

Effects of deuteration on ^{13}C relaxation times in neuro-metabolic compounds: Implications for hyperpolarized spectroscopic imaging

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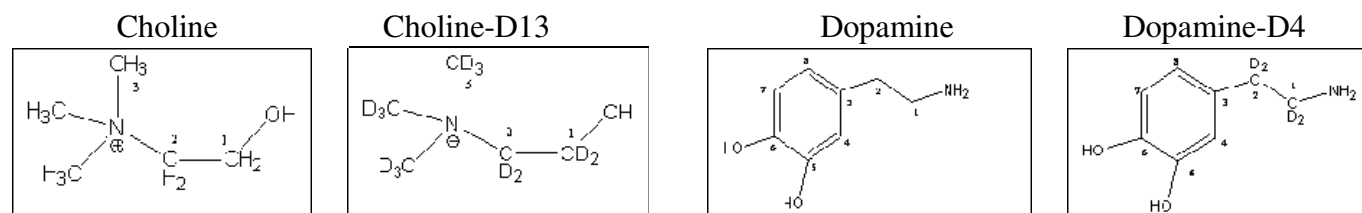
Introduction

Increase of the NMR signal by hyperpolarization techniques is used for many research objectives. The 10,000 fold enhancement¹ of the NMR signal is however limited by the decay of the hyperpolarization from the moment of its generation due to spin-lattice relaxation (T_1 relaxation time). This decay determines the temporal window of opportunity to detect *in vivo* the metabolism of the hyperpolarized compounds. Enrichment of proton positions with Deuterium nuclei is known to prolong the T_1 of an adjacent spin $\frac{1}{2}$ in a manner that is critically dependent on the conformation taken by the molecule in solution². The extent of the deuteration effect on T_1 relaxation time of adjacent carbons ranges between no effect to 7 fold increase per substitution³, which may therefore provide up to 49 fold increase per two substitutions on the same carbon position. However, the extent of this effect in specific molecules is not readily available. Specifically, to the best of our knowledge, the effect of deuteration was not determined in neuro-metabolic compounds such as choline and dopamine, which are of interest due to their potential as diagnostic biomarkers for neurodegenerative disease and disorders.

The choline and dopamine molecules contain both similar (two methylenes) and different (conjugated system in dopamine) chemical moieties (as shown in the schemes below), which could lead to varying degrees of deuteration effects on T_1 . Specifically the T_1 s of the methylene positions in these compounds are of interest due to their proximity to the area of chemical change following metabolic reactions relevant to neurodegenerative diseases and disorders. Although conjugation *per se* does not affect the deuterium induced T_1 prolongation effect⁴, the conformations imposed on the methylene positions due to the conjugated moiety could offset the deuterium effect. To explore the effect of deuteration on the methylene positions in choline and dopamine we compared the carbon T_1 s of either partially or fully deuterated compounds to that of the native, fully protonated compounds.

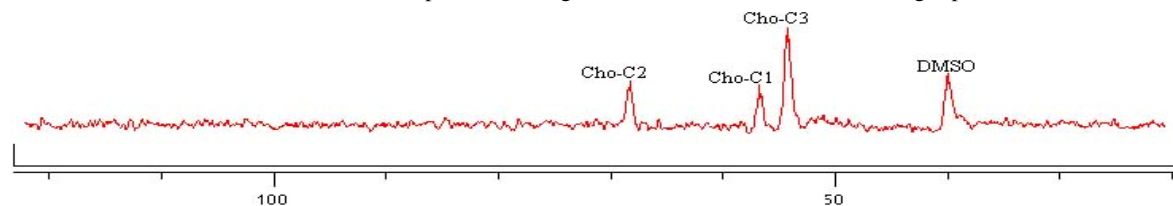
Materials and Methods

Choline Cl⁻ and dopamine HCl were obtained from Sigma-Aldrich (Israel). Choline-D13 Br⁻ and dopamine-D4 were obtained from Cambridge Isotope Laboratories (MA, USA). The molecular structure of these compounds is shown in the schemes below. Carbon-13 signals were detected in these molecules at natural abundance. Inversion recovery studies were carried out at 500 MHz (Varian), equipped with a 5mm direct ^{13}C probe. The number of transitions ranged between 300 to 400 per relaxation delay (τ) with a total scanning time of 12 – 48 hours. The effect of concentration on T_1 was investigated in a concentration range of 20 mM to 20M.



Results

The T_1 s of the non-labeled methylene carbons in choline and dopamine were found to be in the ranges of 3 to 5 seconds and 1 to 2 seconds, respectively. Increase in concentration of choline from 20 mM to 20M led to a slight decrease in the T_1 s between 6 to 16 percent. Full deuteration of choline Cl⁻ resulted in a 7 fold increase in the methylenes' T_1 s. Similarly, the deuteration of the dopamine methylenes resulted in an 8 fold increase in their T_1 . Preliminary studies on hyperpolarized Choline-D13 Br⁻ show the high SNR obtained through DNP of this molecule, suggesting that full deuteration does not interfere with the DNP process. A single transient recorded after an overnight polarization is shown below.



Conclusion

Deuteration of neuro-metabolites leads to profound elongation of the T_1 s and paves the way for *in vivo* hyperpolarized carbon-13 spectroscopic imaging of the deuterated contrast agents. Future kinetic studies of choline enzymatic conversion and double labeling (^{13}C and D) are underway. The effects of full deuteration of the dopamine molecule on the methylene's T_1 , as well as the respective effects on the L-DOPA molecule (the metabolic precursor of dopamine) are currently under investigation.

References

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