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Journal:	*ChemComm*
Manuscript ID:	CC-COM-01-2015-000902.R1
Article Type:	Communication
Date Submitted by the Author:	04-Feb-2015

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Cite this: DOI: 10.1039/x0xx00000x

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/x0xx00000x

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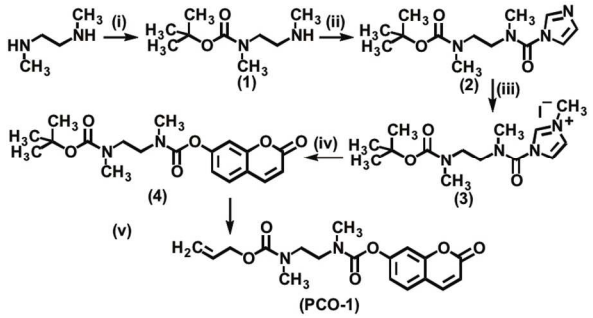
A coumarin-based fluorogenic probe, PCO-1 senses carbon monoxide (CO) selectively in HEPES buffer at pH 8.0 through intramolecular cyclization-elimination pathway based on Pd(0) mediated reaction. The probe exhibits a 'turn-on' response of CO over a variety of relevant reactive oxygen, nitrogen and sulfur species.

Carbon monoxide is well known toxic gas inhaled from common sources e.g. smoke and car exhaust, however various studies have shown that mammalian cells continuously generate carbon monoxide (CO) gas via endogenous degradation of heme by a family of inducible (HO-1) and constitutive (HO-2) heme oxygenase enzymes.[1] Thus CO, a gasotransmitter molecule now considered as a versatile signaling molecule, have shown an essential regulatory roles in a variety of pathophysiological and physiological processes that take place within the nervous, cardiovascular and immune systems.[2] CO produced in the vessel wall by heme oxygenase enzymes possesses vasorelaxing properties, has been revealed to prevent vasoconstriction and also both acute and chronic hypertension through soluble guanylate cyclase stimulation.[3] Endogenous CO appears to control sinusoidal tone in the hepatic circulation,[4] regulates the proliferation of vascular smooth muscle cells[5] and suppress the rejection of transplanted hearts.[6] CO gas has been described to facilitate potent anti-inflammatory effects at concentrations ranging from 10 to 500 ppm.[7] It effectively inhibit human airway smooth muscle cell proliferation,[8] prevent endothelial cell apoptosis,[9] and protects against hyperoxic as well as ischemic lung injury.[10]

Many aspects of CO in chemistry and biological system remain elusive owing to have the significant signal dichotomy because of the lack of ways for selective monitoring this transient small molecule. Recent reports showed that a genetically encoded fluorescent probe,[11a] carbazole-coumarin fused two-photon platform[11b] and some newly designed fluorecent probes[11c-e] are capable of selective detection of CO inside living cell, but still highly selective and sensitive 'turn-on' type systems are greatly desired. Herein, we developed a coumarin-based fluorogenic probe, PCO-1, for the selective detection of CO in HEPES buffer of pH 8.0. The interrogation of CO in fluorogenic platform is achieved using Pd(0)-mediated chemistry through intramolecular cyclization-elimination reaction. PCO-1 represents a unique chemical tool that features a selective 'turn-on' response to CO over reactive oxygen, nitrogen, and sulfur species and thus it can be used to detect this gasotransmitter in aqueous buffer medium.



Scheme 1 Synthetic routes to obtain PCO-1. i) di-tert-butyl dicarbonate, DCM: dichloromethane, 5 °C; ii) *N,N'*-carbonyldiimidazole, THF: tetrahydrofuran; iii) methyl iodide, CH_3CN: acetonitrile, iv) 7-hydroxycoumarin, CH_3CN, NEt_3; v) trifluoroacetic acid, DCM, pyridine, allyl chloroformate, -5 °C.

The stepwise synthetic routes for PCO-1 are shown in Scheme 1 in a satisfactory yield. First, the stability of the probe in reaction buffer [10 mM HEPES, 0.4% DMSO] of pH 8.0 at 37 °C was examined to establish the working ability in the reaction medium. Incubation of the probe in the reaction buffer at 37 °C during 24 h did not show any decomposed and or hydrolysed products because the reversed-phase HPLC analyses displayed only the probe peak (~15.1 min) (Fig. S1, ESI†). This demonstrates sufficient stability of the probe in the reaction condition of interest.

