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MRI Sensing Based on the Displacement of Paramagnetic Ions from Chelated Complexes

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We introduce a mechanism for ion sensing by MRI in which analytes compete with paramagnetic ions for binding to polydentate chelating agents. Displacement of the paramagnetic ions results in alteration of solvent interaction parameters and consequent changes in relaxivity and MRI contrast. The MRI changes can be tuned by the choice of chelator. As an example, we show that calcium-dependent displacement of Mn^{2+} ions bound to EGTA and BAPTA results in a T_1-weighted MRI signal increase, whereas displacement from calmodulin results in a signal decrease. The changes are ion selective and can be explained using relaxivity theory. The ratio of T_2 to T_1 relaxivity is also calcium-dependent, indicating the feasibility of "ratiometric" analyte detection, independent of the probe concentration. Measurement of paramagnetic ion displacement effects could be used to determine analyte ion concentrations with spatial resolution in opaque specimens.

A variety of mechanisms have been proposed for detecting inorganic ions using magnetic resonance imaging (MRI) contrast agents. In the most widely explored approach, analyte binding to a paramagnetic metal chelate withdraws ligands, increasing the number (q) of inner-sphere sites available for water coordination at the paramagnetic center. As a consequence, the longitudinal relaxivity (r_1) of protons on exchangeable solvent molecules in the complex is similarly increased.[1] Here we introduce a new strategy in which the analyte completely releases the paramagnetic ion from a polydentate chelating ligand to form the aqua complex (aq), which serves as an efficient MRI contrast agent with characteristics sharply different from those of the chelate. In this facile and flexible new approach, changes in r_1 can be tuned by the choice of the metal chelate complex (Figure 1a). Measurement of the effect by MRI can then be used to determine the analyte concentration with spatiotemporal resolution.

We tested the applicability of the paramagnetic metal displacement strategy to sense calcium ions by MRI. We chose the relatively labile manganese(II) ion as the paramagnetic reporter because aqueous Mn^{2+} is widely used as a T_1 contrast agent for in vivo imaging.[2] Manganese complexes were assembled with ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), 1,2-bis(*o*-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), and calmodulin (CaM), all metal-binding molecules with selective affinity for Ca^{2+} ions. Structures of Mn^{2+} associated with EGTA[3] and with a protein domain closely related to the four calcium-binding regions of CaM[4] are shown in Figure 1b,c. They suggest that from zero to two coordinated water molecules per Mn^{2+} ion may be present. In contrast, Mn^{2+}(aq) is coordinated by six water molecules.[5] These differences in q, combined with rotational correlation time (τ_R) effects and the time constant for water exchange (τ_M, where $q \neq 0$), contribute to relaxivity changes upon displacement of Mn^{2+} bound by EGTA, BAPTA, or CaM, upon addition of Ca^{2+}.

Mixtures containing 100 μM Mn^{2+} in 1:1 stoichiometry with EGTA or BAPTA, or in 4:1 stoichiometry with CaM, which has four metal binding sites, were imaged in the presence of 0−20 mM $CaCl_2$, using spin-echo pulse sequences in a 4.7 T MRI scanner at room temperature (22 °C). In all cases, progressive changes in the MRI signal were observed as Ca^{2+} concentrations increased (Figure 2a). Controls lacking Mn^{2+} or chelators showed no significant changes (Supporting Information). Intensity data were analyzed to determine relaxivity as a function of the Ca^{2+} concentration for each chelator (Figure 2b). As [Ca^{2+}] increased, values of r_1 increased from 1.7 to 5.2 mM^{-1} s^{-1} for EGTA and from 1.3 to 6.2 mM^{-1} s^{-1} for BAPTA (estimated errors 10%). For CaM, r_1 *decreased* from 10.5 to 5.5 mM^{-1} s^{-1} with increasing [Ca^{2+}]. Changes in the transverse relaxation rate (r_2) were also observed (Figure 2c). Addition of Ca^{2+} to Mn-EGTA or Mn-BAPTA produced r_2 increases from 19 or 8.6 mM^{-1} s^{-1}, respectively, to 92 or 98 mM^{-1} s^{-1}. Addition of Ca^{2+} to Mn_4CaM induced a modest drop in the apparent transverse relaxivity, from

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(1) Li, W.; Fraser, S. E.; Meade, T. J. *J. Am. Chem. Soc.* **1999**, *121*, 1413–1414.
(2) Koretsky, A. P.; Silva, A. C. *NMR Biomed.* **2004**, *17*, 527–531.
(3) Schauer, C. K.; Anderson, O. P. *Acta Crystallogr., Sect. C* **1988**, *44*, 981–986.
(4) Andersson, M.; Malmendal, A.; Linse, S.; Ivarsson, I.; Forsen, S.; Svensson, L. A. *Protein Sci.* **1997**, *6*, 1139–1147.
(5) Lauffer, R. B. *Chem. Rev.* **1987**, *87*, 901–927.

