Study of DNP Polarization of Acetate and Butyrate at 1.11 K

B. Lama, A. Smith, D. Downes, J. Collins, J.R. Long (UF, Biochemistry and Molecular Biology)

Introduction

Dissolution dynamic nuclear polarization (DNP) technique involves the polarization of the low abundance nuclei like ^{13}C by transferring the polarization from the electron of free radicals using microwave radiation at very low temperature, dissolving the hyperpolarized substance in hot solvents and injecting the solution in NMR tube or animals/human for the data acquisition. The signal enhancement of ^{13}C nuclei has been observed up to 12,000 times in solution state.[1,2] Hyperpolarization of substrates like sodium acetate, sodium butyrate, pyruvic acid etc. at low temperature and injection of the hyperpolarized substrates allows the observation and studies of biochemical reactions and metabolisms *in vivo* and *in vitro* at real time.[3] The extent of polarization of the nuclei is dependent upon different factors like the mechanism of polarization transfer, magnetic field strength, microwave sources, temperature etc.[4] Different techniques like cross polarization, formation of long lived singlet order etc. help to enhance the performance of the dissolution DNP.[5]

Experimental

(1-^{13}C) sodium acetate or sodium butyrate (3.0 M) and 50 mM 4-oxo-TEMPO were dissolved in 2:1 v/v D_2O/ethanol-d_6. Glass beads were made using 300 µl solution in liquid nitrogen and polarized at 1.15 K for 2 hours in 5 T magnet (AMRIS) with microwave frequency of 140 GHz with 50 mW power. For the polarization monitoring, first, polarization was killed by applying the RF pulses and then polarization build up was monitored using 30 µsec (5° flip angle, 10 W) pulse for every minute up to 2 hours.

Results and Discussion

Carbonyl carbon has longer relaxation time than the protonated carbons. Therefore polarization of ^{13}C labeled carbonyl carbon of the substrate was monitored. The enhancement for the polarization transfer in NMR spectroscopy is the ratio of the gyromagnetic ratio of two species. Therefore the theoretical maximum enhancement for ^{13}C nuclei is 860 for DNP at 1.11 K. For the measurement of signal enhancement due to DNP, NMR signal from the substrates were compared when the microwave source was turned on and off.

$$Signal\ Enhancement = \frac{\int Signal\ MW_{on}}{\int Signal\ MW_{off}} \qquad [1]$$

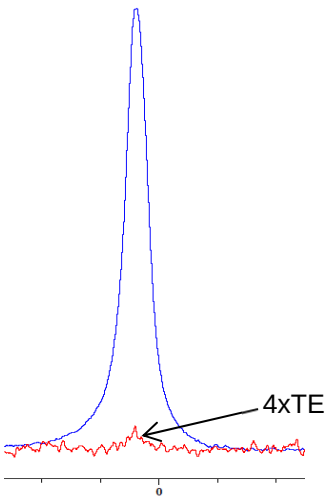

Figure 1. DNP enhanced spectrum of sodium butyrate

The signal enhancement due to DNP (1.1 K, 5 T) is 430 times for sodium acetate and 178 times for sodium butyrate. We are currently altering the sample composition to further improve polarization.

Conclusions

Hyperpolarizations of sodium acetate and sodium butyrate at 1.1 K at 5 T were carried out using TEMPO free radicals. Build up time was monitored and the excellent signal enhancements were obtained. These substrates will be used for the MRI studies of in vivo metabolism in animal models of disease.

Acknowledgements

We gratefully acknowledge the support for this research from the University of Florida-Division of Sponsored Research for the NHMFL DNP initiative.

References

[1] Ardenkjær-Larsen, J.H.,*et al*., *PNAS,* **100**, 10158-10163 (2003).
[2] Lumata, L., *Chem. Eur. J.*, **17**, 10825 – 10827 (2011)
[3] Kurhanewicz, K., *et al*., *Neoplasia,*13, 81-97, (2011).
[4] Ni, Q.Z., *et al*., *Acc. Chem. Res.,* 46, 1933-1941, (2013).
[5] Vasos, P.R.,*et al*., *PNAS,* **106**, 18469-18473 (2009).