Paramagnetic Metal Probes used for the Development of High-Relaxivity Protein Targeted Contrast Agents.

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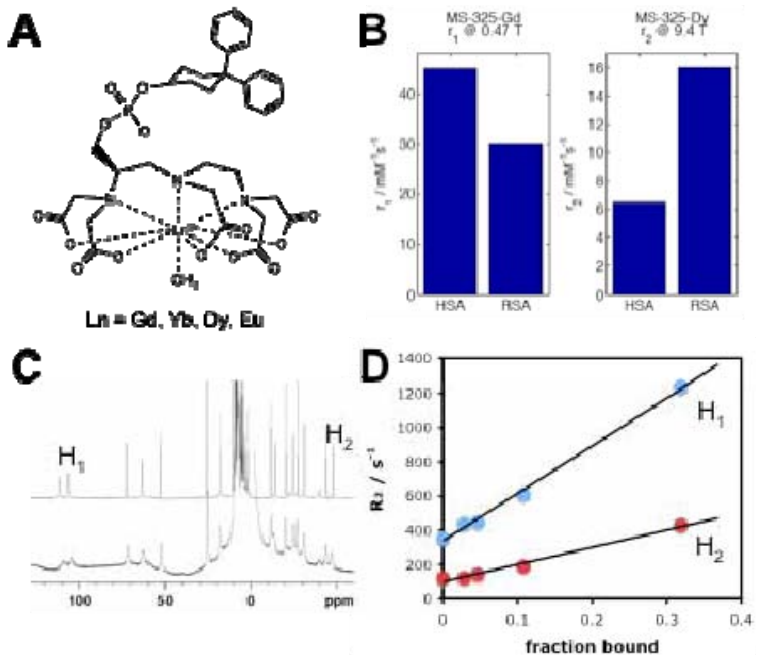
Introduction. The relaxivity of Gd-based MRI contrast agents depends on a variety of parameters: magnetic field, number of coordinated water molecules, water exchange rates, molecular tumbling time and other correlations times. Binding of a contrast agent to a large protein increases relaxivity due to the increase in the molecular tumbling time. MS-325 (Vasovist™, Fig. 1A, Ln = Gd) is a contrast agent for MR angiography which non-covalently binds to serum albumin [1]. Despite the similar size and structure of albumins from various animal species, the relaxivity of MS-325-Gd was found to be significantly higher when bound to human serum albumin (HSA) than when bound to albumins from rabbit (RSA), rat or mouse (Fig. 1B). Potential reasons for this could be differences in (i) local tumbling time of MS-325 in the protein bound state, (ii) changes in water hydration number upon protein binding or (iii) different water exchange times. Lanthanide ions have similar size and charge (+3) and their complexes are often iso-structural, however the ions have diverse physical properties. This paper describes how specific molecular parameters can be addressed individually by replacing the Gd^{3+} in MS-325-Gd with the ions Eu^{3+}, Yb^{3+}, or Dy^{3+} and using various biophysical methods (NMR, relaxometry, luminescence) that exploit the unique physical properties of these ions.

Materials and Methods. ^{1}H-NMR spectra of MS-325-Yb (~4 mM) were measured at 400 MHz (9.4 T) with increasing concentration of albumin (0 – 0.6 mM). The MS-325-Yb fraction bound to albumin was determined by ultrafiltration through a 5 kDa cutoff membrane. Longitudinal relaxivity of MS-325-Gd in 4.5% albumin was measured by inversion recovery at 0.47 T. The transverse relaxivity of MS-325-Dy at 9.4 T was measured using a CPMG sequence. Hydration numbers of MS-325-Eu in albumin were determined by time-resolved fluorescence methods [2]. The bound water chemical shift of MS-325-Dy was obtained from the linear dependence of the bulk water shift in solutions with increasing Dy concentration (0 – 150 mM) using 3-(Trimethylsilyl)-propionic acid as chemical shift reference. All experiments were performed at 37ºC.



Results and Discussion. Fig. 1C shows the NMR spectra of MS-325-Yb in the absence (top) and presence (bottom) of 0.6 mM HSA. Paramagnetic Yb shifts the ^{1}H resonances of the MS-325 ligand away from those of the protein and allows R_2 measurements to be made. The line broadening originates from Curie relaxation induced solely by the increase in tumbling time upon protein binding. Since MS-325-Yb and HSA are in fast chemical exchange, a linear increase in R_2 as function of the MS-325-Yb fraction bound to HSA is found (Fig 1D). This allows estimation of relaxation rates in the bound state. Resonance assignment by 2D NMR and metal-proton distances estimated from X-ray crystallography, allows calculation of a molecular tumbling correlation time of ~10 ns for the HSA bound complex. Similar experiments performed in rabbit albumin (RSA) gave a tumbling time of about 20 ns. Thus, the reduced relaxivity of MS-325-Gd in RSA cannot be caused by larger flexibility of the compound. The luminescence lifetime of MS-325-Eu is virtually identical in HSA and RSA when measured in either H_2O or D_2O, indicating one inner sphere water molecule (q=1) for the protein bound compounds. Transverse relaxivity (r_2) of the bulk water protons in MS-325-Dy solutions (Fig. 1B) is higher in RSA than HSA. Among other parameters, r_2 of the Dy solutions depends on the water exchange rate and molecular tumbling time [3]. Proton relaxation enhancement is more sensitive than ^{17}O and allows determination of water exchange at low concentrations where MS-325-Dy is fully bound (> 90%) to albumin. Using the molecular tumbling time derived above and the bound water shift of δ_M = -650 ppm, the water exchange rate of MS-325-Dy at 37ºC was determined to be $(30 \pm 5) \times 10^6$ s^{-1} in HSA and $(4 \pm 2) \times 10^6$ s^{-1} in RSA. Thus, water exchange depends on the albumin and is slowed down considerably in RSA compared to HSA.

Conclusions. Linewidth measurements of the paramagnetically shifted ligand protons of MS-325-Yb show that albumin binding of MS-325 increases the molecular tumbling time to 10 - 20 ns. Luminescence decay measurements on MS-325-Eu indicate a single metal-bound water in either HSA or RSA. High field transverse relaxivity of MS-325-Dy demonstrates a water exchange rate about 7 times slower when MS-325 is bound to RSA compared to when bound to HSA. Thus, slower water exchange results in a lower r_1 relaxivity for MS-325-Gd in some animal albumins compared to HSA.

References. [1] Caravan *et al.*: *JACS.* (2002) 124, 3152. [2] Horrocks *et al.*: *JACS.* (1979), 101, 334. [3] Pubanz *et al., Inorg. Chem.* (1995) 34, 4447.