Biosensing

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Lighting up Carbon Monoxide: Fluorescent Probes for Monitoring CO in Living Cells**

Lin Yuan, Weiying Lin, Li Tan, Kaibo Zheng, and Weimin Huang*

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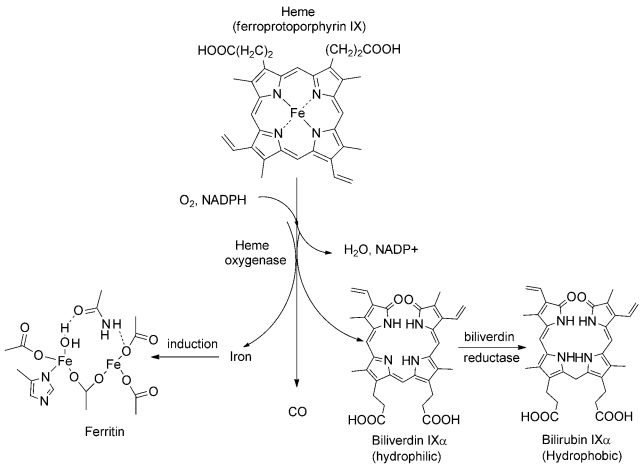
Carbon monoxide (CO) has long been primarily viewed as toxicant, waste, or pollutant because of its strong affinity to hemoglobin.[1,2] As an environmental toxicant, pathological levels of CO can induce both acute and chronic health hazards.[3] However, on the other hand, recent studies indicate that CO, like nitric oxide (NO) and hydrogen sulfide (H_2S), is an essential gaseous second messenger in humans.[4] Indeed, CO is known to mediate signaling processes involved in vasodilation, antiapoptotic, anti-inflammatory, antiproliferative activities, and neurotransmission.[5] The majority of CO (86%) in the body is produced during the oxidative catabolism of heme by heme oxygenase (HO) enzymes (Scheme 1).[3,4,6] Although CO is believed to have a signal–



Scheme 1. HO-catalyzed heme metabolism pathway.[3]

stress dichotomy many aspects of its roles in biological systems remain unclear, thus, sensitive and selective detection techniques which enable tracking CO in complicated biological systems are highly desirable.

Current methods that are available for the detection of CO include chromogenic detection,[7] laser sensor-infrared absorption,[8] electrochemical assays,[9] gas chromatography,[10] and others.[11] However, these techniques are difficult to employ real-time detection of physiological levels of CO inside living cells in a noninvasive manner. Recently, fluorescence sensing and imaging has emerged as one of the most powerful techniques to monitor levels, localization, and movement of biomolecules in living systems. Although a variety of fluorescent probes for other second messengers including H_2S,[12] NO, and H_2O_2[13] have been successfully constructed and applied in biological systems, the development of fluorescent probes for CO suitable for biological settings has largely lagged behind. Only very recently, a significant breakthrough came from the seminal work of He and co-workers[14] and Chang and co-workers,[15] who independently reported two different types of fluorescent CO probes: a biosensor and a small-molecule probe. In this Highlight, these important discoveries are summarized.

Although the fluorescent probes developed by He and co-workers[14] and Chang and co-workers[15] appear to be distinct, the underlying design strategy is very similar, that is, by taking advantage of the unique binding ability of CO to transition-metal ions. The group of He constructed the fluorescent probe COSer for CO by using a genetically encoded approach (Scheme 2). The biosensor is composed of circularly permuted yellow fluorescent protein (cpYFP) as the fluorescent reporter and CooA, a dimeric CO-sensing heme protein from *Rhodospirillum rubrum* as the CO recognition unit. Upon treatment with 10 μM CO for 10 minutes, the probe exhibits a two-fold fluorescence enhancement at $\lambda = 528$ nm. This enhancement is attributed to the conformational change of CooA upon binding to CO. In addition, the probe shows a good selectivity for CO over other relevant heme ligands including H_2S, GSH, NO, O_2, CN^-, and imidazole. Finally, they demonstrated that the probe COSer is capable of imaging CO fluctuations inside living HeLa cells. Although COSer represents the first fluorescent probe for CO, improvement of its properties in several aspects is still needed before it is useful for studies of living systems. It is desirable to increase the signal-to-noise ratio by minimizing the fluorescence background.

Genetic encoding of fluorescent proteins is a very popular technique for fluorescence bioimaging. However, the relatively large molecular sizes of fluorescent proteins could

[*] L. Yuan, Prof. W. Lin, L. Tan, K. Zheng, W. Huang
State Key Laboratory of Chemo/Biosensing and Chemometrics
College of Chemistry and Chemical Engineering, Hunan University
Changsha, Hunan 410082 (P.R. China)
E-mail: weiyinglin@hnu.edu.cn

