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A Smart Magnetic Resonance Contrast Agent for Selective Copper Sensing

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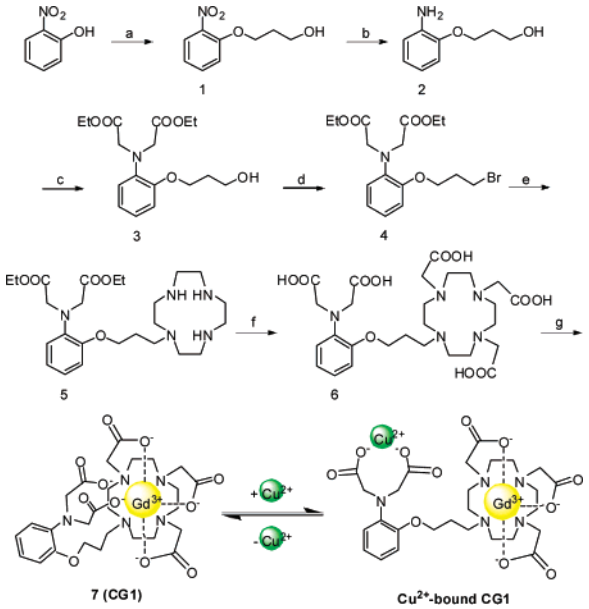
Copper is a required nutrient for human growth and development, but mishandling of copper occurs in many serious human afflictions, including neurodegenerative disesases[1] such as Alzheimer's disease,[2] Menkes and Wilson's diseases,[3] amyotrophic lateral sclerosis,[4,5] and prion diseases.[6,7] The relationships between copper regulation and its physiological or pathological consequences are fascinating but remain insufficiently understood at the molecular level.

To help elucidate the complex contributions of copper to health and disease, we are initiating a broad-based program to develop new chemical approaches to study aspects of its uptake, trafficking, export, and function in living systems.[8,9] In this regard, magnetic resonance imaging (MRI) is a powerful technique that can provide three-dimensional images of organisms with up to cellular resolution.[10] Metal-based contrast agents can enhance MR signals by providing paramagnetic centers to increase the relaxation rates of water protons.[11] High-spin gadolinium(III), with its seven unpaired electrons, is particularly well-suited to this task, and Gd^{3+} coordination complexes are currently used in 40−50% of all clinical MRI applications.[12] We envisioned combining the utility of Gd-based contrast agents with copper-selective recognition elements to provide a new class of chemosensors for molecular imaging of copper biology in living systems. Along these lines, several interesting examples of smart MRI contrast agents have been reported, the majority of which respond to enzymes[13−17] or pH changes.[18−20] In contrast, metal-responsive MR sensors are rare[21−23] and none are specific for copper. In this report, we present the synthesis and properties of Copper-Gad-1 (CG1), a first-generation smart magnetic resonance contrast agent for selective copper sensing. CG1 features good selectivity for Cu^{2+} over abundant biological cations and a 41% relaxivity increase upon Cu^{2+} binding and is sensitive to μM changes in $[Cu^{2+}]$.

Our design strategy for a turn-on, copper-responsive MR sensor relies on modulating the relaxivity of a contrast agent by action of an external analyte or stimulus, a concept inspired by the elegant smart contrast agent for β-galactosidase activity first described by Meade.[13,14] The present sensor construct comprises a Gd^{3+} contrast agent platform coupled to a pendant iminodiacetate site for binding Cu^{2+}, the dominant oxidation state in extracellular fluids. We anticipated that this dianionic, tridentate receptor[24] would afford selectivity for Cu^{2+} over abundant cellular alkali and alkaline earth ions owing to distinct differences in charge and coordination preferences, as well as selectivity for Cu^{2+} over Zn^{2+} due to trends in the Irving−Williams series. In the absence of Cu^{2+}, the hard, anionic carboxylate donors of the copper receptor will hinder inner-sphere water access to the Gd^{3+} core and subsequently minimize relaxivity. Recognition and binding of Cu^{2+} to the pendant receptor will relieve steric congestion around the Gd^{3+} center, leading to increased inner-sphere water access and proton relaxivity.

The synthetic route to CG1 and its proposed action for sensing Cu^{2+} are shown in Scheme 1. Coupling of 2-nitrophenol and 3-bromopropanol provides **1** in 72% yield. Reduction of **1** to aniline

Scheme 1. Synthesis of Copper-Gad-1 (CG1)[a]



[a] Conditions: (a) 3-bromopropanol, NaOH, DMF, 80 °C (72%); (b) H_2 (1 atm), Pd/C, (quant.); (c) ethyl bromoacetate, DIEA, NaI, DMF, 100 °C (87%); (d) CBr_4, PPh_3, CH_2Cl_2, 0 °C (84%); (e) cyclen, $CHCl_3$, rt, (85%); (f) bromoacetic acid, NaOH/H_2O, pH 14, 45 °C (65%); (g) $GdCl_3 \cdot 6H_2O$, H_2O, pH 7, rt (80%).

2 by hydrogenation over Pd/C proceeds in near quantitative yield. Double alkylation of **2** with ethyl bromoacetate affords alcohol **3** in 87% yield. Treatment of **3** with CBr_4 generates bromide **4** in 84% yield. Reaction of **4** with 2.5 equiv of cyclen delivers the monosubstituted macrocycle **5** (85% yield). The final ligand **6** is formed upon addition of bromoacetic acid to **5** under basic conditions (65% yield). Metalation of **6** with $GdCl_3 \cdot 6H_2O$ in water at pH 7 followed by purification by size-exclusion chromatography yields CG1 **7**. CG1 was identified by mass spectrometry, and its Gd^{3+} content was analyzed using ICP-OES.

