MRI-Based Glucose Assay Using Magnetic Nanoparticle Sensors

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Abstract

Glucose sensors for NMR relaxometry and magnetic resonance imaging (MRI) can be used for direct measurement of glucose in turbid biological specimens. Here, we proposed a magnetic glucose sensor based on superparamagnetic iron oxide (SPIO) nanoparticles conjugated to a mannopyranoside derivative and concanavalin A (ConA). The binding of mannopyranoside groups to ConA produced a nanoparticle cluster that was dissociated by competitive binding of glucose to ConA, resulting in changes in the transverse relaxation time ($T_2$) in a glucose-dependent manner. The sensor gave rise to significant $T_2$ changes in physiological glucose levels of 3–8 mM at a nanoparticle concentration of 0.5 nM. Significant $T_2$ responses were observed within 6 min of 5 mM glucose detection. Sensor-based MRI by a benchtop 1 tesla scanner permitted a measurement of multiple samples within 8 min. These results demonstrate that the relaxometric glucose sensor could lead to high throughput direct assay of blood samples by using a compact MRI scanner for point-of-care testing.

Keywords: NMR relaxometry, glucose sensor, magnetic nanoparticles, concanavalin A, MRI

Introduction

Blood glucose level is a crucial factor in estimating the risk of diabetes and diagnosing health conditions. Electrochemical glucose sensors facilitate precise detection of glucose in few μL of blood, and they are generally used for individual diagnosis of diabetes.\textsuperscript{1–3} Optical methods are suitable for high throughput assays of glucose.\textsuperscript{4–6} However, absorption and fluorescence spectroscopy have limitations in direct detection of glucose in the blood because of light absorption, mainly caused by erythrocytes.
NMR relaxometry could be useful in direct glucose measurement in turbid biological specimens because it detects the magnetic relaxation process of water protons excited by radio frequency wave. NMR relaxometry is in fact used for non-disruptive evaluation of water content in foods, rigidity of synthetic polymers and water mobility in contact lens hydrogels. A clinically-used MRI scanner, an advanced relaxometry in other words, allows for simultaneous measurement of multiple specimens with three dimensional information. Therefore, a magnetic sensor that perturbs proton relaxation responding to glucose can be valuable for relaxometric sensing of glucose. Superparamagnetic iron oxide (SPIO) nanoparticles are a promising sensor platform because SPIOs accelerate the transverse relaxation time ($T_2$) of water protons depending on their spatial distribution in a solution. This mechanism has been utilized to develop MRI sensors that undergo aggregation or dissociation responding to calcium, enzyme, and nucleic acid. Although the conventional clinical MRI scanner is too huge and expensive, a combination of the sensor with a compact scanner specifically designed for point-of-care testing could permit high throughput assay of blood glucose. Here, we proposed a glucose sensor that consists of SPIO nanoparticles conjugated to mannopyranoside derivatives and concanavalin A (ConA), and $T_2$-based relaxometric sensing and MR imaging of glucose at physiological level.

**Experimental**

**Reagents**

1,2-distearoyl-$sn$-glycero-3-phosphoethanolamine-$N$-[amino(polyethylene glycol)] sodium salt (PEG MW = 2000) and α-D-mannopyranosylphenyl isothiocyanate were purchased from Yuka Sangyo Co. Ltd. (Tokyo Japan) and Toronto Research Chemicals
(Toronto, ON, Canada), respectively. All the other reagents were obtained from FUJIFILM Wako Pure Chemical Co. (Osaka, Japan), Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), and Sigma-Aldrich Co. LLC. (St. Louis, MO, USA) as the best grade available.

**Apparatus**

Hydrodynamic diameters of nanoparticles were measured using Zetasizer Nano ZS (Malvern Panalytical Ltd., Malvern, Worcestershire, UK) at 25 °C. Transmission electron microscopy (TEM) images were captured using JEM-2100 (JEOL Ltd., Tokyo, Japan) and H-8100 (Hitachi, Tokyo, Japan) operated at 200 kV. MRI was performed with a benchtop 1 tesla MRI scanner with a solenoid radio-frequency coil (ICON, Bruker BioSpin, Ettlingen, Germany).