Our goal is to establish first the probe can reacts selectively with Pd(0) species such that it can be prepared *in situ* during the detection process of CO in a smooth way. Fig. 1 presented fluorescence spectrum of 10 μM PCO-1 centred at λ_{em} = 460 nm (λ_{ex} = 340 nm) in reaction medium (pH 8.0). After being treated with 10 μM $Pd(PPh_3)_4$ this fluorescence intensity enhanced drastically with the progress of time. And finally after 30 mints the intensity was achieved its maximum value with the concomitant increased in relative quantum yield by 130-times (Fig. S2, ESI†). This result clearly indicated that PCO-1 could be introduced to detect Pd(0) through fluorescence study. The probe exhibited very weak fluorescence ($\Phi_F = 0.034 \times 10^{-1}$) as the hydroxyl group of coumarin was functionalized by carbamate derivatives, however it transformed

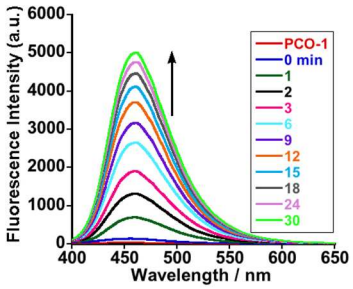
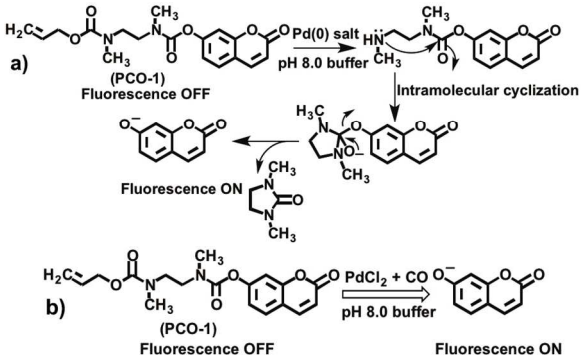


Fig. 1 Fluorescence spectrum of PCO-1 (10 μM) upon treatment with $Pd(PPh_3)_4$ (10 μM) with the progress of time in reaction buffer [10 mM HEPES, 0.4% DMSO] of pH 8.0 at 37 °C (ex: 340 nm).

into a highly fluorescent free coumarin moiety ($\Phi_F = 4.467 \times 10^{-1}$) in the presence of $Pd(PPh_3)_4$. The proposed sensing mechanism was shown in Scheme 2a through intramolecular cyclization-elimination reaction[12] of PCO-1 initiated by Pd(0) species. And 7-hydroxycoumarin molecule was then formed through the entire cyclization-elimination process by the expulsion of *N,N'*-dimethylimidazolidinone. Finally 7-hydroxycoumarin remained in nonprotonated state in the reaction buffer (pH 8.0), owing to have



Scheme 2 a) Proposed mechanistic routes for PCO-1 for the detection of Pd(0) through intramolecular cyclization-elimination reaction. b) Proposed sensing mechanism of CO by the *in situ* generation of Pd(0).

the lower pK_a value (7.7), and thus it became highly fluorescent. The progress of this reaction was studied by the reversed-phase HPLC (Fig. S3, ESI†) that confirmed the elimination of 7-hydroxycoumarin. Isolation of the byproduct, *N,N'*-dimethylimidazolidinone was further confirmed by ESI-MS from the reaction mixture (data not shown). Also the fluorescence intensity drastically enhanced when various concentrations of $Pd(PPh_3)_4$ (1.0 – 16 μM) were incubated for 30 min in reaction buffer (Fig. S4, ESI†). The limit of detection (LOD) was estimated as 7.77 nM from calibration curve (Fig. S5, ESI†) using 3σ method.[13] From the metal ions selectivity study under identical experimental conditions, it was interestingly observed that the introduction of other metal ions including biologically and environmentally relevant ions did not affect the fluorescence intensity as depicted in Fig. S6, S7 (ESI†). The intensity of PCO-1 (10 μM) was found to be unperturbed upon the addition of an excess of 10 equiv (100 μM) of Cr^{3+}, Mn^{2+}, $Fe^{2+/3+}$, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+} and a large excess of (100 equiv) Na^+, K^+, Ca^{2+}, Mg^{2+} etc. Also in the case of various metal ion mixtures [e.g., (Na^+, K^+, Ca^{2+}), (Mn^{2+}, $Fe^{2+/3+}$) and (Cu^{2+}, Zn^{2+}) together with Pd(0), no tolerance of the fluorescence intensities were observed (Fig. S6, ESI†). Other heavy metal sources such as Ru^{3+}, Hg^{2+}, Cd^{2+}, Ag^+, Rh^{3+}, Pt^{2+}, Pb^{2+}, $As^{3+/5+}$, Au^{3+} (10 equiv) showed no contribution in the fluorescence. Also the heavy metals mixtures *viz.* (Ag^++ Pt^{2+}+Au^{3+}), (Ru^{3+}+Rh^{3+}+Pb^{2+}) and (Cd^{2+}+Hg^{2+}+$As^{3+/5+}$) did not hamper the fluorescence initiated by Pd(0) species (Fig. S7, ESI†). Further to explore the reactivity of the probe toward other palladium metal sources having different initial oxidation states *viz.* $PdCl_2$, $Pd(OAc)_2$, $Pd(CH_3CN)_2Cl_2$, $Pd(PPh_3)_2Cl_2$, Na_2PdCl_4 and $(NH_4)_2PdCl_6$ were then examined (Fig. S8, ESI†). To our delight, none of the samples offered similar response as $Pd(PPh_3)_4$ which revealed that the response solely depended on only Pd(0) species. It is an important feature of the present system over the known methods.[14]

