Structural Studies of Membrane-Bound Piscidin by ^{13}C Magic Angle Spinning Solid-State NMR

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Introduction

Piscidin 1 (p1) is a highly potent antimicrobial peptide (AMP) that was discovered in hybrid striped sea bass[1,2]. Its combined broad spectrum antimicrobial action and activity against HIV-1 place it among the top six peptides in a database of more than 2,200 AMPs. Its anti-cancer activity further accentuates its biological importance. In this research, structure-function relationship investigations are carried out on p1 to better understand what molecular properties of the peptide enable it to remain active under varying environmental conditions. Specifically, piscidin is studied under native-like conditions using solid-state NMR. In previous studies, we obtained the backbone structure of piscidin in the presence of two bacterial cell mimics (PDB accession numbers: 2MCU and 2MCV)[3,4,5]. Here, we explore the possibility of obtaining the structure of the side chains so that the role of specific side chains in mediating functionally important peptide-lipid interactions can be better understood.

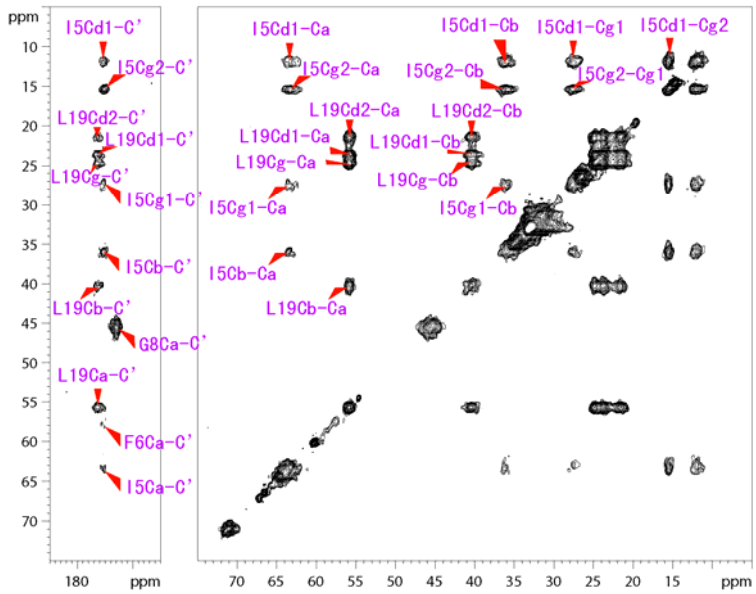

Fig. 1. ^{13}C-^{13}C chemical shift correlation spectrum of ^{13}C/^{15}N-labeled [I5F6G8L19]-p1 in 3:1 DMPC/DMPG at 313 K. The experiments were performed on the 900 MHz NMR spectrometer with a sample spinning of 12 kHz.

^{13}C-^{13}C chemical shift correlation experiments were performed on ^{13}C/^{15}N-[I5F6G8L19] p1 (FFHHIFRGIVHVGKTIH-RLVTG) in the presence of 3:1 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC)/1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG) to mimic bacterial cell membranes.

Experimental

MAS experiments were performed on the NHMFL Ultra-Wide Bore 900 MHz Bruker Avance NMR spectrometer fitted with a state-of-the-art low-E probe for optimum performance.

Results and Discussion

The 2D MAS spectrum obtained on p1 in 3:1 DMPC/DMPG at a 1:20 peptide-to-lipid ratio is provided in Fig. 1 The signals are well resolved and give rise to unique patterns that allow unambiguous assignments to be made.

Conclusions

Using MAS solid-state NMR on backbone and side-chain labeled p1, high-resolution spectroscopy was performed. It is demonstrated that the resolution is excellent, which bodes well in terms of continuing structural work that aims at obtaining a full structure of p1 under biologically relevant conditions.

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References

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