**Synthesis of superparamagnetic iron oxide (SPIO) nanoparticles**

Iron oxide nanoparticle cores were synthesized from iron-oleate using the thermal decomposition method.\(^{22,23}\) Briefly, iron-oleate (4.5 g, 5.0 mmol) and oleic acid (0.71 g, 2.5 mmol) were dissolved in 20 mL of 1-octadecene and evacuated for 30 min with vigorous stirring. The solution was heated to 200 °C under argon and further heated to 320 °C at a rate of 1 °C/min. The reaction was held at 320 °C for 1 h. The reaction solution was then cooled to room temperature and was added to a mixture of ethanol and hexane at a 1:1 ratio. The solution was centrifuged at 6000 g for 10 min and the precipitate was washed thrice with the ethanol-hexane mixture. The black precipitate was dispersed in toluene. Iron concentration was determined via the bathophenanthroline disulfonic acid assay.\(^{24}\) Nanoparticle concentration was calculated from Fe concentration, the diameter determined with TEM, and 5 g/cm\(^3\) of Fe\(_3\)O\(_4\).
density.

Preparation of α-D-mannopyranoside functionalized SPIO (Manno-SPIO)

Total 50 μL of SPIO solution (5 mg Fe/mL in toluene) was mixed with 500 μL chloroform solution containing 5.0 mg of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)] sodium salt (PEG MW = 2000). The mixture was thoroughly evaporated to obtain an even brown lipid film. Five hundred microliters of 10 mM HEPES and 150 mM NaCl buffer (pH 7.4) was added to the lipid film and the mixture was sonicated until the solution became transparent brown. The mixture was applied to a magnetic μ column placed in a μMACS magnetic separator (Miltenyi Biotec, Cambridge, MA, USA) and washed four times with 50 μL buffer aliquots. The purified solution was eluted by removing the column from the magnet and adding 150 μL buffer. Five microliters of 100 mM α-D-mannopyranosylphenyl isothiocyanate in DMSO (approximately 10000 equivalent to the nanoparticle concentration), was added to a mixture of 200 μL of lipid-coated SPIO solution (1 mg Fe/mL) and 500 μL of 0.1 M sodium bicarbonate buffer. The reaction mixture was incubated at room temperature for 16 h and then washed by 100K amicon ultra filtration (MilliporeSigma, Burlington, MA, USA) with 10 mM HEPES buffer (pH 7.4) to obtain Manno-SPIO. The experiment was performed in 10 mM HEPES buffer containing 150 mM NaCl, 1 mM CaCl₂, and 1 mM MgCl₂ (pH 7.4).

TEM sample preparation

ELS-C10 elastic carbon grids (STEM, Tokyo, Japan) were made hydrophilic using an ion coater (IB-2, Eiko, Tokyo, Japan) with 3 mA of plasma current for 40 seconds
before applying sample solution. A 5 μL of the 0.5 nM SPIO solution was dropped on the grid and incubated for 3 min at RT. After the solution was removed by a filter paper, the grid was dried at RT overnight and used for TEM measurement.

NMR relaxation time measurement

The $T_2$ was measured with Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence using Spinsolve ULTRA 43 MHz $^1$H-NMR (Magritek Ltd., Wellington, New Zealand). The parameters in the CPMG pulse sequence were as follows: number of scans = 4, acquisition time = 0.8 s, repetition time = 4 s, CPMG echo time = 1 ms, final echo time = 2 s, number of steps = 20.

MRI measurement

A 100 μL of each sample was added into 0.2 mL PCR tubes and imaged using a solenoid volume coil for transmission and reception (Bruker BioSpin). A multi-slice multi-echo pulse sequence was used to obtain multi-echo images, with parameters including matrix size = 160 $\times$ 256, field of view (FOV) = 2.4 cm $\times$ 3.84 cm, slice thickness = 3 mm, repetition time (TR) = 4,000 ms, echo spacing with echo time (TE) = 10 ms, and number of echoes = 128. The quantitative $T_2$ map was calculated by a non-linear least square fitting using multi-echo imaging.

