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A fast, sensitive and stable fluorescent fiber-optic chemosensor for quantitative detection of Fe^{3+} in real water and HepG2 living cells

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ABSTRACT

Based on the fluorescence quenching mechanism of Fe^{3+} to moxifloxacin (MOX), a fluorescence probe has been found and exhibits high sensitivity, unique selectivity and good stability toward Fe^{3+}. Moreover, the MOX probe can rapidly response to the change of Fe^{3+} concentration in pure aqueous solution. The MOX probe was utilized for quantitative determination of Fe^{3+} in real water sample, showing a good linear relationship ($R = 0.9980$) between (F_0–F) and $[Fe^{3+}]^{1/2}$ at the Fe^{3+} concentration from 0.05–15 μM. The detection limit was found to be 0.032 μM, which is much lower than that of most literature reported. Furthermore, by merging the MOX probe and a Y-tape fiber-optic spectrometer, a fluorescent fiber-optic chemosensor has been developed to quantitatively monitor Fe^{3+} in HepG2 living cells in vivo. The monitoring results are consistence with that obtained from the confocal imaging technique. The established method possesses the advantages of fast responsive, high sensitivity, unique selectivity, very stability and good repeatability for quantitative monitoring Fe^{3+} in real water and HepG2 living cells. In addition, the complex mechanism of MOX toward Fe^{3+} has also been explored by Fourier transform infrared spectroscopy (FT-IR) and mass spectra (MS) in the present work.

1. Introduction

It is well known that Fe^{3+} is an essential ion for maintaining the life of organisms by exhibiting an obligatory role in many biochemical processes at cellular level. Numerous enzymes need to use Fe^{3+} as a catalyst for electron transfer, oxygen metabolism as well as RNA and DNA synthesis [1,2]. Its deficiency or excess may cause hypoferremia or hyperferremia, accordingly. Recently, iron was found to be another important limiting element for phytoplankton primary productivity besides the elements nitrogen, phosphorus and silicon [3,4]. Thus, developing a fast, stable, high sensitive, but simple method for the qualitative and quantitative monitoring of ferric ion in environmental water and cells is critical important to clinic, human health and environmental protection. To date, there exist many procedures for detection of iron or ferric ion, such as inductively coupled plasma mass spectrometry (ICP-MS) [5], atomic absorption spectrometry (AAS) [6], electro-chemical methods [7], colorimetry [8], flow injection [9] and fluorescent approaches [10,11]. Indeed, the classical methods such as ICP-MS and AAS have very high sensitivity and selectivity, however, those methods are costly, requiring a tedious sample pretreatment, and must be performed by well-trained personnel, so they are very difficult to be used for detection of ferric ion in vivo and on-site. In addition, those procedures are difficult to detect different valence state of iron without derivatization.

On the other hand, the fluorescence-based chemosensing holds remarkable advantages over other methods because of its high sensitivity, easy operation, as well as the low cost. Various fluorescent probes and sensors have been constructed recently for direct detection of Fe^{3+} [12–22]. However, there still exist several neck-bottle problems for some present fluorescent probes and sensors such as the harsh preparation conditions, poor hydrophilicity and long responsive time [23–29]. The above disadvantages have greatly hindered their extensive applications in real water samples and cells in vivo.

In present work, a constructive and interesting discovery was found: moxifloxacin (MOX), a famous antibacterial quinolones drug [30,31] with good water-solubility and strong fluorescence property, can be used as an eminent Fe^{3+}-probe. The MOX based probe

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could produce very fast and stable response to Fe^{3+} in aqueous media, showing high sensitivity and unique selectivity against 17 other metal ions. Furthermore, the probe can be regenerated effectively, and reused with high repeatability. In this study, we further investigated in detail the fluorescence quenching mechanism of Fe^{3+} toward MOX by mean of Fourier transform infrared spectroscopy (FT-IR) and mass spectrometry (MS) procedures. Finally, a novel chemosensor, which can provide high sensitive, stable and fast response for Fe^{3+} in real sample, has been successfully developed by integrating a self-assembly fiber-optic fluorescence spectrometer and the MOX based probe. With the established method, we have monitored the reaction process of MOX and Fe^{3+} online in HepG2 cells, and the investigation on the metabolic process of Fe^{3+} in cells in vivo is under way in our lab.

2. Material and methods

2.1. Reagents and apparatus

Moxifloxacin (MOX, 99%) was purchased from Wuhan Dahua Pharmaceutical Co., Ltd (http://www.whdhwy.cn/). Aqueous solutions of metal ions, including Ag^{+}, Al^{3+}, Ba^{2+}, Be^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^{+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}, were prepared from their nitrate or chloride (guarantee reagent), which were purchased from Aladdin Reagent Co., Ltd (Shanghai, China, http://www. aladdin-reagent.com). Fetal bovine serum (FBS), PBS pH 7.4 basic, 0.25% Trypsin-EDTA and dulbecco's modified eagle medium (DMEM) basic were purchased from Gibco (Shanghai, China, http://www.lifetechnologies.com/gibco). Paraformaldehyde fixed fluid (4%) was provided by Boster, Wuhan. Ethylene diamine tetraacetic acid (EDTA) and other reagents used were of analytical grade, and distilled water was used throughout the experiment.

