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Electrode Chemistry Yields a Nanoparticle-Based NMR Sensor for Calcium

Sonia Taktak^{†,§}, Ralph Weissleder^{†,‡}, and Lee Josephson^{*,†}

[†]Center for Molecular Imaging Research, Massachusetts General Hospital and Harvard Medical School, 149 13th Street, Charlestown, Massachusetts 02129

[‡]Center for Systems Biology, Massachusetts General Hospital, Boston, Massachusetts 02114

Abstract

Magnetic nanoparticles (NPs) have been used to obtain NMR-based sensors for analytes ranging from small molecules to viruses by the conjugation of biomolecules (antibodies, proteins, oligonucleotides) to the surface of NPs. In the presence of an analyte, the NPs form clusters that alter the relaxation time of the surrounding water protons. Here, we show that an organic molecule that binds calcium ions of nonbiological origin, rather than a biomolecule, can be employed to modify the surface of a magnetic NP. When calcium ions are added, they induce NP clustering, providing an NMR-based sensor for these ions. Our work suggests that the many chemistries of nonbiological origin, such as those employed for ion-selective electrodes, can be adapted to obtain NMR-based sensors for ions.

Introduction

Biological molecules (proteins, peptides, and oligonucleotides) have often been attached to magnetic nanoparticles (NPs), which enables the resulting NPs to undergo target-mediated changes in aggregation state with changes in the relaxation time of water protons. This principle has been used to assay diverse analytes in a wide variety of assay formats.^{1–5} Alternatively, surface-functionalized NPs used in assays can be enclosed in a device that features a semipermeable membrane that lets small analytes such as glucose enter and leave while the NPs remain inside.⁶ Miniaturized, multireservoir implantable MR-based devices using NPs aggregated by different analytes in different compartments have been described,⁷ and it would be desirable if such devices could measure ions as well as proteins that can currently be determined.

Calcium ions play such a key role in biological processes that a number of approaches have been described to determine their concentration in fluids or to continuously monitor their presence in biological compartments or industrial applications. Recently, the protein calmodium and the M13 peptide were attached to different populations of NPs whose aggregation and effects on T2 were mediated by calcium.³ However, this approach requires sources of calmodium and peptide and is not readily extendable to many ions that lack binding proteins with the requisite affinity and specificity. A plentiful source of chemistry that is nonbiological in origin and might be employed in designing surface-functionalized NPs for ions is found in the considerable literature on ion-selective electrodes. To examine whether an ion-selective electrode chemistry could be adapted to obtain an NP-based assay for ions, we

* Corresponding author. E-mail: josephso@helix.mgh.harvard.edu. Tel: 617-726-6478. Fax: 617-726-5708.

§Current Address: T2 BioSystems, Inc., 286 Cardinal Medeiros Avenue, Cambridge, Massachusetts 02141.

examined the interaction of calcium ions with NPs featuring the diglycolic amide motif because calcium-sensitive electrodes have employed this approach.^{8–10}

Materials and Methods

The synthesis of the Chel-CLIO NP is outlined in Figure 1A. The amino-CLIO (amino-crosslinked dextran-coated iron oxide) nanoparticle was prepared as described.¹¹

Chel^{9,12}

Diglycolic anhydride (1.0 g, 8.6 mmol) was dissolved in 8 mL of 1,4-dioxane. Dibutylamine (1.45 mL, 8.6 mmol) premixed with 0.7 mL of pyridine was added dropwise at 0 °C. After 3 h of reaction at room temperature, solvent was evaporated, and the residue was dissolved in a 1:1 dioxane/HCl solution. After the evaporation of solvent and recrystallization in methanol/water (1:1), 1.39 g of pure compound Chel was obtained as a white powder. The yield after recrystallization was 66%. Structure and purity were confirmed by ¹H NMR and ESI-MS. The compound was very hygroscopic and needed to be lyophilized before each use.

Chel-CLIO

Chel (1.1 mg, 4.5 mmol) in 50 mL of DMSO was added to 1 mg of amino-CLIO NP in MES buffer (50 mM, 0.1 M NaCl) at pH 6.0. Freshly dissolved sulfo-NHS (9.7 mg, 45 mmol) in 500 mL of MES and EDC (8.6 mg, 45 mmol) in 500 mL of DMSO were premixed and added to the mixture in two additions at a 30 min interval. The reaction proceeded for 1 h at room temperature, and the product was purified through a Sephadex G-25 PD10 column (GE Healthcare, Uppsala, Sweden) equilibrated with PBS. The amount of chelator attached was quantified by using the SPDP/TCEP method.⁶ Some 56 Chel molecules were found per NP based on 8000 Fe atoms per NP.¹³ Typical values for NPs were a diameter of 30 nm by laser light scattering with relaxivities per iron of $R1 = 21 \text{ mM}^{-1} \text{ s}^{-1}$ and $R2 = 41 \text{ mM}^{-1} \text{ s}^{-1}$.

Minispec Relaxation Time Measurements—Relaxation times were measured at 0.47 T and 40 °C using a Bruker Minispec mq20 (Bruker Optics Inc, The Woodlands, TX). For T1 relaxation times, an inversion-recovery sequence was used, which consisted of 12 data points with pulse separations ranging from 5 to 1000 ms and 4 scans each. For T2 relaxation times, a cpmg pulse sequence was used, which consisted of 200 data points with a pulse separation of 0.5 ms and 8 scans.

Calcium Detection—All experiments were at 25 °C with Hepes (25 mM, pH 7.2) adjusted with NaOH.