Results and Discussion

We designed a glucose sensor based on SPIO nanoparticles conjugated to phenyl α-D-mannopyranoside groups (Manno-SPIO) and ConA that bind to α-D-mannosyl and α-D-glucosyl moieties (Fig. 1).25–27 We expected that the interactions between Manno-SPIO and ConA would result in nanoparticle clustering, capable of decreasing
$T_2$ compared to that under ConA-free conditions. In the presence of glucose, the clustering is disrupted by competitive binding of glucose to ConA, followed by increase in $T_2$. Therefore, glucose sensor allows $T_2$-based detection of glucose using NMR relaxometry and MRI.

SPIO cores were synthesized using the thermal decomposition method.\textsuperscript{22,23} TEM measurement demonstrated the number average diameter of SPIO core was 16.6 ± 0.6 nm (mean ± SD., $n = 300$), indicating the nanoparticle was uniform. The diameter was in a suitable range to produce an efficient $T_2$ decrease due to the motion average regime.\textsuperscript{16} SPIO core was coated with amino-terminated poly (ethylene glycol) phospholipids to increase water dispersibility, and then conjugated to $\alpha$-D-mannopyranosylphenyl isothiocyanate through the reaction between amine and isothiocyanate to obtain Manno-SPIO. The reported dissociation constant of phenyl $\alpha$-D-Mannopyranoside to ConA is about 10–100 µM, which is a reasonable value to compete with glucose.\textsuperscript{28,29} TEM images of 0.5 nM Manno-SPIO with or without 0.13 µM ConA showed the aggregation occurred only in the presence of ConA (Fig. 2A). The aggregation was dissociated in the presence of 10 mM glucose, indicating the competitive binding of glucose to ConA. Dynamic light scattering (DLS) also showed that the mass distribution peak of 0.5 nM Manno-SPIO significantly increased in the presence of 0.13 µM ConA (Fig. 2B). The addition of 10 mM glucose caused a partial dissociation of the Manno-SPIO and ConA cluster. $T_2$ measurement with 43 MHz NMR demonstrated that the $T_2$ value of 0.5 nM Manno-SPIO solution significantly decreased from 0.745 ± 0.009 s to 0.442 ± 0.013 s in the presence of 0.13 µM ConA (Fig. 2C). The decreased $T_2$ value was recovered to 0.622 ± 0.012 s by the addition of 10 mM glucose. These changes in $T_2$ were consistent with the clustering-dissociation behavior of the sensor.
$T_2$ measurement by NMR relaxometry showed the sensor responses in various concentrations of glucose (Fig 3A). The $T_2$ values increased with an increase in glucose concentration from 0 to 10 mM at the Manno-SPIO concentration of only 0.5 nM. The $T_2$ increased 16% from 3 to 8 mM of glucose, indicating that the sensor has a reasonable response at a physiological glucose level. As a model experiment of the practical use, the glucose concentration in mice serum was quantified by measuring $T_2$ with the sensor (Fig. S1 in the Supporting Information). The calculated glucose concentration was comparable to the values measured by using a commercially available electrochemical sensor, demonstrating the relaxometric sensor may allow for glucose quantification of biological specimens. To evaluate the kinetics of the sensor response, we measured the time course of $T_2$ change rate from glucose to free solution (Fig. 3B). The results demonstrated that the sensor permitted the detection of 1 mM and 5 mM glucose within 12 min and 6 min, respectively ($t$-test, $P < 0.05$). These kinetics are faster than those of the reported relaxometric glucose sensor based on magnetic nanoparticles.\textsuperscript{30} The affinity for other D-saccharides was also tested, although glucose was the most abundant in the blood (Fig. 3C). The addition of 5 mM mannose, fructose, and maltose caused larger $T_2$ increase than the equivalent amounts of glucose. $T_2$ increase with sucrose was comparable to that with glucose, but galactose and lactose caused small increase in $T_2$. These results are consistent with the sugar binding property of ConA. The unmodified hydroxy groups of C-3, C-4, and C-6 of D-glucopyranosyl, D-mannopyranosyl, D-fructofuranosyl rings are essential for binding to ConA.\textsuperscript{25} As the alignments of these hydroxy groups of galactose and lactose did not satisfy the above criteria, galactose and lactose could not cause enough dissociation of the cluster.

