ORGANIC LETTERS

2005
Vol. 7, No. 16
3573−3575

Conjugated Polymer-Based Fluorescence Turn-On Sensor for Nitric Oxide

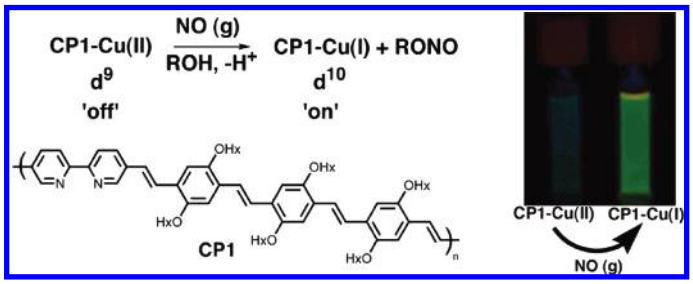
Rhett C. Smith, Andrew G. Tennyson, Mi Hee Lim, and Stephen J. Lippard*

Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

lippard@mit.edu

Received June 14, 2005

ABSTRACT



A turn-on fluorescent sensor for NO (g) in solution was synthesized using a bipyridyl-substituted poly(*p*-phenylene vinylene) derivative (CP1) as the sensory scaffold. The action of NO (g) upon the CP1–Cu(II) complex reduces it to the CP1–Cu(I) complex with a concomitant 2.8-fold increase in emission intensity. The reagent is selective for NO (g) versus other biological reactive nitrogen species, except for nitroxyl, and has a detection sensitivity limit of 6.3 nM.

The discovery that nitric oxide (NO) is the endothelium-derived relaxing factor astonished the scientific community.[1] Since then, an even wider range of biological roles for NO have been elucidated.[2] We are interested in NO as a possible signaling agent in the central nervous system,[3] where it is proposed to mediate synaptic plasticity.[4] The biological chemistry of NO has inspired researchers to devise techniques for its bioimaging,[5] of which turn-on fluorescence is especially attractive because of the demonstrated success of this strategy in real-time monitoring of other cellular signals.[6] Neurological research in particular has benefited from the development of small-molecule fluorescent sensors specific for Ca^{2+} [6b,7] or Zn^{2+}.[8] Here we report a turn-on sensor for NO with nM sensitivity employing a π-conjugated polymer (CP) as the fluorescent reporter.

The enhanced sensitivity of CPs vs small molecule-based sensors, together with their structural and optoelectronic tunability, renders them intriguing scaffolds for the design and construction of detection systems.[9] We therefore investigated this class of materials for imaging NO by turn-on

(1) (a) Ignarro, L. J. *Angew. Chem., Int. Ed.* **1999**, *38*, 1882−1892. (b) Furchgott, R. F. *Angew. Chem., Int. Ed.* **1999**, *38*, 1870−1880. (c) Murad, F. *Angew. Chem., Int. Ed.* **1999**, *38*, 1856−1868.

(2) (a) Richter-Addo, G. B.; Legzdins, P. *Metal Nitrosyls*; Oxford University Press: New York, 1992. (b) Butler, A.; Nicholson, R. *Life, Death and Nitric Oxide*; Royal Society of Chemistry: Cambridge, UK, 2003. (c) Ricciardolo, F. L. M.; Sterk, P. J.; Gaston, B.; Folkerts, G. *Phys. Rev.* **2004**, *84*, 731−765. (d) Cals-Grierson, M. M.; Ormerod, A. D. *Nitric Oxide* **2004**, *10*, 179−193. (e) Toda, N.; Okamura, T. *Pharm. Rev.* **2003**, *55*, 271−324. (f) Wei, C.-C.; Crane, B. R.; Stuehr, D. J. *Chem. Rev.* **2003**, *103*, 2365−2383.

(3) (a) Bon, C. L. M.; Garthwaite, J. *J. Neurosci.* **2003**, *23*, 1941−1948. (b) Pepicelli, O.; Raiteri, M.; Fedele, E. *Neurochem. Int.* **2004**, *45*, 787−797.

