Re-alignment and oblique tilt of the antimicrobial peptide PGLa in lipid membranes observed by solid state ^{19}F-NMR

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Supporting Information

CD-measurement of peptide secondary structure

Circular dichroism (CD) spectra of the peptides were recorded in the presence of small unilamellar vesicles from a mixture of the lipids 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-sn-glycero-3-[phospho-rac-(1-glycerol)] (DMPG), 60:40. The CD spectra at a peptide : lipid = 1 : 50 ratio were very similar to those at 1 : 200 and the helix content was slightly increased (Table S1). We thus concluded that the central part of the peptide remains in an α-helical conformation and the changes in the ^{19}F NMR spectra of the labels result from a different orientation of the peptide.

Table S1. Helix content of PGLa and its CF_3Phg analogs

peptide : lipid ratio epimer	1:50 L-CF_3-Phg	1:50 D-CF_3-Phg	1:200 L-CF_3-Phg
PGLa wild type	66 %	-	60 %
PGLa- Ile9CF_3-Phg	63 %	60 %	53 %
PGLa- Ala10CF_3-Phg	56 %	61 %	48 %
PGLa- Ile13CF_3-Phg	59 %	60 %	54 %
PGLa- Ala14CF_3-Phg	56 %	53 %	48 %

Calculation of peptide orientation from ^{19}F dipolar coupling

We defined a molecular frame in which the z-axis is the helix axis of the peptide (C-terminus towards +z) and the C_α atom of Lys12 lies radially on the y-axis. The tilt angle, τ, and the azimuthal rotation of the helix, ρ, define a set of Euler angles {0, τ, ρ} that describes the orientation of the molecule in the laboratory frame, in which the z-axis is the membrane normal. The relative arrangement of the individual labeled CF_3-groups in the molecular frame is defined by the molecular structure. The dynamic motion of both the molecule with respect to the membrane and the label with respect to the molecular framework are described by an isotropic order parameter, S_{mol}, which scales down the dipolar coupling of the rotating CF_3-group. The signed dipolar couplings of the individual labels are predicted as a function of τ, ρ and S_{mol} as described before.[1] For all labeled positions, the sum of squared deviations to the experimentally determined couplings, χ^2, is determined with MATHEMATICA (http://www.wolfram.com) and compared with the expected experimental error. The ρ/τ plots shown in Figure 2A and B are contour plot of the minimum of χ^2 in the meaningful range $0.4 \leq S_{mol} \leq 0.9$. An example calculation is available as supporting information under the filename PGLa_19F.nb. This file was created by MATHEMATICA, release 4, and can only be read by this or newer versions of MATHEMATICA.

Details of the procedure are described on http://www-ifia.fzk.de/IFIA_Webseiten/Webseiten_Ulrich/publications/docs/JMR-2004-Glaser/orientation_determination_websit.html where further programs and scripts for orientational calculation from ^{19}F-NMR data are publicly available.

The schematic ^{19}F-NMR spectra shown in the figure at the table of content are scaled by an order parameter S_{mol}=0.63, which we found as a result of our calculation.

Variation in the model structure

To determine molecular orientation and dynamics from the time-averaged orientation of individual labels, the relative orientation of the labels in the molecule has to be known. Although we know that the central part of the peptide has an α-helical secondary structure the uncertainty of structural details can be responsible for larger error margins than the experimental uncertainty of dipolar couplings.[1] Therefore, we have compared the experimental ^{19}F dipolar couplings at P:L = 1:50 with possible peptide orientations for a range of different α-helical structures. We used regular helical structures with side chain angles β between 105 and 120° and pitch angles ω between 95 and 102°, and we also used coordinate sets from energy-minimized structures.[1]

All of these structural models showed a minimum of χ^2, i.e. good agreement with experiments, for an obliquely tilted state with a tilt angle τ between helix axis and membrane normal in the range between 120 and 140° and a rotational angle ρ between 80 and 100°. For some structures we found a second minimum of χ^2 at low tilt angle τ (Table S2). The experimentally determined dipolar coupling values of the four L-CF_3-Phg labels alone were therefore not sufficient to safely exclude the possibility that PGLa changes into an inserted transmembrane orientation at high peptide:lipid ratio.

