A Selective Turn-On Fluorescent Sensor for Imaging Copper in Living Cells

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · FEBRUARY 2006

Impact Factor: 12.11 · DOI: 10.1021/ja055064u · Source: PubMed

5 AUTHORS, INCLUDING:

Arnd Pralle
University at Buffalo, The State University of …

52 PUBLICATIONS **2,951** CITATIONS

SEE PROFILE

Published in final edited form as:
J Am Chem Soc. 2006 January 11; 128(1): 10–11.

A Selective Turn-On Fluorescent Sensor for Imaging Copper in Living Cells

Li Zeng[†], **Evan W. Miller**[†], and **Christopher J. Chang**[†]
Department of Chemistry, University of California, Berkeley, California 94720

Arnd Pralle[‡] and **Ehud Y. Isacoff**[‡]
Departments of Molecular and Cell Biology, University of California, Berkeley, California 94720

Copper is an essential element for life, but alterations in its cellular homeostasis are connected to serious neurodegenerative diseases, including Menkes and Wilson diseases,[1-3] familial amyotropic lateral sclerosis,[4,5] Alzheimer's disease,[6] and prion diseases.[7] Because of its essential yet toxic nature, cells exert strict control over intracellular copper distributions,[8-12] and the thermodynamically estimated level of free copper in the cytosol of bacterial model systems is less than one ion per cell.[13] Nevertheless, cellular copper uptake and release events are kinetically rapid; intracellular copper concentrations can increase by up to 20-fold within 1 h upon incubation in growth medium supplemented with micromolar copper,[14] with facile exchange between cytosolic and mitochondrial stores.[15] Many cytosolic, mitochondrial, and vesicular oxygen-processing enzymes require copper as a redox cofactor,[16] but uncontrolled reactions of copper ions with oxygen and reactive oxygen species (ROS) can also trigger oxidative damage to proteins, nucleic acids, and lipids.[4-7]

We are creating new chemical tools to help elucidate the complex physiological and pathological roles of copper and its oxidation biology. In this regard, live-cell optical imaging with copper-selective fluorescent sensors offers a potentially powerful approach for interrogating aspects of labile copper accumulation, speciation, trafficking, and redox function in living systems at the molecular level. Such reagents have greatly facilitated the study of calcium[17] and zinc[18-20] in cell biology, but analogous tools for cellular copper remain underdeveloped. Cu^{+}, which is stabilized within the reducing environment of the cytosol, presents two particular challenges for aqueous sensing: a susceptibility to disproportionate to Cu^{2+} and Cu^{0} in water and a propensity to act as a fluorescence quencher by electron transfer.[21] Examples of small-molecule Cu^{+}-responsive fluorophores are rare[22,23] and require ultraviolet excitation, which can cause damage to living biological samples and interfering autofluorescence from native cellular species; one of these probes has been applied for detecting Cu^{+} in fixed cells.[23] Here, we present the synthesis and properties of Coppersensor-1 (CS1, **4**), a new water-soluble, turn-on fluorescent sensor that exhibits high selectivity and sensitivity for Cu^{+}. This BODIPY-based reagent is the first Cu^{+}-responsive probe with visible excitation and emission profiles and gives a 10-fold turn-on response for detecting this ion. Confocal microscopy experiments further establish that CS1 is membrane-permeable and can be used to monitor intracellular Cu^{+} levels within living cells.

E-mail: chrischang@berkeley.edu
[†]Department of Chemistry.
[‡]Department of Molecular and Cell Biology.

