Solid-state $^{19}$F-NMR relaxometry to monitor the local mobility of membrane-bound peptides

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Summary

We used solid-state $^{19}$F-NMR relaxation to probe the folding and aggregation state of membrane-active peptides in a bilayer environment. Using highly sensitive $^{19}$F-NMR enabled us to measure local relaxation parameters along the amino acid sequence. Two antimicrobial peptides with distinct secondary structures and self-assembly properties were studied this way, the helical peptide PGLa and the $\beta$-strand forming peptide KIGAKI. Whilst $T_r$-relaxation probing fast molecular motions, showed no variance along the peptide sequence, strong variations in $T_r$-relaxation reflect different local mobility on a slower timescale. In particular in the case of KIGAKI, the relaxation experiments were able to reveal self-assembly of the peptide...

Aims

- Where is the peptide folded when bound on the membrane?
- Does the peptide self-assemble oligomerize?
- Which parts of the peptide are involved in oligomerisation?

19F-label

The peptides were labeled in one varied position with trifluoromethyl-bicyclopentanylglycine (CF$_3$-Bpg), an artificial amino acid allowing a direct connection of the $^{19}$F-label with the peptide backbone.

KIGAKI was labeled with both L- and D-CF$_3$-Bpg, which results in different aggregation behavior.

Aggregating $\beta$-strand peptide KIGAKI

The $T_r$-values resemble the values of PGLa, and seem to be system independent, confirming that $T_r$ reports the fast internal motions of the CF$_3$-Bpg sidechain.

Overall, the $T_r$-relaxation times of KIGAKI labeled with L-CF$_3$-Bpg were found to be lower than for PGLa, indicating higher rigidity due to aggregation.

When labeled with D-CF$_3$-Bpg, this aggregation is prevented, reflected in the higher relaxation times. D-CF$_3$-Bpg near the N-terminus in position 2 is not able to prevent aggregation.

$T_r$ relaxation is lower in positions 2, 4, 6 and higher in positions 8, 10, 14. Possibly only the N-terminal half of KIGAKI is involved in aggregates, for example in anti-parallel $\beta$-sheets.

Conclusions

We used solid-state$^{19}$F-NMR relaxation measurements for the first time to scan the local mobility of membrane-bound peptides along the amino acid sequence. This way we were able to reveal a large variability of mobility on the slow time scales monitored by $T_r$-relaxation, and characterize the self-assembly behavior of the KIGAKI peptide.

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Relaxation experiments at the magic angle

Relaxation was measured in mechanically oriented lipid bilayer samples. By aligning the bilayer normal at the magic angle, the rotational diffusion of the peptides averages the anisotropic interactions to zero, giving rise to substantial line narrowing and signal/noise improvement. Because the $^{19}$F-$^{19}$F dipolar coupling within the CF$_3$-label is removed, the measurement of $T_r$-relaxation times is facilitated.

$\alpha$-helical peptide PGLa

GMASKAGAIGKIKVALKA-NH$_{3}$
in DOPC bilayers (peptide/lipid = 1:50)

$T_r$-relaxation:

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The $T_r$-values are almost constant along the amino acid sequence, and might reflect the fast internal motions of the CF$_3$-Bpg sidechain rather than the backbone mobility.

The $T_r$-relaxation times vary strongly along the sequence, in contrast to $T_1$. Variations of $T_r$ along the sequence are larger than sample-to-sample differences, hence there are local differences in mobility on the slow time scale.

Correlation with structure:

No correlation of the $T_r$-relaxation was observed with the $^{19}$F-$^{19}$F dipolar couplings of the CF$_3$-Bpg label (reflecting the sidechain orientation), nor with the angular sidechain position around the helix.

Aggregation of $\beta$-strand peptide KIGAKI

KIGAKI labeled with both L- and D-CF$_3$-Bpg, which results in different aggregation behavior.

$T_r$-relaxation:

The $T_r$-relaxation times of KIGAKI labeled with L-CF$_3$-Bpg were found to be lower than for PGLa, indicating higher rigidity due to aggregation.

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