As the probe senses Pd(0) species selectively among different oxidation states of palladium itself and various other metal ions with no additional reagents, this behavior of the probe has been utilized to detect CO selectively in aqueous medium. This was achieved by the introduction of both $PdCl_2$ and $[Ru(CO)_3Cl(glycinate)]$ (CORM-3) which mixture led to the *in situ* generation of Pd(0) species. We judiciously chosen CO detection by fluorescent method to meet a serious need for new technologies to monitor this gasotransmitter molecule. Our overall strategy for CO detection was based on CO-induced Pd(0) mediated reaction chemistry. CO is better known for its inorganic coordination chemistry and subsequent organometallic reactivity although it is not considered as a particularly nucleophilic or electrophilic species. Palladium has been found to be a catalyst for its extensive use in current organic chemistry. The palladium(II) chloride is reduced into Pd(0) when exposed to CO and thus the *in situ* generation of Pd(0) could be detected by fluorescent method employing PCO-1. We examined the fluorescence of PCO-1 towards

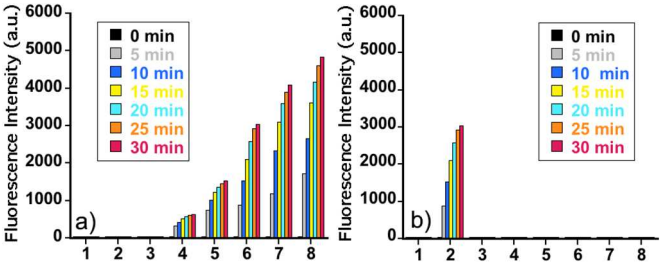


Fig. 2 PCO-1 showed a robust and selective turn-on response to CO in reaction buffer pH 8.0 at 37 °C (ex: 340 nm) mediated by $PdCl_2$. a) Fluorescence responses of PCO-1 (10 μM) to various levels of CORM-3 observe at 0, 5, 10,15, 20, 25 and 30 minutes in presence of 10 μM $PdCl_2$. Legend: (1)-none, (2)-$PdCl_2$, (3)-50 μM CORM-3, (4)-$PdCl_2$+1 μM CORM-3, (5)-$PdCl_2$+5 μM CORM-3, (6)-$PdCl_2$+10 μM CORM-3, (7)-$PdCl_2$+20 μM CORM-3 and (8)-$PdCl_2$+50 μM CORM-3. b) Fluorescence responses of 10 μM PCO-1 to CO in presence of $PdCl_2$ (10 μM) and various species observed at 0, 5, 10, 15, 20, 25 and 30 minutes. Legend: (1)-none, (2)-CORM-3, (3)-NO (source $NOCl_3$), (4)-H_2S (source NaHS), (5)-NaOCl, (6)-H_2O_2, (7)-^{t}BuOOH and (8)-$O_2^{\bullet-}$(source KO_2).

