Xenon-Protein Interaction and Competitive Binding: a Hyperpolarized ^{129}Xe NMR Study

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Introduction: Hyperpolarized ^{129}Xe NMR is a promising technique to study xenon interactions with proteins. Selective polarization transfer to hydrophobic binding sites may be used to "highlight" protein ^{1}H NMR spectra and facilitate peak assignments [1,2]. Moreover, xenon interactions with proteins may determine xenon anaesthetic properites [3,4]. X-ray diffraction has shown that xenon binds to specific sites in protein crystals. Little is known, however, about specificity and dynamics of xenon-protein binding in solution. We propose the use of competitive ligands to study xenon interactions with macromolecules. In this study, we demonstrate that competitive binding affects the NMR parameters of hyperpolarized ^{129}Xe dissolved in bovine serum albumin solution.

Materials and Methods: The optical pumping procedure has been described in detail previously [5]. All NMR experiments were performed using a Siemens Magnetom Vision 1.5T clinical MR system. We used a variable flip angle NMR sequence for measuring ^{129}Xe T_1.

Samples containing 5% w/v bovine serum albumin (BSA) powder (SIGMA Chemicals, Dorset, UK) dissolved in fully deuterated water with low paramagnetic impurity content (Aldrich, Dorset, UK) were prepared with various concentrations of Flucloxacillin Sodium BP (Floxapen; from Beecham Research, Hertfordshire, UK). By using a fully deuterated solvent, we excluded cross-relaxation from xenon to the water protons. Prior to introduction of the hyperpolarized gas, oxygen was removed from these samples by equilibrating the sample (1.5ml) with 100ml of helium for 20 minutes repeatedly in custom-made glassware in which the hyperpolarized xenon could be admitted directly to the liquid. Plasma samples were prepared by centrifuging freshly drawn blood at a temperature of 4°C at 2000 rpm for 10 minutes. The same degassing procedure as for the BSA/D_2O samples was used for the ^{129}Xe T_1 measurements in plasma.

Results and Discussion: We measured the hyperpolarized ^{129}Xe spin-lattice relaxation times in solutions of bovine serum albumin (BSA) in fully deuterated water (D_2O) and various concentrations of flucloxacillin up to 100mM (Fig. 1). Flucloxacillin, a fluorinated drug, is known to bind strongly to BSA and to displace other ligands such as 5-fluorouracil (5FU).

The ^{129}Xe T_1 in pure D_2O is about 1000s, and 13.7s±0.8s in the 5% w/v BSA in D_2O solution. This shows that xenon interactions with BSA provide an effective relaxation mechanism for ^{129}Xe. The ^{129}Xe relaxation time increases with increasing concentraion of flucloxacillin. These findings indicate that xenon and flucloxacillin compete for one or more binding sites on BSA, and that the presence of flucloxacillin prevents xenon from binding to these sites. Consequently, dipolar interaction between xenon and protons of the protein is reduced, which results in a longer spin-lattice relaxation time. The position of the ^{129}Xe signal was shifted by two ppm downfield in the sample containing 100mM concentration of flucloxacillin compared to the 5% w/v BSA in D_2O solution sample. As the position of the ^{129}Xe NMR resonance is dominated by the fast exchange of xenon bound to the protein and xenon dissolved in D_2O, the observed shift is further evidence for the change of the relative compartment sizes upon increasing the concentation of flucloxacillin.

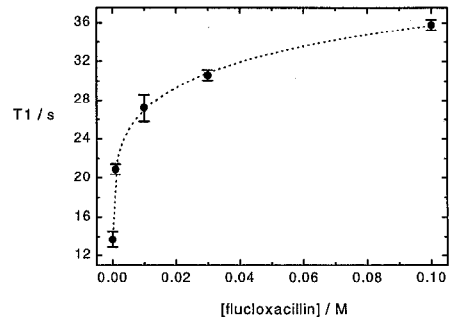

Figure 1: Hyperpolarized ^{129}Xe T_1 in 5% w/v BSA in D_2O for different concentrations of flucloxacillin. Dotted line: guide for the eye.

Plasma contains approximately 5% w/v albumin. The ^{129}Xe T_1 increases from 13.3s±0.1s in pure plasma to 16.2s±0.2s in a sample containing flucloxacillin in 100mM concentration. The position of the ^{129}Xe signal is shifted by one ppm downfield in the sample containing flucloxacillin. Introduction of the drug changes the ^{129}Xe T_1 by about 20%. This indicates that other mechanisms, such as interaction with paramagnetic ions and other proteins, and cross-relaxation to the solvent contribute to xenon relaxation in plasma.

Conclusions: We have demonstrated that flucloxacillin affects xenon interaction with BSA. We have applied the method of competitive binding to identify xenon binding to albumin as one of the ^{129}Xe relaxation mechanisms in plasma. The use of competitive binding is a promising technique to study the specificity of xenon-protein interactions. It could be applied, for instance, to investigate the general anesthetic properties of xenon [3,4] and their origins in more detail.

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