(4) Huang, E. P. *Curr. Biol.* **1997**, *7*, R141−R143.

(5) Nagano, T.; Yoshimura, T. *Chem. Rev.* **2002**, *102*, 1235−1269.

(6) (a) Zhang, J.; Campbell, R. E.; Ting, A. Y.; Tsien, R. Y. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 906−918. (b) Zacharias, D. A.; Baird, G. S.; Tsien, R. Y. *Curr. Opin. Neurobiol.* **2000**, *10*, 416−421.

(7) Miyawaki, A. *Curr. Opin. Neurobiol.* **2003**, *13*, 591−596.

(8) (a) Burdette, S. C.; Lippard, S. J. *Proc. Nat. Acad. Sci.* **2003**, *100*, 3605−3610. (b) Jiang, P.; Guo, Z. *Coord. Chem. Rev.* **2004**, *248*, 205−229. (c) Chang, C. J.; Lippard, S. J. *Metal Ions Biol. Syst.* **2005**, *45*, in press.

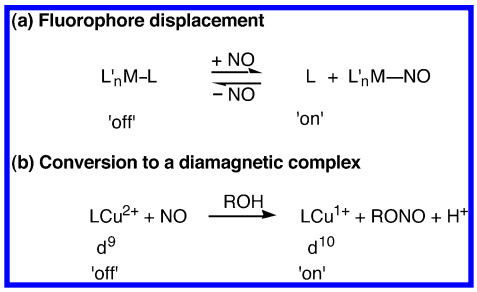
(9) (a) McQuade, D. T.; Pullen, A. E.; Swager, T. M. *Chem. Rev.* **2000**, *100*, 2537−2574. (b) Swager, T. M. *Acc. Chem. Res.* **1998**, *31*, 201−207.

10.1021/ol0513903 CCC: $30.25
Published on Web 07/02/2005

fluorescence, a resistivity-based CP sensor for NO having been previously described.[10] The successful realization of CPs for fluorescence-based detection of NO, in the form of a CP−copper complex that exhibits a turn-on response to NO in solution, represents an advantageous new strategy with many possible applications.

Previously, we reported transition metal-based NO sensors that involved displacement of a fluorescent ligand from a mono- or dimetallic center with attendant turn-on of fluorescence, one of which is reversible (Scheme 1a, L = fluorescent ligand).[11] More recent work unveiled a related approach,[12] involving NO-induced copper redox chemistry,[13] with net conversion of fluorophore-labeled, paramagnetic (quenched) Cu(II) complexes to a diamagnetic (fluorescent) Cu(I) state (Scheme 1b). This mechanism has been considered as a possibility for colorimetric NO sensing.[14]

Scheme 1. Strategies for Fluorescent Detection of Nitric Oxide



In further pursuit of the latter strategy, we prepared a series of CPs integrating copper-binding units at defined intervals and screened their relative fluorescence intensities in both CP−Cu(I) and CP−Cu(II) forms, anticipating turn-on NO detection by the process depicted in Scheme 1b.[15] A poly(*p*-phenylene vinylene) (PPV) derivative incorporating periodic bipyridyl units along the main chain displayed the most suitable properties for further investigation (**CP1**, Figure 1, Hx = *n*-hexyl). A structurally related CP is reportedly quenched in the presence of Cu(II), whereas only moderate quenching occurs with added Cu(I).[16] A careful study of the structural and photophysical characteristics of poly(*p*-phenylene ethynylene)/bipyridyl−Cu(I) complexes has recently been undertaken.[17] This work indicates that stable complexes exist with two bipyridyl units per Cu(I), indicating

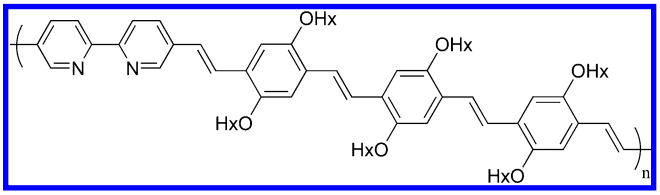


Figure 1. Structure of **CP1**.

that only 0.5 equiv of Cu(I) are necessary to attain maximum quenching of polymer luminescence, as independently observed in the present study (Supporting Information).

