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A new highly copper-selective fluorescence enhancement chemosensor based on BODIPY excitable with visible light and its imaging in living cells

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ABSTRACT

A highly selective fluorescent indicator for copper, substituted with a 1-(furan-2-yl)-N-((pyridin-2-yl) methyl)methanamine (FPA) group at the position 3 or 5 of the BODIPY has been synthesized. The BODIPY indicator forms 1:1 complexes with Cu^{2+} ions producing large bathochromic shifts in the absorption (from 480 nm to 570 nm) and fluorescence spectra (from 550 nm to 600 nm) with a change of solution color and cation-induced fluorescence amplifications. The new boradiazaindacene dye exhibited a high affinity and selectivity for Cu^{2+} over competing metal ions. Confocal microscopy experiments showed that bodipy-FPA can be used for detection of Cu^{2+} levels within living cells.

1. Introduction

Fluorescent probes, which produce quantifiable fluorescence changes upon complexation with suitable guest ions, are widely exploited in biology, biotechnology, molecular recognition, clinical diagnostics, and analytical and life science [1,2]. Of major importance are fluoroionophores targeting transition and heavy metal ions (such as Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}. . .). The design and synthesis of fluorescent chemosensors with high selectivity and sensitivity for metal ions remain an exciting field of biochemistry.

Because of their excellent characteristics, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene [3–5] (better known as BODIPY, difluoro*bo*ron *dipy*rromethene) derivatives may be favored fluorophores in the design of fluorescent probes. Indeed, the valuable qualities of BODIPY [3], comprise relatively high molar absorption coefficients and fluorescence quantum yields, narrow emission bandwidths with high peak intensities, robustness toward light and chemicals. Furthermore, BODIPY dyes are excitable with visible light (above 500 nm), have narrow emission bandwidths with high peak intensities, and are amenable to structural modification.

Copper ions, as the third most abundant essential trace element after iron and zinc in biological and ecological systems, play a vital role in various physiological processes [6,7]. The total copper content of the adult human body contains typically is about 70–80 mg of copper under normal circumstances [8]. However, copper ions in abnormal levels are noxious and can cause oxidative stress and neurological disorders, including Alzheimer's, Parkinson's, Wilson's and other diseases [9,10]. Thus, it is highly necessary to design and synthesize novel sensors for the measurement and detection of copper ions.

Since paramagnetic Cu^{2+} is a notorious fluorescence quencher, only few ratiometric fluorescent chemosensors for Cu^{2+} are available in the literature [11–14]. Certainly, some manuscripts show fluorescence amplifications with Cu^{2+}, but Cu^{2+} have interference with other metals as well. The first fluorescent probe for Cu^{2+} based on BODIPY as reporter subunit (K_d = 3 μM) was showed in 2006 [15], which had 8-hydroxyquinoline as receptor and showed significant fluorescence quenching in the presence of Cu^{2+} and Hg^{2+} with markedly higher selectivity and sensitivity for Cu^{2+} than Hg^{2+}. Yoon et al. described a BODIPY derivative as off/on fluorescent chemosensor and chemodosimeter which displayed a chelation enhanced fluorescence effect with Cu^{2+} (K_d = 20 μM), Pb^{2+} (K_d = 0.1 mM), and Zn^{2+} (K_d = 2 mM) [16]. A colorimetric and NIR turn-on fluorescent chemosensor based on BODIPY with DPA

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as a chelator with high selectivity for Cu^{2+} among many transition metal ions has been reported by Boens [17]. Bitter and coworkers reported that a thiacalix[4]crown appended BODIPY off/on fluoroionophore exhibiting pronounced fluorescence enhancement upon addition of Cu^{2+} (K_d = 40 μM), Hg^{2+} (K_d = 42 μM), and Fe^{3+} (K_d = 87 μM) [18]. Jiao et al. had described a membrane-permeable Cu^{2+}-selective water-soluble, BODIPY-based fluorescent probe (K_d = 0.1 μM) which published in 2009 [19]. Latex nanoparticles functionalized with 1,4,8,11-tetraazacyclotetradecane (cyclam) as a Cu^{2+} chelator and doped with a BODIPY fluorophore showed fluorescence quenching in the presence of Cu^{2+}, while no interferences were observed with Zn^{2+} and Ni^{2+} [20]. Therefore, the rapid monitoring of Cu^{2+}, especial the ratiometric and colorimetric detection, is very important.

Bis(pyridin-2-ylmethyl)amine [commonly known as di(2-picolyl)amine, DPA] is a chelator of several metal ions, including Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Ag^{+}, Cd^{2+}, Hg^{2+} and Pb^{2+} [21]. The derivatives of 1-(furan-2-yl)-N-((pyridin-2-yl) methyl)methanamine (FPA) likely have the similar chelating properties as DPA since FPA may form complexes with not only N-atoms, but also with O atom. To the best of our knowledge, fluorescent metal ions sensors with 1-(furan-2-yl)-N-((pyridin-2-yl) methyl)methanamine (FPA) chelator have not yet been reported before. Above all, in this paper, FPA to bind Cu^{2+} is not quenching but enhancing fluorescence. In this context, we studied the interaction of the BODIPY derivatives **1** (BODIPY-FPA), with transition metal ions Cu^{2+}. The interaction of **1** with Cu^{2+} was investigated through UV–visible spectrophotometric, steady-state and time-resolved fluorescence in 4:1 MeCN-HEPES buffer (v/v, 20 mM, pH 7.2).

