figure 2) were assigned by considering "PISA" wheel fits[13] for residues 13−17

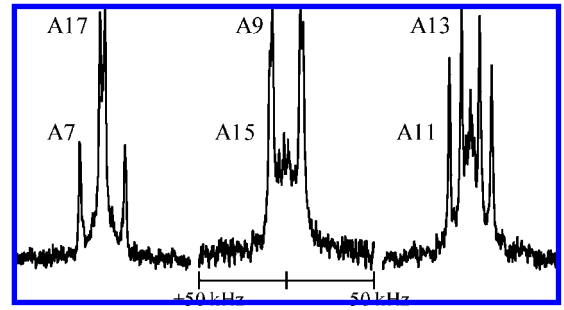

Figure 1. ^{2}H NMR spectra of labeled alanines in GWALP23/DLPC.

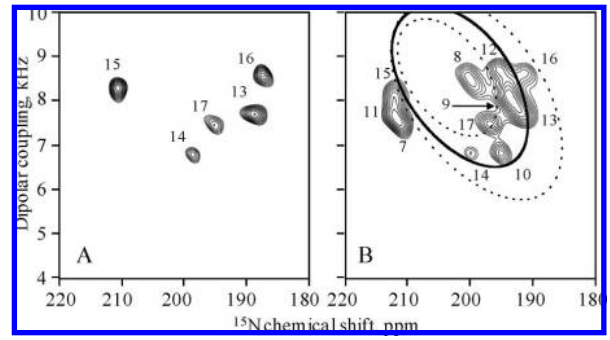

Figure 2. PISEMA spectra for GWALP23/DLPC with (A) 5 labels or (B) 11 labels, with peak assignments. The theoretical PISA helical wheels represent τ of 7.8° or 10.8° (best fit; solid curve, with $\rho = 346°$) or 13.8°. Spectra were recorded using 2.08 ms of total evolution time in t1, and 5.12 ms of acquisition time in t2, with the ^{1}H frequency centered at 4.7 ppm [see Supporting Information for a (τ, ρ) contour plot].

(Figure 2A), and for the remaining six residues 7−12, using a difference spectrum. Backbone dihedral (ϕ,Ψ) angles of (−65, −40), appropriate for "membrane coils,"[16] were used for the PISA wheel fits. A unique "connection" from Leu^{12}−Ala^{13} is observed to link the two spectra (Figure 2).

With assignments in hand (Table 1), a best-fit PISA wheel for all 11 residues in the GWALP23 core helix could be determined (Figure 2B), yielding tilt τ of 10.8° and rotation ρ of 346° for GWALP23 in DLPC. (The definition of τ is unambiguous between the GALA and PISEMA analyses; for ρ, we use the GALA definition with Gly[1] as origin.[5] For the best fit, the rmsd is 15.6 ppm (0.8 kHz) for the ^{15}N chemical shift and 0.9 kHz for the ^{15}N−^{1}H dipolar coupling.) The PISEMA fits are sensitive to the dipolar coupling constant $\nu_{||}$[17] and to the magnitudes of the principal elements of the ^{15}N chemical shift tensor, particularly σ_{33},[13] averaged over the sequence of labeled residues. We could obtain low rmsd fits using σ_{33} within the range 210−214 ppm. For the

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10.1021/ja803734k CCC: $40.75

Table 1. Measured NMR Parameters for GWALP23 in DLPC.[a]

residue	$-CD_3$ $\Delta\nu_q$ (kHz)	^{15}N chemical shift (ppm)		$^{15}N-^{1}H$ coupling (kHz)	
Ala-7	25.8	209		7.4	
Leu-8		198		8.5	
Ala-9	16.0	193		8.1	
Leu-10		194		6.9	
Ala-11	24.3	210		7.8	
Leu-12		195		8.7	
Ala-13	10.3	192[c]	188[b]	7.8[c]	7.8[b]
Leu-14		199	198	6.9	6.9
Ala-15	18.4	210	209	8.2	8.3
Leu-16		191	187	8.6	8.6
Ala-17	2.7	196	194	7.6	7.5

[a] $\beta = 0°$ sample orientation. [b] Figure 2A. [c] Figure 2B.

