T_2-labelling of phosphate by ^{17}O – a method with potential for *in vivo* studies of phosphorus metabolism

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Introduction

^{31}P NMR has been used since the inception of biological magnetic resonance to study bioenergetics in *in vivo*, *ex vivo*, and *in vitro* systems, most commonly to report on the concentration of high-energy phosphate-containing compounds (ATP, ADP, PCr, Pi, etc.) in tissue. As ^{31}P has 100% natural abundance and is the only stable isotope of phosphorus, tracer studies to investigate metabolic rates and pathways analogous to metabolism studies performed with ^{13}C are not possible. However, magnetisation transfer studies to investigate rates of phosphate exchange have been widely employed (for example, (1)). As ^{31}P T_1 values are on the order of seconds, such measurements are restricted to relatively fast exchange reactions. Metabolic rates and pathways of phosphate-containing compounds have also been studied by the addition of a stable oxygen isotope to phosphate groups. ^{18}O causes a change in chemical shift of ^{31}P, and is thus detectable with NMR (2). Mass spectrometry has also been employed to follow the progression of ^{17}O or ^{18}O-labelled phosphate through metabolic processes, for example to study bioenergetic rearrangements in heart tissue following ischemic insult (3). As the ^{18}O chemical shift isotope effect is rather small, and mass spectrometry is a destructive technique, neither method is well suited to *in vivo* studies. We report here a method to detect ^{17}O-labelled phosphate via the T_2 relaxivity effect of ^{17}O on ^{31}P, caused by scalar coupling interactions between ^{17}O and ^{31}P. The method allows an MR spectrum of ^{17}O-labelled phosphate to be generated, and unlike previous MR and mass-spectrometry techniques the method is well suited to *in vivo* studies. The method may play a role in future *in vivo* bioenergetics studies, allowing labeled phosphate groups to be followed through metabolic pathways to provide information on metabolic rates and reaction paths.

Materials and Methods

^{17}O-labelled sodium phosphate was synthesized by reacting phosphorus pentachloride with a large excess of ice-cold 20 atom-% ^{17}O-enriched water, heating the resultant solution to 95 ºC, then pH-neutralizing with sodium hydroxide. This produced an approximately 100 mM sodium phosphate solution with an estimated 67 % of phosphate molecules containing with one or more ^{17}O nuclei (based on the water ^{17}O-enrichment). MR data were acquired on a 11.1 T 40-cm diameter horizontal bore magnet and spectrometer equipped with one proton and two X-nucleus transmit channels. ^{17}O-labelled phosphate solutions were placed in 3-mm diameter glass tubes within orthogonally-oriented ^{31}P and ^{17}O Rf coils. ^{17}O-decoupled ^{31}P spin-echo datasets were acquired into 16 averages with a repetition time of 5 s, data were analysed with Matlab (The MathWorks, Natick MA, USA).

Results

Figure 1 shows a plot of ^{31}P signal decay due to T_2 relaxation, measured by arraying the echo time of a ^{31}P spin echo experiment. Data in the figure were acquired with (blue) and without (red) ^{17}O decoupling during the echo period. Monoexponential T_2 relaxation is observed when ^{17}O decoupling is applied as ^{31}P nuclei in labeled and unlabeled phosphate molecules experience the same magnetic environment. Non-monoexponential T_2 relaxation is observed in the absence of ^{17}O decoupling as labeled phosphate molecules experience T_2 relaxation effects due to scalar coupling interactions between ^{17}O and ^{31}P nuclei. These data show that an echo time of 4 ms is sufficient to attenuate signal from ^{17}O-labelled phosphate, but causes little effect on the magnitude signal from unlabelled phosphate. Figure 2 shows data from a ^{31}P spin-echo acquisition with an echo time of 4 ms. Figure 2A shows a spectrum acquired without ^{17}O decoupling during the echo period, signal in this spectrum originates from unlabeled phosphate only, as signal from labeled phosphate has undergone T_2 relaxation during the echo period. Figure 2B shows data acquired with ^{17}O decoupling applied during the echo period, showing signal from both labeled and unlabeled phosphate. Subtraction of the data in Figure 2A from that in Figure 2B produces a ^{31}P spectrum originating from ^{17}O-labeled phosphate only (Figure 2C).

Discussion and Conclusions

Our data demonstrate that the T_2 relaxivity effect of ^{17}O on ^{31}P can be used as a labeling scheme for phosphate-containing molecules. Decoupling at the ^{17}O frequency allows this T_2 relaxivity effect to be switched on and off, permitting identification of ^{17}O-labelled phosphate. Unlike previous studies employing ^{18}O-labelled phosphate, our method is applicable to *in vivo* bioenergetics studies. Future studies will explore the use of this technique in other phosphate-containing molecules and in living systems.

References and Acknowledgements

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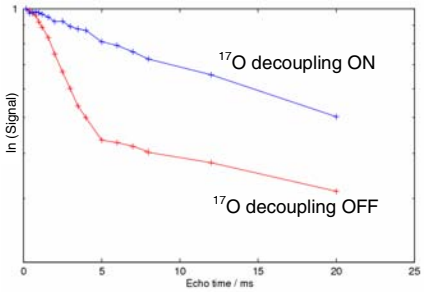

Figure 1 (left): Plot of log(signal) against echo time for a sample containing both unlabelled and ^{17}O-labelled phosphate, acquired with (blue) and without (red) ^{17}O decoupling.

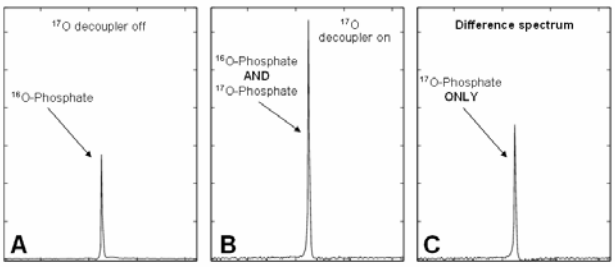

Figure 2 (right): Spectra from spin-echo experiments from the same sample, acquired without (A) and with (B) ^{17}O decoupling. Plot C shows a difference spectrum produced from A and B.