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Combination of a rhodamine derived chemosensor and up-conversion excitation nanocrystals for cysteine detection

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ABSTRACT

This paper developed a rhodamine-based chemosensor for cysteine optical sensing. Aiming at improved photostability, up-conversion excitation nanocrystals were constructed, serving as excitation source. After being modified by α-cyclodextrin, these excitation nanocrystals became water dispersible. It was found that their up-conversion emission overlapped well with absorption of our chemosensor, resulting in an efficiency energy transfer between them which was confirmed by emission decay lifetime analysis. Further analysis suggested that the sensing mechanism between our chemosensor and cysteine was a simple complexation one with binding stoichiometry of 1:1. Sensing performance of Nanocrystal:Chemosensor system showed emission "turn-on" effect towards cysteine with good photostability and high selectivity over competing amino acids and thiols.

1. Introduction

As a biological thiol and sulfide source in human body, cysteine (Cys) has been proved relevant to some vital physiological and pathophysiological processes. A series of diseases could be triggered upon abnormal levels of cellular Cys concentration, such as hair depigmentation, weakness, edema, lethargy, growth slow, muscle and fat loss, skin lesion, liver damage, neurotoxicity and even Alzheimer's disease [1–3]. As a consequence, Cys detection and recognition have harvested much research attention in health and medical care recently. Among the numerous paths for Cys detection, optical sensing has been found promising owing to its advantages of fast response, non-destructive detection, low need for apparatus and simple pretreatment [4–6]. For performance optimization and multifunctional purpose, hybrid structures are usually adopted since they hold and preserve features of each component [7]. A representative hybrid structure is usually composed of two components: a chemosensor and a supporting host [8–10]. In this case, chemosensor is responsible for recognizing and quantifying analyte by showing sensing signals. Supporting host offers a good matrix for chemosensor owing to its good mechanical strength and stability.

Literatures have demonstrated optical sensing systems of hybrid structure for Cys detection. Their chemosensors are usually excited by UV-light, leading to photobleaching and background light interference [7–10]. To overcome this drawback, up-conversion lattice has been proposed as excitation nanocrystals for chemosensors. In this case, an up-conversion lattice harvests IR radiation and emits proper excitation wavelength for chemosensor, so that chemosensor photobleaching and background light interference can be avoided [11,12]. $NaYF_4$ lattice has been highly recommended owing to its virtues of high efficiency, good match with biological window, long lifetime, low biological toxicity and high photostability, which makes itself a promising excitation source for chemosensors [13,14]. It is assumed that the narrow rare-earth-based emission, long lifetime and large Stokes shift may lead to efficient energy transfer to chemosensors. There may be other potential advantages such as non-invasive and deep penetration of excitation light, low background light interference from autofluorescence of biological tissues and feasibility of multiple labeling under the same excitation. Regardless of these advantages, the combination of up-conversion lattice and organic fluorescent chemosensors has not been well and fully developed, even though there are many superior organic fluorescent chemosensors for Cys detection [15].

There is a technical problem to be solved for these $NaYF_4$ crystals. They are prepared using oleic acid as stabilizing reagent [16]. The resulting $NaYF_4$ crystals are thus covered by oleic acid chains, showing hydrophobic surface which is incompatible with hydrophilic biological systems. Consequently, surface treatment should be performed on these oleic acid covered $NaYF_4$ crystals. It seems that α-cyclodextrin (α-CD) can well meet this requirement. Here, α-CD has both hydrophilic rim and hydrophobic cavity in its structure and thus has been explored as a phase transfer for nanoparticles [16]. In this effort, we try to modify $NaYF_4$ crystals with α-CD, as depicted by Scheme 1. It is anticipated that the oleic