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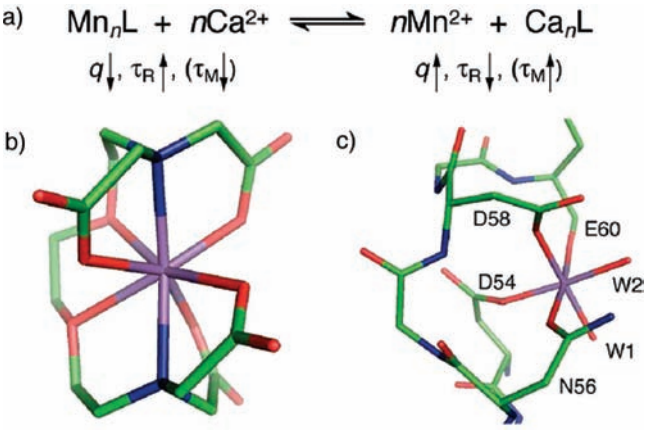

Figure 1. Mechanism of MRI calcium sensors based on displacement of Mn^{2+} from chelated complexes. (a) Upon addition of excess Ca^{2+}, Mn^{2+} ions are released from ligand molecules (L) including EGTA, BAPTA, and CaM. Dissociated Mn^{2+} ions have higher q, lower τ_R, and, for L = CaM, higher τ_M than chelated manganese, all factors that influence longitudinal relaxivity. (b) In the complex with EGTA, Mn^{2+} (purple) is octacoordinate with $q = 0$.[2] (c) The crystal structure of Mn^{2+} in the complex with a canonical calcium binding site, loop 2 of calbindin,[4] shows two bound waters (W1 and W2); this binding loop has 71% sequence identity to the corresponding seven residues in the first EF hand of CaM, suggesting that Mn^{2+} ions in the complex with CaM may similarly exhibit $q = 2$.

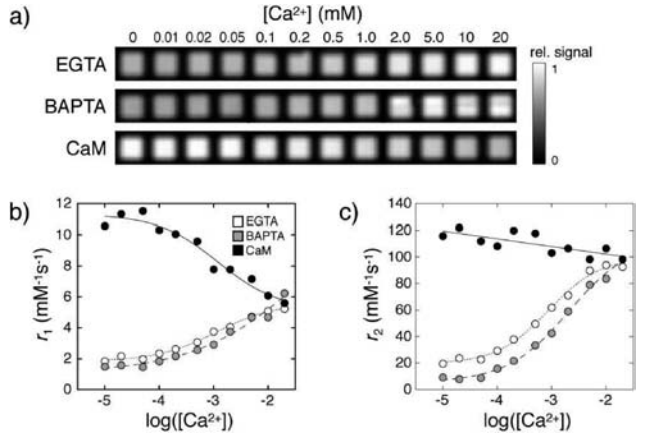

Figure 2. MRI and relaxivity changes upon paramagnetic displacement from chelators. (a) Progressive T_1-weighted MRI signal changes observed as a function of $[Ca^{2+}]$ added to mixtures of 100 μM Mn^{2+} with 100 μM EGTA, 100 μM BAPTA, or 25 μM CaM, all in MOPS, pH 7. Intensity changes were normalized to the maximal MRI signal across the titration range. (b) Longitudinal relaxivities (r_1) computed from T_1-weighted data, as a function of calcium concentrations for Mn-EGTA (open circles), Mn-BAPTA (gray circles), and Mn_4CaM (black circles). Fitted curves are shown for visualization purposes. (c) Transverse relaxivity (r_2) for the same samples.

