Exchange Dynamics of a Cryptophane-based Xenon Molecular Sensor

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Introduction

Optical pumping is capable of producing very large signal enhancements of ^{129}Xe (1). However, the inert nature of xenon makes targeted imaging a challenge since it cannot be covalently coupled to a targeting moiety. This had led to the development of biosensors based on cryptophane-A (CryA) (2), which act as a molecular host for xenon atoms, and can themselves be functionalized and targeted towards specific analytes. Since xenon associated with CryA (Xe@CryA) gives rise to a unique chemical shift in the ^{129}Xe spectrum, biosensor localization can be determined by monitoring that peak directly or, alternatively, via chemical exchange saturation transfer (CEST). The latter technique, termed hyperCEST (3), allows for the detection of sub-nanomolar quantities of cryptophane-based xenon biosensors (4). The exchange dynamics of xenon with cryptophane are important because they impose a limit on the obtainable contrast in a CEST experiment. For instance, once Xe@CryA spins are saturated they must dissociate and be replaced by polarized spins from Xe@water before more contrast can be generated. Several studies have addressed these dynamics with analogues and derivatives of CryA (5-9). However, given the wide-ranging conclusions of those studies, it was prudent to investigate our specific CryA construct. *The objective of this study was to elucidate the dynamics of the reversible complexation of xenon with a CryA host molecule, in particular the time scale on which xenon atoms dissociate from CryA as this is an inherent limitation of CEST contrast.*

Methods

A monoacid derivative of CryA was modified to introduce a pentamer peptide (KEEEE) which facilitated aqueous solubility, and this construct was dissolved in water containing 5% v/v isopropanol. Hyperpolarized xenon was prepared by spin-exchange optical pumping with a MITI XenoSpin polarizer (Nycomed Amersham) containing rubidium vapor, using a xenon gas mixture (2% Xe natural abundance, 10% N_2, 88% He). After polarization (~5%), xenon was bubbled through a capillary tube placed in a 5mm phantom containing 600 µL of the CryA solution. NMR experiments were conducted on a 300 MHz Varian ^{UNITY}INOVA spectrometer using a commercial 5mm dual tuned (^{1}H, ^{129}Xe), temperature controlled RF probe. The hyperpolarized gas mixture was bubbled for 25 s at 0.5 SLM, which was sufficient to saturate the solution with xenon, followed by a 2 s wait period to allow bubbles to clear. A continuous wave saturation pulse was applied prior to signal excitation and acquisition (spectral width = 25 kHz, acquisition time = 500 ms). The saturation pulse frequency was set either on-resonance with the Xe@CryA frequency, or off-resonance at an equal distance from the Xe@water frequency but on the opposite side. A range of saturation pulse durations was chosen for each combination of xenon partial pressure (P_{xe}), temperature, CryA concentration, and saturation intensity in order to produce good dynamic range for the signal decay, and Xe@water signal intensity was recorded as a function of saturation duration and frequency for each set of parameters. Measured signal intensities and known physical and NMR parameters were input into MATLAB (version 7.10, The MathWorks) and a nonlinear least squares routine was used to model the data against the McConnell-Bloch equations for two-site exchange, yielding dissociation rates (k_{rev}) for each set of parameters P_{Xe}, temperature, and CryA concentration. The equilibrium constant for complexation, K_{eq}, was determined by the relative signal intensities of Xe@water and Xe@CryA.

Results

Simulated CEST curves for each parameter combination agreed well with experimental data (Fig. 1 & 2). Using the dissociation rates, Xe@CryA residence times were found to be 20 – 40 ms for 20 °C samples, and 5 – 7 ms for 37 °C samples (Fig. 3). Temperature had the most significant impact on the xenon exchange rate, while physical constants such as cryptophane concentration and xenon pressure had a much smaller effect.

