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Colorimetric and luminescent dual-signaling responsive probing of thiols by a ruthenium(II)-azo complex

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ABSTRACT

A dinuclear ruthenium(II) complex linked via a reducible azo group $[Ru(bpy)_2(azobpy)Ru(bpy)_2]Cl_4$ (Ru_2azo, bpy = 2,2′-bipyridine, azobpy = 4,4″-azobis (2,2′-bipyridine)) was adopted as a probe for thiols. Results showed that Ru_2azo could selectively and effectively react with biological thiols (such as cysteine, homocysteine and glutathione) with a 10^{-7} M detection limit. After it reacted with thiols, the original gray color of Ru_2azo solution immediately turned yellow and the luminescence significantly enhanced, showing "naked-eye" colorimetric and "off–on" luminescent dual-signaling response for thiols. Mechanism studies demonstrated that Ru_2azo reacted with thiols undergoing a two-electron transfer process, forming the azo^{2-} anion product.

1. Introduction

Among all the amino acids, cysteine (Cys) combined with homocysteine (Hcy) and reduced glutathione (GSH) compose the primary biological thiols which are responsible for the redox homeostasis for their participation in the process of reversible redox reactions. Biological thiols can protect the cells from oxidative stress by trapping free radicals that induce DNA and RNA damage. The abnormal level of those thiols has been proven to be directly linked to a series of bio-functional chaos [1], such as hematopoiesis decrease, muscle and fat loss, and psoriasis, and Hcy is also a risk factor for cardiovascular [2] and Alzheimer's disease [3,4].

The design of highly selective and sensitive reporters for detecting biological thiols levels has attracted much attention in the past decades. A variety of analytical methods [5–16] have been developed and many compounds [17–25] have been designed to detect thiol levels. Very recently, Yoon et al. have reviewed the recent progress in chemosensors for detection of thiols [26,27]. Among all these chemosensors, most of them are single signaling responsive [17–22,28–31], either luminescent responsive or colorimetric responsive, or else. Only a few of them are dual signaling responsive [23,32–34]. To develop the thiol molecular probe, some thiol-specifically acceptors, such as aldehyde groups [22], disulfide bond [24], maleimide [28] and sulfonate ester [34], are often used as the thiol-recognition moieties for the structural design of the probes. In recent years, more and more modified gold nanoparticles are designed as thiol probes, considering the strong bind affinity of sulfide atom with gold [9,17,30].

Ru(II) polypyridyl complexes, due to their intense polarized luminescence, large Stokes shifts, high chemical and photo-stability, low energy absorption and relatively long lifetimes, have emerged as novel and promising probes in the recognition and detection of anions [35–37], metal ions [38,39], small molecules [34,40,41] and biomacromolecules [42–44]. However, study involving the probing of biothiols is rare. Recently, Yuan [22] and Chen [45] have developed some Ru(II) complexes containing aldehyde group that show good luminescent selectivity towards Cys and Hcy, but the detection limits for Cys or Hcy were only at the micro molar level, which hinder their further application. Yuan [34] and Zhao [46] improved the detection sensitivity for biothiols by using Ru(II) complex as the luminophore which prior protected by 2,4-dinitrobenzenesulfonyl (DNBS), however, such a probe will releases an environmental toxic and health-harmful gas SO_2 after the degradation of sulfonate ester. Even worse, DNBS was found to induce colonic damage in animal and human body [47,48]. In this work, we present a molecular probe $[Ru(bpy)_2(azobpy)Ru(bpy)_2]Cl_4$ (Ru_2azo, bpy = 2,2′-bipyridine, azobpy = 4,4″-azobis(2,2′-bipyridine), Scheme 1) [49,50] with specific selectivity towards thiols, on which the redox-active azo group will react with biological reductants Cys, Hcy and GSH, resulting in naked-eyed colorimetric and "off–on" luminescent dual response for biothiols.

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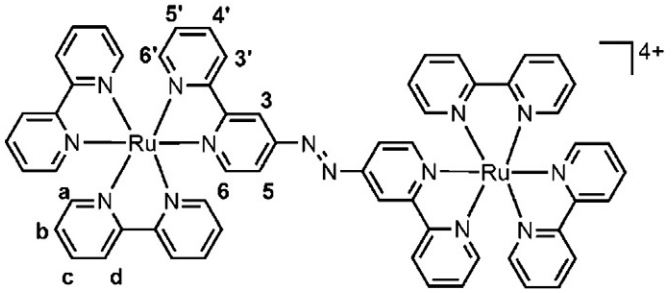

Scheme 1. Chemical structure of complex Ru_2azo.

2. Experimental

2.1. Materials and measurements

The $Ru(bpy)_2Cl_2$ [51] and azobpy ligand [52] were synthesized according to the literature methods. Unless otherwise stated, all the amino acids and other reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments.

Microanalysis (C, H and N) was carried out with a Perkin-Elmer 240Q elemental analyzer. Infrared spectra were recorder on a Bruker VECTOR22 spectrometer in KBr pellets over a range of 400–4000 cm^{-1}. ^1H NMR spectra were recorded on Varian-300 spectrometer. All chemical shifts are given relative to tetramethylsilane (TMS). Electrospray mass spectra (ES–MS) were recorded on a LCQ system (Finnigan MAT, USA). The electronic absorption spectra were recorded using Perkin Elmer Lambda850 spectrometer. Emission spectra were recorded on a Perkin-Elmer L55 spectrofluorophotometer. The pH measurements were carried out in the Sartorius PB-10 pH-meter. Cyclic voltammetry measurements were performed on a CHI 660A Electrochemical Workstation. All samples dissolved in CH_3CN were purged with Ar prior to measurements and 0.1 M tetrabutylammonium perchlorate (TBAP) was used as a supporting electrolyte. A standard three-electrode system comprising a glassy carbon working electrode, Pt-wire auxiliary electrode and a saturated calomel reference electrode (SCE) was used. The scan rate was 0.02 V/s unless otherwise noted. The ESR spectra were measured using a Bruker e-scan ESR spectrometer. The electrolysis experiments were carried out in deaerated CH_3CN containing 0.1 M TBAP as supporting electrolyte under certain electrolysis potential using DJS-292B potentiostation (Shanghai Shengke Instrument Equipment Co. Ltd (China)).

