Hyperpolarized N-15 of choline - potential for observing phospholipid metabolism in cancer

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Introduction

Dynamic Nuclear Polarization (DNP) is becoming a widely accepted and successful method for generating highly non-equilibrium populations of nuclear spin states in a variety of molecules in solution state and thereby to enhance the magnetic resonance (MR) signal by up four orders of magnitude (1). The potential to carry out real-time metabolic imaging using ^{13}C pyruvate is showing enormous potential (2). Our ability to probe this and other metabolic processes using DNP may yield information that has hitherto been inaccessible to MR. In this abstract we present the first attempt to hyperpolarize the quaternary ^{15}N in a sample of isotopically enriched choline. DNP employing a HyperSense® polariser was found to achieve a polarization of about 6%. The T_1 was found to be of the order 200s in the presence of free radical. Furthermore the chemical shifts of the parent choline and its metabolic product phosphocholine can be distinguished in the ^{15}N spectrum. Changes in choline metabolism have been observed in a variety of cancers and are known to reflect differences in phospholipid metabolism. These differences provide information on malignancy (3) and on treatment response (4,5). Clearly the existence of quaternary nitrogen nuclei in organic compounds is scarce. However in some specific circumstances this may prove a viable alternative to ^{13}C.

Methods and Results

Choline chloride was purchased from Sigma Aldrich (98% 15N). This was dissolved in a solution containing 15 mM of free radical in DMSO and was polarized using two different free radicals, OX63 and Finland. The T_1 was measured by recording a spectrum every 30s employing a 10 deg flip angle. The decay of the magnetisation was fitted taking account of the loss of polarization from the RF pulse. The sample was polarized overnight in a HyperSense® DNP polarizer at low temperature (1.4K) in a superconducting magnet (3.35T) with microwave irradiation at 94 GHz. The polarized sample was dissolved in a 4ml EDTA solution and transferred to a high field NMR (9.4T) prior to detection of ^{15}N. The spectrum on the left in Fig 1 shows a hyperpolarized ^{15}N signal from choline (20mM final concentration after dissolution) employing a single 30 deg flip angle after 4mins. The spectrum on the right shows the signal recorded after 23mins and under identical conditions. The polarization was calculated to be ~6% with respect to the thermal signal, extrapolated back to t = 0. The ^{15}N T_1 values have been measured under a number of conditions. In the absence of free radical the relaxation times were measured by inversion recovery and found to be T_1 = 218s for 100mM ^{15}N choline in 100% D_2O, T_1 = 232s for 83mM choline in 84%H_2O/16%D_2O. Conversely in the presence of the free radical and monitoring the decay of the hyperpolarized signal, the relaxation time was found to be T_1 = 203s for 2mM choline in OX63 and T_1 = 165s for 18mM choline using the Finland free radical. These results suggest that the 15N T_1 is not greatly perturbed by the presence of the free radical.

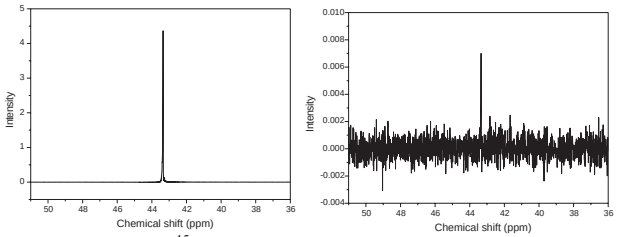

Fig 1. Left shows ^{15}N spectrum of hyperpolarized choline recorded with a single scan 4mins after dissolution. Right shows the ^{15}N spectrum from the same sample after 23mins and under identical conditions.

Discussion

Fig. 2 shows a ^{15}N spectrum of a concentrated solution of PCho and Cho (natural abundance ^{15}N) recorded at 11.7T. The chemical shift difference between the parent choline and its metabolic product phosphocholine is about 0.1ppm and is clearly resolved at high field. This may prove more difficult in-vivo but suggests that one should be able to distinguish not only the parent compound but also the metabolic products of the phosphorylation reaction in-vitro. This may provide a novel and sensitive way to probe the early stages of cellular choline metabolism.

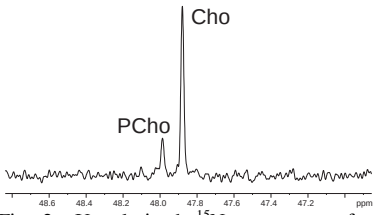

Fig 2. Unpolarized ^{15}N spectrum of a mixture of PCho and Cho (natural abundance ^{15}N) recorded with 800 scans, experiment time 13hrs.

Conclusions

We have presented preliminary data for the hyperpolarization of ^{15}N in choline. The polarization achieved was ~6%. The T_1 was found to be of the order 200s in the presence of the free radical. The exceptionally long T_1 combined with the potential to observe a metabolic process implicated in the pathology of cancer make these preliminary experiments highly encouraging.

Acknowledgements

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References

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