JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

A Reaction-Based Fluorescent Probe for Selective Imaging of Carbon Monoxide in Living Cells Using a Palladium-Mediated Carbonylation

Brian W. Michel,[†] Alexander R. Lippert,[†,§] and Christopher J. Chang*[,†,‡]

[†]Department of Chemistry and [‡]Howard Hughes Medical Institute, University of California, Berkeley, California 94720, United States

Supporting Information

ABSTRACT: Carbon monoxide is a member of the gasotransmitter family, which also includes NO and H_2S, and has been implicated in a variety of pathological and physiological conditions. Whereas exogenous therapeutic additions of CO to tissues and whole animals have been well-studied, the real-time spatial and temporal tracking of CO at the cellular level remains an open challenge. Here we report a new type of turn-on fluorescent probe for selective CO detection based on palladium-mediated carbonylation reactivity. CO Probe 1 (COP-1) is capable of detecting CO both in aqueous buffer and in live cells with high selectivity over a range of biologically relevant reactive small molecules, providing a potentially powerful approach for interrogating its chemistry in biological systems.

Carbon monoxide is best known as a toxic gas inhaled from common sources such as smoke and car exhaust, but emerging studies have shown that this reactive small molecule is also continuously produced in the body via the breakdown of heme by heme oxygenase enzymes.[1−3] Similar to the other major gasotransmitter molecules NO and H_2S, CO is proposed to play significant roles in modulating responses to both chemical and physical stresses.[4−7] In one example, exogenous and endogenous CO can provide protection against tissue damage during myocardial ischemia/reperfusion,[8] and to this end, CO-releasing molecules (CORMs) based on transition-metal carbonyl complexes have been developed as potential therapeutics that allow for more targeted release of CO in comparison with direct gas inhalation.[4,9,10]

Despite the important signal/stress dichotomy of CO, many aspects of its chemistry in biological systems remain elusive, in part because of the lack of ways to track this transient small molecule selectively within intact, living biological specimens. Indeed, the primary methods for interrogating the biological effects of CO to date involve detecting a gross anatomical change of some observable parameter, such as infarct size in studies of the effects of CO on ischemia/reperfusion or offline extracellular measurements using myoglobin[11,12] or dirhodium-supported particles[13] for colorimetric readouts. We reasoned that the development of a CO-responsive small-molecule fluorescent probe would meet a critical need for new technologies to monitor this reactive small molecule in biological systems with spatial and temporal information. This approach has proved useful for studying the contributions of a variety of small signal/stress molecules in biological settings,[14−18] including NO,[19−22] H_2S,[23−29] and H_2O_2,[30−40] but there have been no reports of analogous indicators for CO. Herein we present the design, synthesis, and biological evaluation of a new type of chemical reagent for selective CO detection in living cells based on palladium-mediated carbonylation chemistry. CO Probe 1 (COP-1) represents a unique first-generation chemical tool that features a robust turn-on response to CO with selectivity over reactive nitrogen, oxygen, and sulfur species and can be used to detect this gasotransmitter in aqueous buffer and in live-cell specimens.

