Solid-State NMR Based Monitoring of Permeation of Cell Penetrating Peptides into Skin

Desai, P.R.; Shah, P.P.; Patlolla, R.R.; Singh, M. (Florida A&M U., Pharmaceutical Sciences); Cormier, A.R.; Paravastu, A.K. (Florida State U., Chemical and Biomedical Engineering)

Introduction

Skin acts like a protective barrier against foreign agents penetrating into the body, making drug delivery into deep layers challenging. Cell penetrating peptides (CPPs) are known to effectively permeate into the skin, but tracking CPP penetration in deep skin layers is challenging. We have shown that CPP penetration can be detected using ^{31}P solid state NMR without the need for fluorescent tags required for fluorescence based methods.

Experimental

Hairless rat skin was used for the study. Four peptides were permeated into the skin at various concentrations and permeation time periods using Franz diffusion cells. The skin was then sectioned using a cryotome at different depths. For each skin sample, ^{31}P solid state NMR spectra were collected under magic angle spinning using the Ernst angle technique. Confocal laser scanning microscopy was used as a complementary tool to track these CPP's across the skin.

Results and Discussion

We observed ^{31}P NMR chemical shifts that depended systematically on peptide type, peptide concentration, skin depth, and permeation time (Fig 1). Significant changes were observed for TAT, R11, R8, and YKA peptides. Furthermore, TAT, which is known to be a highly effective CPP, produced the largest shifts in the ^{31}P NMR signal. YKA, which is not a CPP, produced the smallest shift in the NMR spectra. Observed effects decreased with skin depth up to 200 µm, as confirmed by a decrease in observed fluorescence in confocal laser scanning microscopy images. Our results indicate that CPP permeation affects the local chemical environment experienced by skin lipids, suggesting that solid state NMR could be used to study mechanisms of CPP permeation.

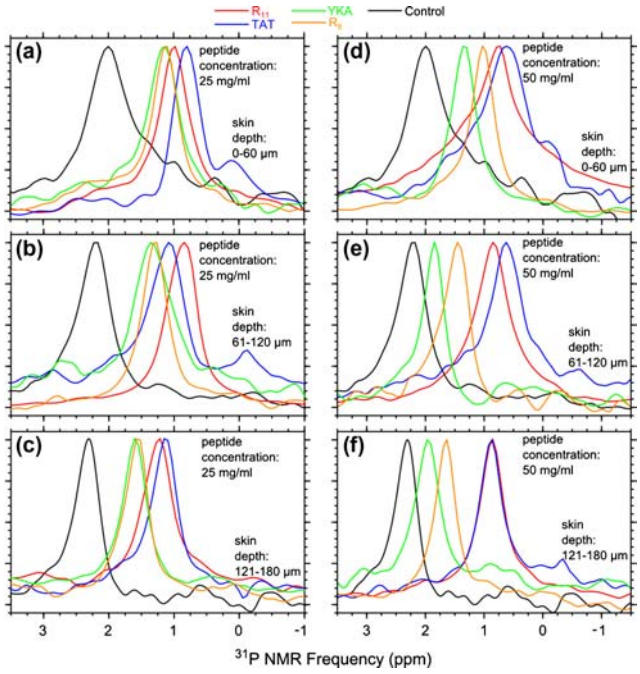

Fig 1: ^{31}P NMR spectra from skin sections at different depths (a and d: 0-60 µm; b and e: 61-120 µm; c and f: 121-180 µm), for 16 hr permeation of solutions with different peptide concentrations (a, b, and c: 25 mg/ml; d, e, and f: 50 mg/ml).

Conclusions

Solid state NMR was shown to be capable of tracking the permeation of peptides into the deep layers of skin. Unlike established fluorescence based methods, the NMR data indicate effects on local organization of the lipids. The recorded NMR spectral shifts represent a novel basis for CPP tracking and the potential to attain insight into how CPPs interact with the skin lipids while permeating from the stratum corneum to the deep layers.

Acknowledgements

The authors acknowledge Dr. Geoffrey Strouse and Steve Hira, Department of Chemistry, Florida State University for their kind help in confocal microscopic studies and also the financial assistance provided by RCMI (NIH) Grant Number G12RR003020. A portion of this work was performed at the NHMFL, which is supported by National Science Foundation (DMR-0654118), the State of Florida, and the U.S. Department of Energy.

References

[1] Desai, P.R., *et al.,* European Journal of Pharmaceutics and Biopharmaceutics, **86**,190–199 (2014).
[2] Ernst, R.R., *et al.,* Review of Scientific Instruments, **37**, 93 (1966).
[3] Afonin, S., *et al.,* Chemphyschem, 2134-2142 (2006).
[4] Verly, R.M., *et al.,* Biophy, 2194-2203 (2009).