Results and Discussion

As shown in Figure 1A, diglycolic anhydride was reacted with dibutylamine in the presence of a base, followed by conjugation to amino-CLIO NPs using standard EDC/sulfo-NHS chemistry as described.¹⁴ The proposed bridging of two or three Chel-CLIO NPs by a calcium ion is shown in Figure 1B. This bridging gives rise to the aggregation of Chel-CLIO and the T₂ change, as shown in Figure 1C.

To demonstrate that the Chel-CLIO NP would respond to calcium ions, the concentrations of calcium chloride were varied between 1 and 100 mM at Chel-CLIO concentrations of 0.1, 0.2, and 0.4 mM Fe (Figure 2A). The response of Chel-CLIO was dependent on the NP concentration. This behavior permitted the dynamic range of T₂ changes to be fit to the concentrations of calcium. For example, to obtain a Chel-CLIO-based system that responded to 3 to 4 mM Ca, a relatively high concentration of NPs (0.4 mM Fe) was employed. To obtain

a system that responded to 10–30 mM Ca, the NP concentration was reduced to 0.2 mM Fe. Finally, to obtain a system that responded to between 60 and 100 mM Ca a NP concentration of 0.1 mM Fe was employed. The NP aggregates formed upon Ca^{2+} addition were between 50 and 500 nm, values typically obtained when NP aggregates are formed by reactions with avidin,¹⁵ peroxidase-mediated covalent linkages,¹⁶ viruses,¹⁷ and oligonucleotides.¹¹

To examine the reversibility of the system, the calcium chelating agent EDTA (70 mM) was added after calcium, as shown in Figure 2B. Here the y axis is given by T2/T1 rather than T2 in order to correct for small amounts of precipitation that may occur in the calcium-induced, aggregated state of the NPs. The theory and benefits of this correction method have been described in detail elsewhere.¹⁵ Briefly, this correction is possible because of the independence of T1 and dependence of T2 on the aggregation state of NPs. With respect to T1, NP aggregates behave as porous, fractal aggregates that permit water access to the NP surface.¹⁵ Because surface contact is the basic mechanism through which NPs reduce the T1 of water and because NP surface area is not affected by aggregation formation, T1 is independent of the NP aggregation state. The dependence of T2 on the NP aggregation state results from so-called outer-sphere diffusion theory, the basic theory through which magnetic spheres create small inhomogeneities within the uniform applied magnetic field. The diffusion of water through the inhomogeneities reduces T2. For a recent discussion of outer-sphere theory and the size of magnetic particles, see ^{ref 18}.

The selectivity of the sensor-interfering ions was checked, as shown in Figure 3 (0.2 mM Fe, 28 mM Ca). The sensor failed to respond to Li, NH_4 , or K (28 mM), but Mg produced a partial response that is a common interfering ion for calcium sensors.^{8–10}

The prototype NMR calcium sensor described here may undergo further optimization to obtain a fully commercial design. However, the range of calcium concentrations in extracellular biological fluids is roughly 0.3–30 mM, depending on the organism, fluid, and physiological state. The response of the current sensor, which can be adjusted by changing NP concentrations, is within the range of extracellular calcium concentrations. If necessary, interfering free magnesium ions, also present in the millimolar concentration range in biological fluids, can be removed by the addition of a magnesium-selective chelator.^{9,19}

Here, we show that the chemistry developed for use with electrodes can be employed for the design of an ion-selective magnetic NP using the diglycolic amide motif^{8–10} to obtain an assay for calcium ion. Other ion-selective electrode chemistry, for example chemistry that uses the malonamide group for the detection of magnesium ions^{9,19–21} or that uses the thiodiglycolic amide group for the detection of copper ions,²² might be attached to amino-CLIO or other magnetic particles or NPs, to obtain ion-mediated changes in the particle aggregation state. The potential to adapt “graft” electrode chemistry to the surfaces of magnetic NPs may open up new prospects for developing MR-based assays for nonmagnetic (diamagnetic) anions or cations. In addition, surface functionalized NPs might be enclosed in semipermeable devices and, using MR as a detection method rather than electric conductivity, form the basis of a new generation of ion sensors.

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Figure 1.

Synthesis and reactions of calcium chelating NPs. (A) Synthesis of Chel-CLIO, the calcium chelating NPs. Amino-CLIO is reacted with the diglycolic amide acid precursor using EDC/NHS chemistry. (B) Binding of calcium to the diglycolic amide motif. Calcium forms links between two or three Chel-CLIOs. (C) Calcium ions induce Chel-CLIO aggregation and a T₂ decrease.

Figure 2.

Response of Chel-CLIO NP to calcium. (A) The response of Chel-CLIO to calcium is sensitive to the NP concentration. This provides a way of adjusting the dynamic range of the method. (B) Reversibility of the Chel-CLIO aggregation. At 0.2 mM Fe, T₂ decreases with increasing Ca, an effect reversed by the addition of the calcium chelator EDTA. Because T₁ is determined by NP concentration rather than the aggregation state of Chel-CLIO (see below), plotting T₂/T₁ corrects for possibility of a slight precipitation, which may occur because of the extended incubation time employed during the reversibility experiment.

Figure 3.

Response of Chel-CLIO to different ions. The response to K^+ , Mg^{2+} , Li^+ , and NH_4^+ is shown (black bars). The response to Ca (28 mM) in the presence of K^+ , Mg^{2+} , Li^+ , and NH_4^+ is shown (white bars).