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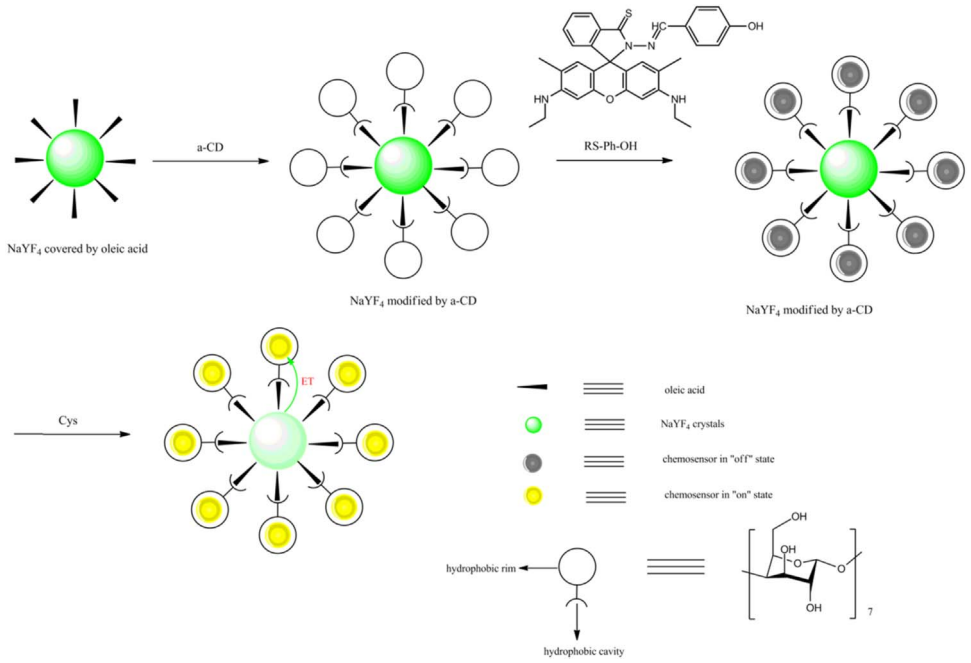

Scheme 1. Synthetic route and sensing strategy of α-CD modified $NaYF_4$ crystals and RS-Ph-OH.

chains on $NaYF_4$ crystal surface are connected with hydrophobic cavity of α-CD, leaving the hydrophilic rim for water phase (see Scheme S1, Supporting information for more details). Aqueous solubility of α-CD modified $NaYF_4$ crystals is expected to be improved. A rhodamine derived chemosensor is chosen here to recognize Cys, aiming at a sensing system of high sensitivity, good selectivity and minimal photobleaching.

2. Experimental section

2.1. Information for reagent and equipment

All starting compounds, including rhodamine 6G, 4-hydroxybenzaldehyde, Lawesson's Reagent and α-cyclodextrin (α-CD), were provided by Alfresa Pharma Corporation and used with no further purifications. General reagents, including absolute ethanol, anhydrous hydrazine (95 wt%), cyclohexane, hexane, acetonitrile, oleic acid, NaOH, concentrated HCl, 1-octadecene, rare earth salts and other inorganic salts, were supplied by Eryong Chemical Company (Shanghai, China). Organic solvents were purified and redistilled before usage. Solvent water was deionized.