CP1 is a bright red-orange solid (λ_{max} = 462 nm) with strong fluorescence emission centered at 542 nm (Φ = 0.30). Upon addition of 1 equiv of $Cu(OTf)_2$ to a solution of **CP1**, the integrated fluorescence intensity was quenched 4-fold, whereas addition of 1 equiv of $[Cu(NCMe)_4][BF_4]$ decreased the fluorescence by ~30% (Figure 2).[15] Adding >0.5 equiv of Cu(II) afforded little additional emission quenching.

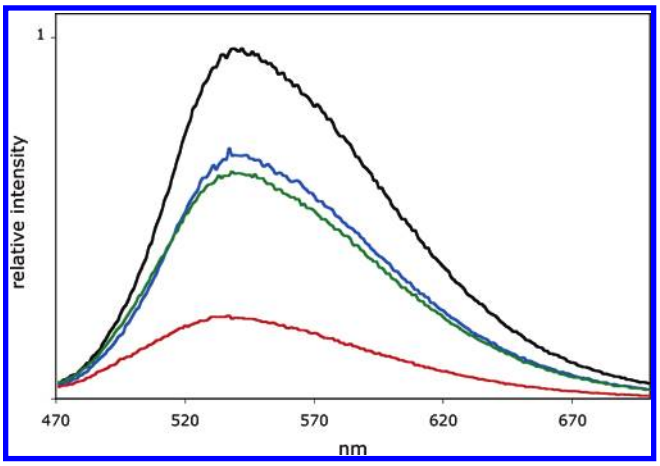


Figure 2. Emission spectra of **CP1** (black), **CP1**−Cu(I) (green), **CP1**−Cu(II) (red), and **CP1**−Cu(II) immediately following addition of 300 equiv of NO (g). All measurements were made in 4:1 CH_2Cl_2/EtOH.

Introduction of 300 equiv of NO (g) to the **CP1**−Cu(II) complex rapidly (<1 min) increased the integrated emission by 2.8-fold, producing a fluorescence spectrum similar to that of the **CP1**−Cu(I) complex (Figure 2). NO (g) did not alter the fluorescence of **CP1** in the absence of Cu(II).

In the proposed mechanism, Scheme 1b, protons are formed that could affect the fluorescence of **CP1**. Protons alone, however, decrease the emission from bipyridyl-PPVs.[18] Using a handheld UV lamp, a decrease in emission was qualitatively confirmed in the current case following addition of 3 μL of glacial acetic acid to 5 mL of a 2 μM solution of **CP1** in 4:1 CH_2Cl_2/EtOH.

(10) Shioya, T.; Swager, T. M. *Chem. Commun.* **2002**, 1364−1365.
(11) (a) Franz, K. J.; Singh, N.; Lippard, S. J. *Angew. Chem., Int. Ed.* **2000**, *39*, 2120−2122. (b) Franz, K. J.; Singh, N.; Spingler, B.; Lippard, S. J. *Inorg. Chem.* **2000**, *39*, 4081−4092. (c) Hilderbrand, S. A.; Lim, M. H.; Lippard, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 4872−4878. (d) Lim, M. H.; Lippard, S. J. *Inorg. Chem.* **2004**, *43*, 6366−6370.
(12) Lim, M. H.; Lippard, S. J. **2005**, submitted for publication.
(13) Tran, D.; Skelton, B. W.; White, A. H.; Laverman, L. E.; Ford, P. C. *Inorg. Chem.* **1998**, *37*, 2505−2511.
(14) Dacres, H.; Narayanaswamy, R. *Sens. Act. B* **2003**, *90*, 222−229.
(15) Details of the full set of polymers examined will be reported elsewhere.
(16) Wang, B.; Wasielewski, M. R. *J. Am. Chem. Soc.* **1997**, *119*, 12−21.
(17) Kokil, A.; Yao, P.; Weder, C. *Macromolecules* **2005**, *38*, 3800−3807.
(18) Eichen, Y.; Nakhmanovich, G.; Gorelik, V.; Epshtein, O.; Poplawski, J. M.; Ehrenfreund, E. *J. Am. Chem. Soc.* **1998**, *120*, 10463−10470.

