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Magnetic Resonance Imaging Detects a Specific Peptide−Protein Binding Event

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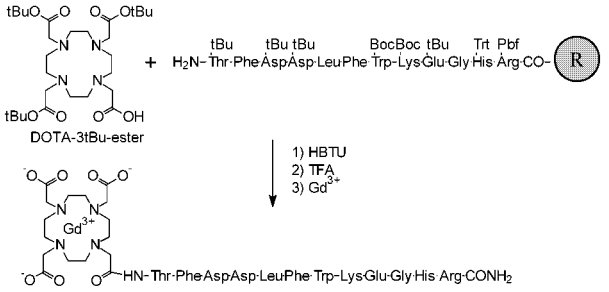
Contrast agents (CA) have become important diagnostic tools for clinical MRI studies. These paramagnetic metal complexes function by accelerating the relaxation rate of bulk water protons. Any image contrast produced by the agent typically reflects nonuniform distribution of the complex in different tissues, a crude physical process. A major step forward in the development of contrast agents would be to devise molecules whose ability to relax water protons is triggered or enhanced greatly by recognition of a particular biomolecule. This would open up the possibility of developing MRI tests specific for biomarkers indicative of particular disease states.

Meade and co-workers[1] have reported a novel strategy toward this end in which the paramagnetic ion is encased within a restrictive cavity that can be cleaved by a particular enzyme. In the absence of enzyme, water coordination to the metal is restricted while in its presence the coordination site is unblocked and the relaxation rate is enhanced. Another strategy is to take advantage of the increase in water relaxivity (r_1) that occurs upon slowing molecular rotation (τ_R) of a small paramagnetic complex, by either binding to a macromolecule[2,3] or polymerization of the agent itself.[4] One common targeting protein is human serum albumin because it displays a rather wide range of binding capabilities. Typically, interactions between low molecular weight Gd^{3+} complexes and albumin are rather weak.[3,5−8] One approach to increase specificity and binding interactions is to design a Gd^{3+} chelate that binds at the active site of an enzyme as an inhibitor.[2] Here, we report a somewhat different approach that has potential for screening a variety of biomolecules by MRI, a peptide-based CA that is activated upon binding to a specific target protein.

We previously reported the isolation of a 20 residue peptide from a combinatorial library that binds to the yeast transcription repressor protein Gal80 with excellent specificity.[9] The K_A of the fluorescein-labeled peptide·Gal80 complex was approximately 3×10^7 M^{-1} (determined by fluorescence polarization). Subsequent work revealed that only 12 residues were necessary for specific binding (TFDDLFWKEGHR; Y. Han and T. K., unpublished observations). A DOTA moiety was added to the N-terminal threonine of this 12-mer by direct coupling of DOTA-tris(*tert*-butyl ester) (Scheme 1) in an automated peptide synthesizer with use of a standard Fmoc solid-phase peptide synthesis protocol. The unmodified peptide

Scheme 1. Solid-Phase Peptide Synthesis of Gd^{3+}-G80BP



(G80BP) and Gd-DO3A-peptide (Gd^{3+}-G80BP) were purified by HPLC and characterized by electrospray mass spectrometry (ES-MS).

To ensure that conjugation of GdDOTA to the peptide did not compromise binding to Gal80, a competitive binding experiment was performed by displacing the fluorescein-labeled 12-mer with either G80BP or Gd^{3+}-G80BP. Analysis of these data (not shown) gave K_A values of 2×10^6 and 5×10^5 M^{-1} for G80BP and Gd^{3+}-G80BP binding to Gal80, respectively. This indicates that the hydrophobic fluorescein group on the N-terminal position enhances binding (∼15-fold) while the more hydrophilic GdDO3A inhibits binding (∼5-fold) relative to the parent peptide. Nevertheless, the Gd^{3+}-G80BP·Gal80 binding constant is much larger than that typically found for low molecular weight Gd^{3+}-chelates interacting with proteins. In comparison, it is ∼20 to 80-fold larger than the MS-325-albumin constant,[5,6] ∼650-fold larger than the GdBOPTA-albumin[7] or GdEOB-DTPA-albumin constants,[8] and even ∼3-fold larger than the inhibitor-directed, sulfonamide-GdDTPA-carbonic anhydrase interaction constant.[2]