110 to 98 mM^{-1} s^{-1}. All three ligands showed substantial calcium-dependent changes in r_2/r_1 (Supporting Information), suggesting that these reagents and their derivatives might be used to probe concentration-independent "ratiometric" quantification of $[Ca^{2+}]$ or other divalent cations.[6,7]

The titration curves produced by adding Ca^{2+} to Mn^{2+} mixtures with EGTA, BAPTA, or CaM converged on relaxivity values similar to those measured using Mn^{2+}(aq) complexes ($r_1 = 5.6 \pm 0.1$ mM^{-1} s^{-1} and $r_2 = 98 \pm 3$ mM^{-1} s^{-1}), supporting the conclusion that the relaxation changes were caused by the Ca^{2+}-dependent Mn^{2+} displacement mechanism. A 20 mM Ca^{2+} concentration brought about almost complete Mn^{2+} dissociation in all cases. This result is in reasonable agreement with reported values of $K_d(Mn^{2+})/K_d(Ca^{2+})$ for EGTA of $10^{-1.3}$ and for BAPTA of 10^{-2}.[8,9] The Ca^{2+}-induced r_1 increases observed for Mn-EGTA and Mn-BAPTA were also consistent with the anticipated effects of increasing q of Mn^{2+} from zero to six following displacement of the ion from the chelators. Relaxivities of Mn-EGTA and Mn-BAPTA complexes measured in the absence of calcium were comparable to outer-sphere relaxivities reported at different fields for other Mn^{2+} complexes having $q = 0$.[10−12] Ironically, the low relaxivities and relatively labile nature of many $q = 0$ Mn^{2+} complexes have historically been considered a limitation to their use as contrast agents in clinical MRI, but these very properties have been exploited here to enable calcium sensing based on displacement of Mn^{2+} from Mn-EGTA and Mn-BAPTA.

The structure and nuclear magnetic relaxation properties of Mn_4CaM have not been previously studied, but inner-sphere contributions to r_1 of the CaM complex can be estimated, in conjunction with experimentally justified values for τ_M and τ_R (see the Supporting Information). The modeling studies indicate that the T_1 relaxivity per bound water (r_1/q) for Mn_4CaM is approximately 10-fold higher than for Mn^{2+}(aq). Both a short τ_M, as generally reported for $q \neq 0$ Mn^{2+} complexes,[5] and long τ_R for Mn_4CaM probably account for its strong relaxation effects and counterbalance its reduced q, compared to Mn^{2+} (aq). The shape of the CaM titration curve thus reflects multiple determinants of r_1, in addition to contributions of Mn^{2+}/Ca^{2+} exchange at all of the four protein metal-binding sites. The CaM titration data were well approximated ($r = 0.98$) by a monophasic binding curve with a Hill coefficient of 0.8, suggesting that metal substitution at each site was approximately independent and identically distributed.

To explore the analyte selectivity, we measured relaxivity changes of Mn^{2+} complexes in the presence of diamagnetic competitor ions of biological relevance. Excesses of Zn^{2+}, Mg^{2+}, or K^+ (each 1 mM) were added to 100 μM Mn^{2+} in stoichiometric mixtures with metal-binding sites in EGTA, BAPTA, or CaM. As predicted by reported affinity constants[8,9] and the Irving−Williams series, Zn^{2+} produced strong r_1 changes in Mn-EGTA and Mn-BAPTA and abolished calcium sensitivity, but Mg^{2+} and K^+ had little effect (Figure 3a−c). Calcium-induced longitudinal relaxation (T_1) changes on Mn_4CaM persisted in the presence of the competitor ions, but both Zn^{2+} and Mg^{2+} reduced calcium-dependent effects. Competitive effects on calcium-dependent transverse relaxation (T_2) changes followed a similar pattern

(6) Grynkiewicz, G.; Poenie, M.; Tsien, R. Y. *J. Biol. Chem.* **1985**, *260*, 3440–3450.
(7) Aime, S.; Fedeli, F.; Sanino, A.; Terreno, E. *J. Am. Chem. Soc.* **2006**, *128*, 11326–11327.
(8) Cheng, K. L.; Ueno, K.; Imamura, T. *CRC Handbook of Organic Analytical Reagents*; CRC Press: Boca Raton, FL, 1982.
(9) Yuchi, A.; Tanaka, A.; Hirai, M.; Yasui, T.; Wada, H.; Nakagawa, G. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 3377–3381.
(10) Elizondo, G.; Fretz, C. J.; Stark, D. D.; Rocklage, S. M.; Quay, S. C.; Worah, D.; Tsang, Y. M.; Chen, M. C.; Ferrucci, J. T. *Radiology* **1991**, *178*, 73–78.
(11) Troughton, J. S.; Greenfield, M. T.; Greenwood, J. M.; Dumas, S.; Wiethoff, A. J.; Wang, J.; Spiller, M.; McMurry, T. J.; Caravan, P. *Inorg. Chem.* **2004**, *43*, 6313–6323.
(12) Wang, S.; Westmoreland, T. D. *Inorg. Chem.* **2009**, *48*, 719–727.

