Imaging of bound water in the frozen rat brain

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Introduction

Evidence has accumulated in the past 40 years for the existence of an important proportion of highly structured water in biological samples [1,2]. The term 'bound water' is generally used for this component which is believed to consist of water molecules in a thin hydration layer around biological macromolecules. This layer is in intimate contact with the macromolecules and moderates the transfer of relaxation from the solid-like protons 'frozen' in the macromolecules to the bulk water [3-5]. Given the large surface of interaction, the properties of bound water closely reflect any possible changes in the structure of the macromolecules [1] and this could provide a good indicator of cell health. Additionally, early NMR studies on rat liver tissue during carcinogenesis have clearly shown changes in the amount of the bound water fraction and especially of its T_1 relaxation time which is specific to the tumour only [6] (several other parameters, such as the total water content, are indicative of liver disease, but not specific for cancerous tissue). The bound water has strikingly different physical - and NMR - properties from those of the bulk water. Among other things, the bound water remains unfrozen at temperatures substantially below 0^{o}C; it is also present as a thin unfrozen layer on the surface of ice [1]. In the following, we make use of this property to investigate the unfrozen bound water by NMR and MR imaging of a rat brain. This study shows that imaging of bound water is feasible, to estimate the concentration of bound water relative to 'free' water in the frozen rat brain and to gain information about its spatial distribution.

Materials and methods

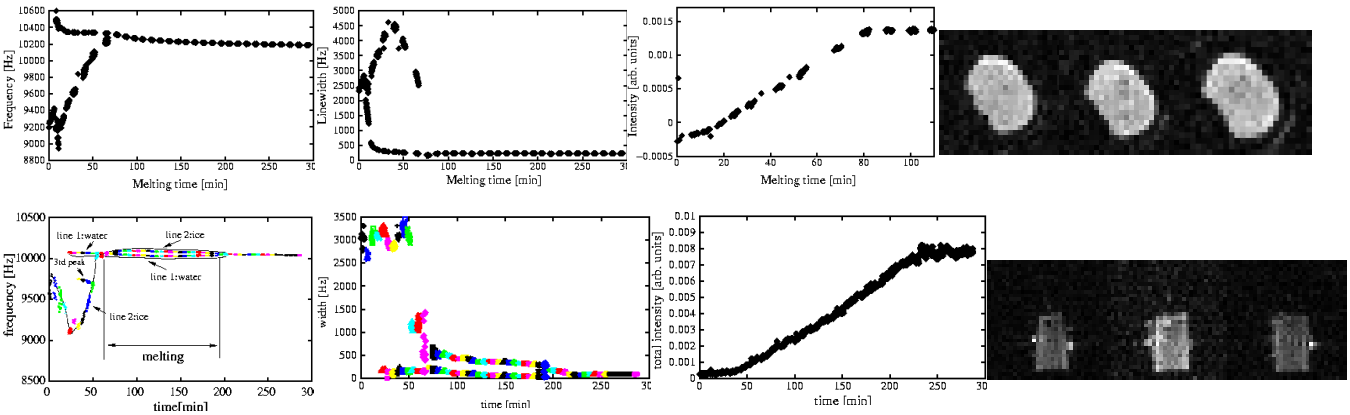
Measurements were performed on a 4T whole-body scanner (Varian UnityInova). An insert gradient coil with an inner diameter 120 mm and maximum strength of 400 mT/m was used for the measurements. A surface coil of 3cm inner diameter was used for RF transmission and reception.

Changes in the NMR response of a rat brain with temperature have been investigated and compared to results obtained from a pure water phantom. The brain of a male Sprague-Dawley rat was excised and put in a cylindrical plastic container with dimensions of 2x3cm. It was measured in the MR scanner for two hours immediately following excision, then shock frozen with liquid isopentane at -50^{o}C, placed in a deep freezer at constant temperature of -80^{o}C for 5 hours, then measured again whilst thawing. A cylindrical plastic container of the same dimensions as the one containing the rat brain was filled with tap water. The water was frozen by gradually immersing its container into liquid nitrogen; the process was, however, not slow enough, so that several cracks were produced in the ice. It was then transferred to the MR coil and imaged whilst thawing freely due to contact with the air at room temperature and RF power absorption. In both cases, shimming, power calibration and sequence parameters optimization were performed on the unfrozen samples. The frozen samples were imaged using these optimized conditions.

We investigated the changes in the NMR response with a simple pulse-acquire sequence. A square pulse of 4μs duration was applied corresponding to an angle of 25^{o}, and 2048 complex points were acquired for the FID. MR imaging was performed using a fast 3D single point imaging sequence, SPRITE [7], which is ideally suited for imaging of fast relaxing species. Each datum point was collected 300μs following a short (6μs) hard pulse corresponding to a flip angle of 6^{o}. Both the excitation and the read-out were performed in the presence of gradient fields (three phase encoding gradients). SPRITE images with 1 mm resolution were obtained from a FOV of 64x64x32 mm and matrix size 64x64x32, TR of 4 ms, TE=0.3ms, 1 average. The total acquisition time for SPRITE was 5 ½ min, and 200s for each set of 20 FIDs. The imaging and NMR sequences were alternatly applied and the evolution of the frozen samples was monitored over a period of 3-6 hrs.

Results

Changes in the position, intensity and shape of the different proton lines were monitored as a function of time. The peaks were fit using a Lorentzian shape with Gauss admixture. Except for the very first spectrum, corresponding to the lowest temperature, the Gaussian contribution to the line shape was found to be zero. The temperature of the sample was not directly monitored, but inferred by comparison with the results obtained on the water phantom. The dependence of the intensity of the proton peak on time is shown in Fig.1 The broad peak in both spectra was identified as belonging to ice (frozen 'free' water). Melting in the ice phantom was identified as the time interval where the linewidth of the ice peak is close to that of water; the peaks coexist and practically no changes in the NMR lines were observed. Analogously, in the rat brain, melting was presumed to take place after the two lines merged. The narrow peak, before melting starts, is expected to consist either entirely of, or have a very large component from, the bound water. The SPRITE image of the rat brain, shown in Fig. 1 was acquired 25 minutes after the monitoring of melting started; those of the ice phantom were acquired after 32 min. By dividing the intensity of the narrow peak just before melting starts to the total intensity of the free water (the plateau seen on the plot of intensity vs. time), the amount of bound water can be estimated. It is roughly 6% in the ice sample and 12% in the rat brain.



Conclusions

Imaging of bound water in the frozen rat brain has been demonstrated using SPRITE – an imaging sequence for fast relaxing species. The bound water was identified as being the unfrozen water component and the temperature was inferred by comparison with a water phantom. The amount of bound water in both samples was estimated to be approximately 6% for the ice sample and 12% for the rat brain.

References

[1] R. Mathur-de-Vre Prog. Biophys. Molec. Biol. 15:103-134(1979); [2] D. Pouliquen et al., Anticancer Res. 15, 321-330(1995); [3] S. Koenig, Acad. Radiol. 3, 597-605(1996); [4] R.G. Bryant, D.A. Mendelson and C.Coolbaugh Lester, Magn. Reson. Med. 21, 117-126(1991); [5] B. Halle, V.P. Denisov and K. Venu, Biol. Magn. Reson. 17, 419-449(1999); [6] Pouliquen et al., Anticancer Research 13:49-56(1993); [7] Balcom BJ et al., Magn. Reson. A. 1996 123(1):131-4