A Calcium Sensitive Magnetic Resonance Contrast Agent

Wen-hong Li, Scott E. Fraser and Thomas J. Meade
Beckman Institute, 139-74, Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA

Introduction

As part of our efforts to study cell signaling and regulation in intact animals, we are developing MR contrast agents that provide information about physiological species and biochemical events.[1] Here, we report the first MRI contrast agent DOPTA-GD (Figure 1) whose relaxivity is selectively modulated by Ca^{2+} concentration.[2] Ca^{2+} is an important intracellular secondary messenger of signal transduction and regulates many cellular functions. A noninvasive technique for measuring Ca^{2+} changes in living organisms will serve as an important tool for biomedical research.

Results and Discussion

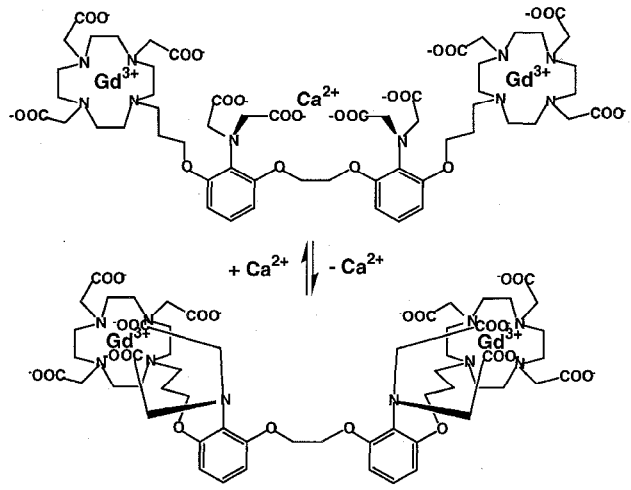
This new MR agent modulates access of water to a chelated Gd^{3+} ion in the presence and absence of Ca^{2+}. The design of the agent is based on the synthesis and characterization of several model systems that ultimately led to the macrocyclic dimer shown in Figure 1. 1,2-bis(o-aminophenoxy)ethane-N,N,N',N'-tetra-acetic acid (BAPTA) binds Ca^{2+} with a 10^5 fold selectivity versus the divalent metal ion Mg^{2+}, and is relatively insensitive to pH fluctuations at physiological conditions.[3] 1,4,7-tris(carboxymethyl)-1,4,7,10-tetra-azacyclododecane (DO3A) chelates lanthanides with high affinity to form a thermodynamically stable and kinetically inert complex.[4] DOPTA-GD was designed to possess two limiting conformational states with respect to calcium concentration ($[Ca^{2+}]$). In the absence of Ca^{2+}, each pair of aromatic iminoacetates of BAPTA interacts with Gd^{3+} through ionic attractions. In the presence of Ca^{2+}, the aromatic iminoacetates of BAPTA rearrange to bind Ca^{2+} thereby allowing water to bind directly to Gd^{3+}.

DOPTA-GD was synthesized from nitroresorcinol in 8 steps.[2] The effect of $[Ca^{2+}]$ on the relaxivity of DOPTA-GD was assessed by T_1 measurements. The relaxivity of DOPTA-GD in Ca^{2+} free buffer = 3.26 $mM^{-1}sec^{-1}$, and increased with increasing $[Ca^{2+}]$. The change in relaxivity is mostly striking in the $[Ca^{2+}]$ range of 0.1 μM to 10 μM, and levels off at higher level of $[Ca^{2+}]$ reaching a maximum of 5.76 $mM^{-1}sec^{-1}$. Hill plot analysis of the measured relaxivities at varying $[Ca^{2+}]$ result in a dissociation constant of the complex = 0.96 μM.

The increase in observed relaxivity of DOPTA-GD (~80%) that is induced by an increase in $[Ca^{2+}]$ corresponds to a 80% relaxivity change of each Gd^{3+} unit and is significantly higher then our previously reported enzyme-reporter class of agents.[1] In addition, the relaxivity of DOPTA-GD is relatively insensitive to $[Mg^{2+}]$ change. Increasing Mg^{2+} concentration from 0 to 10 mM changed the relaxivity of DOPTA-GD less than 8%. Intracellular $[Mg^{2+}]$ is approximately 1 mM and its fluctuation is less dynamic than $[Ca^{2+}]$, and therefore interference on the $[Ca^{2+}]$ measurements by DOPTA-GD should be minimal. Further, changing the pH from 6.80 to 7.40 changed the measured T_1 of DOPTA-GD by less than 3% (in the presence or absence of Ca^{2+}). Therefore, within physiological pH ranges, H^+ should not interfere with the relaxivity of the complex with respect to $[Ca^{2+}]$.

In summary, we have synthesized a MR contrast agent where the relaxivity of the complex is controlled by the presence or absence of the divalent ion Ca^{2+}. By structurally modulating inner-sphere access of water to a chelated Gd^{3+}ion we observe a substantial change in T_1 upon the addition of Ca^{2+}. Importantly, the agent is selective for binding Ca^{2+} ions versus Mg^{2+} and H^+. An immediate application of this agent is to study the cellular Ca^{2+} activity changes during the embryogenesis. The agent can be conveniently injected inside cells at the early developmental stage. Both the cell movements and the Ca^{2+} fluctuations during the development can be followed over long time. The experiments may help to resolve some uncertainties of measuring Ca^{2+} activity of the interior cell layers not accessible to light microscopy.

Figure 1. Schematic of DOPTA-GD representing the proposed conformational dependence on Ca^{2+}.



References: **1**. Moats, R. A., Fraser, S. E., Meade, T. J. *Angew. Chem. Int. Ed. Eng. 36*, 726, **1997**. **2**. Li, W. H., Fraser, S. E., Meade, T. J. *J.Am.Chem.Soc.* In press. **3**. Tsien, R. Y. *Biochemistry 19*, 2396, 1980. **4**. Kumar, K., Chang, C. A., Tweedle, M. F. *Inorg. Chem. 32*, 587,1993.