Following initial trials with NO (g), we examined the selectivity of the sensor for nitric oxide vs other biologically relevant reactive nitrogen species (RNS). In parallel with ongoing work in our laboratory,[19] we evaluated a nitrosothiol (SNAP, *S*-nitroso-*N*-acetylpenicillamine), a nitroxyl (HNO) donor (Angeli's salt, $Na_2N_2O_3$),[20] and a nitrosonium (NO^+) source ($NOBF_4$) for their ability to alter the emission spectra of **CP1**, **CP1**−Cu(I), or **CP1**−Cu(II). These donors were selected on the basis of their commercial availability in high purity and well-studied kinetics of RNS formation.[22] None of the donors (50 equiv of SNAP, 50 equiv of $NOBF_4$, or 16 equiv of Angeli's salt, $Na_2N_2O_3$) induced a change in the emission spectra of **CP1** or **CP1**−Cu(I). When 50 equiv of SNAP was added to **CP1**−Cu(II), a 1.5-fold turn-on of fluorescence occurred slowly over 2 h, in accord with the known ability of cupric ion to catalyze the release of NO from nitrosothiols.[22e] The same effect was observed upon reversing the order of Cu(II) and SNAP addition, in which case the expected 4-fold quenching occurred immediately upon Cu(II) addition, followed by a slow turn-on. This control indicates that the presence of excess SNAP does not interfere with Cu(II) binding by **CP1**. A full 24 h was required following addition of SNAP before a turn-on response (2.1-fold) similar to that evoked by NO (g) was attained. Because of the significantly longer time for SNAP (24 h) vs NO (<1 min) to elicit a fluorescence response, nitrosothiols should not be considered as seriously interfering analytes.

The addition of 50 equiv of $NOBF_4$ did not affect the fluorescence of solutions of **CP1** or of preformed **CP1**−Cu(I) or **CP1**−Cu(II). When Cu(II) was added to a solution of **CP1** containing 50 equiv of $NOBF_4$, the emission spectrum was the same as that of **CP1**−Cu(II). In the presence of EtOH, $NOBF_4$ will form EtONO. The solution resulting from addition of $NOBF_4$ to **CP1**−Cu(I) was therefore expected to contain the same species present in the reaction of **CP1**−Cu(II) with NO. These two solutions exhibited identical emission spectra.

Although no response was elicited by nitroxyl with **CP1**−Cu(I), it is noteworthy that an immediate 2.8-fold increase occurred upon reaction of **CP1**−Cu(II) with 50 equiv of nitroxyl, formed from decomposition of Angeli's salt. The spectrum produced was nearly identical to that exhibited by **CP1**−Cu(I), suggesting that nitroxyl may reduce Cu(II) to Cu(I).

The effect of O_2 was also investigated as another possible interfering biological species. Aeration of a cuvette containing a nitrogen-purged **CP1**−Cu(II) solution slightly decreased the integrated fluorescence (~6%). This response is presumably due the ability of O_2 to serve as a collisional quencher and is within the error of detection.

The sensitivity of the **CP1**−Cu(II) complex to NO was evaluated by addition of progressively lower concentrations of SNAP to a 630 nM solution of the sensor. On the basis of multiple measurements versus an emission standard, NIST-issued quinine sulfate dihydrate, we determined a 10% increase in integrated emission to be the lowest quantifiable change discernible at our instrumental detection limit. By using this method, we computed a sensitivity of about 6.3 nM for the **CP1**−Cu(II) system.