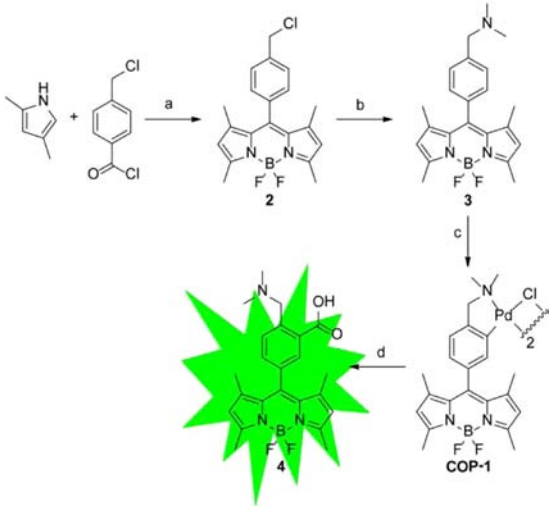
Our overall strategy for imaging CO in live biological systems relies on exploiting selective CO-induced reaction chemistry for its detection. Recent work from our laboratory on reaction-based fluorescent probes for imaging of H_2O_2[30−38] and H_2S[23] have taken advantage of the respective nucleophilic oxidative and reductive abilities of these small molecules. In contrast, CO is not a particularly nucleophilic or electrophilic species and is better known for its inorganic coordination chemistry and subsequent organometallic reactivity. Therefore, we envisioned metal-mediated carbonylation chemistry as a potential means to design a reaction-based fluorescent CO probe, as covalent incorporation of CO into a dye scaffold can significantly alter its electronic characteristics. In particular, we turned our attention to palladium because of the established reactivity of this metal in catalytic carbonylation reactions[41−47] as well as recent reports demonstrating the compatibility of organometallic reactions with cellular systems, including Ahn's indicators for palladium/platinum[48] and Meggers' ruthenium-induced allyl carbamate cleavage,[49] where cell viability was maintained. Additionally, Bradley and co-workers elegantly illustrated the use of palladium-functionalized microspheres to elicit both deallylation and cross-coupling reactions in live cells.[50] On the basis of these considerations, we designed and synthesized the cyclopalladated species COP-1, anticipating that the presence of palladium would quench the fluorescence of the borondipyrromethene difluoride (BODIPY) core via heavy-atom electronic effects and that upon binding of CO, a carbonylation reaction would concomitantly release reduced Pd(0) and a more fluorescent species. To this end, we prepared COP-1 by alkylation of benzyl chloride **2** with dimethylamine and subsequent cyclometalation with $Pd(OAc)_2$, which was then converted to the chloride dimer (Scheme 1).

With COP-1 in hand, we tested its fluorescence properties and CO reactivity in aqueous solution buffered to physiological pH. For the present in vitro analysis, we utilized the water-

Received: July 17, 2012
Published: September 13, 2012

   dx.doi.org/10.1021/ja307017b | J. Am. Chem. Soc. 2012, 134, 15668−15671

Scheme 1. Synthesis and Reactivity of COP-1[a]



[a]Reagents and conditions: (a) CH_2Cl_2, reflux, 2 h, then solvent swap (~10:1 toluene:CH_2Cl_2), Et_3N, $BF_3{\cdot}Et_2O$, 50 °C, 1 h. (b) Me_2NH, K_2CO_3, KI, CH_3CN, microwave, 80 to 100 °C, 1 h. (c) $Pd(OAc)_2$, benzene, 50 °C, 14 h, then solvent swap (acetone), LiCl. (d) Wet CH_2Cl_2, CO, 31 °C, 14 h.

soluble complex $[Ru(CO)_3Cl(glycinate)]$ (CORM-3) as an easy-to-handle CO source.[51,52] As expected, COP-1 was weakly fluorescent in Dulbecco's phosphate-buffered saline (DPBS) buffered to pH 7.4 (λ_{em} = 503 nm, Φ = 0.01; see Figure S2 in the Supporting Information). Addition of 50 μM CORM-3 to a solution of COP-1 at 37 °C triggered a robust fluorescence turn-on response due to CO-induced formation of the carbonylation product **4**, which was synthesized and characterized independently for verification (λ_{max} = 499 nm, ε = 23 000 M^{-1} cm^{-1}, λ_{em} = 507 nm, Φ = 0.44; see Figure S1). Interestingly, this acid was the major product in aqueous solution, in contrast to the dealkylative amide product typically observed in organic solutions.[45] Within 60 min of reaction under these conditions, COP-1 produced a 10-fold increase in fluorescence (Figure 1a). Moreover, we observed a dose-dependent response of COP-1 to CORM-3 down to 1 μM (~28 ppb CO) levels (Figure S3). Furthermore, the fluorescence turn-on response for COP-1 was found to have good selectivity over other biologically relevant reactive oxygen, nitrogen, and sulfur species, including H_2O_2, *tert*-butyl hydroperoxide (*t*BuOOH), hypochlorite (OCl^-), superoxide ($O_2^{\bullet-}$), NO, peroxynitrite ($ONOO^-$), and H_2S, as exposure of COP-1 to these molecules did not trigger fluorescence responses to the same extent as exposure to CO (Figure 1b).

