Size-dependent chemical exchange saturation transfer (CEST) in liposomes

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INTRODUCTION

Liposomes are a versatile platform for the delivery of drugs and contrast agents because they provide biocompatibility for in vivo applications. Recently, Aime et al. [1] designed a liposomal MRI contrast system by encapsulating paramagnetic shift agents [2,3] in liposomes (lipoCEST). They reported high MR sensitivities using liposome concentrations as low as 90 pM. We studied the mechanism responsible for the observed lipoCEST contrast. In particular, we elucidate the effect of liposome size on the CEST enhancement, in order to show that smaller liposomes can generate better lipoCEST contrast.

MATERIALS & METHODS

Liposomes were prepared using the rapid extrusion method [4]. We mixed phosphatidylcholine (PC) and cholesterol in a 1:1 molar ratio. Liposomes formed during the extrusion process passively encapsulated 100mM Thulium 1,4,7,10-tetraaza-1,4,7,10-tetrakis(carboxymethyl)cyclododecane (Tm-DOTA) in phosphate buffered saline (PBS). We used size exclusion chromatography to separate the liposomes from unencapsulated Tm-DOTA. Liposome sizes were characterized with dynamic light scattering (DLS). The average exchange rate from intra-liposomal water to bulk water is [5]

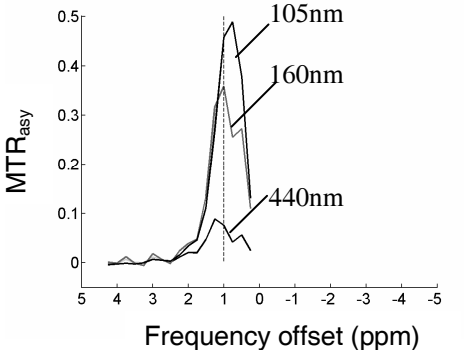
$$\bar{k}_{lw} = N_l P_l \bar{S}_l / (x_l V_w) \approx 3P_l / \bar{r}_l \qquad (1)$$

where N_l, $\bar{S}_l$, $\bar{r}_l$ are total number, average surface area, and average radius of liposomes; P_l and and x_l are the liposomal permeability and total water fraction, respectively. The approximation in Eq. (1) assumes that the liposome concentration is low ($x_l << 1$) and that the size distribution is narrow ($\bar{S}_l \approx 3\bar{V}_l / \bar{r}_l$). All experiments were done on a Bruker Avance 11.7T system at room temperature of 23±1°C. A 1D presaturation spin-echo pulse sequence was used to obtain saturated water magnetization as a function of saturation frequencies. The magnetization transfer ratio (MTR) is obtained from MR signals with and without presaturation at the resonance frequency of intraliposomal water: MTR = 1 − [S(on)/S(off)]. The MTR asymmetry is the difference between negative and positive offset frequencies with respect to water: $MTR_{asy} = MTR_{+\Delta\omega} - MTR_{-\Delta\omega}$. The pulse sequence parameters are: presaturation frequency offsets Δω = −4.35 ppm to 4.25 ppm with a 0.25 ppm stepsize, amplitude = 1.8μT, TE = 2ms, TR = 8s + T_{sat} (presaturation time).

RESULTS & DISCUSSION

We prepared liposome samples with three different sizes: 105nm , 160nm , and 440nm. MTR_{asy} vs. presaturation pulse frequency are shown in the **Fig**. The trend is that smaller liposomes correspond to larger MTR_{asy}. Their physical and magnetic properties are summerized in the Table below. The concentrations were adjusted such that the intraliposomal water fraction is kept constant at 0.72%. We measured the exchange rates by measuring MTR_{asy} as a function of T_{sat} using saturation times from 25ms to 10 s [6]. From Eq. (1), the membrane permeability P_l can be calculated from the ratio of the bulk water exchange rate and $1/\bar{r}_l$ (see Table). As expected, P_l is a physical property of the lipid composition and is independent of liposome size. Taken together, our data show that smaller liposome sizes have a larger water average transmembrane exchange rate [7], suggesting that saturated liposomal water is more efficiently transferred outside to bulk water because of a larger surface-to-volume ratio.

diameter (nm)	[liposome] (nM)	x_l	$3/\bar{r}_l$ (μm^{-1})	$\bar{k}_{lw}$ (s^{-1})	P_l (μm/s)	MTR_{asy} @ 1ppm
105±30	32	0.72%	57	92	1.61	0.46
160±30	5.5	0.72%	38	60	1.58	0.34
440±200	0.38	0.72%	13.6	22	1.62	0.13



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