The Influence of Bovine Serum Albumin on the T1 Relaxation of [1-13C]Pyruvate – A Study at Low Fields

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INTRODUCTION: [1-^{13}C]pyruvate has become the most important molecule in hyperpolarized MRI/S studies. One of the reasons of its success is that the T_1 at high field in [1-^{13}C]pyruvate is long, which minimizes loss of the large signal enhancement produced by DNP. The T_1 of carbon-1 has typically been reported in the 45-55 second range[1], but we have observed drastically reduced T_1 values at low field in the presence of bovine serum albumin (BSA). Albumin, the most abundant protein in plasma, plays a vital role in both transport and maintenance of osmotic pressure, and is often a requisite component in perfusion media. This depolarization effect, caused by BSA binding to pyruvate, has been usefully noted by Moreno *et al.*[2], but was only studied at high field (14.1 T). We show here a more dramatic effect as the field is reduced to below 30 gauss. Due to the importance of perfused organ and cell experiments to hyperpolarized research, we believe that this information is of considerable practical utility.

METHODS: [1-^{13}C]pyruvic acid was polarized to ~30% in a HyperSense DNP polarizer. 15 mM OX063 trityl radical served as the source of electron spin, and two sample sizes were used to vary the radical concentration of the final sample. The polarized pyruvic acid was dissolved in ~4 mL of an isotonic Tris-buffered solution containing 100 mg/L Na_2EDTA as a metal-ion chelating agent. Dilution in an equal volume of 2x Krebs-Henseleit buffer containing a variable concentration of BSA yielded a solution of sodium pyruvate at pH = 7.4±2. Prior to dilution, the buffer had been allowed to warm and equilibrate with air, ensuring that differences in dissolved O_2 did not affect our relaxation measurements. After dilution, the final sample was rapidly transferred to a solenoidal coil placed within a variable magnetic field generated by 61-cm Helmholtz coils. Small flip-angle pulses were applied every 3 seconds, and the signal decay was fit to a single exponential. The minimal RF loss was accounted for in the calculation of T_1. Two sets of T_1 measurements were made, one at 27.0 G (28.94 kHz ν_L) and another at 15.8 G (16.95 kHz ν_L). In each set, one experiment was performed with 50 µM radical concentration, and the remainder of the experiments were carried out with 100 µM radical concentration and the following concentrations (w/v %) of BSA: 0, 0.50, 1.0, 2.0, and 3.0.

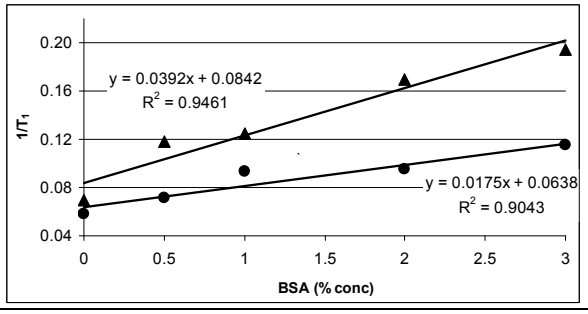
RESULTS: The observed T_1's of [1-^{13}C]pyruvate at different experimental conditions are given below in Table 1. The observed T_1 can be separated into individual contributions from different relaxation mechanisms as follows:

$$\frac{1}{T_1^{obs}} = \frac{1}{T_1^{rad}}[radical] + \frac{1}{T_1^{BSA}}[BSA] + \frac{1}{T_1^{pyr}},$$

where $(T_1^{rad})^{-1}$ and $(T_1^{BSA})^{-1}$ are the relaxation rates attributable to interaction with the trityl radical and BSA, respectively, and T_1^{pyr} is the T_1 of pyruvate alone. All three relaxation rates are functions of magnetic field. Lines were fit to the observed relaxation rate $(1/T_1^{obs})$ as a function of the varying concentrations of radical and BSA, as shown in Figure 1. The fitted equations and correlation coefficients are listed in the graphs. Assuming linearity for $(T_1^{rad})^{-1}$ and extrapolating back to zero radical concentration, the T_1 of pyruvate should be 28.7 sec and 39.7 sec at 15.8 G and 27.0 G, respectively.

	50 µM OX063	100 µM OX063	0.5% BSA	1% BSA	2% BSA	3% BSA
27.0 G	24.0	17.2	14.0	10.7	10.5	8.7
15.8 G	19.1	14.4	8.5	8.0	5.9	5.1

Table 1. T_1's (sec) of [1-^{13}C]pyruvate at various experimental conditions.



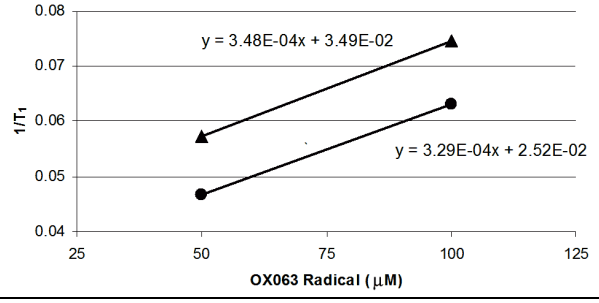


Figure 1. Relaxation rate as a function of BSA and OX063 radical concentration. (●) 27.0 G and (▲) 15.8 G

CONCLUSIONS: At low field, the combined effects of BSA and OX063 trityl radical can lead to a dramatically low T_1 for [1-^{13}C]pyruvate – e.g., 5 sec at 3% BSA, which is typical for perfusion experiments. Radical-induced relaxation appears to be relatively independent of field at the two fields studied here. On the other hand, the relaxation effect of BSA is significantly more effective at 15.8 G than at 27.0 G, suggesting that even faster relaxation could occur at lower fields. At 100 mM OX063 radical concentration, which is typical for pyruvic acid DNP protocols, BSA becomes the dominant source of relaxation at 1% and 2% concentration for 15.8 G and 27.0 G, respectively. This strongly suggests for a cautious use of BSA, and care should be taken to either (1) avoid the presence of BSA during transfer from polarizer to magnet, or (2) transfer in an increased magnetic field environment, or (3) minimize the transfer time.

REFERENCES: [1] Golman et al., *Mag Res Med*, 59:1005–1013 (2008); [2] Moreno et al., *Am J Physiol Heart Circ Physiol* 298:1556-1564 (2010)