Finally, we evaluated the ability of COP-1 to visualize changes in CO levels in live cells using confocal microscopy. HEK293T cells were incubated with either CORM-3 (5 or 50 μM) or a vehicle control, and then the cells were treated with 1 μM COP-1 (Figure 2). A significant and dose-dependent increase in intracellular fluorescence was observed in CORM-3-treated cells (Figure 2b,c,e) over vehicle control samples (Figure 2a,e). In addition, we performed two independent types of assays to show that the palladium-based probe and its reactivity are nontoxic to the cellular specimens and compatible with live-cell imaging over the course of these experiments. First, we acquired bright-field images and overlaid them with fluorescence images of the cells stained with Hoescht 33342 nuclear stain that clearly showed intact and viable nuclei

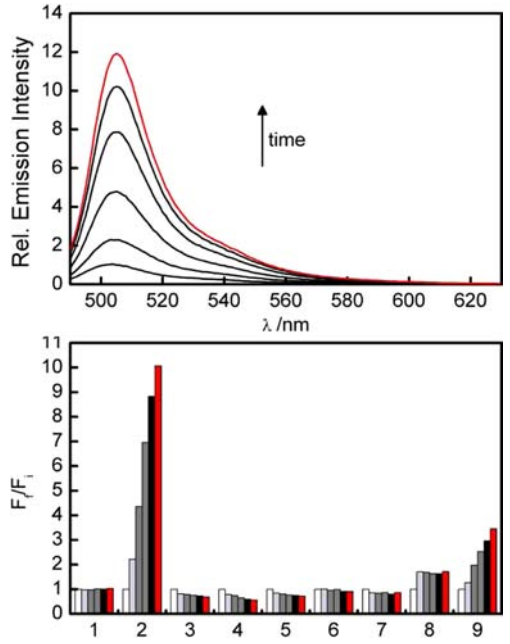


Figure 1. COP-1 shows a robust and selective turn-on response to CO in buffered aqueous solution. (a) Turn-on fluorescence response of 1 μM COP-1 to 50 μM CORM-3 in pH 7.4 DPBS at 37 °C (λ_{ex} = 475 nm, emission collected at 490–630 nm). Time points are represented by spectra taken 0, 5, 15, 30, 45, and (red) 60 min after the addition of CORM-3. (b) Fluorescence responses of 1 μM COP-1 to CO and biologically relevant reactive oxygen, nitrogen, and sulfur species. Bars represent normalized integrated fluorescence intensity responses between 490 and 630 nm with λ_{ex} = 475 nm for the respective analytes (50 μM) at t = 0, 5, 15, 30, 45, and (red) 60 min. Data were acquired in pH 7.4 DPBS buffer at 37 °C. Legend: (1) control; (2) CORM-3; (3) H_2O_2; (4) *t*BuOOH; (5) NaOCl; (6) $O_2^{\bullet-}$; (7) NO; (8) $ONOO^-$; (9) H_2S.

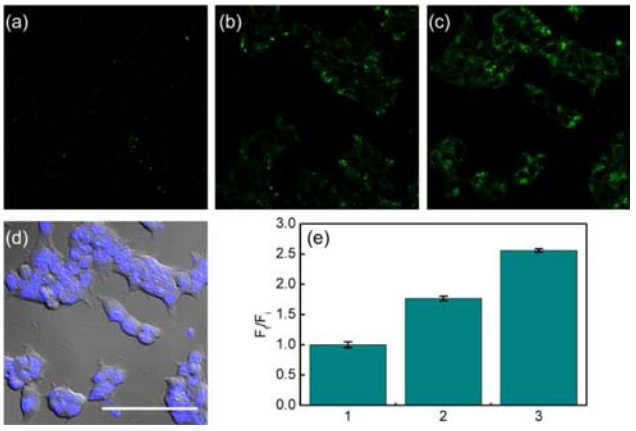


Figure 2. Confocal microscopy images of CO detection in live HEK293T cells using COP-1. (a) HEK293T cells incubated with COP-1 for 30 min at 37 °C. (b) HEK293T cells incubated with 5 μM CORM-3 for 45 min at 37 °C and 1 μM COP-1 for the final 30 min. (c) HEK293T cells incubated with 50 μM CORM-3 for 45 min at 37 °C and 1 μM COP-1 for the final 30 min. (d) Bright-field image of the cells in (c) overlaid with images of 1 μM Hoescht 33342-stained cells. The scale bar represents 100 μM. (e) Mean fluorescence intensities of representative images with (1) 1 μM COP-1, (2) 1 μM COP-1 and 5 μM CORM-3, and (3) 1 μM COP-1 and 50 μM CORM-3.