The ability of Cu^{2+} to modulate the longitudinal relaxivity of CG1 was determined at 25 °C using T_1 measurements at a proton frequency of 400 MHz. Spectroscopic measurements were carried out under simulated physiological conditions (phosphate-buffered saline, PBS, pH 7.4). The compound shows good stability in PBS solution for up to 5 days (<4% hydrolysis) but loses an acetate arm over longer time scales (75% hydrolysis after 30 days).[25] In the absence of Cu^{2+}, the relaxivity of CG1 in PBS is 3.76 mM^{-1} s^{-1}. The addition of Cu^{2+} triggers a 41% relaxivity enhancement to 5.29 mM^{-1} s^{-1}, and a plot of relaxivity vs $[Cu^{2+}]$ shows that the observed relaxivity reaches a maximum value at 1 equiv Cu^{2+} and levels off at higher added Cu^{2+} concentrations (Figure 1a). A Hill plot gives a slope of 1.15 ± 0.10, indicating a simple binding process with no cooperativity. The apparent K_d for the 1:1 Cu^{2+}/CG1 complex is 167 ± 48 μM. Binding of Cu^{2+} to CG1 is

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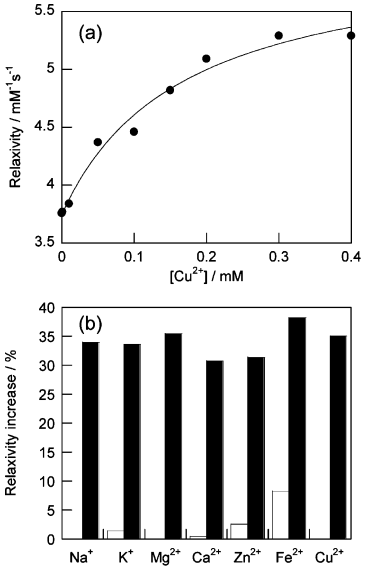

Figure 1. (a) Relaxivity response of 0.21 mM CG1 to various concentrations of Cu^{2+}. T_1 measurements were acquired in PBS, pH 7.4, at a proton frequency of 400 MHz. (b) Relaxivity responses of CG1 to various metal ions. Bars represent the percent change in relaxivity over CG1 alone. White bars represent the addition of an excess of the appropriate metal ion (10 mM for Na^+, 2 mM for K^+, Mg^{2+}, and Ca^{2+}, and 0.2 mM for Fe^{2+} and Zn^{2+}) to a 0.21 mM solution of CG1. Black bars represent the subsequent addition of 0.2 mM Cu^{2+} to the solution. Spectra were acquired in PBS, pH 7.4, at a proton frequency of 400 MHz.

reversible; treatment of a solution containing CG1 and Cu^{2+} with excess EDTA restores CG1 proton relaxivity back to baseline levels. CG1 can potentially detect biologically relevant changes in Cu^{2+} concentrations. For example, the addition of 30 μM Cu^{2+}, the estimated level of copper released during neural activity,[2] to a 0.21 mM solution of CG1 gives a 9% relaxivity increase, a value which may be suitable for some in vivo imaging applications on the time scale of MR measurements.[26]

The relaxivity response of CG1 shows good selectivity for Cu^{2+}. Figure 1b displays the response of 0.21 mM CG1 to several biologically relevant metal ions. CG1 exhibits excellent selectivity for Cu^{2+} over abundant cellular alkali and alkaline earth cations at physiological levels; the addition of 10 mM Na^+ or 2 mM K^+, Mg^{2+}, and Ca^{2+} do not elicit CG1 relaxivity enhancements or interfere with its Cu^{2+} response. Moreover, 0.2 mM Fe^{2+} or Zn^{2+} trigger much lower CG1 relaxivity responses and do not affect its ability to sense 0.2 mM Cu^{2+}. Addition of 10-fold excess Zn^{2+} (300 μM) over Cu^{2+} (30 μM) does show a partially disrupted response (3% increase) over Cu^{2+} alone (9% increase). We also tested the effect of pH on CG1 relaxivity and observed no change in relaxivity for CG1 in the physiologically relevant pH range of 6.8−7.4.

Finally, we sought to probe the mechanism of Cu^{2+}-induced CG1 relaxivity enhancements. To achieve this goal, we measured the luminescence lifetimes of the Tb^{3+} analogue of CG1 in PBS-buffered H_2O and D_2O in the absence and presence of Cu^{2+}. Analysis of this data using Horrock's method[27] shows that the estimated values for q, the number of inner-sphere water molecules, for CG1 goes from 0.31 in the absence of Cu^{2+} to 1.07 after Cu^{2+} addition. The luminescence data support the idea that Cu^{2+} binding to CG1 increases relaxivity in part by increasing q.

To close, we have described a new approach to selective copper sensing using MR detection. CG1 provides a prototype candidate for potential MR-based copper imaging in living systems, displaying selectivity for Cu^{2+} over a range of competing cellular metal ions, a 41% increase in relaxivity upon Cu^{2+} recognition, and sensitivity to μM changes in Cu^{2+} levels. Luminescence data for the CG1 Tb^{3+} analogue support a mechanism involving q-modulation by Cu^{2+} binding. Goals for the design of next-generation copper-specific MR sensors include greater turn-on responses, increased sensitivity to lower Cu concentations, and higher selectivity for Cu^{2+} in the presence of excess Zn^{2+}.

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Supporting Information Available: Synthetic and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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