Supporting Information Available: Synthetic and experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

Properties required for an effective Cu^+ sensor in biological environments include selectivity over abundant cellular metal ions, with the added challenge of discriminating Cu^+ over Cu^{2+}, compatibility with biological samples, including water solubility and membrane permeability, long-wavelength excitation and emission profiles to minimize sample damage and native cellular autofluorescence, and a turn-on or ratiometric fluorescence response for superior spatial resolution. CS1 combines a BODIPY fluorescent reporter having favorable optical properties and biological compatibility with a thioether-rich receptor for selective and stable binding of soft Cu^+ in water. A related phenyl-bridged BODIPY with a thioether macrocycle has been reported for detecting Cu^{2+} in acetonitrile solution.[24] The synthesis of CS1 is outlined in Scheme 1. BODIPY **1** is obtained in a one-pot, two-step procedure via condensation of 2,4-dimethyl-3-ethylpyrrole with chloroacetyl chloride followed by treatment with BF_3•OEt_2. The overall yield is 16% for two steps. The tetrathia receptor **3** is also delivered in two steps. Conversion of ethyl 2-hydroxyethyl sulfide with thiourea and HBr proceeds smoothly to generate thiol **2** in 84% yield. Treatment of **2** with bis(2-chloroethyl)amine hydrochloride under basic conditions furnishes the azatetrathia receptor **3** in 79% yield. Coupling of **1** and **3** in refluxing acetonitrile affords CS1 (**4**) in 22% yield after workup and purification.

CS1 was evaluated under simulated physiological conditions (20 mM HEPES, buffer pH 7). The probe displays optical features characteristic of the BODIPY chromophore. The apo probe exhibits two absorption bands in the visible region with peaks at 510 nm ($\varepsilon = 2.6 \times 10^4\ M^{-1}\ cm^{-1}$) and 540 nm ($\varepsilon = 3.0 \times 10^4\ M^{-1}\ cm^{-1}$) and a corresponding emission maximum at 566 nm. CS1 displays weak fluorescence in its apo form ($\Phi = 0.016$) due to efficient photoinduced electron transfer (PET) quenching by the azatetrathia receptor. Upon addition of Cu^+, the fluorescence intensity of CS1 increases by ca. 10-fold ($\Phi = 0.13$, Figure 1a) with a slight blue shift of the emission maximum to 561 nm. The absorption spectrum of Cu^+-bound CS1 displays a single major visible absorption band at 540 nm ($\varepsilon = 4.0 \times 10^4\ M^{-1}\ cm^{-1}$) with a shoulder at 530 nm. Binding analysis using the method of continuous variations (Job's plot) establishes that a 1:1 Cu^+:CS1 complex is responsible for the observed fluorescence enhancement, and the apparent K_d for Cu^+ binding to CS1 is $3.6(0.3) \times 10^{-12}$ M (Supporting Information).

The emission response of CS1 is highly Cu^+ selective. Figure 1b displays the fluorescence responses of a 2 μM CS1 to the presence of various biologically relevant metal ions. The emission profiles of apo or Cu^+-bound CS1 are unchanged in the presence of 2 mM Ca^{2+}, Mg^{2+}, or Zn^{2+}, indicating excellent selectivities for Cu^+ over these abundant cellular cations. Other first-row transition metal ions, including 50 μM Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, or Cu^{2+}, produce no discernible changes in emission intensities for the apo probe and do not interfere with its Cu^+ response. Especially notable is the observed selectivity for Cu^+ over Cu^{2+}, rendering CS1 potentially valuable for studying specific copper-mediated redox events in biological systems.

We next sought to evaluate the ability of CS1 to operate within living cells. HEK 293 cells incubated with 5 μM CS1 for 5 min at 25 °C show faint intracellular fluorescence (Figure 2a). Supplementing cells with 100 μM $CuCl_2$ in the growth medium for 7 h at 37 °C and then staining with CS1 under the same loading conditions results in a marked increase in observed intracellular fluorescence, as determined from scanning confocal microscopy on live samples (Figure 2b). Control experiments without dye give no background fluorescence, and pretreatment of copper-supplemented cells with the membrane-permeable Cu^+ chelator, 3,6,12,15-tetrathia-9-monoazaheptadecane (500 μM for 5 min at 25 °C), before CS1 staining also shows faint intracellular fluorescence (Figure 2c). Brightfield measurements after copper and CS1 treatment confirm that the cells are viable throughout the imaging experiments (Figure 2d). Taken together, these experiments show that CS1 is cell-permeable and can respond to changes in intracellular [Cu^+] within living cells.

