

Adiabatic modulation of the longitudinal and transverse relaxations, T1ρ and T2ρ, of Gd-Fullerenol contrast agent: application for the cellular imaging.ρ

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Introduction: Rotating frame transverse relaxation (T2ρ) is the dominant relaxation mechanism during an adiabatic Carr-Purcell (CP) spin-echo pulse sequence with no delays between refocusing pulses [1]. Rotating frame longitudinal relaxation (T1ρ) is the dominant relaxation mechanism during adiabatic pulses placed prior to the excitation pulse [2]. The influence of exchange and dipolar interactions on the rotating frame relaxation rate constants, R1ρ and R2ρ (=1/T1ρ and 1/T2ρ, respectively) depend on the modulation functions of the adiabatic pulses used. This property of adiabatic pulses was utilized to generate the T2ρ and T1ρ contrasts in the human occipital lobe ¹H₂O at 4T magnetic field [1,2]. It was shown that adiabatic contrast methods provide an enhanced sensitivity to chemical exchange and dipolar relaxation pathways and provide a method to generate MR relaxation contrast based on the differences of the modulation functions of AFP pulses of the HSn (n=1,4) family.

Following the pioneering discovery by Kroto and Smalley of buckminsterfullerene C60, metallofullerene derivative Gd@C82 became an attractive candidate for the tracking studies of viable cells including stem cells. Recent studies showed that Gd@C82 fullereneol exhibit an increased cell uptake with protamine sulfate transfection, high R1 relaxivity and significant R2 magnetic field strength dependency (3). Here we measured the T2ρ and T1ρ of the Gd@C82 fullereneol and the cells labeled by this compound using different HSn pulses and investigated the mechanisms of uptake of the Gd@C82 fullereneol by the HeLa cells. Theory of the nuclear spin 1/2 - electron spin interactions in the rotating frame combined with two-site exchange was applied for the data analysis.

Methods: Spectroscopy studies of Gd@C82 fullereneol / water solution and HeLa cells labeled in culture with Gd@C82 were conducted with a 9.4T MRI/MRS system. HS1 and HS4 AFP pulses (R=20) of the hyperbolic secant HSn family were inserted prior to excitation with an adiabatic half passage (AHP) pulse for measuring T1ρ and after the AHP pulse for measuring T2ρ. LASER localization was used. T1ρ and T2ρ measurements were acquired incrementing the number of AFP pulses. Different AFP pulse lengths (Tp = 1-10 ms) were used for the measurements. 10M HeLa cells cultured in DMEM media in flasks were labeled as described in [3] with 2.25 ml 0.05 μmole/mL Gd, Gd-fullereneol (American Hightech Materials, Davis, CA), 18 μg Protamine sulfate and 6.75 ml DMEM. Cells were incubated overnight at 37 C, then washed with PBS and 7 units/mL heparin in PBS.

Results and discussion: The paramagnetic relaxation enhancement in proton spin due to presence of unpaired electron was presented in the well-known Solomon-Bloembergen-Morgan theories [4]. Here, we used the formalism by Goldman [5] for the nuclear-electron dipolar interactions accommodated for the time-dependent precession angle and the frequency sweep during adiabatic rotation. The relaxation rates are pulse modulation function dependent, as represented in Figure. 1. These plots predict relaxation enhancement during the HS4 pulse as compared to the HS1 pulse for the R1ρ relaxation, and decrease of the R2ρ during the HS4 as compared to the HS1 pulse.

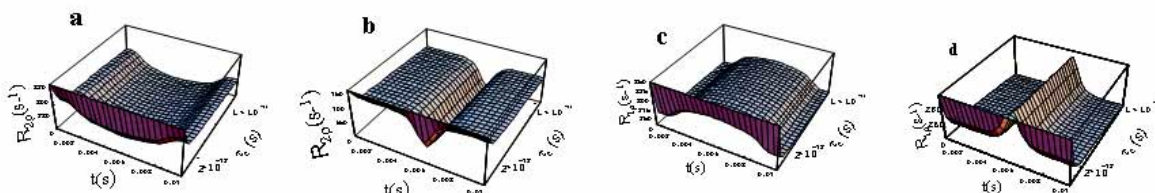
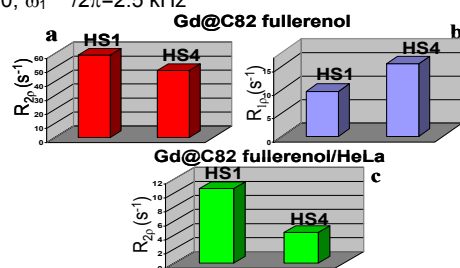


Figure 1. a,b Calculated transverse relaxation rates due to electron-nuclear dipolar interactions during the a) HS4 and b) HS1 pulses and the longitudinal relaxation rates c) HS4 and d) HS1 as a function of the rotational correlation time (τ_c). The pulse parameters used for calculation were: pulse length $T_p=10$ ms, $R=20$, $\omega_1^{\max}/2\pi=2.5$ kHz



In Figure 2a,b the relaxation rate measurements of the Gd@C82 fullereneol/water (0.1 μMol) using $T_p=2$ ms are presented. These plots show the effects of the adiabatic pulse modulation functions on R1ρ and R2ρ. The ratio R1ρ (HS4)/R1ρ (HS1)≈1.6 and R2ρ (HS4)/R2ρ (HS1)≈1.22 was obtained. As expected, this ratio is significantly pulse length dependent (data not shown). A significant effect on the T2ρ relaxations was observed with the HeLa cells labeled by Gd@C82 fullereneol (Figure 2c).

Figure 2. a,b T1ρ (a) and T2ρ (b) measurements of the Gd@C82 fullereneol/water; c) T2ρ of the Gd@C82 -fullereneol/HeLa sample. Measurements were performed using HS1 and HS4 AFP pulses, $R=20$, pick-power $\omega_1^{\max}=2.5$ kHz.

In experiments, T2ρ was strongly attenuated by the HS4 pulse as compared to the HS1 pulse, suggesting sensitivity to the compartmentalization of paramagnetic agent in the cell (observed on electron microscopy) and the change of exchange relaxation parameters (e.g. apparent populations and apparent relaxation rate constants of the exchanging sites, P' and R'2ρ) of water molecules in and outside the cells. This work suggests that adiabatic T1ρ and T2ρ measurements provide a method to assess the relaxation parameters of the sample and promising for the relaxation studies of MR contrast agents.

References

1. Michaeli S, Sorce DJ, Idiyatullin D, Ugurbil K, Garwood M. *J Magn Reson* 169:293-299 (2004); 2. Michaeli S, Sorce DJ, Springer C, Ugurbil K, Garwood M. *PISMRM*, 2005, 3. (a) Anderson S., Lee K., Frank JA, *Invest. Radiol*, in press; Mikawa M., 4. I. Solomon I., *Phys. Rev.* 99, 559 (1955); Bloembergen N., Morgan L., *J. Chem. Phys.* 842, 1961. 5. Goldman M., *Quantum description of High-Resolution NMR in Liquids*, 1988.

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