Discussion

Xenon's interaction with CryA was modeled with the McConnell-Bloch equations to ascertain k_{rev}, the dissociation rate of xenon from CryA, as a function of PXe, temperature, and CryA concentration. This rate is dictated by the relative amounts of signal in each of the two pools, which in turn depends on the equilibrium constant of Xe-CryA complexation. Here, K_{eq} was calculated to be 200 - 400 M^{-1}, which is lower than previously published reports (5-9). Early studies of Xe-cryptophane dynamics noted that K_{eq} was dependent upon the size of the internal cavity and reported values anywhere from 800 - 3900 M^{-1} (5, 6), but these experiments were performed in degassed organic solvents. Subsequent studies have incorporated carboxylic acid functional groups to improve aqueous solubility of CryA, with reported binding constants between 1000 and 30 000 M^{-1} (7, 8). However, those studies may not accurately describe our system because of differences in cryptophane modification and experimental protocol, specifically the use on non-degassed solvents. Indeed, attempting to use reported equilibrium constants fails to produce quality fits to the experimental data collected. The disparity between values reported here and elsewhere is likely the result of competition between small molecules and ions in solution which also possess an affinity for CryA. Bartik and coworkers postulated (5) that N_2, O_2, water, and perhaps other impurities were bound to CryA when they observed ^{1}H NMR resonance lines that were broader for non-degassed samples compared to degassed ones. Furthermore, Berthault notes (9) that chemical modification of cryptophane and the presence of counterions can drastically affect the observed equilibrium constant, and hence the association-dissociation dynamics, for Xe-cryptophane complexation. Knowing the xenon exchange dynamics for these biosensors is important because the rate of chemical exchange is a primary factor that influences the amount of contrast generated in a CEST experiment. With knowledge of the CryA residence time it is conceivable that better saturation pulses or schemes can be devised that are more efficient than the current continuous wave pulse schemes, with potentially reduced SAR exposure that sacrifice neither contrast nor sensitivity. Future work will aim to apply these results to the development of such strategies.

References

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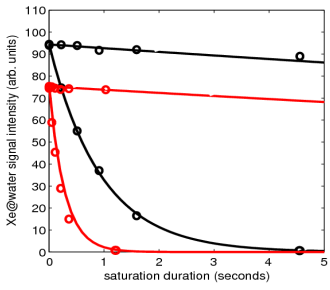

Figure 1: Representative CEST curves. [CryA] = 160 µM, P_{Xe} = 10.3 kPa. Circles are experimental data, lines are simulated data. Red: 37C Black: 20C.

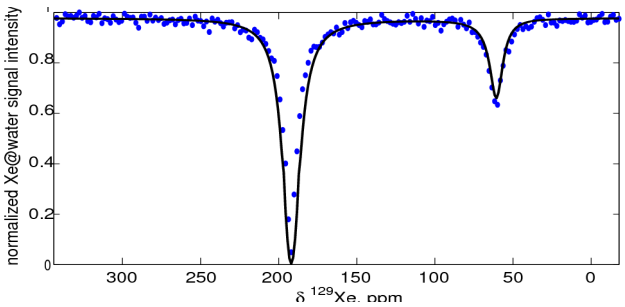

Figure 2: Using the derived Xe-CryA dissociation rate (k_{rev}), a z-spectrum was simulated (black) and compared to experiment (blue). [CryA] = 40 µM, P_{Xe} = 10.3 kPa. Saturation pulse : 1.4 s, B_1 = 431 Hz.

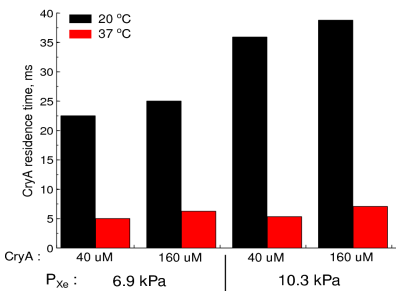

Figure 3: Xe@CryA residence time. Values were obtained by fitting CEST signal decay to the McConnell-Bloch equations.