To close, we have described the synthesis, properties, and cellular applications of CS1, a new fluorescent chemosensor for imaging labile Cu^{+} in living biological samples. CS1 is the first Cu^{+}-selective fluorescent probe with visible excitation and emission profiles, gives a 10-fold turn-on response for detecting this ion, and features excellent selectivity for Cu^{+} over biologically relevant metal ions, including Cu^{2+}. Moreover, confocal microscopy experiments establish that CS1 can be used for detecting Cu^{+} levels within living cells. Applications of CS1 and related chemical tools to study copper oxidation biology in living systems are underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgment

We thank the University of California, Berkeley, the Camille and Henry Dreyfus Foundation, the Arnold and Mabel Beckman Foundation, and the American Federation for Aging Research for funding this work. E.W.M. was supported by a Chemical Biology Interface Training Grant from the National Institutes of Health (T32 GM066698). C.J.C. thanks Prof. David Rorabacher for helpful discussions.

References

1. Waggoner DJ, Bartnikas TB, Gitlin JD. Neurobiol. Disease 1999;6:221–230.
2. Vulpe C, Levinson B, Whitney S, Packman S, Gitschier J. Nat. Genet 1993;3:7–13. [PubMed: 8490659]
3. Bull PC, Thomas GR, Rommens JM, Forbes JR, Cox DW. Nat. Genet 1993;5:327–337. [PubMed: 8298639]
4. Valentine JS, Hart PJ. Proc. Natl. Acad. Sci. U.S.A 2003;100:3617–3622. [PubMed: 12655070]
5. Bruijn LI, Miller TM, Cleveland DW. Annu. Rev. Neurosci 2004;27:723–749. [PubMed: 15217349]
6. Barnham KJ, Masters CL, Bush AI. Nat. Rev. Drug Discovery 2004;3:205–214.
7. Brown DR, Kozlowski H. Dalton Trans 2004:1907–1917. [PubMed: 15252577]
8. O'Halloran TV, Culotta VC. J. Biol. Chem 2000;275:25057–25060. [PubMed: 10816601]
9. Rosenzweig AC, O'Halloran TV. Curr. Opin. Chem. Biol 2000;4:140–147. [PubMed: 10742187]
10. Huffman DL, O'Halloran TV. Annu. Rev. Biochem 2001;70:677–701. [PubMed: 11395420]
11. Puig S, Thiele DJ. Curr. Opin. Chem. Biol 2002;6:171–180. [PubMed: 12039001]
12. Arnesano F, Banci L, Bertini I, Ciofi-Baffoni S. Eur. J. Inorg. Chem 2004:1583–1593.
13. Rae TD, Schmidt PJ, Pufahl RA, Culotta VC, O'Halloran TV. Science 1999;284:805–808. [PubMed: 10221913]
14. Herd SM, Camakaris J, Christofferson R, Wookey P, Danks DM. Biochem. J 1987;247:341–347. [PubMed: 3426541]
15. Cobine PA, Ojeda LD, Rigby KM, Winge DR. J. Biol. Chem 2004;279:14447–14455. [PubMed: 14729672]
16. Linder MC, Hazegh-Azam M. Am. J. Clin. Nutr 1996;63:797S–811S. [PubMed: 8615367]
17. Tsien RW, Tsien RY. Annu. Rev. Cell Biol 1990;6:715–760. [PubMed: 2177344]
18. Kikuchi K, Komatsu K, Nagano T. Curr. Opin. Chem. Biol 2004;8:182–191. [PubMed: 15062780]
19. Lim NC, Freake HC, Brückner C. Chem.—Eur. J 2005;11:38–49.
20. Burdette SC, Lippard SJ. Proc. Natl. Acad. Sci. U.S.A 2003;100:3605–3610. [PubMed: 12655069]
21. Rapisarda VA, Volentini SI, Farias RN, Massa EM. Anal. Biochem 2002;307:105–109. [PubMed: 12137786]
22. Cody J, Fahrni CJ. Tetrahedron 2004;60:11099–11107.
23. Yang L, McRae R, Henary MM, Patel R, Lai B, Vogt S, Fahrni CJ. Proc. Natl. Acad. Sci. U.S.A 2005;102:11179–11184. [PubMed: 16061820]
24. Rurack K, Kollmannsberger M, Resch-Genger U, Daub. J. Am. Chem. Soc 2000;122:968–969.

