Biosensors and Bioelectronics 50 (2013) 1–7

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Highly sensitive and selective phosphorescent chemosensors for hypochlorous acid based on ruthenium(II) complexes

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ARTICLE INFO

Article history:
Received 4 March 2013
Received in revised form
13 May 2013
Accepted 4 June 2013
Available online 15 June 2013

Keywords:
Phosphorescent chemosensor
Ruthenium(II) complex
Hypochlorous acid
Luminescence imaging

ABSTRACT

Microbicidal activity of hypochlorous acid (HOCl) plays pivotal roles in many biological processes, and suitable methods for the *in vivo* detection of HOCl are highly desirable. Herein we report the development of two novel ruthenium(II) complex-based phosphorescent chemosensors for HOCl, $[Ru(bpy)_2(DNCA\text{-}bpy)](PF6)_2$ (bpy: 2,2′-bipyridine; DNCA-bpy: 4-N-(2,4-dinitrophenyl)carboxamide-4′-methyl-2,2′-bipyridine) and $[Ru(bpy)_2(DNCH\text{-}bpy)](PF6)_2$ (DNCH-bpy: 4-N′-(2,4-dinitrophenyl)carbohydrazide-4′-methyl-2,2′-bipyridine). The two sensors are almost non-luminescent due to the intramolecular photoinduced electron transfer (PET) process from the Ru(II)-bpy moiety to dinitrophenyl group, but specific reaction of the sensors with HOCl in aqueous media can afford a highly luminescent derivative, $[Ru(bpy)_2(COOH\text{-}bpy)](PF6)_2$ (COOH-bpy: 4-carboxylic acid-4′-methyl-2,2′-bipyridine), accompanied by 190-fold and 1100-fold turn-on luminescence signal enhancement for $[Ru(bpy)_2(DNCA\text{-}bpy)](PF6)_2$ and $[Ru(bpy)_2(DNCH\text{-}bpy)](PF6)_2$, respectively. By taking advantage of high specificity and sensitivity of the chemosensor and excellent photophysical property of Ru(II) complex, $[Ru(bpy)_2(DNCH\text{-}bpy)](PF6)_2$ was applied for visualizing the endogenous HOCl generation in living macrophage cells during the processes of stimulation and phagocytosis via the red-emitting luminescence.

1. Introduction

Hypochlorous acid (HOCl), one of the important reactive oxygen species (ROS), plays crucial roles in the immune defense against microorganisms and also in inflammation (Sugiyama et al., 2007). In living organisms, HOCl is produced by the peroxidation of chloride ions catalyzed by a heme enzyme, myeloperoxidase (MPO), which localizes mainly in leukocytes, including neutrophils, macrophages, and monocytes (Yap et al., 2007). With a pKa of 7.463 at 35 °C, under physiological conditions, approximately half of HOCl dissociates to the hypochlorite anion (ClO^-), an extensively used disinfectant and bleaching agent (Shepherd et al., 2007; Zhou et al., 2012; Chen et al., 2011a; Yang et al., 2009). Although HOCl functions mainly in the prevention of microorganism invasion (Lin et al., 2009; Zhan et al., 2010; Huo et al., 2012), its excessive or misplaced production caused by variations of MPO levels is implicated in many human diseases (Chen et al., 2010; Panizzi et al., 2009; Chen et al., 2011b), such as hepatic ischemia-reperfusion injury, atherosclerosis, lung injury, cardiovascular diseases, neuron degeneration, arthritis and even cancers (Kim et al., 2011; Sun et al., 2008; Yan et al., 2010; Q. Zhao et al., 2011). Deeper insights on the physiological functions of HOCl in living organisms are fundamentally important to human health and disease.

