A multifunctional nanosensor based on silica nanoparticles and biological applications in living cells

Wenyu Wu, Zhihong Sun, Ye Zhang, Jun Xu, Huisheng Yu, Xiao Liu, Qin Wang, Weisheng Liu and Yu Tang*

Received 18th August 2012, Accepted 24th September 2012
DOI: 10.1039/c2cc36686j

A multifunctional nanosensor based on silica nanoparticles has been designed by importing metal binding sites and hydrogen bonds directly attached to the chromophore. It works well in the recognition of Hg^{2+}, H_{2}PO_{4}^{-}, S^{2-} via different combination mechanisms, and intracellular imaging.

Various concepts to enhance the sensitivity, selectivity, and biocompatibility of sensors have been proposed. However, the field of nanosensor technology, in terms of a general approach for the reliable detection of multiple analytes from biological and environmental samples in a complex environment containing various interferents compared with the traditional single species analysis still encounters major challenges. Present issues for biological samples such as poor intracellular uptake and organelle sequestration remain and require more contributions. In recent years, multifunctional receptors which provide multiple binding sites and some geometrical preference have seen progress. The design of these receptors with multiple binding sites for simultaneous multiple ion detection is laborious, because the sites require a suitable scaffold that can anchor them in close proximity positions without interacting with each other. Based on the aforementioned notion, this study investigates a potential device that operates on a single-type receptor unit for multi-ion analysis, including metal cations and anions.

Silica nanoparticles possessing low toxicity, good membrane permeability, and water dispersibility properties have been widely used as the matrix of nanosensors. Therefore, rhodamine-functionalized silica nanoparticles (RFSNP) have been designed with the rhodamine unit acting as both metal binding site and hydrogen bond donor (Scheme 1). This study aims to achieve the following objectives: to investigate the ability of the assembled nanoparticles to recognize, bind, and sense metal cations and anions on the functionalized surface of inorganic supports, and to design a binding model for multi-ion sensing by importing the metal binding site and the hydrogen bond directly attached to the chromophores.

The fluorescent nanosensor was synthesized using a modified Stöber–Van Blaaderen method (Fig. S1, ESI†). TEM imaging and the dynamic light scattering (DLS) pattern of the nanoparticles immobilized with and without organic ligands revealed no significant size change (25 nm ± 5 nm; Fig. S2, ESI†). FT-IR bands at 2922, 2854, and 1338 cm⁻¹ of the sample RFSNP corresponded to the vibrations of the aliphatic C–H and C–N bonds, whereas bands at 1633, 1580, and 1473 cm⁻¹ were attributed to the stretching vibration of the ≡C–H bond in the phenyl group of the rhodamine (Fig. S3, ESI†). The RFSNP chromophore concentration of 10.4 wt% was determined by thermogravimetric analysis (Fig. S4, ESI†) and elemental analysis (Table S1, ESI†).

The binding of RFSNP to metal ions was demonstrated by the emission spectral response in the absence and presence of excess metal ions (Fig. 1a). The emission band at 565 nm of RFSNP, with quantum yield (Φ) ca. 0.04, was weak for the rhodamine unit in the spirocyclic form. Upon addition of Hg^{2+}, it was enhanced by approximately thirteen fold (Φ = 0.24) with a 13 nm red-shift to 578 nm that accompanied an obvious emission color change to red, which could be observed by the naked eye (Fig. S6, ESI†). Notably, the rhodamine unit had undergone a ring-opening reaction as a result of Hg^{2+} binding (Scheme 1). RFSNP showed high selectivity toward Hg^{2+} ions over other competing metal ions (Fig. 1a). Most coexisting ions, such as K⁺, Na⁺, Ca^{2+}, Mg^{2+}, and Fe^{3+}, which exist at high concentrations in living cells, have negligible responses in inducing detection (Fig. 1b).

Scheme 1 A schematic representation of the detection systems of RFSNP nanoparticles for multi-ion sensing.