Scheme 1.
Synthesis of Coppersensor-1 (CS1)

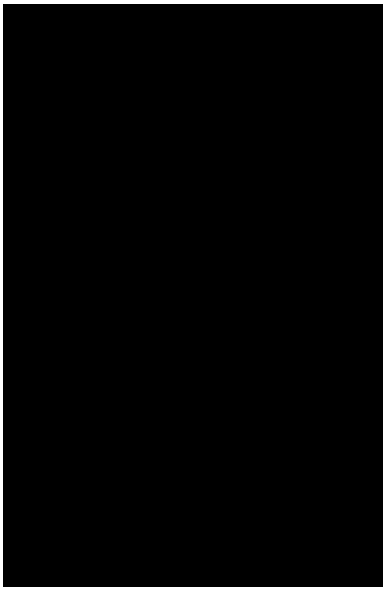

Figure 1.

(A) Fluorescence response of 2 μM CS1 to Cu^{+}. Spectra shown are for buffered [Cu^{+}] of 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, and 2.0 μM. Spectra were acquired in 20 mM HEPES, pH 7, with excitation at 540 nm. (B) Fluorescence responses of CS1 to various metal ions. Bars represent the final integrated fluorescence response (F_f) over the initial integrated emission (F_i). Initial spectra were acquired in 20 mM HEPES, pH 7. White bars represent the addition of an excess of the appropriate metal ion (2 mM for Ca^{2+}, Mg^{2+}, and Zn^{2+}, 50 μM for all other cations) to a 2 μM solution of CS1. Black bars represent the subsequent addition of 10 μM Cu^{+} to the solution. Excitation was provided at 540 nm, and the emission was integrated over 550–650 nm.

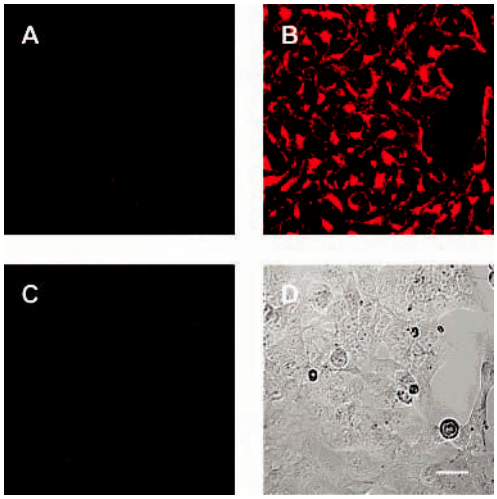

Figure 2.

Confocal fluorescence images of live HEK 293 cells. (A) Cells incubated with 5 μM CS1 for 5 min at 25 °C. (B) Cells supplemented with 100 μM $CuCl_2$ in the growth media for 7 h at 37 °C and stained with 5 μM CS1 for 5 min at 25 °C. (C) $CuCl_2$-supplemented cells pretreated with 500 μM of the competing Cu^+ chelator, 3,6,12,15-tetrathia-9-monoazaheptadecane, for 5 min at 25 °C before staining with 5 μM CS1 for 5 min at 25 °C. (D) Brightfield image of live HEK 293 cells shown in panel B, confirming their viability. Scale bar = 25 μm.