CO reactivity in reaction buffer (pH 8.0) at 37 °C. Water soluble complex $[Ru(CO)_3Cl(glycinate)]$ (CORM-3) has been used as an easy-to-handle CO source.[15] The proposed sensing mechanism of CO *viz.* Scheme 2b clearly indicated the simple redox reaction initiated by $PdCl_2$. The progress of this reaction with CO was investigated by the reversed-phase HPLC (Fig. S3, ESI†) in support of the elimination of 7-hydroxycoumarin as it was found in the case of earlier Pd(0) sensing method. This result confirmed the reaction of CO being initiated by the *in situ* generated Pd(0) species. Addition of 50 μM CORM-3 to a solution of 10 μM PCO-1 in the presence of 10 μM $PdCl_2$ at 37 °C produced a robust fluorescence 'turn-on' response by more than 150-fold higher in fluorescence. Variable pH dependency study of fluorescence indicated that the maximum enhancement was observed in the range of pH 8 (Fig. S9, ESI†). Moreover we observed the dose dependent responses toward CORM-3 down to 1 μM (~28 ppb CO) level (Fig. 2a). The limit of detection (LOD) of CO was calculated as 8.49 nM by 3**σ** method (Fig. S10, ESI†). In addition, the fluorescence 'turn-on' response to CORM-3 shows good selectivity over a variety of biologically

relevant reactive nitrogen, sulfur and oxygen species, including NO, H_2S, NaOCl, H_2O_2, *tert*-butyl hydroperoxide (^{t}BuOOH) and superoxide (O_2^-). The introduction of these molecules did not trigger any fluorescence enhancement as it happened in case of CO (Fig. 2b).

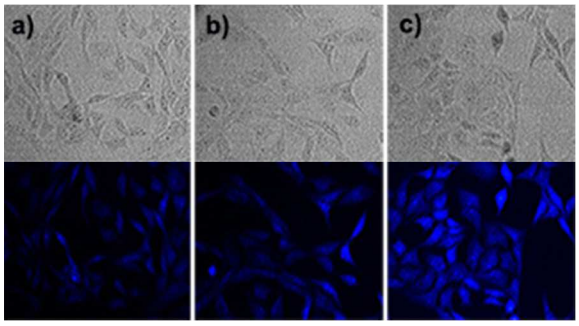


Fig. 3 Fluorescence microscopy images of A549 cells for CO detection using PCO-1 (10 μM) in presence of 10 μM $PdCl_2$ with the incubation of a) 10 μM, b) 20 μM and c) 50 μM of CORM-3 in the reaction buffer at 37°C (ex: ~340 nm). The first and second row represented the phase contrast and fluorescence images respectively.

Finally to visualize CO levels in live cells we examined fluorescence microscopy experiment with PCO-1 (Fig. 3). A549, human lung carcinoma cells were incubated with 10 μM PCO-1 and CORM-3 (10, 20 and 50 μM) in presence of 10 μM $PdCl_2$ (Fig. 3) at 37ºC. A dose-depended intracellular fluorescence was observed in case of CORM-3 over the control experiment (Fig. S11, ESI†). The fluorescence images with the corresponding phase contrast images also demonstrated the different fluorescence distribution of CO molecule inside the cells using the various concentration of CORM-3. In addition to that, 10 μM of PCO-1 did not show any significant cytotoxic effect (Fig. S12, ESI†) on A549, human lung carcinoma cells for at least up to 4 h of its treatment though there was significant cytotoxicity for higher doses after 4 h onward. These results indicated that the fluorogenic probe, PCO-1 is an efficient candidate for monitoring changes in intracellular CO.

In summary, we introduced a new approach for CO detection in aqueous buffer through intramolecular cyclization-elimination reaction based on Pd(0) mediated reaction by the synthesis of a new coumarin-based fluorogenic probe PCO-1. PCO-1 triggered a 'turn-on' fluorescence response to CO with a concomitant increase of fluorescence intensity by 150 times. The response is selective over a variety of relevant reactive nitrogen, sulfur and oxygen species and can be used to image CO in living cells

Financial assistance from Department of Science and Technology (DST), New Delhi, govt. of India for providing Fast track research grand (vide project no. SB/FT/CS-142/2012) is gratefully acknowledged.

Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details and additional figures (Fig. S1-S12). See DOI: 10.1039/c000000x/

