Temperature and oxygenation dependence of haemoglobin and hemocyanin relaxation times at 9.4T

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Introduction

Proton relaxation rates of blood are beneath field strength depending on physiological parameters like haematocrit, ion compositon, oxygen saturation, pH and on physical variables like temperature (1, 2). Knowledge of correct T_1 and T_2 values are of considerable importance for quantification of fMRI signals, blood flow and perfusion in MR experiments (3, 4, 5) as well as for the quantification of tissue oxygenation levels in determining correct oxy/desoxy haemoglobin ratios (3). Furthermore, analysis of the proton relaxation times of water in the blood will give additional important physiological information and further insights into the bioenergetics of organisms (1).

Since MRI and NMR studies using ectothermic animals as model organisms are becoming more and more frequent (e.g. 6), correct T_1 and T_2 values under these temperature conditions (in a temperature range of 0°C-20°C) are crucial for quantitative measurements of flow and perfusion with MRI in these organisms. Recently, it was shown that cephalopods are ideal model organisms for MR imaging and spectroscopy studies in neurological research under patho-physiological conditions (7). The potential use of these organisms for functional MRI studies relying on BOLD contrast, information on the magnetic properties of the cephalopod blood pigment haemocyanin depending on the oxygenation state is indispensable. To address this question the aim of this study was to determine the T_1 and T_2 values of water proton signals from purified haemoglobin and haemocyanin solutions at different temperature and oxygenation states.

Materials and methods

For the NMR studies on haemoglobin, blood (around 1 mL/sample) was taken from the caudal vein of North Sea cod acclimated at 10°C (*Gadus morhua*, n=3). Heparin (57.8 mg/1mL blood) was added used to prevent coagulation. Haematocrit values were around 30 Vol. % in all samples. T_1 and T_2 NMR studies were conducted on whole blood samples as well as on purified haemoglobin. Samples were washed, lysed, and centrifuged. The lysate was then applied to a SEPHADEX column (Pharmacia biotech, Sweden).

Haemocyanin samples were taken from cephalopod blood from the Atlantic (*Sepia officinalis*, at 15°C water temperature, Arcachon, France). Haemocyanin is an extracellular blood pigment, therefore the blood samples were only centrifuged and supernatants were taken directly for the NMR studies.

Fully oxygenated blood pigment samples were equilibrated by aerating the samples on a stirrer for 30 minutes at least prior the NMR experiments. For the NMR measurements ca. 450 mL of blood were transferred into a 5 mm NMR tube under aeration and sealed immediately. Blood pigment samples were deoxygenated by adding a few grains of sodium dithionite into the NMR tube right after the first set of NMR experiments. Figures 1a+b shows examples of oxygenated and deoxygenated blood pigments prior NMR experimentation. The clearly visible colour differences indicate the oxygenation state of the blood pigments. Oxygenated haemoglobin is cherry red and turns to dark red when deoxygenated (Fig. 1a), whereas fully oxygenated haemocyanin ranges from yellow to blue (depending on the functional units of the haemocyanin protein) and becomes colourless when deoxygenated (Fig 1b). **Figure 1:**

All NMR spectroscopy studies were performed on a wide bore Bruker 400 AVANCE system operating at 9.4T using an air cooled inverse 5mm broadband probe. Temperature was controlled with a standard Bruker Temperature control unit resulting in a temperature stability of ±0.1°C inside of the probe. T_1 proton relaxation rates from the water signal were determined using a classical inversion-recovery method taken from the Bruker NMR sequence library. T_2 proton relaxation rates were calculated from the water signal from ^{1}H NMR spectra collected with a classical Carr-Purcell-sequence (Bruker library). T_1 and T_2 values were determined for all samples at temperatures between 5 and 20 °C in 5 °C steps.

Results and Discussion

A clear linear dependency of T_1 proton relaxation time from purified haemoglobin could be observed with temperature (Figure 2a). Additionally, the T_1 value of whole blood for 15°C (red hash) is added in figure 2 indicating no differences between blood and purified haemoglobin. A linear temperature dependency of T_1 values could be observed in bovine blood between 24°C and 38°C at 4.7T as well (5), although T_1 increase with temperature is two-fold higher in this study. This can be explained with the lower haematocrit values of fish blood (30 Vol.%) in comparison to bovine blood (40 Vol.%) in the former study. Lu et al. found an inverse linear relationship between T_1 relaxation times and haematocrit levels. The oxygenation state of haemoglobin did not affect the temperature dependency of T_1 values as observed on rat haemoglobin before (1), but T_1 is dependent on oxygenation levels (5).

Figure 2b presents the change in T_1 values from oxygenated (red) and deoxygenated (blue) haemocyanin with temperature. Interestingly, the linear increasing rate is in good correlation to fish haemoglobin, although haematocrit values could not even be compared (under 10 Vol.%). The oxygenation state of haemocyanin did not influence T_1 relaxation rates as indicated in figure 2b. Furthermore, fully oxygenated haemocyanin showed only a slight increase of maximal 0.6 s in their T_2 relaxation rates in comparison to deoxygenated haemocyanin (data not shown). This implies that differences in BOLD contrast in potential functional MRI studies on cephalopods could only be minimally elaborated, therefore functional studies should be performed with MR techniques relying on an increase of perfusion rather than BOLD contrast.

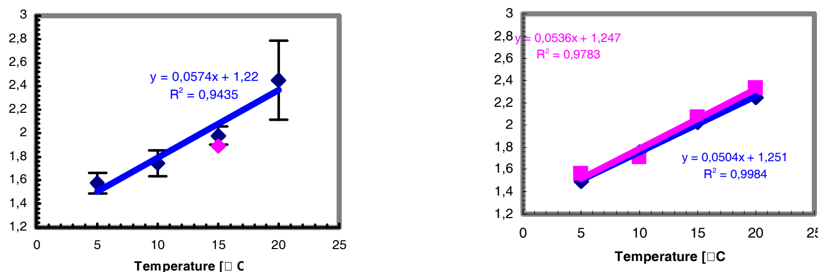

Figure 2: Temperature dependency of T_1 relaxation times from haemoglobin (a) and haemocyanin (b)

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