Rational design of hyperpolarized xenon NMR molecular sensor for the selective and sensitive determination of zinc ions

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A B S T R A C T

Although Zn²⁺ ions are involved in large numbers of physiopathological processes, non-invasive detection of Zn²⁺ ions in opaque biological samples remains a huge challenge. Here, we developed a novel zinc-responsive hyperpolarized (HP) ¹²⁹Xe-based NMR molecular sensor. This HP ¹²⁹Xe-based NMR molecular sensor was synthesized by attaching 2-(diphenylphosphino) benzenamine as ligand for zinc ions to the xenon-binding supramolecular cage, cryptophane. The ¹²⁹Xe NMR spectroscopy of such molecular sensor was shifted up to 6.4 ppm in the presence of Zn²⁺ ions, which was nearly four times larger than that of the reported similar sensor. The application of the sensor would benefit low concentration detection by using indirect NMR/MRI method. The response exhibited high sensitivity and selectivity as discriminated from other six potentially competing metal ions. The application of this sensor in the analysis of zinc ions in the rat serum samples was also evaluated. The strategy is generally applicable in developing sensitive and selective sensors for quantitative determination of zinc ions.

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1. Introduction

Zinc is an essential trace element in the human body [1] and is the second most abundant transition metal in the living organisms [2]. Zn²⁺ ions not only constitute many enzymes [2] and proteins [3], but also play a key role in various physiological processes [4]. Either a deficiency or excess of Zn²⁺ ions will cause a physiological dysfunction of the organism. Thus, it is of great significance to develop a highly sensitive and specific method for the detection of Zn²⁺ ions.

There are many chemosensors for detection of Zn²⁺, which are mostly based on quinoline, benzazole or fluorophores. However, the major chemosensors are unsuitable to offer real-time monitoring of the Zn²⁺ distribution in different tissues of the body. Moreover, NMR molecular sensors could provide a potential tool for detecting the Zn²⁺ ions in the living organisms because of its non-invasive and non-destructive their characters. However, the conventional NMR sensors are not as sensitive as most of their peers such as fluorescent sensors [5,6]. The application of NMR molecular sensors has been therefore limited by their intrinsically low sensitivity at thermal equilibrium. In contrast, the use of hyperpolarized ¹²⁹Xe-based NMR molecular sensor suggests a possible solution [7]. The nuclear spin of the xenon atom can be hyperpolarized by a spin-exchange optical pumping (SEOP) technique [8–10], and the resulted nuclear spin polarization could be enhanced by four orders of magnitude in comparison to the Boltzmann polarization [11], leading to an amplification of the NMR sensitivity by a factor of 10,000 [12].

Cryptophanes are an important class of supramolecular hosts that can form stable inclusion complexes with small molecules [13–16], and cryptophane-A is considered as one of the most suitable host molecules for xenon [17]. A Xe-cryptophane complex can be formed by self-assembly of xenon with a cryptophane, and the ¹²⁹Xe NMR spectra display two dramatically different chemical shifts for the encapsulated Xe and free Xe in solution [16]. As Xe has large, polarizable electron cloud, the chemical shift of encapsulated Xe was extremely susceptible to the surrounding environment. A Xe-based sensor is usually formed by the combination of the cryptophane and a ligand via a molecular linker, and when the ligand interacts with the targeting molecule, it will affect the electron density experienced by the encapsulated Xe, resulting in the change of chemical shift. Therefore, hyperpolarized ¹²⁹Xe-based NMR molecular sensor could be an exceptional tool for the sensitive detection of targeting molecules. Such Xe-based sensor strategy has already been used for the detection of a variety of biological systems, including proteins [7], enzymes [18], nucleic acids [19], metal ions [20] and in-cell biological targets [21] 2-(diphenylphosphino) benzenamine and its derivatives are a group of ligands [22–24], which are able to chelate with zinc ions [22]. It is widely used as a highly efficient and selective ligand, due to its feasible preparation, stability in air, and great selectivity.
In this study, a HP $^{129}$Xe-based NMR molecular sensor exploiting 2-(diphenylphosphino)benzenamine as a ligand for the capture of zinc ions was synthesized. The synthesized sensor was composed of a cryptophane moiety as a Xe-based NMR signal reporting part, 2-(diphenylphosphino)benzenamine as the chelating moiety, and a molecular linker (Scheme 1). The detection of Zn$^{2+}$ ions by the new HP $^{129}$Xe-based NMR molecular sensor was conducted, and the HP $^{129}$Xe-based NMR molecular sensor responded to Zn$^{2+}$ ions with a high selectivity. It is worth noting that the chemical shift difference of the encapsulated xenon is nearly four times larger than that of the reported similar sensor, which is important for the low concentration detection by using indirect NMR/MRI method, such as Hyper-CEST [25], and the feasibility of the application of the indirect NMR/MRI method has also been improved because of the largely enhanced chemical shift difference.

