CF3-groups as labels in solid-state 19F-NMR of biomembranes: fundamental aspects and experimental strategies

Carsten Sachsea, Ralf W. Glaserb, Ulrich H.N. Dürrb, c and A.S. Ulrichb,c
Friedrich-Schiller-Universität Jena, b) University of Karlsruhe and c) Forschungszentrum Karlsruhe
ulrich.duerr@fz-juelich.de

Introduction

Our group has established 19F-labeling of biomolecules as a strategy for the structure elucidation of peptides in biomembranes. So far, amino acid analogues carrying a single 19F-nucleus had been employed, which proved to possess expected favourable NMR properties, namely high sensitivity and very little or no distortion of biomembrane structure and function (cf. paper by P. Wadhwani).

To enlarge the set of viable fluorine labels, CF3-groups may be inserted into membrane-active molecules by peptide synthesis. Amino acid derivatives used so far include CF3-L-α-amino-isocarbostyril acid and 4-CF3-phenylglycine. The latter is used extensively in the antimicrobial peptide PGLa, depicted on the left.

Besides a further increase in sensitivity, we expect additional favourable NMR properties from the CF3-group, especially the possibility to analyze the orientation dependence of the dipolar coupling. However, spectral analysis of the CF3-group is rather complex due to the presence of both dipolar couplings and CSA interactions, which are on the same order of magnitude.

Due to the very broad lineshapes encountered in solid-state 19F-NMR, echo pulse sequences have to be employed, which separate the nuclear signal from the proton ring-down noise. The Hahn echo sequence (top) is used to refocus the chemical shift anisotropy (CSA) as well as heteronuclear dipolar interactions, while a quadrupolar echo sequence (bottom) is necessary to refocus the homonuclear dipole coupling.

In the CF3-group both interactions are present. Therefore, none of the mentioned echoes will be adequate for the CF3-group. Antonijevic and Wimperis have suggested a special phase cycling scheme for the quadrupolar echo sequence, designed to refocus all interactions.

Another option is to suppress the CSA contribution to the spectra altogether, using the CPMG multipulse sequence. Thereby dipolar splitting can be measured at high accuracy, although without sign. Additionally, the CSA is available from echo spectra and yields the sign of the splitting.

Pulse sequences

The lineshape becomes considerably less complicated when peptides carrying CF3-groups are incorporated into macroscopically aligned lipid bilayer samples. The two extreme positions marked in the previous panel (corresponding to vertical and parallel orientation with regard to the magnetic field) can now be accessed individually by tilting the oriented sample. Note that the triplet's spectral shift due to chemical shift anisotropy allows determination of the sign of dipolar splitting.

For future applications, we expect to gain accuracy by applying the Antonijevic and Wimperis phase cycle and their CPMG multipulse sequence, which suppresses CSA interaction and allows for a more exact determination of the dipolar interaction.

Oriented lipid samples

The lineshape becomes considerably less complicated when peptides carrying CF3-groups are incorporated into macroscopically aligned lipid bilayer samples. The two extreme positions marked in the previous panel (corresponding to vertical and parallel orientation with regard to the magnetic field) can now be accessed individually by tilting the oriented sample. Note that the triplet's spectral shift due to chemical shift anisotropy allows determination of the sign of dipolar splitting.

We tested different pulse sequences on the oriented lipid samples, as depicted on the left. Due to the considerably narrower spectra of the oriented samples, one-pulse experiments turned out to be practically feasible in liquid crystalline samples.

Conclusions

In summary, we have given an overview of the lineshape distortions expected in the spectral analysis of CF3-groups under different echo sequences. It was possible to predict the complex lineshapes for the expected powder spectra by SIMPSON simulations. Using oriented samples and special pulse sequence schemes, these problems can be overcome in the study of membrane-active peptides.

From a number of CF3-labeled analogues of the antimicrobial peptide PGLa it was possible to measure the sign of the dipolar splitting. The acquired data allowed determination of the peptide's orientation in the lipid bilayer. In that analysis we found a highly interesting re-alignment process presented in detail on Erik Stradinsberg's poster.

The authors wish to thank Iasa Antonijevic and Stephen Wimperis for discussing their phase cycle prior to publication.

CF3-groups as solid state NMR labels

The figure shows two fluorine analogues of the non-natural amino acid phenylglycine (Phg). It has the advantage of supplying a rigid connection of the 19F-reporter to the protein backbone. Therefore, determination of the 19F-CSA orientation will give direct information on the global molecular alignment in the lipid membrane.

In the mononitrinated 4-F-phenylglycine (far left), which was used in a number of previous studies, the CSA tensor has a high asymmetry γ, whose analysis requires additional information on the sidechain torsion angle ψ.

In contrast, the CF3-group in 4-CF3-phenylglycine (left) rotates freely, thus giving an axially symmetric CSA tensor independent of ψ. Moreover, it exhibits strong intra-group homonuclear dipolar coupling, which may be utilized in CPMG experiments.

Model CF3-system under different echoes

To gain some heuristical insight into the spectral distortions that have to be expected, we calculated the parameters of a hypothetical CF3-spin system under the different echo sequences. We used the simulation package SIMPSON to generate the model spectra depicted below.

CSA parameters were chosen as a=−60ppm for the total width and γ=0.0 for the asymmetry parameter, reflecting averaging due to the CF3-group's rotation. The isotropic chemical shift position was chosen as δ=65ppm, according to MAS studies of 4-CF3-Phg, and a maximum dipolar splitting of δd=8kHz was assumed.

The pulse sequences used on-ideal t 1 pulses with a 90° width of 6kHz. The carrier frequency TOF was set to different values (cf. figure) to allow evaluation of off-resonance effects. Echo delay times were τ1=τ2=25µs. A B0 field strength of 470 MHz fluorine resonance frequency was used.

The simulations clearly show that the quadrupolar echo using Antonijevic and Wimperis' phase cycle is well-suited to retrieve the ideal spectrum depicted to the left. This could otherwise only be expected from a—technically impossible—experiment with a single, ideal 90°-puls. The picture also shows the NMR parameters characterizing the spectrum. The values of both the CSA (a) and the dipolar coupling (δd) reflect the spatial orientation of the CF3-group, as indicated by the arrows in the figure.

CF3-labeled membrane-active peptide

As a system of biological relevance, we studied the antimicrobial α-helical peptide PGLa, a member of the magain family. Its amino acid sequence is NH2-GAGASKGAAIKGKVKALKALCOOH. We synthesized four analogues carrying CF3-phenylglycine mutations in four different hydrophobic positions. The spectra obtained in single-pulse experiments on macroscopically aligned bilayer samples are presented in the left figure.

The spectra taken at different peptide/lipid ratios show dramatic changes in the dipolar splittings observed at low and high peptide concentrations. The table on the right shows the signed values of the dipolar splittings extracted from the 1-pulse spectra. These values are used as input to calculate the peptide's orientation in the lipid bilayer. The analysis demonstrates that PGLa is aligned flat on the membrane surface at low peptide lipid ratio. At high peptide concentration, however, it flips its alignment to make a 60° angle with respect to the membrane normal. The details and biological implications of this model are presented in detail on Erik Stradinsberg's poster.

References

2 Antonijevic, and S. Wimperis, accepted for publication in J. Magn. Reson.