As a proof of concept of high throughput assay, we obtained multi-echo images and calculated a quantitative $T_2$ map in different concentrations of glucose by using a
benchtop 1 tesla MRI scanner (Fig. 4). The $T_2$ map showed significant changes among 6 specimens in PCR tubes depending on glucose concentrations. Measurement time of one scan took only 8 min for eight samples including two standard samples, indicating the possibility of high throughput direct assay of turbid samples. For future practical use, NMR instruments equipped with a sample changer can be used as high throughput relaxometers. In addition, a portable and compact scanner equipped with a permanent magnet has attracted attention in recent years.\textsuperscript{31} Combination of the relaxometric sensor with a scanner specifically designed for point-of-care testing could lead to high throughput direct assay. Other types of magnetic sensing devices may also be useful for the future application.\textsuperscript{32,33}

**Conclusions**

We developed a glucose sensor based on the magnetic nanoparticles conjugated to phenyl $\alpha$-D-mannopyranoside and ConA. The sensor formed a cluster owing to the interaction between mannopyranosyl groups and ConA, causing substantial changes in $T_2$ from non-clustering state. The sensor showed significant $T_2$ increase in the range of physiological glucose level and could function at a nanoparticle concentration of 0.5 nM. The sensor kinetics were faster than those of the other relaxometric glucose sensors. Sensor-based quantitative MRI permitted simultaneous measurement of multiple samples with various glucose concentrations. These results can be a precedent for application of relaxometric sensors to high throughput direct assay of turbid specimens by MRI.

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Supporting Information
Additional method and figure about the comparison of glucose concentration measured by the relaxometric sensor with a commercially available electrochemical sensor. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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Figure Captions

Fig. 1 Design of glucose sensor based on Manno-SPIO and ConA.

Fig. 2 (A) TEM images of Manno-SPIO (M), M with ConA (M + C), and M + C with glucose (M + C + G). Scale bar = 50 nm. (B) Hydrodynamic diameter histograms of M, M + C, and M + C + G. (C) $T_2$ values measured with 43 MHz $^1$H-NMR (mean ± SEM, $n$ = 3). [Manno-SPIO] = 0.5 nM, [ConA] = 0.13 μM, [glucose] = 10 mM.

Fig. 3 (A) A glucose titration curve of $T_2$ (mean ± SEM, $n$ = 3). (B) $T_2$ change course of the sensor at different concentrations of glucose (mean ± SEM, $n$ = 3). Glucose was mixed in solution at 0 min. (C) $T_2$ changes in the presence of 5 mM monosaccharides and disaccharides (mean ± SEM, $n$ = 3). [Manno-SPIO] = 0.5 nM, [ConA] = 0.13 μM.

Fig. 4 $T_2$ map of the sensor solution at different concentrations of glucose. $T_2$ map was produced from 128 multi-echo images with different TEs measured by a 1T MRI scanner. [Manno-SPIO] = 0.5 nM, [ConA] = 0.13 μM.
Fig. 1

(A)

(B)

(C)

Fig. 2
Fig. 3

(A) $T_2 / s$ vs [Glucose] / mM

(B) $\Delta T_2$ (%) vs Time / min

(C) $T_2 / s$ for different sugars

Fig. 4

$[\text{Glucose}]$ / mM

$T_2$ (s)
Graphical Index

Magnetic nanoparticle

[Glucose] / mM

0 1 3 5 8 10

T₂ (s)

0.40

0.20

T₂ (s)

[Glucose] / mM

0 1 3 5 8 10