2. Experimental

2.1. Materials

2-(Diphenylphosphino)benzenamine was purchased from VsciChem™ Technology (Beijing) CO., Ltd. Thionyl chloride was purchased from Shanghai Jin Shan Ting Xin™ Chemical Reagent Factory. Deuterated toluene-d$_8$ was obtained from Landisville™ NJ Norell Inc. USA, and 1,1,2,2-tetrachloroethane was commercially available from Sinopharm™ Chemical Reagent Beijing Co., Ltd.

2.2. Apparatus

A tailor-designed xenon hyperpolarizer was used in this study [26]. The high performance liquid chromatographs (HPLC) were produced by the Scientific Software International, Inc. The $^1$H NMR and $^{129}$Xe NMR spectra were obtained with a Bruker™ AVANCE 500 spectrometer and Bruker™ AVANCE III 400 spectrometer, respectively, and the mass spectra were produced by a Bruker™ microOTOFQ spectrometer.

2.3. Synthesis of sensor 1

2.3.1. Synthesis of compound 3

To conjugate the alkyl linker to Cryptophane-Cage 2, 881 mg (1 mmol) compound 2 was dissolved in 30 mL acetone. Excess amounts of anhydrous potassium carbonate and ethyl bromoacetate were added to the solution, and the reaction was allowed to initiate and sustain by stirring and refluxing for 5 h, followed by a series of processes including cooling down to room temperature, filtering,
and solvent evaporation. The recovered substance was purified by silica gel column chromatography to yield product 3, which was a white solid substance, and the eluent used was chloroform: hexane 4:1 (v/v), resulting a 81% yield. The synthesis was performed under the guiding of Ref. [27].

2.3.2. Synthesis of compound 4

Compound 3 was dissolved in 30 mL tetrahydrofuran, followed by the addition of 4 M sodium hydroxide aqueous. The reaction was allowed to stir overnight with refluxing. The solution was then cooled down to room temperature, extracted by chloroform, and evaporated under vacuum. The white recovered substance was subject to a silica gel column chromatography to produce a pure product 4, which was a white solid substance. The eluent used was chloroform: hexane 4:1 (v/v), resulting a 95% yield of product 4. The preparation of compound 4 was carried out by referring to the procedure described in ref [27].

2.3.3. Synthesis of cryptophane-2-(diphenylphosphino)benzenamide (sensor 1)

Compound 4 (30 mg, 0.032 mmol) was dissolved in 20 mL of dry dichloromethane in a dry flask, where 2 mL of dry thionyl chloride was added. The reaction was stirred overnight with reflux under a nitrogen atmosphere. The solution was then evaporated under vacuum to obtain compound 5. 9 mg (~0.03 mmol) of 2-(diphenylphosphino)benzenamine was dissolved in 10 mL dry dichloromethane, to which 12 mg dry triethylamine was added afterwards. The compound 5 was dissolved in 10 mL dry dichloromethane, to which 0.03 mmol) of 2-(diphenylphosphino)benzenamine was dissolved in 30 mL tetrahydrofuran, followed by the addition of 4 M sodium hydroxide aqueous. The reaction was subject to a silica gel column chromatography to yield product 3, which was a white solid substance. The eluent used was chloroform: hexane 4:1 (v/v), resulting a ~81% yield. The synthesis was performed under the guiding of Ref. [27].

2.4. Preparation of hyperpolarized xenon

The hyperpolarized xenon was prepared via the spin-exchange optical pumping method [8,9,28] by using an in-house-made xenon polarizer, which was developed based on our own-site equipment and techniques [11,26,29]. The gas mixture comprised 2% Xe, 10% N2, and 88% He; the temperature in the pumping cell was 438 K and the pressure was 74 psi. The flow rate of xenon gas mixture was 0.1 standard litre per minute with an average xenon spin polarization of ~20%. After the polarization process, the hyperpolarized xenon was transferred into a 10-mm NMR tube containing the sample for NMR spectrometry.

2.5. 129Xe NMR experiments

All the 129Xe NMR experiments were conducted on a BrukerTM AVANCE III 400 spectrometer equipped with a 10-mm double tuned liquid probe. The Lamor frequency of 1H and 129Xe was 400.17 MHz and 110.69 MHz, respectively. The amplitude of radio frequency-B1 field was set at 8.9 kHz for 129Xe NMR. The sample was placed into a tailor-made NMR tube for the transferring of hyperpolarized Xe gas from the polarizer. All the xenon chemical shifts in solution were evaluated by referring to the xenon gas signal, which was calibrated at 0 ppm.

3. Results and discussion

The 129Xe NMR experiments clearly distinguished sensor 1 and its chelate with Zn2+ ions (Fig. 1). In the absence of Zn2+ ions, there were two signals presenting in 129Xe NMR spectroscopy, which were assigned to dissolved free xenon at δ = 212.9 ppm and xenon caged in 1 (Xe@1) at δ = 74.2 ppm. The addition of Zn2+ ions resulted a change of chemical shift of Xe@1 from δ = 74.2 ppm to δ = 67.8 ppm, which demonstrated that the Zn2+ ions were chelated with the ligand, and this in turn modified the electron density experienced by the encapsulated Xe and consequently its chemical shift.