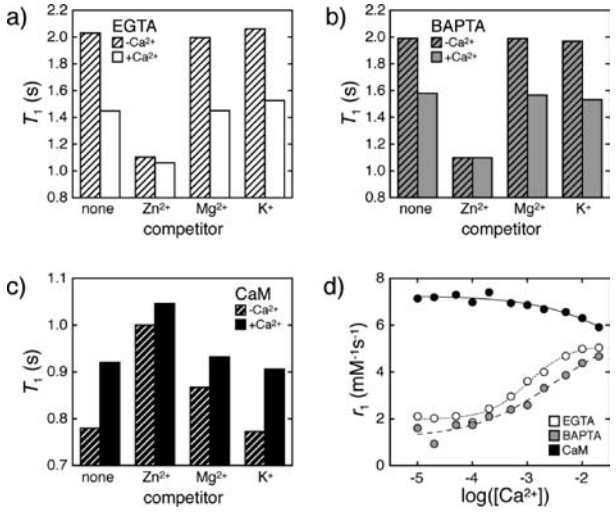

Figure 3. Calcium-dependent T_1 changes recorded in the presence of competitor ions. Data obtained from 100 μM Mn^{2+} mixtures with (a) 100 μM EGTA, (b) 100 μM BAPTA, or (c) 25 μM CaM, in the absence or presence of competing diamagnetic cations (each 1 mM) and in the absence (striped bars) or presence (solid bars) of 1 mM Ca^{2+}. (d) Titration of Ca^{2+} against Mn-EGTA, Mn-BAPTA, or Mn_4CaM in artificial cerebrospinal fluid (aCSF), showing the effects of biomimetic ion concentrations on titration curves. Mn^{2+}, Ca^{2+}, and binding ligand concentrations as in Figure 2b. Note that 1 mM Ca^{2+} did not bring about maximal relaxivity changes for any of the ligands. 1 mM Zn^{2+} was more effective at displacing Mn^{2+} than 1 mM Ca^{2+}, and therefore resulted in larger relaxation changes for all three chelators. The biologically relevant competitors Mg^{2+} and K^+ did not alter calcium res;ponses observed for Mn-EGTA and Mn-BAPTA, however, and Mg^{2+} only partially attenuated the Mn_4CaM response to 1 mM Ca^{2+}.

for all three Mn^{2+} complexes (Supporting Information). As an additional test, we examined the calcium-induced relaxation changes induced in aCSF, a biomimetic solution approximating the interstitial environment of the brain (Figure 3d). The paramagnetic Mn^{2+} displacement effect for EGTA and BAPTA was minimally perturbed by the presence of competing solution components. For Mn_4CaM, Ca^{2+}-dependent r_1 changes were reduced and occurred at higher [Ca^{2+}], perhaps reflecting the relatively high concentration (0.8 mM) of Mg^{2+} in aCSF.

These results indicate that the Mn^{2+} displacement mechanism for measuring Ca^{2+} or other ion concentrations by MRI provides strong contrast changes and could be useful for applications in solution, as well as in relatively simple naturalistic environments where few extraneous metal ligands are present.[13] A particular strength of the approach is the potential for ratiometric measurements. An obvious limitation in complex contexts is the possibility that paramagnetic metal ions, once released from the chelators, might diffuse away or be sequestered by binding partners unassociated with a sensing mechanism. This problem could be particularly serious where reversible or dynamic measurements are desired. A possible solution would be to use steady-state infusion or dialysis methods to maintain the concentration of paramagnetic complexes to a fixed level, ensuring that any analyte-induced metal dissociation and MRI contrast changes are reversed over time. In cases where the displaced paramagnetic ion is relatively nontoxic, these approaches might conceivably be applied in live animals. We note that the sensitivity range of the complexes studied here is ideally suited to detecting millimolar calcium concentrations present in extracellular compartments like the cerebral interstitium.[14] In the future, extensions of the paramagnetic metal displacement mechanism might include sensing of other ions, such as Zn^{2+} or Mg^{2+}, or the design of novel paramagnetic ion/chelator pairs, for example based on engineered CaM variants, with improved selectivity and sensitivity for detecting analytes by MRI.

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Supporting Information Available: Experimental details, additional results from control relaxation measurements, ratiometric data, relaxivity modeling, and selectivity data. This material is available free of charge via the Internet at http://pubs.acs.org.

(13) Manz, B.; Hillgartner, M.; Zimmermann, H.; Zimmermann, D.; Volke, F.; Zimmermann, U. *Eur. Biophys. J.* **2004**, *33*, 50–58.

(14) Angelovski, G.; Fouskova, P.; Mamedov, I.; Canals, S.; Toth, E.; Logothetis, N. K. *ChemBioChem* **2008**, *9*, 1729–1734.