---

“Key Laboratory of Nonferrous Metal Chemistry and Resources Utilization of Gansu Province, State Key Laboratory of Applied Organic Chemistry, College of Chemistry and Chemical Engineering, Lanzhou University, Lanzhou, P. R. China. E-mail: tangyu@lzu.edu.cn; Fax: (+86) 931-891-2582; Tel: (+86) 931-891-2552

Institute of Cancer Biology and Drug Screening, School of Life Science, Lanzhou University, Lanzhou, P. R. China.

† Electronic supplementary information (ESI) available: Experimental and characterization details. See DOI: 10.1039/c2cc36686j"
Fig. 1 (a) Fluorescence responses of RFSNP (20 μM) in the presence of various metal ions (150 mM K⁺, 10 mM Na⁺, 0.5 mM Mg²⁺, 1 mM Ca²⁺ and others 100 μM) in H₂O-ethanol (1 : 1, v/v) at pH 7.2 with an excitation at 523 nm. (b) Competition experiments. The black bars (A) represent the addition of an excess of metal ions to a solution of RFSNP, and the chromatic bars (B) represent the subsequent addition of 40 μM Hg²⁺ ions to the foregoing solution. Inset of (a): corresponding linear regression plot of 1/(F-F₀) vs. 1/[Hg²⁺] M⁻¹, Y = -1.27×10⁻³ + 3.37×10⁻⁸ X (R² = 0.9952), and visual emission color change after the addition of Hg²⁺.

According to the linear Benesi–Hildebrand expression, the measured [(1/[F – F₀]) at 578 nm varied as a function of 1/[Hg²⁺] in a linear relationship (R² = 0.9952) (Fig. 1a inset), indicating that the 1 : 1 stoichiometry between RFSNP and Hg²⁺ ion corresponded with the Job plot (Fig. S7a, ESI†). The association constant for RFSNP with Hg²⁺ (K(Φ)) (±10%) was 4.43×10⁶ M⁻¹. Under optimized conditions, the detection limit for Hg²⁺ ions was 60 nM (12 ppb) (Fig. S7b, ESI†), which is sufficient to sense the Hg²⁺ concentration in blood with respect to EPA limit (10 μg L⁻¹) at a physiological pH environment (6.0–8.0) (Fig. 2a). The fluorescence intensity of RFSNP–Hg²⁺ showed a continuous decrease under alkaline conditions (pH > 8.1) (Fig. 2a). The S-shaped curve has a linear response range between pH 8.1–11.8. This range suggests a potential application for pH determination in abnormal physiological phenomena.¹²

The selectivity of RFSNP–Hg²⁺ for a variety of anions was evaluated (Fig. 2b) to assess the value of the nanohybrid as a multi-ion sensor. A remarkable fluorescence enhancement and an obvious red to purple emission color change were observed when H₂PO₄⁻ was added to the neutral solution of Hg²⁺ bound to the cation binding site on RFSNP. After the addition of H₂PO₄⁻ anions, the emission band blue-shifted by 19 nm, from 578 nm to 559 nm. Another 6-fold fluorescence enhancement with a binding constant of 1.28×10⁴ M⁻² was measured (Fig. S10, ESI†), and the fluorescence quantum yield increased to a factor of 1.5 (Φ = 0.35). The Job plot conducted between RFSNP–Hg²⁺ and H₂PO₄⁻ determined that the binding stoichiometry was 2 : 1 (Fig. S12a, ESI† inset). The pH value changed from 7.02 to 6.89 when the solution of H₂PO₄⁻ was added. It excludes the possibility that pH value changes resulting from the addition of anions could lead to the obvious fluorescence change.

