A fluorescent sensor array based on ion imprinted mesoporous silica

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ABSTRACT

We report an ion imprinted mesoporous silica based fluorescence turn-on sensor array for discrimination of metal ions. A novel fluorescent functional monomer containing an 8-hydroxyquinoline (8-HQ) moiety in combination with one-pot co-condensation method was employed to prepare fluorescent ion imprinted mesoporous silica. The imprinted materials for Zn^{2+} and Cd^{2+} as well as a control blank non-imprinted material (NIM) were synthesized. With the covalently anchored organic fluorophore in the inorganic mesoporous silica matrix, the binding of metal ions to the imprinting site was directly transformed into fluorescence signals. Both the imprinted materials displayed faster binding kinetics toward metal ions than NIM. Apparent binding constants (adsorption constants) for the materials with the metal ions in water were determined by a Langmuir-type analysis of the spectrofluorimetric titration data. The sensor array composed of the three materials allows discriminating the two template metal ions (Zn^{2+} and Cd^{2+}) plus three non-template metal ions (Mg^{2+}, Ca^{2+} and Al^{3+}) at two different concentrations. This work proves that using a simple fluorescent receptor can provide a pattern based fluorescent sensing system by taking advantage of the imprinting effect.

1. Introduction

Chemical sensor arrays have been demonstrated to be highly effective tools for molecular recognition (Albert et al., 2000; Jurs et al., 2000; Lavigne and Anslyn, 2001; Wright and Anslyn, 2006). Unlike chemical sensors suffering from interference of structurally or chemically similar species, differentially responsive arrays take advantage of such interference by deliberately attempting to use the nonspecific response patterns for discrimination. As long as each component of an array responds to each analyte to a different degree, unique diagnostic patterns for individual analytes will be generated. Up to now, inspired by the superb performance of cross-responsive mammalian olfactory system, considerable efforts have been made to develop sensor arrays for various kinds of analytes such as metal ions (Mayr et al., 2002, 2003; Lee et al., 2006; Palacios et al., 2007a, 2008; Basabe-Desmonts et al., 2007a), anions (Palacios et al., 2007b; Zyryanov et al., 2007), amino acids (Buryak and Severin, 2005a; Folmer-Andersen et al., 2006), peptides (Buryak and Severin, 2005b), proteins (Zhou et al., 2006), nucleic acids (McCleskey et al., 2003) and carbohydrates (Schiller et al., 2007; Edwards et al., 2007). Compared with conventional methods, molecular imprinting technique (Cormack and Mosbach, 1999; Haupt and Mosbach, 2000; Whitcombe and Vulfson, 2001; Alexander et al., 2006) provides an alternative approach to sensor arrays.

Molecularly imprinted polymers (MIPs), also called plastic antibodies, are highly cross-linked polymer matrices prepared in the presence of a template molecule. MIPs have many advantages over natural antibodies in terms of storage, reusability and harsh environment utility. They can also be rapidly and inexpensively generated making them amenable to an array setting. The major drawbacks associated with MIPs, relatively low overall affinities and high levels of cross-reactivity can actually be advantageous for differential sensor arrays. As long as signals are generated for an analyte from one or more MIP sensors a characteristic fingerprint for this analyte can be obtained (Wright and Anslyn, 2006; Basabe-Desmonts et al., 2007b). To design a highly selective imprinting sensor for a single analyte, hard work of optimization is usually needed to eliminate the interference from some structurally related species which are highly similar to the template in size, shape and functionality. While for the cross-responsive sensor arrays, we can take advantage of such interference to establish an imprinting sensor array to discriminate the template and its structurally related species.

Although imprinted materials are theoretically promising candidates for sensor arrays, there are few works to date focusing on the design of imprinting sensor arrays (Hirsch et al., 2003;

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Greene et al., 2004; Greene and Shimizu, 2005; Takeuchi et al., 2007). As an initial demonstration of the utility of MIPs in sensor arrays, Greene et al. (2004) developed an eight channel MIP sensor array that was able to differentiate six aryl amine analytes. The response from the MIP array was measured using the UV absorbance of the unbound analyte. A drawback of this method is the necessity of employing analytes with an optical handle for evaluation of the binding between the MIPs and analytes. Takeuchi et al. (2007) synthesized six protein-imprinted polymers using three template proteins with acidic or basic functional monomers. The fingerprints for protein profiling were obtained by determination of the unbound protein with HPLC. In these two works, the analysis depending on the detection of unbound analyte is more similar to a binding assay rather than a sensor and is limited to analytes that have their inherent detectable properties (e.g., UV-active). To eliminate this limitation, Greene and Shimizu (2005) prepared a colorimetric MIP sensor array for seven aromatic amines using a dye-displacement strategy. Although this strategy can give levels of accuracy in classifying analytes similar to those of direct measurements using UV-active analytes, it is still an indirect measure of binding.

Among the several transduction mechanisms to report the chemical recognition event, fluorescence has become one of the most powerful ones with many advantages (Basabe-Desmonts et al., 2007b). However, to the best of our knowledge, to date no fluorescent imprinting sensor array has been reported. Herein, we report a fluorescence turn-on imprinting sensor array based on ion imprinted mesoporous materials. Imprinted materials for Zn^{2+} and Cd^{2+} together with a control blank non-imprinted material (NIM) were synthesized. To visualize the binding events, 8-hydroxyquinoline (8-HQ) moiety was appended to the traditional functional monomer 3-aminopropyltriethoxysilane (APTES) and hence a novel fluorescent functional monomer was obtained. Fluorescent mesoporous silica was then prepared by a conventional one-pot method with the co-condensation of such fluorescent functional monomer and the cross-linker tetraethoxysilane (TEOS). The design, synthesis and characterization of such ion imprinted mesoporous materials and their applicability to fluorescent sensor array for metal ions were described and discussed in detail.