Since the chemical shift of xenon is extremely sensitive to the chemical environment, to confirm these results, it is necessary to examine the direct influence of the free Zn2+ ions on Xe@1. To investigate the influence of free Zn2+ ions in solution, 1680 μM Zn2+ ions were added into 100 μM compound 4, which had cryptophane cage without the modification of chelating moiety (Fig. 2b). The chemical shift of the encapsulated xenon did not have an obvious change with the addition of Zn2+ ions (Fig. 2), indicating that Zn2+ ions could not directly affect the chemical shift of the caged xenon. This result also proved that the chelating moiety modified cryptophane cage was chelated to Zn2+ ions through a chelating reaction, which was the reason for the chemical shift change of Xe@1.

Furthermore, the affinity of the sensor to Zn2+ ions was evaluated by adding Zn2+ ions at four different concentrations into 100 μM sensor 1 (Fig. 3). No significant change in the chemical shift of Xe@1 was observed for the sample containing 1.68 equiv (Fig. 3a) Zn2+ ions. Whereas, in the case of the sample added with 3 equiv Zn2+ ions, an altering was observed for the signal of Xe@1 at the signal bottom (Fig. 3b). As the Zn2+ ions concentration increased to 8 equiv (Fig. 3c), the peak of Xe@1 was split into two, which were assigned to Xe@1 with and without chelated Zn2+ ions. When the Zn2+ ions at the concentration of 16 equiv were used (Fig. 3d), the signal shifted up-field by 6.4 ppm as compared with the signal of the none Zn2+ ions sample. Through the deconvolution of spectra, the dependence of the chelated sensor on the concentration of the added Zn2+ ions was evaluated. Through the simulation of the stability constant equation, the stability constant
was determined $K=8.27 \times 10^6 \text{M}^{-2}$. Normally, a high affinity is preferable during the development of the molecular sensor to increase the detection sensitivity. The low affinity caused by a relatively high Zn$^{2+}$ ions concentration in contrast to the case of sensor 1 was inevitable to result in a distinct chemical shift change of the caged Xe. Fortunately, this potential problem of suppressed sensitivity has been overcome by the ultrahigh sensitivity because of hyperpolarization, allowing a greatly enhanced sensitivity more than 10,000 times higher than that of the traditional NMR.

Further, to demonstrate the specificity of the sensor to Zn$^{2+}$ ions, we investigated the response of sensor 1 to calcium(II), magnesium(II), mercury(II), palladium(II), copper(I), and silver(I) ions, with the $^{129}$Xe NMR spectra shown in Fig. 4. No noticeable influence of the added metal ions on the NMR signal was observed, except for Zn$^{2+}$. The obtained results indicated that the sensor developed in this study was highly specific to the detection of Zn$^{2+}$ ions among potentially competing metal ions.

The magnitude of the chemical shift difference of Xe@1 after the chelating with the metal ions demonstrated the sensitivity of sensor to the targeted ions. A large chemical shift difference would alleviate the resolution requirement of the NMR/MRI, as the resolution for the detection of the complex biological samples could be very low due to the field-shimming problem. Furthermore, a relatively large chemical shift would be extremely important for some high sensitivity indirect measurement methods, e.g. Hyper-CEST [25] and MT. Hyper-CEST possesses the highest sensitivity among the NMR/MRI methods known so far, and sensitivity is of vital importance for its application in low-concentration detection [25]. However, because of its intrinsic indirect measuring method, its frequency resolution was much lower than that of the conventional methods, and this leads to a high requirement for the chemical shift sensitivity of the sensor. In this work, the $^{129}$Xe NMR based sensor 1 showed a 6.4 ppm chemical shift change in respect to that of the Zn$^{2+}$ ions, which was much larger than 1.7 ppm of the similar sensor as reported [20].

In order to investigate the reproducibility of this method, the inter-assay precision was estimated by determining the response of 100 $\mu$M sensor 1 for six times, which were immersed in 400 $\mu$M zinc ions. The coefficients of variation (CV) were calculated to be 1.7%, indicating acceptable fabrication reproducibility. The intra-assay precision of the sensors was evaluated by assaying one sensor 1 for six times, which were immersed in 400 $\mu$M zinc ions, and the CV was 0.87% at its initial response, indicating an acceptable stability.

To further demonstrate the practicality of the proposed sensor, the recovery test was studied by adding different amounts of zinc ions in 400 $\mu$M rat serum samples. The recoveries were from 96.7% to 103.5%. The average precision was ± 6.0% (Table 1). The results indicated that the proposed method was highly accurate, precise and reproducible. It can be used for direct analysis of practical samples.

### 4. Conclusion

In this article, we have shown the rational design of the HP $^{129}$Xe-based NMR molecular sensor and the specific determination
of Zn$^{2+}$. More importantly, this $^{129}$Xe-based NMR molecular sensor possesses excellent chemical shift sensitivity, which is desirable for the low concentration indirect NMR/MRI measurement. By taking the advantages of the magnetic resonance molecular imaging, this zinc-activated HP $^{129}$Xe-based NMR molecular sensor suggests a great potential to be used in the monitoring of Zn$^{2+}$ ions and investigating Zn$^{2+}$ ions related physiopathological processes in biological organisms in the foreseeable future.

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