(Figure 2d). Second, as a further validation of cell viability, a water-soluble tetrazolium salt (WST) cell proliferation assay

dx.doi.org/10.1021/ja307017b | J. Am. Chem. Soc. 2012, 134, 15668–15671

was performed over a range of probe concentrations (500 nM to 10 μM) with and without the addition of CORM-3 (100 μM). Cell viability remained constant over the range of probe concentrations evaluated (Figure S4). We note that the concentrations of CORM-3 employed are well within the therapeutic window,[53] as up to 500 μM CORM-3 has been shown not to alter cell viability, and the added CORM-3 in these present experiments was 1−2 orders of magnitude less than this upper limit.[54] Estimates of CO fluxes produced by heme oxygenase and other endogenous sources are cell-type-dependent and remain a subject of debate,[55] and we hope that expanding the toolbox of CO detection technologies will help address this important issue.

In summary, we have presented a new approach to biological CO detection through the synthesis and application of COP- 1, a cyclopalladated probe that interacts with CO to trigger a fluorogenic carbonylation reaction. COP-1 shows a robust turn-on fluorescence response to CO that is selective over a variety of reactive nitrogen, oxygen, and sulfur species and can be used to image CO in living cells. Current efforts are focused on designing indicators with improved sensitivity and greater turn-on or ratiometric response, adapting this reaction-based strategy to other imaging modalities, and using COP-1 and next-generation chemical tools to study CO function in a variety of biological models.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, including synthesis and characterization, selectivity assays, spectroscopic methods, cellular imaging methods, and cell viability assays. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

chrischang@berkeley.edu

Present Address

[§]Department of Chemistry, Southern Methodist University, Dallas, Texas 75275-0314, United States.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by Amgen, Astra Zeneca, Novartis, and the Packard Foundation. C.J.C. is an Investigator of the Howard Hughes Medical Institute. We thank Ann Fischer (UC Berkeley Tissue Culture Facility) for expert technical assistance.