Luminescent imaging technique using luminescent chemsensors offers a unique approach for visualizing HOCl in intercellular systems (Yuan et al., 2012a; Koide et al., 2011) due to its high sensitivity, selectivity, simplicity of data collection, and high spatial and temporal resolutions. In recent years, several synthetic chemsensors that exhibit fluorescence responses to HOCl were developed (Chen et al., 2008; Yuan et al., 2011), and some of them were used for the monitoring of HOCl generation in living biological samples (Sugiyama et al., 2007; Yuan et al., 2012b). Unfortunately, little attention was paid to the design of phosphorescent chemosensors with transition-metal complexes as signaling moieties (N. Zhao et al., 2011). The luminescent transition metal complexes, especially those with ruthenium (II), are an increasingly important class of sensor materials due to their extraordinary photophysical properties (Balasingham et al., 2011; Lo et al., 2008; Li et al., 2012), such as strong visible absorption and emission, large Stokes shift, and high photo-, thermal and chemical stabilities (Komatsu et al., 2013; J.X. Zhang et al., 2012). In addition, it is well known that the emission of Ru(II) complexes can be easily modulated by a photoinduced

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http://dx.doi.org/10.1016/j.bios.2013.06.005

electron transfer (PET) mechanism (Zhang et al., 2010b; Q. Zhao et al., 2011; Liu et al., 2013), which offers a useful strategy for developing Ru(II) complex-based phosphorescent chemosensors (Fernández-Moreira et al., 2010; Lo, 2007).

Herein we describe the design and synthesis of two Ru(II) complex-based phosphorescent chemosensors, $[Ru(bpy)_2(DNCA\text{-}bpy)](PF_6)_2$ and $[Ru(bpy)_2(DNCH\text{-}bpy)](PF_6)_2$, for the detection of HOCl in living cell. As shown in Scheme 1, the sensors consist of three parts: a Ru(II) complex as the signaling unit; a strong electron acceptor, dinitrophenyl (DNP) as the quencher; and an amide linkage as the specific reaction moiety. In this "signaling unit-recognition linker-quencher" sandwich approach, the intrinsic photophysical attributes of the Ru(II) complex can be withheld by the PET process from Ru(II) center (a potent electron donor) to the DNP moiety (R. Zhang et al., 2012; W. Zhang et al., 2012; Ji et al., 2010). We envisioned that HOCl might promote the oxidation reaction of the amide linkage to generate a –N–Cl species (Slates et al., 1964), which can further undergo a hydrolysis process to form a highly luminescent complex, $[Ru(bpy)_2(COOH\text{-}bpy)](PF_6)_2$ (Leffler and Bond, 1956), resulting in the remarkable enhancement of red luminescence signals. The results of high sensitivity, selectivity, and rapid response of $[Ru(bpy)_2(DNCH\text{-}bpy)](PF_6)_2$ to HOCl promoted us to use this sensor to visualize exogenous HOCl in living HeLa cells, and the endogenous HOCl generation in living macrophage cells during the processes of stimulation and phagocytosis, respectively.

2. Experimental section

2.1. Reagents and apparatus

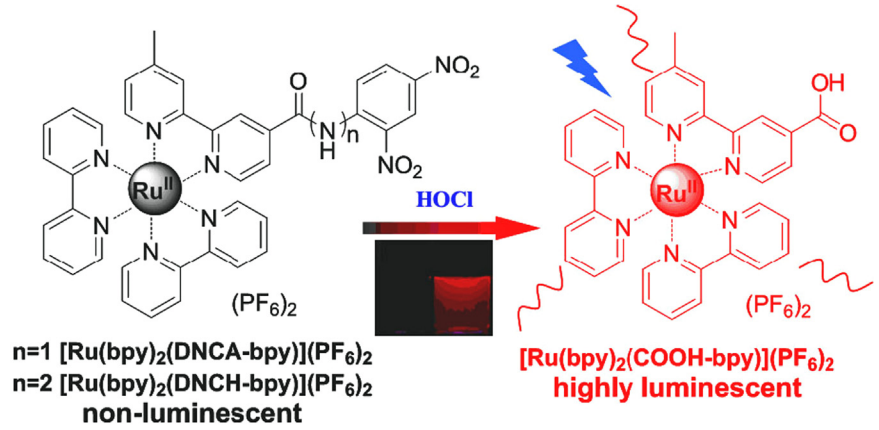
4β-Phorbol-12-myristate-13-acetate (PMA), lipopolysaccharide (LPS), interferon-γ (IFN-γ) 4-aminobenzoic acid hydrazide (4-ABAH), 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazolium bromide (MTT), 3-morpholinosydnonimine (SIN-1), zymosan A from *Saccharomyces cerevisiae*, 2,4-dinitroaniline, and 2,4-dinitrophenylhydrazine were purchased from Sigma-Aldrich. 4-Carboxylic acid-4′-methyl-2,2′-bipyridine (Aldridge et al., 2006), *cis*-Ru(II)$(bpy)_2Cl_2 \cdot 2H_2O$ (Sullivan et al., 1978) and 1-hydroxy-2-oxo-3-(3-aminopropyl)-3-methyl-1-triazene (NOC-13) (Hrabie et al., 1993) were synthesized by using the literature methods. The stock solution of hypochlorite was prepared by dilution of the commercial sodium hypochlorite solution, and stored according to the literature method (Chen et al., 2008). The hypochlorite concentration was determined by using its molar extinction coefficient of 391 $M^{-1}\,cm^{-1}$ at 292 nm before use (Chen et al., 2008). The cultured HeLa and RAW 264.7 macrophage cells were obtained from Dalian Medicine University. Unless otherwise stated, all chemical materials were purchased from commercial sources and used without further purification. Deionized distilled water was used throughout.