The water proton relaxivity of Gd^{3+}-G80BP in buffer is 8.3 ± 0.2 mM^{-1} s^{-1} (pH 7.4, 20 MHz, 25 °C), similar to that of a GdDTPA derivative linked to tri-lysine.[2] Upon addition of Gal80 to 14 μM Gd^{3+}-G80PB (Figure 1), the relaxation rate ($1/T_1$) of bulk water increases substantially. This is clearly a specific effect because little change was seen when an equivalent amount of BSA was added. The lower curve was fit to a 1:1 binding model by fixing K_A to 5×10^5 M^{-1} and r_{1free} to 8.3 mM^{-1} s^{-1} while allowing r_{1bound} to vary. The calculated r_{1bound} value (44.8 ± 1.7 mM^{-1} s^{-1}) is comparable to that measured for other $q = 1$ complexes when bound to albumin, a protein of similar size ($r_{1bound} = 48.9 \pm 3.5$ mM^{-1} s^{-1} for MS-325-albumin[5] and 39.0 ± 4.4 mM^{-1} s^{-1} for GdEOB-DTPA-albumin[8]). Given the binding constant for Gd^{3+}-G80BP plus Gal80 and the water relaxivities of the free and bound peptides, one can easily estimate the ΔT_1 and corresponding change

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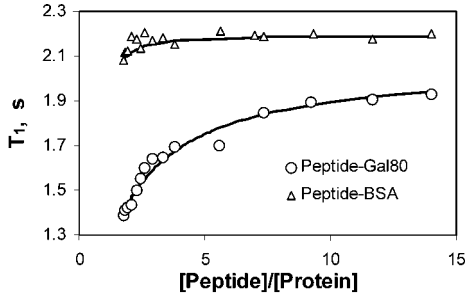

Figure 1. Water proton spin−lattice relaxation times for a 14 μM solution of Gd^{3+}-G80BP upon addition of either BSA (△) or Gal80 (○). In each case, the protein concentration was varied from 1 to 8 μM.

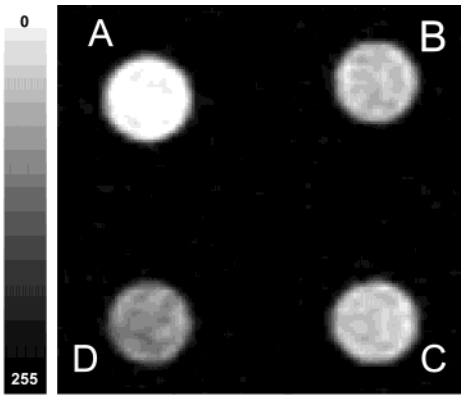

Figure 2. T_1-weighted MR images of four tubes (3 mm OD) containing 14 μM Gd^{3+}-G80BP plus (A) 11 μM Gal80, (B) 11 μM BSA, and (C) no protein. Sample D contained only buffer. Images were collected at room temperature on a Philips 1.5T Clinical Imager using the body coil as transmitter and a knee coil as receiver. A single 4 mm slice was acquired centered at the sample height (15 mm). The FOV was 50 × 50 mm and the matrix size was 256 × 256 points. A standard clinical T_1-weighted spin−echo image sequence with TR/TE = 300/16 ms and two averages was used.

in MR image intensity one would anticipate upon mixing these samples. Upon mixing 11 μM Gal80 and 14 μM Gd^{3+}-G80BP, ~59% of the peptide would be bound to Gal80 and the ΔT_1 would be 0.78 s. This is a much larger change in T_1 than one would get for previously reported systems at these same concentrations. For example, for the vascular contrast agent, MS-325, and its protein binding partner, HSA, at these same concentrations, only 6% of the agent would be bound to HSA and ΔT_1 would be 0.13 s. This illustrates the importance of using high affinity contrast agents capable of binding low concentrations of protein.

To demonstrate the feasibility of using this molecularly targeted agent at such low concentrations, MRI experiments were performed on a phantom using a standard 1.5T clinical imaging system. The images in Figure 2 show that free Gd^{3+}-G80BP can be detected in buffer at 14 μM (compare intensities of D versus C) and no further enhancement was observed when 11 μM BSA was included (B versus C). In comparison, a large enhancement was observed for the sample containing the specific binding partner, Gal80. A 10-fold difference in image intensity was observed in samples A versus C (Figure 2).

The Gal80-binding peptide employed in this study was isolated by using phage display to screen a combinatorial peptide library. This is a very general technique that can be used to isolate peptides with relatively high affinities for virtually any biomaterial. Thus, the approach that we have employed here should be a general paradigm for the development of protein-specific, binding-activated contrast agents. Furthermore, it should be possible to also extend this approach to non-peptide ligands. In particular, more "drug-like" cell-permeable protein-binding compounds conjugated to the appropriate Gd^{3+} complex could be interesting in vivo imaging agents. We anticipate that by optimizing the imaging equipment[10] and the water exchange kinetics of the Gd^{3+} complex,[11] the technique could be extended to proteins at sub-μM concentrations.

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(10) The images shown were collected by using a standard T_1-weighted clinical imaging sequence without optimizing for T_1 differences between samples. Even so, the image intensity of sample A was 10-fold higher than that of sample C.
(11) Given that the water relaxivity of the Gd^{3+} complex used here (a monoamide) is known to be limited by water exchange,[12] a complex having optimal water exchange and a 2- to 3-fold increase in bound relaxivity would easily bring this technique into the sub-μM range.
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