The interaction between anions and organic receptors is primarily through the formation of hydrogen bonds with receptors, such as amides, ureas, imidazoles, and pyrazoles.¹³ The addition of H₂PO₄⁻ most likely resulted in a hydrogen bonding interaction with the imino group near the pyridine ring (Scheme 1). Electron transfer from the oxygen atom of H₂PO₄⁻ to the imino nitrogen atom further enhanced the intramolecular charge transfer (ICT) process, which led to emission blue shift, along with an increase in fluorescence degree. XPS spectra (Fig. S11, ESI†) were recorded to explore the status of chromophores during the H₂PO₄⁻ bonding process on the matrix in order to prove the direct bonding of H₂PO₄⁻ with the chromophores rather than coordinating with the Hg²⁺ ions. The high resolution N 1s spectrum showed a main peak at 400.0 eV, a binding energy shoulder at 401.1 eV, and a higher peak at 406.8 eV which was due to NO₃⁻ peaks at 400.0 eV, a binding energy shoulder at 401.1 eV, and a higher peak at 406.8 eV which was due to NO₃⁻ ions, suggesting that H₂PO₄⁻ did not squeeze out the NO₃⁻ ions participating in the coordination with Hg²⁺. The middle peak accounts for the presence of a species bearing a protonated nitrogen atom as a hydrogen bond donor during the H₂PO₄⁻ coordination steps.

Furthermore, the original spectrum of RFSNP was restored upon the addition of S²⁻ to the RFSNP–Hg²⁺ system (Fig. 2b, Fig. S12b, ESI†), implying the decomposition of Hg²⁺ by S²⁻ and a subsequent spirolactam ring closure reaction.

For biological applications, the cytotoxicity in living cells (HEK 293T and HeLa cells) was determined by conventional MTT assays (Fig. S14, ESI†). Upon exposure to concentrations of 5–100 μM (0.562 g L⁻¹) RFSNP for 24 h, ~82% of the HEK 293T and ~91% of the HeLa cells remained viable indicating low toxicity in the cellular environment. Also, the cytotoxicity decreased significantly after covalent grafting of the organic ligands onto the nanoparticles (Fig. S15, ESI†). RFSNP can be used as a viable nanosensor in biological samples, for their great appetency with phospholipids.¹⁴ It is known that the accumulation of intracellular mercury can lead to neurotoxicity, nephrotoxicity, DNA damage and consequently many health problems.¹⁵

Confocal microscopy experiments were carried out to demonstrate the value of the nanosensor as an imaging agent for the detection of Hg²⁺ ions in living cells. HeLa cells incubated with only the nanosensor exhibited almost no intracellular fluorescence (Fig. S17a and d, ESI†). After incubation with 20 μM RFSNP in phosphate buffered solution (PBS) for 12 h at 37 °C, followed by incubation with 20 μM Hg²⁺ for 0.5 h (Fig. 3), intracellular red fluorescence was observed, localized in the perinuclear area of the cytoplasm. HeLa cells treated with different concentrations of Hg²⁺ (0–20 μM) showed brighter fluorescence in comparison to untreated cells with no or very little fluorescence (Fig. S18, ESI†). As mercury binds to proteins,
we measured the free concentration of Hg$^{2+}$ to be 5.89 μM (Table S2, ESI†), lower than 20 μM. However, due to the presence of H$_2$PO$_4^-$, the nanosensor can be triggered by even lower concentrations of mercury ions for cell imaging. Z-scan fluorescence imaging could detect the distributions of RFSNP in different layers inside the living cells incubated with RFSNP and Hg$^{2+}$ (Fig. 4), indicating that the uptake of RFSNP into living cells occurred. All the biological results establish that the multifunctional nanosensor can penetrate the cell membrane and is capable of intracellular imaging.

Upon exposure to S$^2-$ ions, a substantial fluorescence decrease was observed (Fig. S17c and f, ESI†), which indicates the reversibility of binding between RFSNP and Hg$^{2+}$ ions.

In conclusion, a nanosensor with the potential capability to detect multiple ions, including Hg$^{2+}$ and H$_2$PO$_4^-$ by enhancement and S$^2-$ by quenching of fluorescence intensity, has been established successfully. It has low cytotoxicity and good cell permeability, and can be applied for the imaging and biosensing of multiple ions in living cells. In addition, the RFSNP-Hg$^{2+}$ system has a potential application in pH determination between 8.1–11.8. We expect that this approach is applicable for the construction of various other kinds of sensing device by importing receptors that contain appropriate multiple binding sites and hydrogen bonding donors to monitor intracellular metal ions, anions, and other species in living cells.

We thank the National Natural Science Foundation of China (Project 21071068, 20931003) and the Fundamental Research Funds for the Central Universities ( lzujbky-2012-k08) for support.

Notes and references