Disruptive Effects of Antimicrobial Piscidin on Cancer Cell Mimics: A Solid-State Deuterium NMR Study

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Introduction

Piscidin 1 (p1) is a highly potent antimicrobial peptide (AMP)[1,2] that has also anti-cancer properties against HT1080 and HeLa cells[3]. Its combined activity against a broad spectrum of bacteria and HIV-1 places it among the top six peptides in a database containing more than 2,200 AMPs. In this research, we are investigating physico-chemical properties of p1 that impart the peptide its potency against cancer cells. Specifically, we have been interested in the effects of p1 on membrane fluidity and order. Using solid-state NMR and bilayers mimicking cancer cell membranes, we have obtained the order of lipid acyl chains in the presence and absence of piscidin and characterized the level of disruption induced by the peptide.

Experimental

^{2}H solid-state NMR experiments were conducted at 32 ºC using a quadrupolar echo pulse sequence on a 400 MHz Bruker NMR spectrometer, which was fitted with a low electrical field probe built at the NHMFL. Parameters included a 90º-pulse width of 3.05 μsec and an echo time of 30.0 μsec.

Results and Discussion

To mimic cancer cells, bilayers were made using a 2:1:1 mixture of POPC (palmitoyl-oleoyl phosphatidylcholine), POPS (palmitoyl-oleoyl phosphatidylserine), and cholesterol (chol). To characterize the change in order parameter of the lipid acyl chains resulting from the presence of piscidin, we included lipids deuterated in their palmitoyl chains (Fig. 1) and carried out ^{2}H solid-state NMR on bilayers containing either POPC-d31 or POPS-d31. When piscidin partitions at the water-bilayer interface, it induces disordering of the lipid acyl chains. Spectroscopically, this effect leads to a decrease in the quadrupolar splittings of the deuterons in methylene and terminal methyl groups of the ^{2}H-labeled lipids. Our deuterium ssNMR data recorded at the NHMFL (Fig. 1) indicate that the order parameter decreases by 3.5% and 9.0% in the plateau (C2-C7) and methyl (C16) regions, respectively, when POPC-d31 is used while these respective values are 8.5% and 17% when POPS is ^{2}H-labeled.

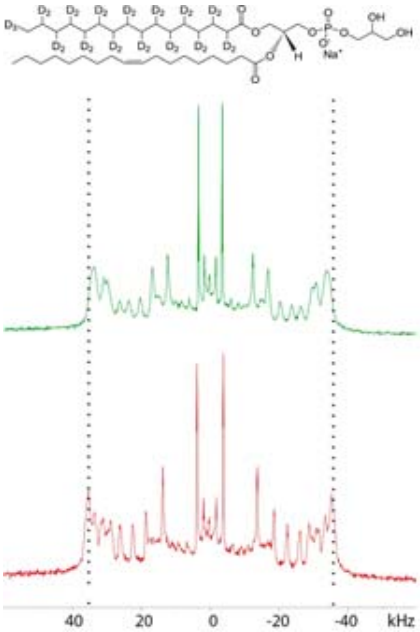

Fig. 1. ^{2}H NMR spectra of POPC-d31 in oriented 2:2:1 POPC/POPS/Chol. Spectra recorded at 305 K.

Conclusions

These results show that p1 is more effective at disrupting the anionic (POPS) than the zwitterionic (POPC) lipid. This knowledge is helpful in determining specific peptide-lipid interactions that are critical in allowing the peptide to perform its membrane disruptive effects.

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References

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