NMR spectra were recorded on a Bruker Avance spectrometer (400 MHz). Mass spectra were measured on HP1100LC/MSD MS and LC/Q-TOF MS spectrometers. Absorption spectra were measured on a Perkin-Elmer Lambda 35 UV–vis spectrometer. Elemental analysis was carried out on a Vario-EL analyzer. Luminescence spectra were measured on a Perkin-Elmer LS 50B luminescence spectrometer with the conditions of excitation wavelength, 456 nm; emission wavelength, 626 nm; excitation slit, 10 nm; and emission slit, 10 nm. HPLC analysis was carried out on a SinoChrom ODS-BP 5 μm ($4.6 \times 250\ mm^2$) column using a HPLC system composed of two pumps (P230) and a detector (UV 230+). All bright-field and luminescence imaging measurements were carried out on a Nikon TE2000-E luminescence microscope. The microscope, equipped with a 100 W mercury lamp, a Nikon B-2 A filters (excitation filter, 450–490 nm; dichroic mirror, 505 nm; emission filter, >520 nm) and a color CCD camera system (RET-2000R-F-CLR-12-C, Qimaging Ltd.), was used for the luminescence imaging measurements with an exposure time of 5 s for both HeLa cells and RAW 264.7 macrophage cells. The relative luminescence intensities of images were analyzed by using an ImageJ software.

2.2. Luminescent titrations of $[Ru(bpy)_2(DNCA\text{-}bpy)](PF_6)_2$ and $[Ru(bpy)_2(DNCH\text{-}bpy)](PF_6)_2$ with HOCl

The details of the syntheses and characterizations of $[Ru(bpy)_2(DNCA\text{-}bpy)](PF_6)_2$ and $[Ru(bpy)_2(DNCH\text{-}bpy)](PF_6)_2$ are shown in the Supplementary material.

The reactions of $[Ru(bpy)_2(DNCA\text{-}bpy)](PF_6)_2$ (10 μM) and $[Ru(bpy)_2(DNCH\text{-}bpy)](PF_6)_2$ (10 μM) with HOCl were carried out in 0.1 M phosphate buffer of pH 7.4 at room temperature. Typically, the samples containing different concentrations of HOCl were stirred with ($[Ru(bpy)_2(DNCA\text{-}bpy)](PF_6)_2$) for 30 min or ($[Ru(bpy)_2(DNCH\text{-}bpy)](PF_6)_2$) for 10 min at room temperature, and then subjected to the luminescence measurements. The calibration curve was derived from the luminescence intensity of $[Ru(bpy)_2(DNCH\text{-}bpy)](PF_6)_2$ reacted with HOCl in 0.1 M phosphate buffer of pH 7.4 against the HOCl concentration. To determine the detection limit, the luminescence intensity of $[Ru(bpy)_2(DNCH\text{-}bpy)](PF_6)_2$ in the absence of HOCl was measured 20 times and the standard deviation of the blank measurement was calculated. Then the detection limit was calculated according to the reported method defined by IUPAC (Mocak et al., 1997).



Scheme 1. Proposed phosphorescence turn-on strategy of the chemosensors for the HOCl detection.