Fluorescence spectra were recorded with a Hitachi FL-2500 fluorescence spectrophotometer (Hitachi, Japan) and a self-assembly fiber-optic spectrometer (Ocean optics USB2000+). The IR spectra were obtained using a FT-IR (Nicolet 6700, USA). Cell imaging was pictured by inverted research microscope (Nikon eclipse Ti, Japen). AAS and ICP-AES were purchased from ThermoFisher, IRIS Intrepid II XSP, America.

2.2. HepG2 Cell culture

The human hepatocellular liver carcinoma cell line (HepG2) was provided by the Animal Experimental Center of Sun Yat-sen University. Cells were plated on 18 mm glass cover slips and allowed to adhere for 24 h and then cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 mg/mL streptomycin and 100 U/mL penicillin) at 37 °C in the humidified atmosphere under an atmosphere of 5% CO_2.

2.3. Fluorescence characterization

2.3.1. Procedures for fluorescence measurement

Generally, 0.0401 g MOX was dissolved in 100 mL distilled water to get a concentration of 1 mM MOX aqueous solution as the stock solution for subsequent use. All the concentrations of metal ions and EDTA stock solution were 10 mM. For selectivity and interference investigation, the final concentration of MOX and 18 different metal ions were 10 μM (MOX) and 20 μM (metal ions) in the mixed solution. Then the fluorescence of the mixtures was determined immediately using a Hitachi FL-2500 fluorescence spectrophotometer (Hitachi, Japan). The excitation wavelength of fluorescence spectrometry was set at 295 nm and the voltage was 400 V in the fluorescence test except for the quantitative determination (700 V). For the reversibility test of MOX probe, 4 μL Fe^{3+} stock solution (10 mM) was added into 2 mL MOX solution (10 μM) to make the final concentration of Fe^{3+} to be 20 μM. The fluorescence intensity of the mixture was monitored by FL-2500 fluorescence spectrophotometer immediately. Then 4 μL of EDTA solution was added to the above MOX–Fe^{3+} solution. The fluorescence intensity was detected again. The above operation was performed repetitively for several times.

2.3.2. The operation of fiber-optic spectrometer for fluorescence monitoring

A self-fixed Y-tape fiber-optic spectrometer (LED source light: 365 nm and 405 nm) has been applied for real-time monitoring of the fluorescence changes during reaction. The excitation wavelength of fluorescence spectrometry was set at 365 nm. The fiber-optic detector was perpendicular to the quartz cuvette to emit an exciting light and at the same time to receive the emitted fluorescent signal. All the experiments were carried out at room temperature and the fluorescent signals were recorded in real-time by a computer.

2.3.3. Fluorescence imaging of HepG2 Cell

The cells cultured in DMEM were treated with 2 μL of 10 mM MOX (final concentration: 20 μM) and incubated at 37 °C for 30 min. The treated cells were washed with PBS to remove the residual MOX on the surface of cell, and then dispersed in 2 mL PBS. Different volume of 10 mM Fe^{3+} solution (0 μL, 2 μL, 4 μL, 8 μL, 16 μL) was added into the above solution to react for 15 min. Cell imaging was pictured by inverted research microscope (Nikon eclipse Ti, Japen) and the blue channel window was chosen as a signal output. The relative fluorescence was calculated with an Image-Pro Plus software.

2.4. FT-IR and MS characterization

For FT-IR characterization, 0.0401 g MOX was mixed with excessive $FeCl_3$. And the mixture was ground in mortar and placed into vacuum drying oven for drying overnight (about 12 h). Then MOX and the reaction products of MOX with $FeCl_3$ were characterized by FT-IR (NICOLET 6700) and MS (LCQ Deca XP MAX).

2.5. FAAS and ICP-OES characterization

Zhujiang River water, Yanhu Lake water and tap water were collected in Guangzhou. The water samples were filtered 2 times through 0.22 μm membrane filter before use and then analyzed under the optimal conditions by FAAS and ICP-OES. Standard concentration curve of iron ion was 0 ppb, 11.2 ppb, 22.4 ppb, 44.8 ppb and 100 ppb, respectively.

3. Results and discussion

3.1. Fluorescence characterization and selectivity of MOX toward Fe^{3+}

In the preliminary experiment, it was found that no obvious color change can be observed when MOX solution was mixed with 17 metal ions of Ag^{+}, Al^{3+}, Ba^{2+}, Be^{2+}, Ca^{2+}, Cd^{2+}, Cr^{3+}, Co^{2+}, Cu^{2+}, Fe^{2+}, K^{+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}, respectively under natural light. However, the color of the MOX solution changed immediately to brown due to the mixing of MOX and Fe^{3+} (Fig. 1a). This experimental result promoted us to further investigate the fluorescent properties of MOX. As expected, only the mixture of MOX and Fe^{3+} displayed the phenomena of fluorescence quench