2.2. Synthesis

The dinuclear Ru(II) complex was synthesized according to the previously reported method [52] with some modification. A mixture of $Ru(bpy)_2Cl_2$ (0.1 g, 0.21 mmol) and azobpy ligand (0.034 g, 0.1 mmol) was suspended in 20 mL EtOH/H_2O (1:1, v/v) and heated at 85 °C under Ar. After 8 h reflux, the mixture was evaporated under reduced pressure, and the crude product was purified by column chromatography on alumina with CH_2Cl_2-EtOH (10:1, v/v) as eluent. Yield: 77 mg, 60%. Anal. Calcd. for $C_{60}H_{46}N_{14}Cl_4Ru_2$: C 55.13%, H 3.55%, N 15.00%. Found: C 55.01%, H 3.47%, N 15.15%. ES-MS [CH_3OH, m/z]: 291.0 ($[M]^{4+}$), 400.1 ($[M+Cl^-]^{3+}$). ^1H NMR (300 MHz, DMSO-d_6): δ 9.06 (s, 2H), 8.84 (d, J = 8.4 Hz, 2H), 8.62 (d, J = 8.1 Hz, 8H), 7.95 (m, 10H), 7.82 (d, J = 6.3 Hz, 2H), 7.66 (d, J = 5.4 Hz, 2H), 7.60 (d, J = 7.2 Hz, 2H), 7.56 (d, J = 5.4 Hz, 2H), 7.50 (d, J = 4.8 Hz, 6H), 7.40–7.29 (m, 10H).

2.3. UV–visible (UV–vis) spectral responses of Ru_2azo toward thiols

The UV–vis spectral responses of Ru_2azo toward different amino acids were measured in a 10 mM HEPES (HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer of pH 7.5 at room temperature. When adding different concentrations of Cys, Hcy or GSH into Ru_2azo (10 μM) solution, the mixture was stirred for 5 min and then the absorption spectra were acquired on the Perkin Elmer Lambda850 spectrometer.

2.4. Luminescent responses of Ru_2azo toward thiols

The luminescent responses of Ru_2azo toward different amino acids were measured in a 10 mM HEPES buffer of pH 7.5 at room temperature. When adding different concentrations of Cys, Hcy or GSH into Ru_2azo (10 μM) solution, the mixture was stirred for 5 min and then the luminescent spectra were acquired on the Perkin-Elmer L55 spectrofluorophotometer.

3. Results and discussion

3.1. pH effect

To evaluate the effects of pH on the stability of Ru_2azo, the electronic absorption spectra of Ru_2azo were measured at different pH conditions ranging from 1.97 to 12.0. To minimize the errors produced by the baseline shift under different pH, we used the ratio of absorbance in 562 nm via in 439 nm as an index for estimating the stability of complex. The value of $A_{562\ nm}/A_{439\ nm}$ was changed little from pH 1.97 to 12.0 (as shown in Fig. S1), demonstrating that the Ru_2azo was stable in weakly acidic, neutral and weakly basic conditions. This result basically indicated that Ru_2azo can work well as a probe for biological thiols such as Cys, Hcy and GSH among all the amino acids.

3.2. UV–vis spectral responses of thiols

Fig. 1a showed a ratio of $A_{562\ nm}/A_{439\ nm}$ upon addition of different amino acids, and Fig. 2a gave photographs directly illustrating the colorimetric response of complex upon additions of Cys, Hcy and GSH among different amino acids. The UV–vis spectrum of Ru_2azo was recorded in HEPES buffer solution (10 mM, pH 7.5) in the absence or presence of 20 equiv. of different amino acids (shown in Fig. S2). No detectable change in absorption spectra was observed upon the addition of amino acids including Ala, Arg, Asp, Asn, Glu, Gln, Gly, His, Leu, Ile, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, and Val. However, upon addition of Cys, the absorption band at 562 nm of Ru_2azo (attributed to low energy MLCT (d → azobpy π*)) significantly decreased, and the hyperchromic effect and red shift of absorption at 439 nm (attributed to MLCT (d → bpy π*)) were also observed. Similar cases were observed upon addition of Hcy and GSH, while no change in absorption happened upon addition of cystine (Cyss) which was as oxidative form of Cys and linked by disulfide bond. At the same time, the color of the complex solution changed immediately from gray to yellow after adding Cys, Hcy or GSH. The colorimetric effect could be directly observed by naked eyes. Among all the amino acids, Ru_2azo was specifically responsive of thiol-containing amino acids, acting as a "naked-eye" probe for thiols. These results demonstrated that complex was characteristic of high selectivity toward thiols over other proteinogenic amino acids.

To further investigate the interaction of Ru_2azo and thiols, an absorption titration experiment was carried out. Fig. 3 displayed the changes in absorption spectra of complex (10 μM) in the presence of different concentrations of Cys in the HEPES buffer. Upon addition of Cys, the absorbance at 562 nm decreased gradually, and the absorption band at 439 nm increased and exhibited a red-shift to