1 (*a*) R. Tenhunen, H. S. Marver and R. Schmid, *Proc. Natl. Acad. Sci. U.S.A.*, 1968, **61**, 748; (*b*) M. D. Maines, *FASEB J.*, 1988, **2**, 2557; (*c*) S. W. Ryter, J. Alam and A. M. K. Choi, *Physiol. Rev.*, 2006, **86**, 583; (*d*) R. Tenhunen, H. S. Marver and R. M. Schmid, *J. Biol. Chem.*, 1969, **244**, 6388; (*e*) M. D. Maines, *Annu. Rev. Pharmacol. Toxicol.*, 1997, **37**, 517.
2 A. Verma, D. J. Hirsch, C. E Glatt, G. V Ronnett and S. H. Snyder, *Science*, 1993, **259**, 381.
3 (*a*) D. Sacerdoti, B. Escalante, N. G. Abraham, J. C. McGiff, R. D. Levere and M. L. Schwartzman, *Science*, 1989, **243**, 388; (*b*) G. S. Marks, J. F. Brien, K. Nakatsu and B. E. McLaughlin, *Trends Pharmacol Sci.*, 1991, **12**, 185.
4 M. Suematsu, N. Goda, T. Sano, S. Kashiwagi, T. Egawa, Y. Shinoda and Y. Ishimura *J. Clin. Invest.*, 1995, **96**, 2431.
5 T. Morita, S. A. Mitsialis, H. Koike, Y. X. Liu and S. Kourembanas, *J. Biol. Chem.*, 1997, **272**, 32804.
6 K. Sato, J. Balla, L. Otterbein, R. N. Smith, S. Brouard, Y. Lin, E. Csizmadia, J. Sevigny, S. C. Robson, G. Vercellotti, A. M. Choi, F. H. Bach and M. P. Soares, *J. Immunol.*, 2001, **166**, 4185.
7 L. E. Otterbein, F. H. Bach, J. Alam, M. Soares, H. Tao Lu, M. Wysk, R. J. Davis, R. A. Flavell and A. M. Choi, *Nat. Med.*, 2000, **6**, 422.
8 R. Song, R. S. Mahidhara, F. Liu, W. Ning, L. E. Otterbein and A. M. Choi, *Am. J. Respir. Cell Mol. Biol.*, 2002, **27**, 603.
9 S. Brouard, L. E. Otterbein, J. Anrather, E. Tobiasch, F. H. Bach, A. M. Choi and M. P. Soares, *J. Exp. Med.*, 2000, **192**, 1015.
10 (*a*) L. E. Otterbein, L. L. Mantell and A. M. K. Choi, *Am. J. Physiol.*, 1999, **276**, L688; (*b*) T. Fujita, K. Toda, A. Karimova, S. F. Yan, Y. Naka, S. F. Yet, D. J. Pinsky, *Nat. Med.*, 2001, **7**, 598.
11 (*a*) J. Wang, J. Karpus, B. S. Zhao, Z. Luo, P. R. Chen and C. He, *Angew. Chem. Int. Ed.*, 2012, **51**, 9652; (*b*) K. Zheng, W. Lin, Li Tan, H. Chen and H. Cui, *Chem. Sci.*, 2014, **5**, 3439; (*c*) L. Yuan, W. Lin, L. Tan, K. Zheng and W. Huang, *Angew. Chem. Int. Ed.*, 2013, **52**, 1628; (*d*) B. W. Michel, A. R. Lippert and C. J. Chang, *J. Am. Chem. Soc.*, 2012, **134**, 15668; (*e*) S. H. Heinemann, T. Hoshi, M. Westerhausend and A. Schiller, *Chem. Commun.,* 2014, **50**, 3644.
12 W. S. Saari, J. E. Schwering, P. A. Lyle, S. J. Smith and E. L. Engelhardt, *J. Med. Chem.*, 1990, **33**, 97.
13 (*a*) S. Pal, B. Sen, S. Lohar, M. Mukherjee, S. Banerjee and P. Chattopadhyay, *Dalton Trans.*, 2015, **44**, 1761; (*b*) S. Pal, M. Mukherjee, B. Sen, S Lohar and P. Chattopadhyay, *RSC Adv.*, 2014, **4**, 21608.
14 (*a*) B. Zhu, C. Gao, Y. Zhao, C. Liu, Y. Li, Q. Wei, Z. Ma, B. Du and X. Zhang, *Chem. Commun.*, 2011, **47**, 8656; (*b*) H. Li, J. Fan, J. Du, K. Guo, S. Sun, X. Liu and X. Peng, *Chem. Commun.*, 2010, **46**, 1079; (*c*) A. L. Garner and K. Koide, *Chem. Commun.*, 2009, 86; (*d*) A. L. Garner and K. Koide, *J. Am. Chem. Soc.*, 2008, **130**, 16472; (*e*) F. Song, A. L. Garner and K. Koide, *J. Am. Chem. Soc.*, 2007, **129**, 12354; (*f*) B. A. Sparano, S. P. Shahi and K. Koide, *Org. Lett.*, 2004, **6**, 1947.
15 J. E. Clark, P. Naughton, S. Shurey, C. J. Green, T. R. Johnson, B. E. Mann, R. Foresti and R. Motterlini, *Circ. Res.*, 2003, **93**, e2.