Effect of Binding on Hyperpolarized MR Signals

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INTRODUCTION: In drug discovery studies, the question of binding of the drug to its target of interest is of great importance. It is well known that in fast exchange systems, changes in observed magnetization, chemical shift, and spin relaxation rates (T_1, T_2, $T_{1\rho}$ etc…) can be used to estimate equilibrium constants such as the binding constant in a ligand-host system[1]. One such well-characterized system, with published binding constants, is that of benzoic acid (ligand) and β-cyclodextrin (host)[2,3]. In the presence of β-cyclodextrin, the aromatic ring of benzoic acid preferentially inserts itself into the inner-core of the cyclodextrin molecule by hydrophobic interactions[4]. Typically NMR studies of these compounds at low concentrations in solution can require long experiment times and complicated pulse sequences. As a result of the dramatically improved signal-to-noise ratio (SNR) provided by hyperpolarized ^{13}C spectroscopy, natural abundance spectra can be acquired in one pulse[5]. The goal of this study was to use hyperpolarized ^{13}C spectroscopy in the benzoic acid - β-cyclodextrin system to understand the relationship between binding and loss of hyperpolarized signal.

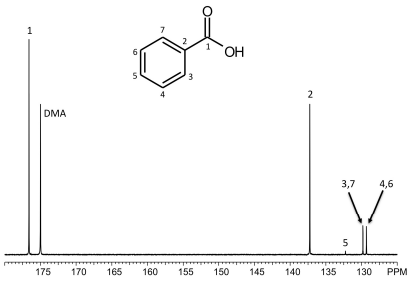

Figure 1. Single 5° pulse spectrum of 36.4mM natural abundance benzoic acid (310K, pH=7.8). All carbons are observed: C1 – 176.6 ppm, C2 – 137.3 ppm, C3,C7 – 129.8 ppm, C4, C6 – 129.3 ppm and C5 – 132.2 ppm.

METHODS: Samples of natural abundance benzoic acid were dissolved in dimethylacetamide (DMA) to a concentration of 3.6M. 2mg of the Finland radical per 100mg of benzoic acid was used as the organic free radical. Prepared samples were polarized for approximately 1 hour (at 94.094 Ghz) in a Hypersense® (Oxford Instruments, Oxford UK) and dissolved in 5mL of 100 mM phosphate buffer (pH=7.8). For assessment of binding, varying hyperpolarized benzoic acid solution (11 to 54mM) was mixed with 0.5mM β-cyclodextrin in the same 100mM phosphate buffer. All NMR studies were conducted at 310°K in a 10mm broadband probe on a 500Mhz (125Mhz for ^{13}C) Varian INOVA NMR spectrometer. All data were acquired using a 5° flip angle, 3s repetition time and 1 transient. Apparent T_1 relaxation times were fit to a mono-exponential equation in Matlab (Mathworks) as previously described[6,7].

RESULTS AND DISCUSSION: As shown below (**Figure 1**), hyperpolarized allowed the observation of all natural abundance ^{13}C carbons in benzoic acid in one 5° degree pulse. Hyperpolarized benzoic acid was then mixed in increasing concentrations relative to 0.5 mM β-cyclodextrin to assess the changes in T_1 due to binding. Figure 2 demonstrates the change in hyperpolarized signal with time for a solution of benzoic acid alone (**Figure 2a**) and 10 mM benzoic acid combined with 0.5mM β-cyclodextrin. At this benzoic acid to β-cyclodextrin ratio there was a visible decrease in the hyperpolarized signal of all benzoic acid carbons over time. The apparent T_1 relaxation times for the C_1 and C_2 carbons of benzoic acid in **Figure 2** decreased from 34.9 secs and 19 secs (Figure 2a) to 28.8 secs and 16.4 secs, respectively (**Figure 2b**). The T_1 relaxation time of the other protonated ring carbons of bezoic acid were too short to accurately measure a change with binding. **Figure 3** is a plot of constant β-cyclodextrin divided by **Δ**Obs, defined as $1/T_{1obs}$-$1/T_{1free}$, versus increasing concentrations of benzoic acid. The y-intercept of the linear fit is the binding constant K[1]. The log K derived from the fits of the C_1 and C_2 T_1 changes were 1.74 and 1.68, respectively. These binding constants were within the range of published log K measurements of benzoic acid (1.5-2.2) with β-cyclodextrin[2].

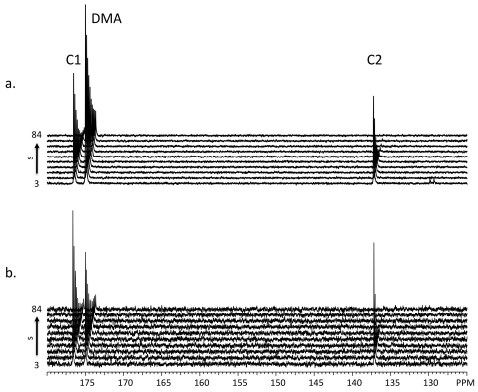

Figure 2. (a) Hyperpolarized benzoic acid and (b) benzoic acid with the addition of 0.5mM β-cyclodextrin are shown. Every third spectrum of the array is shown (a temporal resolution of 9 secs).

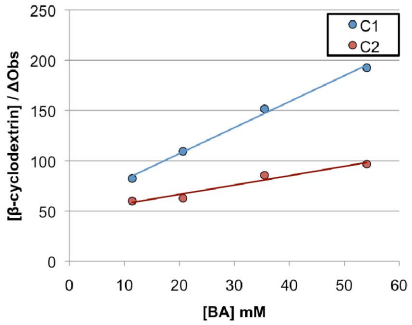

Figure 3. Plot of [β-cyclodextrin]/**Δ**Obs in as a function of increased benzoic acid [BA] concentration. The R^2 of the C_1 and C_2 fits were 0.995 and 0.953 respectively.

CONCLUSIONS: This study demonstrates that hyperpolarized signals from natural abundance ^{13}C carbons in a ligand decrease with the degree of binding to a host and can be used to determine the binding constant. This finding has implications for using *in vitro* hyperpolarized spectroscopy for the rapid screening of binding constants of small molecular weight drugs and for the improved understanding of the impact of enzymatic binding on the *in vivo* T_1 of hyperpolarized metabolic probes. Additionally, changes in T_1 of different ligand carbons with binding could provide structural information about the binding site.

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