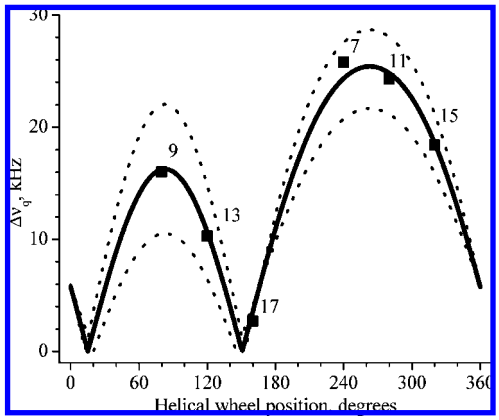

Figure 3. GALA fits to ^2H NMR data. The best-fit solid curve shows $\tau = 12.6°$, $\rho = 306°$ (with $\varepsilon_{||} = 58.7°$, rmsd = 0.74 kHz).[5] Other curves represent $\tau = 9.6°$ (lower) and $\tau = 15.6°$ (upper) [see Supporting Information for a (τ, ρ) contour plot].

simulations in Figure 2B we used σ_{33} of 212 ppm and a motionally averaged $\nu_{||}$ of 10.4 kHz.[17] If a smaller σ_{33} of 210 ppm is assumed, then τ becomes about 1° smaller.

We independently determined τ and ρ from GALA analysis of the ^2H NMR spectra (Figure 3). Using a principal order parameter S_{zz} of 0.87, which is typical for WALP peptides,[5] we find once again that GWALP23 is moderately tilted in DLPC. The agreement between the GALA and PISEMA methods is close, with GALA indicating a best fit of (12.6, 306) to (τ, ρ), compared to (10.8, 346) for PISEMA. The PISEMA analysis is likely less sensitive to molecular motion than is GALA.[11] Indeed, if S_{zz} of 1.0 is used, the GALA fit to (τ, ρ) is (10.9, 306), even closer to the PISEMA prediction for τ.

The duplicate measurements for residues 13−17 in Figure 2A and 2B permit an estimate of experimental reproducibility. For these two independently synthesized samples of GWALP23 in DLPC, we observe deviations of up to 4 ppm (0.2 kHz) in the ^{15}N chemical shift, and up to 0.1 kHz in the $^{15}N-^{1}H$ dipolar coupling (Table 1). The uncertainty in hydration and mechanical alignment to achieve $\beta = 0°$ is a significant source of experimental error (see Supporting Information for ^{31}P NMR spectra). The GALA and PISEMA results are in good agreement, with the two independent methods predicting τ values of 12.6° and 10.8°, respectively. The rmsd values are less than 1 kHz for each observable parameter: ^2H quadrupolar splitting, ^{15}N chemical shift, $^{15}N-^{1}H$ dipolar coupling. Both methods are less sensitive in determining ρ compared to τ.[5,13] Yet even the ρ values, which define direction of peptide tilt with respect to C_α of Gly^1 (reference point), are similar, being 306 ± 10° for GALA and 346 ± 10° for PISEMA. The sensitivities of the methods to molecular motion are expected to be different, because the average $C_\alpha-C_\beta$ bond direction—with respect to the helix axis—is close to the magic angle, whereas the average N−H direction is far from the magic angle. Within this context, the experimental agreement is striking.

In light of the experimental agreement between the inherently different GALA and PISEMA methods on the tilt of GWALP23, ~10° to 13° in hydrated bilayers of the short lipid DLPC, questions remain about the predictions of larger tilt angles when using MD simulations.[8−12] GWALP23 itself has not yet been simulated by MD. For WALP23, the MD simulations have indicated a tilt of 32.7 ± 8.5°, using an implicit membrane model with a 23 Å hydrophobic thickness,[8] or 33.5 ± 9.0° using a DMPC bilayer model[11] (or 31 ± 12° for WLP23 in DMPC);[12] compared to 5.5° experimentally using GALA.[6] For WALP19, the MD-simulated tilt has been reported as 15.5 ± 6.7° for an implicit membrane,[8] or 12.5 ± 7.5° in DMPC,[9] compared to an experimental GALA tilt of 3.6°.[4] Several possible explanations come to mind: (a) Perhaps the case of GWALP23 in DLPC is atypical, showing only "accidental" concurrence of the PISEMA and GALA experimental methods. Clearly, more peptide and lipid samples warrant testing. (b) Further systematic refinements of the computational methods may be needed. (c) Different hydration levels could affect bilayer thickness and peptide tilt.[18] (d) Complex motional averaging scenarios, which could compromise the ^2H quadrupolar splittings and GALA analysis,[11] could in principle also compromise the $^{15}N-^{1}H$ dipolar couplings and PISEMA analysis. Resolution of the underlying issues will await further experiments and further calculations. We note that the design of GWALP—with its single Trp anchors—should offer advantages for interpreting the anchor residue dependence of the tilt and other biophysical properties.

Acknowledgment. This work was supported in part by NIH grants P20RR15569 and R01GM70971 and utilized the Biomedical Technology Resource for NMR Molecular Imaging of Proteins, supported by grant P41EB002031.

Supporting Information Available: ^{31}P NMR spectra to show sample alignment. Contour (τ, ρ) plots for GALA and PISEMA. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA803734K