Equipments for chemosensor and sample characterization are summarized as follows. NMR, MS, IR and elemental analysis data were taken by a Varian INOVA 300 spectrometer, a Agilent 1100 MS series/AXIMA CFR MALDI/TOF MS spectrometer, a Bruker Vertex 70 FTIR spectrometer (400–4000 cm^{-1}, KBr pellet technique) and a Vario Element Analyzer, respectively. UV–vis and photoluminescence spectra were taken by a Shimadzu UV-3101PC spectrophotometer and a Hitachi F-4500 fluorescence spectrophotometer, respectively. XRD patterns were collected on a Rigaku D/Max-Ra X-ray diffractometer ($\lambda = 1.5418$ Å). Sample morphology analysis was finished by a Hitachi S-4800 microscope (SEM) and a JEM-2010 transmission election microscope (TEM), respectively. Emission decay dynamics were recorded on a two-channel TEKTRONIX TDS-3052 oscilloscope with a tunable laser as excitation source ($\lambda_{ex} = 980$ nm). For sample characterization, nanocrystals were dispersed in phosphate buffer (PBS, pH = 7.0, 5 mg in 10 mL) to form a turbid liquid, then chemosensor was added and exposed to ultrasonic bath for 5 min. For emission stability monitoring, samples were exposed to continuous laser radiation generated by a laser light source LOD-BLD-0980-5W-C/P ($\lambda_{ex} = 980$ nm, excitation power density = ~50 W/cm^2). High performance liquid chromatography (HLPC) was finished on a Waters 600 instrument with a XAqus C18 5 μM 100 Å column and an Alltech ELSD 2000 ES evaporative light-scattering detection.

2.2. Synthesis of RS-Ph-OH

(E)-3′,6′-bis(ethylamino)-2-((4-hydroxybenzylidene) amino)-2′,7′-dimethylspiro [isoindoline-1,9′-xanthene]-3-thione (RS-Ph-OH) was synthesized according to a three-step procedure described as follows [17] (see Scheme S2, Supporting information, for more details). First, rhodamine 6G (5 g) was dissolved in ethanol (40 mL), then anhydrous hydrazine (20 mL) was slowly added. The resulting mixture was heated to 80 °C and kept for 12 h under N_2 protection. Solvent and excess hydrazine were removed by rotary evaporation under reduced pressure. Crude product was recrystallized in ethanol/water (V/V = 4:6) to give 2-amino-3′,6′-bis (ethylamino)-2′,7′-dimethylspiro [isoindoline-1,9′-xanthen]-3-one (R6-NH_2, Yield 68%). ^{1}HNMR ($CDCl_3$), δ (ppm): 1.27–1.31 (t, 6H, NCH_2CH_3), 1.92 (s, 6H, xanthene-CH_3), 3.25–3.27 (q, 4H, NCH_2CH_3), 4.83 (s, N-NH_2), 5.38 (s, $NHCH_2CH_3$), 6.15 (s, 2H, xanthene-H), 6.47 (s, 2H, xanthene-H), 7.14 (dd, 1H, Ar-H), 7.59 (dd, 2H, Ar-H), 8.23 (dd, 1H, Ar-H). MS *m/z*: $[m]^+$ calc. for $C_{26}H_{28}N_4O_2$, 428.2; found, 428.4.

Second, R6-NH_2 (5 mmol) and Lawesson's reagent (6 mmol) were dissolved in anhydrous toluene (50 mL). This mixture was heated to 120 °C and kept for 8 h under N_2 protection. Solvent was removed by rotary evaporation under reduced pressure. Crude product was purified on a silica gel column using with CH_2Cl_2 as eluent to give 2-amino-3′,6′-bis (ethylamino)-2′,7′-dimethylspiro [isoindoline-1,9′-xanthene] -3-thione (RS-NH_2, Yield 81%). ^{1}HNMR ($CDCl_3$), δ (ppm): 1.27–1.30 (t, 6H, NCH_2CH_3), 1.94 (s, 6H, xanthene-CH_3), 3.24–3.26 (q, 4H, NCH_2CH_3), 4.78 (s, N-NH_2), 5.35 (s, $NHCH_2CH_3$), 6.17 (s, 2H, xanthene-H), 6.46 (s, 2H, xanthene-H), 7.17 (dd, 1H, Ar-H), 7.55 (dd, 2H, Ar-H), 8.25 (dd, 1H, Ar-H). MS *m/z*: $[m]^+$ calc. for $C_{26}H_{28}N_4OS$, 444.2; found, 444.4.

Third, the mixture of RS-NH_2 (2 mmol), 4-hydroxybenzaldehyde (3 mmol) and ethanol (30 mL) was heated to