Table S2. Best fitting orientations for various model structures of PGLa

Structure	Orientation						
	ω (°)	α (°)	β (°)	χ^2 (kHz^2)[c]	τ (°)	ρ (°)	S
α-helix, standard[a]	99.8	53.2	121.8	0.16	122.5	84.6	0.63
α-helix, energy-minimized[b]	91-7-98.4	43.6-51.9	109.8-111.6	0.18 1.22	130.6 4.0	100.3 69.7	0.64 0.9
regular α-helix	100	47	110	0.17 1.54	133.4 5.2	90.8 100.5	0.63 0.9
regular α-helix	96	47	110	0.19	129.8	91.6	0.67
regular α-helix	102	47	110	0.11 1.16	135.0 5.4	90.6 101.6	0.64 0.9
regular α-helix	100	47	105	0.19 0.15	139.5 12.0	91.8 96.4	0.63 0.9
regular α-helix	100	47	115	0.16	128.9	90.9	0.63

[a] secondary structure of poly-Ala chains as constructed in SYBYL with φ=-58°/ψ=-47°. [b] α-helix after 500 steps of energy-minimization. [c] All local minima with $\chi^2 < 2$ kHz^2 are shown.

Orientational information from D-CF_3-Phg epimers

As a by-product of synthesis and purification of CF_3-Phg labeled PGLa analogs we also obtained peptides with the NMR label in D-conformation.[1] ^{19}F-NMR spectra of these peptides were recorded to obtain additional qualitative information about the peptide orientation and dynamics (Table S3). The inclusion of these data confirmed the obliquely tilted orientation and it also excluded the possibility of a transmembrane orientation of the PGLa helix. The chemical shift of the ^{15}N label at Gly11 provides a more convincing evidence for the tilted orientation, however, since effects of the ^{15}N label on orientation can be excluded.

Table S3. ^{19}F dipolar couplings [in kHz] of D-CF_3Phg-labeled epimers of PGLa

position labeled with D-CF_3-Phg	Ile9	Ala10	Ile13	Ala14
P:L ratio 1:200	0	12.1	-3.5	8.2
P:L ratio 1:50	-4.7	10.7	-4.1	7.6

^{15}N experiments

^{15}N NMR experiments were performed at 50.68 MHz on a Bruker Avance 500 MHz NMR spectrometer, using a ramped cross polarization sequence with a CP power of 40 kHz, 2.5 s relaxation delay time, 100 kHz spectral width, 2048 data points, and tppm20 proton decoupling. In hydrated samples the contact time was 1 ms, in dry peptide powder a 2 ms contact time was used. Between 30,000 and 200,000 scans were collected. Spectra were referenced to $^{15}NH_4NO_3$ by setting the signal of solid $^{15}NH_4Cl$ to 18.0 ppm.

The ρ/τ plots shown in Figure 2C and D were obtained in a similar way as described above by comparison of experimentally determined chemical shift values (Fig. 2E/F) and calculated data. For calculation of the ^{15}N chemical shift of the oriented peptide we used the values σ_{11}=14, σ_{22}=33 and σ_{33}=197 ppm resulting from a fit to the experimentally determined tensor spectrum of the peptide powder. We used an orientation of the ^{15}N tensor of the labeled glycine with σ_{33} tilted 17° from N-H toward the N-C' bond, and σ_{11} normal to the plane of the peptide bond. The order parameter was set to S=0.7. There was no agreement of the ^{15}N chemical shift data with a transmembrane I-state orientation for any order parameter 0<S<1.

REFERENCES

(1) Glaser, R. W.; Sachse, C.; Dürr, U. H. N.; Wadhwani, P.; Ulrich, A. S. *J. Magn. Reson.* **2004**, *168*, 153-163.