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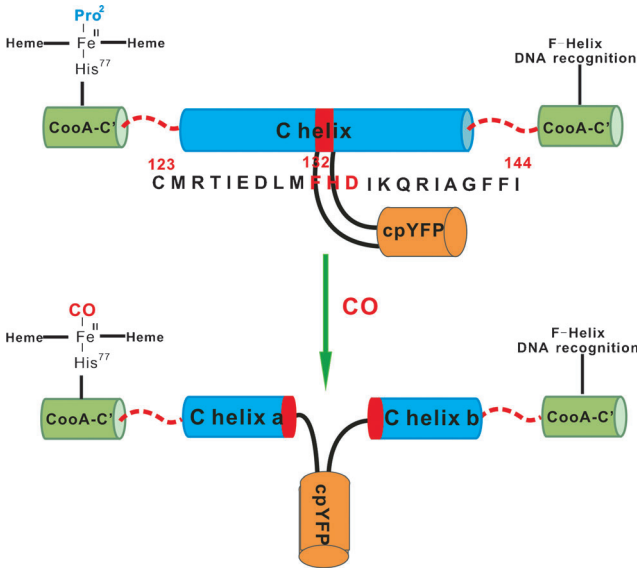
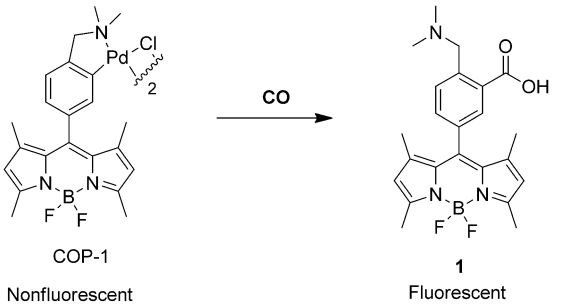


Scheme 2. The sensing process of the probe COSer for CO. Upon CO binding, the long C helix of CooA is broken into two parts (C helix a and b) and connected through residues 132 to 134.



Scheme 3. Structure of probe COP-1 and the sensing mechanism for CO.

potentially interfere with the structure or function of the protein to which they are fused. By contrast, small-molecule fluorescent probes offer the advantages of small size, membrane permeability, low fluorescence background, and operational simplicity. Interestingly, almost at the same time the report from He and co-workers came out,[14] the group of Chang disclosed the first small-molecule fluorescent probe, COP-1, for imaging CO in living cells based on palladium-mediated carbonylation chemistry (Scheme 3).[15] COP-1 was designed by exploiting the inorganic coordination chemistry and subsequent organometallic reactivity of CO. They reasoned that the free probe may have low fluorescence because of the heavy-atom electronic effects of palladium. By contrast, upon reaction with CO, COP-1 could be transformed into the compound **1**, which is highly fluorescent as the palladium atom is released. Indeed as designed, COP-1 exhibits almost no fluorescence in Dulbecco's phosphate-buffered saline (DPBS) buffered to pH 7.4 ($\lambda_{em} = 503$ nm, $\Phi = 0.01$).[15] However, a 10-fold fluorescence enhancement was observed in the presence of CO. In addition, the fluorescence enhancement is dose-dependent and the probe can detect CO down to level of 1 μM. Furthermore, the probe is not reactive with other biologically relevant reactive oxygen, nitrogen, and sulfur species such as H_2O_2, *tert*-butylhydroperoxide (*t*BuOOH), hypochlorite (OCl^-), superoxide ($O_2^{-\bullet}$), NO, peroxynitrite ($ONOO^-$), and H_2S. Importantly, they further demonstrate that the palladium-based probe is not only nontoxic and biocompatible, but also suitable for monitoring CO in living cells. Although this small-molecule probe is promising for practical applications, the response time is relatively long. It takes about an hour to reach the highest level of fluorescence enhancement. This may render the probe difficult to use for the real-time detection of transient CO in living cells.

The properties of the genetically encoded fluorescent probe COSer and small-molecule fluorescent probe COP-1 are summarized in Table 1. Although both the probes are capable of monitoring CO in living cells, they exhibit different advantages and disadvantages. The small-molecule-based fluorescent probe displays a larger fluorescent enhancement (10-fold) than the genetically encoded fluorescent probe COSer (2-fold) in vitro. However, the biosensor is much faster than the small-molecule-based probe in terms of response time. Another notable difference between these probes is that the COSer probe functions by a reversible mechanism whereas the probe COP-1 operates by an irreversible reaction-based approach. The reversible COSer is more favorable for real-time detection of CO. By contrast irreversible COP-1 is more suitable for monitoring low levels of CO because of signal accumulation.

In conclusion, the recent noteworthy reports on the fluorescent probes for the detection of CO in living cells have been highlighted. These novel probes display the potential to be powerful tools for imaging of CO in living systems. Despite these critical advances, significant challenges remain in the development of fluorescent CO probes for tracking endogenous CO in living cells, tissues, and even animals. The next generation of fluorescent CO probes with improved properties including fast response, large dynamic range, high selectivity, and reversibility may pave the way for ultimately unraveling the mysteries of endogenous CO in

Table 1: Summary of fluorescent probes for the detection of CO.

Probe	Probe platform	Detection mechanism/ Detection limit	Fluorescenceenhancement in vitro/ time	Fluorescence enhancement in living cells
COSer	Genetically encoded	Reversible binding with CooA/0.5 μM	2-fold at 10 μM CO in pH 7.4 MOPS/10 min	ca. 58% after incubation with 5 μM CO-RM2 (a CO donor)
COP-1	Small molecule	Irreversible carbonylation reaction/1 μM	10-fold at 50 μM CORM-3 (a CO donor) in pH 7.4 DPBS at 37°C/60 min	ca. 1.8 (2.5)-fold after incubation with 5 (50) μM CORM-3

complicated biological systems. In addition, the development of fluorescent probes for detecting CO gas in other settings including cars and industrial facilities is also needed.

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