Novel Gd-based MRI contrast agents binding to plasma proteins: synthesis and characterization

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Introduction

Gd-based MR blood-pool contrast agents have been prepared, that rely on non-covalent binding to plasma proteins. In order to obtain more efficacious compounds, we have focused part of our effort to increasing the number of exchangeable water molecules. Indeed, all other parameters being equal, the relaxivity of a contrast agent is directly proportional to the number of bound water molecules. We present here the preparation and full characterization of Gd-L1, a macrocyclic Gd(III) chelate with two bound water molecules, designed to bind to HSA, as compared to two other chelates Gd-L2 and Gd-L3.

Materials and Methods

Ligands L1, L2 and L3 and their chelates were synthesized, purified, and characterized. Relaxivity was measured in the absence and in the presence of HSA (50 mM buffer, pH = 7.4, 37°C, 0.5 and 1.4T). NMRD were recorded at 4 different temperatures. Luminescence lifetime measurements were recorded in water and D_2O. Quantitative ENDOR data were obtained following a new protocol described in another poster.

Results

The substituents on L1 were selected in order to provide HSA-binding capability (biphenyl moiety) as well as 3 Gd coordinating groups for a total of 7 Gd donor groups on the ligand. The sulfonatopropyl moiety provides a negative charge, which should decrease the propensity of q = 2 compounds for anion binding, while, at the same time, being a poor lanthanide binding group. As a comparison, ligand L2 was prepared. The Gd-L2 chelate is the HSA-binding equivalent of Gd-DOTA. As such, it should behave like a q = 1 Gd complex. Similarly Gd-L3 was synthesized as a comparison (q = 0) (Fig. 1). As shown in Fig. 2, Gd-L1 has about twice the relaxivity of Gd-L2 in buffer, as can be expected for a q = 2 chelate vs. a q = 1 compound. Similarly, the low relaxivity of Gd-L3 can be accounted for if no water molecule is present in the first coordination sphere. On the other hand, if rotation is slowed down by binding to HSA, the measured relaxivities, shown in Fig. 2, do not reflect the same observation. Indeed, the relaxivity of bound Gd-L1 is exceedingly low compared to that of Gd-L2. This observation can be explained by a slowing-down of the water exchange rate or more likely by an exclusion of water from the first coordination sphere of this q = 2 complex. Quantitative ENDOR measurements, as well as fluorescence lifetime measurements (Fig. 3), were performed in buffer and in the presence of HSA. They are in agreement with the loss of the coordinated waters from Gd-L1 upon binding to the protein, most likely replaced by amino acid side chains from the protein.

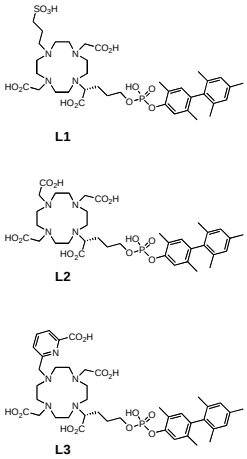

Fig. 1: ligands L1, L2, L3

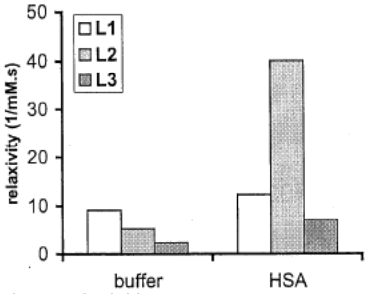

Fig. 2: relaxivities at 0.5T

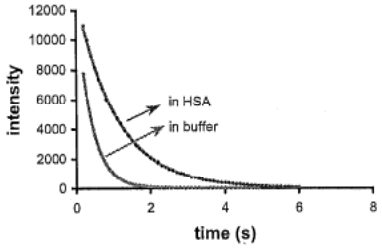

Fig. 3: luminescence data for Eu-L1

Conclusions

The design of new protein-binding high-relaxivity MRI contrast agents has been tackled by preparing a compound with two inner-sphere water molecules as compared to q = 1 and q = 0 chelates. Unfortunately, the binding of this chelate to HSA was shown to result in a low relaxivity as a consequence from the exclusion of the inner-sphere waters as demonstrated by ENDOR and fluorescence measurements. The combination of relaxivity, ENDOR and fluorescence measurements allowed for a full characterization of Gd chelates both in the presence and in the absence of protein. This better insight proves very useful in the design of better protein-binding MRI contrast agents.