Indirect Detection of Enzymatic Processes by Hyperpolarized NMR: Temporal Information, Enhanced Spectral Resolution and Slow Spin Relaxation

T. Harris[1], P. Giraudeau[1], and L. Frydman[1]
[1]Chemical Physics, Weizmann Institute of Science, Rehovot, Israel

Introduction

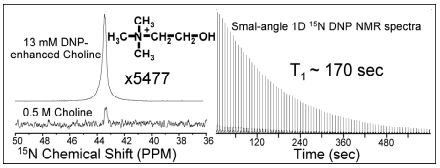
Continuous efforts are invested in developing improved NMR techniques to characterize metabolism. Particularly pressing is the need to enhance NMR's sensitivity. The last years have witnessed what promises to become a major development in this area, with the advent of *ex situ* dynamic nuclear polarization (DNP).[1] The long transfer times needed by this *ex situ* approach imply that only sites with long T_1s will retain a useful hyperpolarization; for this reason, DNP-enhanced studies have focused principally on non-protonated low-γ nuclei like ^{13}C of carbonyls or ^{15}N of quaternary amines.[2,3] This study describes a new approach capable of endowing *ex situ* DNP with an indirect-detection capability, whereby controlled "aliquots" of the low-g-nuclide hyperpolarization are repeatedly transferred to neighboring protons using spatially-selective principles. This method maximizes the efficiency of the transfer, and provides the temporal dimension needed for carrying out spectral characterizations of metabolism. ^1H MRS detection also implies maximum sensitivity and an oft-improved resolution. The usefulness of this new approach is illustrated with hyperpolarized measurements of two Choline related enzymatic reactions involved in cancer metabolism and neuronal transmission.

Methods

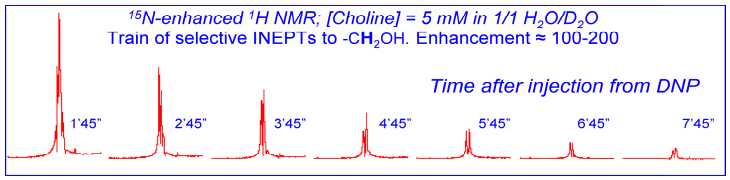
DNP was carried out on an OIMBL Hypersense® operating at ≈1.5 K and at an electron Larmor frequency of ca. 94 GHz; NMR spectra were measured on a 500 MHz Varian spectrometer equipped with an inverse triple-axis gradient probe. Typical DNP runs utilized 5-20 µL aliquots of 1.5 M ^{15}N-labeled Choline (Sigma) or 0.5 M of ^{15}N-Acetylcholine (synthesized in house) dissolved in 1/1 D_2O/d_6DMSO together with 20 mM of OX063. Samples were transferred using 3 mL of pressurized (10 bar) H_2O into 5 mm tubes to give samples of ≈2 mL volume and varying concentrations of metabolites. For the enzymatic experiments Choline Kinase or Acetylcholine Esterase (Sigma) were prepared in concentrated buffers. To ensure homogeneous mixing of the hyperpolarized solution with the enzyme solution, samples were mixed outside the NMR magnet and then injected into the test tube.

Results

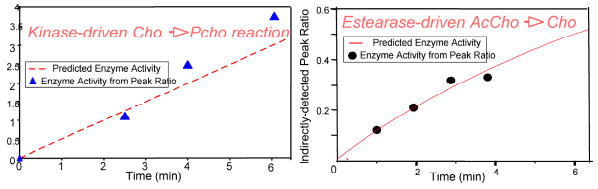
When viewed as a potential target for *ex situ* DNP hyperpolarization Choline shows great promise, possessing a non-protonated nitrogen that has been shown to undergo very efficient enhancement and is endowed with very long T_1 relaxation times.[4] The Figure on the right gives an idea of the sensitivity enhancement that can be observed for this site.



In spite of this potential, Choline's ^{15}N site is ill-posed for following the acetylation, deacetylation or phosphorylation reactions that one might be interested to quantify in a metabolic setting. All of these processes take place on the molecule's hydroxyl site, resulting in the minor shift effects for the distant ^{15}N peak. Larger shifts arise on the proton sites of these molecules, closer as they are to the epicenter of the metabolic transformations. Exploiting this within a DNP-enhanced setting could be possible by transferring the ^{15}N hyperpolarization using the small but non-negligible J_{NH} couplings, for instance via INEPT-based sequences.[5,6] This strategy, however, is ill- suited for acquiring the multiple scans necessary for extracting metabolic information, which requires that hyperpolarization be transferred multiple times. To deal with this constraint we explored the integration of the DNP-enhanced indirect-detection INEPT sequence with spatial-encoding techniques.[7,8] These enabled us to run a *series* of ^1H NMR experiments like the one illustrated on the left; leading to a clean set of methylene ^1H NMR spectra, hyperpolarized and characterized by very slow decays owing to the extreme T_1s of the ^{15}N site acting as their source of signal.



With this capability at hand, we set out to explore the usefulness of this approach for monitoring enzymatic processes. The Figure on the right illustrate some of these with results arising from the phosphorylation of Choline by Choline Kinase, showing a decrease in the initial Choline-derived ^1H NMR peak and an concomitant increase in the Phosphocholine one. Also summarized are results arising from the hydrolysis of Acetylcholine by an Esterase. Notice that in this case the ^{15}N peaks of reactant and product are shifted by only 0.05 ppm (2.5 Hz at 11.7 T) and it would be difficult or impossible to resolve these two species by direct ^{15}N detection; yet by transferring polarization to the neighboring ^1Hs, the kinetics of this reaction could be clearly followed.



References

[1] Ardenkjaer-Larsen, J.H. *et al* (2003) *Proc Natl Acad Sci USA* **100,** 10158-10163. [2] Golman, K. *et al* (2006) *Cancer Res.* **66,** 10855-10860. [3] Kohler, S.J. *et al* (2007) *Magn. Reson. Med.* **58,** 65-69. [4] Gabellieri, C. *et al* (2008) *J. Am. Chem. Soc.* **130,** 4598-4599. [5] Morris, G. A. & Freeman, R. (1979) *J. Am. Chem. Soc.* **101,** 760-762. [6] Sarkar R. *et al* (2010) *J. Am. Chem. Soc.* ja9021304. [7] Frydman, L., Scherf, T. & Lupulescu, A. (2002) *Proc Natl Acad Sci USA* **99**, 15858-15862. [8] Mishkovsky, M & Frydman, L. (2009) *Ann. Rev. Phys. Chem.* **60**, 429-448.

Financial support: Israel Science Foundation ISF 447/2009; Helen Kimmel Award for Innovative Research.