■ REFERENCES

(1) Tenhunen, R.; Marver, H. S.; Schmid, R. *Proc. Natl. Acad. Sci. U.S.A.* **1968**, *61*, 748−755.
(2) Maines, M. D. *FASEB J.* **1988**, *2*, 2557−2568.
(3) Ryter, S. W.; Alam, J.; Choi, A. M. K. *Physiol. Rev.* **2006**, *86*, 583−650.
(4) Motterlini, R.; Otterbein, L. E. *Nat. Rev. Drug Discovery* **2010**, *9*, 728−743.
(5) Mann, B. E. *Top. Organomet. Chem.* **2010**, *32*, 247−285.
(6) Bilban, M.; Haschemi, A.; Wegiel, B.; Chin, B. Y.; Wagner, O.; Otterbein, L. E. *J. Mol. Med.* **2008**, *86*, 267−279.
(7) Wu, L.; Wang, R. *Pharm. Rev.* **2005**, *57*, 585−630.
(8) Fujimoto, H.; Ohno, M.; Ayabe, S.; Kobayashi, H.; Ishizaka, N.; Kimura, H.; Yoshida, K.-i.; Nagai, R. *Arterioscler., Thromb., Vasc. Biol.* **2004**, *24*, 1848−1853.
(9) Motterlini, R.; Clark, J. E.; Foresti, R.; Sarathchandra, P.; Mann, B. E.; Green, C. J. *Circ. Res.* **2002**, *90*, e17−e24.
(10) Alberto, R.; Motterlini, R. *Dalton Trans.* **2007**, 1651−1660.
(11) Morimoto, Y.; Durante, W.; Lancaster, D. G.; Klattenhoff, J.; Tittel, F. K. *Am. J. Physiol.* **2001**, *280*, H483−H488.
(12) Marks, G. S.; Vreman, H. J.; McLaughlin, B. E.; Brien, J. F.; Nakatsu, K. *Antioxid. Redox Signaling* **2002**, *4*, 271−277.
(13) Moragues, M. E.; Esteban, J.; Ros-Lis, J. V.; Martínez-Máñez, R.; Marcos, M. D.; Martínez, M.; Soto, J.; Sancenón, F. *J. Am. Chem. Soc.* **2011**, *133*, 15762−15772.
(14) Yang, Y.; Zhao, Q.; Feng, W.; Li, F. *Chem. Rev.* **2012**, DOI: 10.1021/cr2004103.
(15) Kim, H. N.; Lee, M. H.; Kim, H. J.; Kim, J. S.; Yoon, J. *Chem. Soc. Rev.* **2008**, *37*, 1465−1472.
(16) Cho, D.-G.; Sessler, J. L. *Chem. Soc. Rev.* **2009**, *38*, 1647−1662.
(17) Jun, M. E.; Roy, B.; Ahn, K. H. *Chem. Commun.* **2011**, *47*, 7583−7601.
(18) Du, J.; Hu, M.; Fan, J.; Peng, X. *Chem. Soc. Rev.* **2012**, *41*, 4511−4535.
(19) Kojima, H.; Nakatsubo, N.; Kikuchi, K.; Kawahara, S.; Kirino, Y.; Nagoshi, H.; Hirata, Y.; Nagano, T. *Anal. Chem.* **1998**, *70*, 2446−2453.
(20) Lim, M. H.; Xu, D.; Lippard, S. J. *Nat. Chem. Biol.* **2006**, *2*, 375−380.
(21) Yang, Y.; Seidlits, S. K.; Adams, M. M.; Lynch, V. M.; Schmidt, C. E.; Anslyn, E. V.; Shear, J. B. *J. Am. Chem. Soc.* **2010**, *132*, 13114−13116.
(22) Kojima, H.; Hirotani, M.; Nakatsubo, N.; Kikuchi, K.; Urano, Y.; Higuchi, T.; Hirata, Y.; Nagano, T. *Anal. Chem.* **2001**, *73*, 1967−1973.
(23) Lippert, A. R.; New, E. J.; Chang, C. J. *J. Am. Chem. Soc.* **2011**, *133*, 10078−10080.
(24) Sasakura, K.; Hanaoka, K.; Shibuya, N.; Mikami, Y.; Kimura, Y.; Komatsu, T.; Ueno, T.; Terai, T.; Kimura, H.; Nagano, T. *J. Am. Chem. Soc.* **2011**, *133*, 18003−18005.
(25) Liu, C.; Pan, J.; Li, S.; Zhao, Y.; Wu, L. Y.; Berkman, C. E.; Whorton, A. R.; Xian, M. *Angew. Chem., Int. Ed.* **2011**, *50*, 10327−10329.
(26) Peng, H.; Cheng, Y.; Dai, C.; King, A. L.; Predmore, B. L.; Lefer, D. J.; Wang, B. *Angew. Chem., Int. Ed.* **2011**, *50*, 9672−9675.
(27) Qian, Y.; Karpus, J.; Kabil, O.; Zhang, S.-Y.; Zhu, H.-L.; Banerjee, R.; Zhao, J.; He, C. *Nat. Commun.* **2011**, *2*, 495.
(28) Yu, F.; Li, P.; Song, P.; Wang, B.; Zhao, J.; Han, K. *Chem. Commun.* **2012**, *48*, 2852−2854.
(29) Montoya, L. A.; Pluth, M. D. *Chem. Commun.* **2012**, *48*, 4767−4769.
(30) Lippert, A. R.; Van de Bittner, G. C.; Chang, C. J. *Acc. Chem. Res.* **2011**, *44*, 793−804.
(31) Chang, M. C. Y.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2004**, *126*, 15392−15393.
(32) Miller, E. W.; Albers, A. E.; Pralle, A.; Isacoff, E. Y.; Chang, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 16652−16659.
(33) Miller, E. W.; Tulyanthan, O.; Isacoff, E. Y.; Chang, C. J. *Nat. Chem. Biol.* **2007**, *3*, 263−267.
(34) Srikun, D.; Miller, E. W.; Domaille, D. W.; Chang, C. J. *J. Am. Chem. Soc.* **2008**, *130*, 4596−4597.
(35) Dickinson, B. C.; Chang, C. J. *J. Am. Chem. Soc.* **2008**, *130*, 9638−9639.
(36) Miller, E. W.; Dickinson, B. C.; Chang, C. J. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 15681−15686.
(37) Srikun, D.; Albers, A. E.; Nam, C. I.; Iavarone, A. T.; Chang, C. J. *J. Am. Chem. Soc.* **2010**, *132*, 4455−4465.
(38) Dickinson, B. C.; Peltier, J.; Stone, D.; Schaffer, D. V.; Chang, C. J. *Nat. Chem. Biol.* **2011**, *7*, 106−112.
(39) Abo, M.; Urano, Y.; Hanaoka, K.; Terai, T.; Komatsu, T.; Nagano, T. *J. Am. Chem. Soc.* **2011**, *133*, 10629−10637.
(40) Song, D.; Lim, J. M.; Cho, S.; Park, S.-J.; Cho, J.; Kang, D.; Rhee, S. G.; You, Y.; Nam, W. *Chem. Commun.* **2012**, *48*, 5449−5451.
(41) Dupont, J.; Consorti, C. S.; Spencer, J. *Chem. Rev.* **2005**, *105*, 2527−2571.

