Direct determination of the water coordination number of MRI contrast agents by ENDOR spectroscopy.

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Synopsis

*E*lectron-*N*uclear-*Do*uble-*R*esonance (ENDOR) spectroscopy provides a novel methodology for direct determination of the water coordination number (*q*) of Gd based MRI contrast agents for both aqueous solutions and protein bound complexes. It is shown that, depending on the structure of the ligand, the hydration number can be significantly different in aqueous solution compared to compounds bound to a protein target. This explains the unexpectedly low relaxivity for some protein bound complexes.

Introduction

The relaxivity of Gd-based MRI contrast agents depends on a variety of parameters, such as the magnetic field, water exchange rates and other correlations times. It is, however, directly proportional to the number of water molecules (*q*) ligating the metal center, making *q* an essential experimental parameter. Direct information on *q* has been obtained previously e.g. by X-ray crystallography. However, single crystals are not necessarily reflective of conditions in aqueous solutions and are very difficult to obtain for contrast agents bound to protein targets. Indirect information on *q* can be obtained from ^{17}O chemical shift studies but these are not practicable on protein bound compounds because of the high concentrations required. Alternative methods, such as fluorescence quenching require metals different from Gd(III) and thus may raise concerns on metal induced structural changes of the complex. Recently, ENDOR spectroscopy has been proven to be well suited for determination of the Gd-H_{water} distance for various MRI contrast agents [1-3]. Here we present a novel methodology to obtain direct information on *q* based on ENDOR spectroscopy.

Materials and Methods

^{1}H-Mims-ENDOR spectra [1-3] corresponding to the $-^1/_2 \Leftrightarrow +^1/_2$ electronic transition of Gd(III) have been obtained at ~18 GHz electron spin resonance frequency at low temperatures (~8 K) on ~ 1 mM solutions of compound with either H_2O/CD_3OH or D_2O/CD_3OD as solvent mixtures. The difference between the spectra obtained in protonated and deuterated media represents the spectrum of exchangeable protons which is dominated by the proton hyperfine coupling tensor assigned to the protons of the directly coordinating water molecule. Spectra of the protein bound complexes have been obtained at ~1.5 mM compound concentration in ~2 mM Human serum albumin (HSA) solution in either H_2O or D_2O based HEPES buffer (50 mM, pH 7.4).

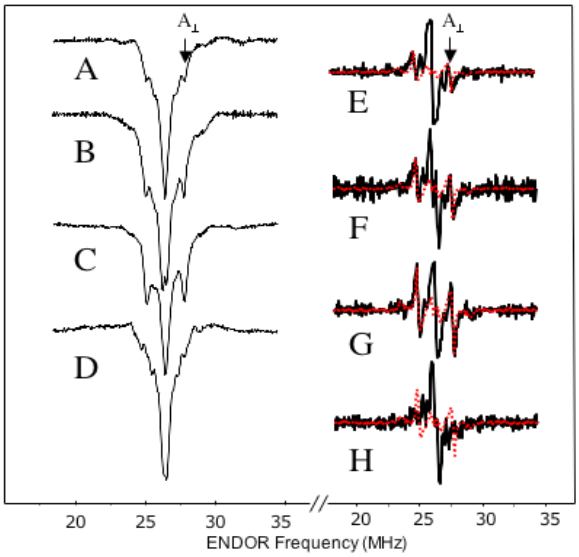

Fig. 1: ENDOR spectra of MRI contrast agents.

Results and Discussion

Fig. 1A shows the proton ENDOR spectrum for exchangeable protons of MS-325-d_{10}, a novel MRI contrast agent developed for blood vessel imaging. From the principal values of the hyperfine coupling tensor $A_\perp$, a proton-Gd distance of 3.1 Å can be obtained [1]. Fig. 1E shows the first derivative of Fig. 1A (black), superimposed with the spectrum of the $Gd^{3+}(H_2O)_8$ aqua complex scaled by 1/8 (red). The similar intensity of the spectral features at $A_\perp$ indicate q=1, which is in agreement with previous studies. For MS-325-d_{10} bound to HSA (Fig. 1B and F), the same hydration number (q=1) is obtained, indicating that binding of the compound to the protein does not perturb the metal coordination sphere. Fig. 1C shows the ENDOR spectrum of a DO3A derivative (**L1**) measured in aqueous solution. Comparison of the signal intensity of the exchangeable protons (Fig. 1G, black) with $Gd^{3+}(H_2O)_8$ (scaled by 1/4, red) yields q=2. In contrast, if **L1** is bound to HSA, no directly coordinated water is detected (Fig. 1D and H). Consequently, upon protein binding both water molecules are replaced (most likely by amino acid side chains). This is in line with the unexpectedly low relaxivity of this compound when bound to HSA.

Conclusions

Proton ENDOR spectroscopy is a useful method for the direct determination of the water hydration number of metal complexes such as Gd(III)-based MRI contrast agents in frozen solutions. The method requires only low sample amount (< 500 nmol) and can be applied to protein bound complexes thus making it a valuable tool for the development of protein targeted high relaxivity compounds.

References

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