2. Experimental

2.1. Instrument and reagent

2-Aminomethylpyridine and furaldehyde were procured from Heowns. All of the other chemical solvents and reagents were obtained from Bailingwei. ^1H and ^{13}C NMR spectra were recorded with a Bruker DRX-400 and DRX-400/4 spectrometer with TMS as an internal standard and $CDCl_3$ as solvent. Chemical shift multiplicities are reported as s = singlet, d = doublet, t = triplet and m = multiplet. ^{13}C spectra were referenced to ppm with the $CDCl_3$ (77.67 ppm) signal. Mass spectra were recorded in E.I. mode. Melting points were obtained with an X-4 precise micro melting point cryoscope (Beijing Fukai Instrument Co.) and are uncorrected. All measurements were performed at room temperature.

2.2. General method for measurements of photophysical properties

UV–vis absorption spectra were recorded on a Varian UV-Cary5000 spectrophotometer and for the corrected steady-state excitation and emission spectra, a FLS920 spectrofluorometer and a F7000 spectrofluorometer was employed. Freshly prepared samples in 1 cm quartz cells were used to perform all UV–vis absorption and emission measurements. For the determination of the fluorescence quantum yields ϕ_f of **1**, only dilute solutions with an absorbance below 0.1 at the excitation wavelength (λ_{ex} = 480, 530 or 560 nm) were used. Rhodamine 6G in ethanol (ϕ_f = 0.91) was used as fluorescence standard [22,23]. In all cases, correction for the solvent refractive index was applied. All spectra were recorded at room temperature. The titration experiments with copper were carried out by adding small quantities of a stock solution of metal perchlorate salts in 4:1 MeCN-HEPES buffer (v/v, 20 mM, pH 7.2) to a much larger volume (25 mL) of solutions of **1**.

2.3. Crystal structure determination

Crystals of **1** were obtained by slow evaporation of the ethyl acetate in air over two weeks, yielding red stick crystals with approximate dimensions of 0.38 mm × 0.37 mm × 0.23 mm suitable for X-ray diffraction. The crystals belonged to the monoclinic space group *P*-21 (number 4) with cell dimensions a = 8.3557(16) Å, b = 12.6690(18) Å, c = 22.148(4) Å, α = 90°, β = 90°, γ = 90°, V = 2344.5(7) Å^3, Z = 4, ρ_{calc} = 1.388 g cm^{-3}, $2\theta_{max}$ = 42.00°, μ(Mo$K\alpha$) = 0.227 mm^{-1}.

The data for **1** was measured on a Bruker Smart APEX II CCD diffractometer at 297 ± 3 K equipped with graphite-monochromatized Mo$K\alpha$ radiation (λ = 0.71073 Å). The structures were solved by direct methods. All non-hydrogen atoms were subjected to anisotropic refinement by full-matrix least-squares methods on F^2 by using the program package SHELXS-97 [24]. Hydrogen atoms were placed at calculated positions. Final R indices [$I > 2\sigma(I)$] were R_1 = 0.1447, wR_2 = 0.2241; max./min. residual electron density 0.38/−0.33e$^-$ Å^{-3}.

Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 1411450 for **1**. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: +44 01223 336033 or e mail: deposit@ccdc.cam.ac.uk or www.ccdc.cam.ac.uk/conts/retrieving.html.

2.4. Response parameter and determination of binding constant

The ground-state association constants K of the complexes between the probe **1** and Cu^{2+} were determined in 4:1 MeCN-HEPES buffer (v/v, 20 mM, pH 7.2) by absorption titration data. If **1** binds with Cu^{2+} to form a complex with a complexing ratio of 1:1, one can describe the equilibrium as follows [25]:

$$[\mathbf{1}] + [Cu^{2+}] \overset{K}{\rightleftharpoons} [1 - Cu^{2+}] \quad (1)$$

Where K is complex association constant, the relative absorbance α is defined as the ratio of free **1**, $[\mathbf{1}]_f$, to the total amount of **1**, $[\mathbf{1}]_t$ in 4:1 MeCN-HEPES buffer (v/v, 20 mM, pH 7.2). It can be experimentally determined by measuring the absorbance values in the presence of different concentrations of Cu^{2+}:

$$\alpha = \frac{[\mathbf{1}]_f}{[\mathbf{1}]_t} = \frac{(A_t - A)}{(A_t - A_0)} \quad (2)$$

Where A_0 and A_t are the limiting absorbance values for α = 1 (in the absence of Cu^{2+}) and α = 0 (sensor **1** is completely complexes with Cu^{2+}), respectively. According to the derivation following the mass law reported elsewhere [26], the relationship between the α and the Cu^{2+} concentration can be represented as follows

$$\frac{\alpha}{(1-\alpha)} = \frac{1}{K[Cu^{2+}]} \quad (3)$$

It is apparent from Eq. (3) that the relative absorbance α has a distinct functional relationship with the concentration of Cu^{2+} and the association constant K, which provides the basis for the detection of the K value. The experimental data were fitted to Eq. (3) by adjusting the K value [27].

2.5. Time-resolved fluorescence spectroscopy

Fluorescence lifetimes were measured by a FLS920 at room temperature. The samples and copper complex were dissolved in 4:1 MeCN-HEPES buffer (v/v, 20 mM, pH 7.2) and the concentrations were adjusted to have optical densities at the excitation wavelength <0.1. The monitored wavelength was 560 nm, 580 nm,