(42) Aguilar, D.; Cuesta, L.; Nieto, S.; Serrano, E.; Urriolabeitia, E. P. *Curr. Org. Chem.* **2011**, *15*, 3441−3464.
(43) Fuchita, Y.; Tsuchiya, H.; Miyafuji, A. *Inorg. Chim. Acta* **1995**, *233*, 91−96.
(44) Barr, N.; Bartley, J. P.; Clark, P. W.; Dunstan, P.; Dyke, S. F. *J. Organomet. Chem.* **1986**, *302*, 117−126.
(45) Dupont, J.; Pfeffer, M.; Daran, J. C.; Jeannin, Y. *Organometallics* **1987**, *6*, 899−901.
(46) Ryabov, A. D. *Synthesis* **1985**, 233−252.
(47) Li, H.; Cai, G.-X.; Shi, Z.-J. *Dalton Trans.* **2010**, *39*, 10442−10446.
(48) Santra, M.; Ko, S.-K.; Shin, I.; Ahn, K. H. *Chem. Commun.* **2010**, *46*, 3964−3966.
(49) Streu, C.; Meggers, E. *Angew. Chem., Int. Ed.* **2006**, *45*, 5645−5648.
(50) Yusop, R. M.; Unciti-Broceta, A.; Johansson, E. M. V.; Sanchez-Martin, R. M.; Bradley, M. *Nat. Chem.* **2011**, *3*, 239−243.
(51) Clark, J. E.; Naughton, P.; Shurey, S.; Green, C. J.; Johnson, T. R.; Mann, B. E.; Foresti, R.; Motterlini, R. *Circ. Res.* **2003**, *93*, e2−e8.
(52) Motterlini, R.; Mann, B. E.; Johnson, T. R.; Clark, J. E.; Foresti, R.; Green, C. J. *Curr. Pharm. Des.* **2003**, *9*, 2525−2539.
(53) Vadori, M.; Seveso, M.; Besenzon, F.; Bosio, E.; Tognato, E.; Fante, F.; Boldrin, M.; Gavasso, S.; Ravarotto, L.; Mann, B. E.; Simioni, P.; Ancona, E.; Motterlini, R.; Cozzi, E. *Xenotransplantation* **2009**, *16*, 99−114.
(54) Desmard, M.; Davidge, K. S.; Bouvet, O.; Morin, D.; Roux, D.; Foresti, R.; Ricard, J. D.; Denamur, E.; Poole, R. K.; Montravers, P.; Motterlini, R.; Boczkowski, J. *FASEB J.* **2009**, *23*, 1023−1031.
(55) Foresti, R.; Bani-Hani, M. G.; Motterlini, R. *Intensive Care Med.* **2008**, *34*, 649−658.