2. Materials and methods

2.1. Materials and chemicals

All reagents used were of at least analytical grade. Ultrapure water (18.2 MΩ cm) obtained from a WaterPro water system (Labconco Corporation, Kansas City, USA) was used throughout the experiments. 8-HQ, chlorosulfonic acid, n-propylamine, cetyltrimethylammonium bromide (CTAB) and TEOS were purchased from Guangfu Fine Chemical Research Institute (Tianjin, China). APTES was obtained from Wuhan University Silicone New Material Co., Ltd. (Wuhan, China). Aqueous stock solutions of Zn^{2+}, Cd^{2+}, Mg^{2+}, Ca^{2+} and Al^{3+} (5 mM) were prepared from $Zn(NO_3)_2 \cdot 6H_2O$, $CdCl_2 \cdot 2.5H_2O$, $MgCl_2 \cdot 6H_2O$, $Ca(NO_3)_2 \cdot 4H_2O$ and $Al(NO_3)_3 \cdot 9H_2O$, respectively. CH_2Cl_2 was distilled with calcium hydride. Unless stated otherwise, commercial grade chemicals were used without further purification.

2.2. Instrumentation

^1H NMR and ^{13}C NMR spectra were recorded on a Mercury VX-300 spectrometer (Varian, USA) using tetramethylsilane (TMS) as an internal standard. High resolution mass spectra (HRMS) with an electronic spray ionization (ESI) source were determined on an IonSpec 7.0T FT-ICR mass spectrometer (IonSpec, USA). Elemental analyses (EA) were carried out with a Vario EL analyzer (Elementar, Germany). IR spectra (4000–400 cm^{-1}) in KBr were recorded on a Magna-560 spectrometer (Nicolet, USA). Scanning electron microscopy (SEM) images were taken on an SS-550 microscope (Shimadzu, Japan) operated at 15 kV. N_2 adsorption–desorption isotherms were recorded on a NOVA 2000e surface area and pore size analyzer (Quantachrome, USA) at 77 K. UV–vis absorption spectra were measured with a UV-3600 UV–vis spectrophotometer (Shimadzu, Japan). Fluorescence spectra were measured with an F-4500 Fluorescence spectrophotometer (Hitachi, Japan).

2.3. Preparation of fluorescent ion imprinted mesoporous materials

Synthesis of Zn^{2+} imprinted material (ZnIM): $Zn(NO_3)_2 \cdot 6H_2O$ (2.97 g) (template) and CTAB (1.75 g) were dissolved in water (75 mL), to which **1a** (see the synthesis and characterization of compounds **1a** and **1b** in Supplementary Material) (142 mg, 0.2 mmol) was added. After stirring the mixture for 1 h, TEOS (8.9 g, 40 mmol, dissolved in 10 mL of MeOH) and NaOH aqueous solution (1 M, 20.0 mL) were added. The mixture was stirred for 48 h and then refluxed at 90 °C for 24 h. The solid product was recovered by filtration and dried in vacuum at 65 °C for 24 h. The sol–gel material was crushed, grounded into powder, suspended in acetone and wet sieved through a 76 μm stainless steel sieve. The fractions passed through the sieve were collected. The resultant fine sol–gel powder was collected by filtration, air dried, Soxhlet extracted by ethanol for 48 h to remove the surfactant templates and washed with copious 0.1 M EDTA solution to remove metal ion templates. To ensure the complete removal of the surfactant and metal ion templates, the material was finally extracted with 1 M solution of HCl in ethanol, washed with copious water and dried in vacuum. The Cd^{2+} imprinted material (CdIM) was synthesized in the same way except the addition of $CdCl_2 \cdot 2.5H_2O$ (2.28 g) as template. The control blank NIM was prepared in the same way without addition of any metal ion template.

2.4. Fluorescence measurements

For the spectrofluorimetric titrations, a set of 10 mL mixture solutions were prepared by adding various volumes of 5 mM stock solution of metal ions to the suspension of 5 mg of the materials in 20 mM HAc/NaAc buffer solution (pH 5.0) (see details of fluorescence measurements in Supplementary Material).

For the array study, a set of 10 mL mixture solutions were prepared by adding 100 μL/200 μL of 5 mM stock solution of metal ions to the suspension of 5 mg of the materials in 20 mM HAc/NaAc buffer solution (pH 5.0) and the five metal ions (Zn^{2+}, Cd^{2+}, Mg^{2+}, Ca^{2+} and Al^{3+}) were tested against the array (NIM, ZnIM and CdIM) at two different concentrations (5×10^{-5} and 10^{-4} M) with five replicates, so that two "3 materials × 5 analytes × 5 replicates" data matrixes of fluorescence intensity were generated for the two concentration levels. The raw data matrixes were processed using PCA in Matlab (The MathWorks Inc., USA).

Except the kinetics study, all the fluorescent tests were carried out 3 h after the addition of metal ions to guarantee the complete equilibrium of the fluorescence intensity.

3. Results and discussion

3.1. Design and synthesis of the fluorescent ion imprinted mesoporous silica

Traditional metal ion imprinted materials are always used as sorbents for selective solid-phase extraction of metal ions (Dai et al.,