Although previous work has provided strong evidence for the mechanism presented in Scheme 1b,[13] we carried out a number of additional checks to confirm its validity in the present context. The strongest supporting evidence is the near perfect match of emission spectra derived from **CP1**−Cu(I), **CP1**−Cu(I)/NO^+, and **CP1**−Cu(II)/NO. Exposure of **CP1**−Cu(II) to NO (g) in the absence of EtOH, which is required to form RONO in the proposed mechanism, did not produce an increase in fluorescence. Finally, the expected diminution of the EPR signal of **CP1**−Cu(II) occurred upon addition of 1 equiv of NO, confirming reduction to Cu(I). When 1 equiv of $Cu(OTf)_2$ was added to a solution of **CP1** (0.5 equiv bind) followed by 1 equiv of NO (g), the integrated signal decreased by 45%, indicating that only **CP1**-coordinated Cu(II) is reduced to Cu(I). No bands attributable to stable copper nitrosyls were observed by IR spectroscopy.

Despite the strong evidence and precedence for the proposed mechanism, we note that, in addition to the $d^9 \rightarrow d^{10}$ transformation that occurs upon reduction, some degree of ligand rearrangement or other structural alteration, to which CP emission is very sensitive,[17,21] could play a role in the observed sensory response. Work in progress with small-molecule model compounds will elucidate such possibilities.

The system reported here represents an early manifestation of a new strategy for the fluorescent detection of NO. To the best of our knowledge, it also signifies the first fluorescence-based sensor for NO employing a conjugated polymer scaffold.[10] Studies have commenced to identify additional transition metal-conjugated polymer complexes for improved sensory response, devise specificity for NO over nitroxyl, and prepare highly fluorescent water-soluble derivatives for biological imaging of nitric oxide.[21]

Acknowledgment. This work was supported by NSF Grant CHE-0234951. R.C.S. and A.G.T. thank the NIH and NSF for Fellowships. The MIT DCIF NMR spectrometer was funded through NSF Grants CHE-9808061 and DBI-9729592.

Supporting Information Available: Experimental details, absorption and emission spectra, EPR spectra, and details of sensitivity and selectivity measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0513903

(19) Tennyson, A. G.; Lippard, S. J. Manuscript in preparation.

(20) Bartberger, M. D.; Lin, W.; Ford, E.; Miranda, K. M.; Switzer, C.; Fukuto, J. M.; Farmer, P. J.; Wink, P. A.; Houk, K. N. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10958−10963.

(21) (a) Wang, D.; Gong, X.; Heeger, P. S.; Rininsland, F.; Bazan, G. C.; Heeger, A. J. *Adv. Funct. Mater.* **2003**, *13*, 463−467. (b) Kuroda, K.; Swager, T. M. *Chem. Commun* **2003**, 26−27. (c) Xu, Q.-H.; Gaylord, B. S.; Wang, S.; Bazan, G. C.; Moses, D.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 11634−11639.

(22) (a) Bonner, F. T.; Ravid, B. *Inorg. Chem.* **1975**, *14*, 558−563. (b) Bazylinski, D. A.; Hollocher, T. C. *Inorg. Chem.* **1985**, *24*, 4285−4288. (c) Singh, R. J.; Hogg, N.; Joseph, J.; Kalyanaraman, B. *J. Biol. Chem.* **1996**, *271*, 18596−18603. (d) Williams, D. L. H. *Met. Enzymol.* **1996**, *268*, 299−308. (e) Smith, J. N.; Dasgupta, T. P. *Nitric Oxide* **2000**, *4*, 57−66. (f) Chen, Y.; Irie, Y.; Keung, W. M.; Maret, W. *Biochem.* **2002**, *41*, 8360−8367.