Communication

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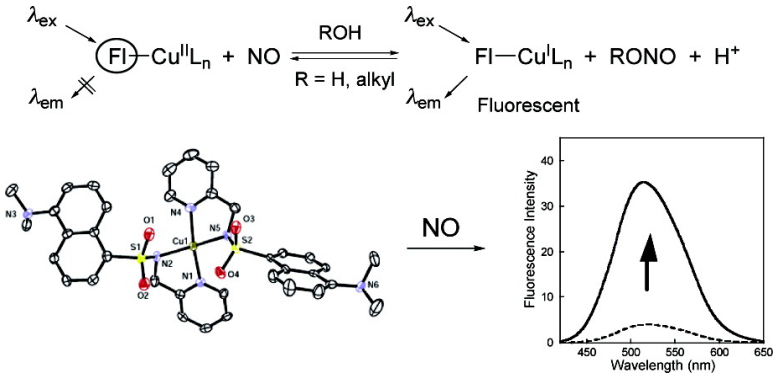

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Copper Complexes for Fluorescence-Based NO Detection in Aqueous Solution

Mi Hee Lim and Stephen J. Lippard*

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

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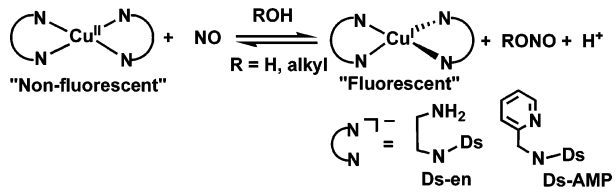
Nitric oxide (NO)[1] has captured the attention of chemists, biologists, and medical researchers since its identification as a signaling molecule in humans.[2−4] NO regulates both beneficial and harmful biological processes, depending on a variety of not yet fully delineated factors.[2,3,5−12] Elucidation of the biological roles of NO would benefit substantially from a probe that could detect the molecule directly in living cells. Fluorescence-based methodology offers one possible approach to satisfy these requirements.[13,14] Here, we describe two copper complexes that sense NO by turn-on emission with nanomolar sensitivity in aqueous media.

Recently, our laboratory has devised fluorescence-based NO sensors comprising transition metal complexes with coordinated fluorophores. In particular, a fluorophore-displacement strategy was discovered[15] and exploited utilizing cobalt,[15−17] ruthenium,[18] and dirhodium complexes.[19] For biological applications, however, these systems are limited by low sensitivity (∼4 μM) and their lack of water-compatibility. Organic molecule-based NO sensors, most notably *o*-diaminofluoresceins and related molecules, can detect intracellular NO,[13,14,20] but they do not react directly with the molecule and monitor only products of its oxidation. A fluorescence response may therefore depend on the kinetic and thermodynamic properties of NO oxidation by O_2.[13,14] Iron complexes can sense NO in aqueous environments; however, some display diminished fluorescence, which is not preferred for biological imaging.[21,22] Others show fluorescence enhancement, but are air-sensitive and display only modest turn-on emission with NO.[23]

Detailed mechanistic studies of the reduction of Cu(II) to Cu(I) by NO[24] suggested an approach for nitric oxide detection. A fluorescent ligand quenched by coordination to a paramagnetic Cu(II) center might emit upon NO-induced conversion to diamagnetic Cu(I). In the present investigation, we applied this strategy to achieve small molecule-based fluorescent NO sensing with nanomolar sensitivity in organic and, significantly, buffered aqueous solutions (Scheme 1). The only previously described Cu(II) system that exhibits NO-triggered fluorescence enhancement does so in aqueous methanol by a different mechanism, nitrosylation-induced ligand dissociation from the reduced Cu(I) center.[25] The present sensors provide substantial improvement in sensitivity over our previous compounds and demonstrate their enhanced potential for studying the biology of NO.

The Cu(II) complexes [Cu(Ds-en)$_2$] (**1**)[26,27] and [Cu(Ds-AMP)$_2$] (**2**), where Ds-en and Ds-AMP are the conjugate bases of dansyl ethylenediamine (Ds-Hen) and dansyl aminomethylpyridine (Ds-HAMP), respectively, were prepared for fluorescence-based NO detection in aqueous solution. The Cu(II) complex **2** was obtained in methanol from a 1:2 ratio of Cu(OAc)$_2$ and the ligand Ds-HAMP with added KOH. This compound was designed to provide greater stability for the pseudotetrahedral Cu(I) species anticipated as the NO reaction product. As shown in Figure 1, the dihedral angle, Θ, between the five-membered N−Cu−N chelate ring planes in **2** is 39.3°, compared to the Θ value of 3.9° in **1**.[27] Cyclic voltammetric

Scheme 1



studies of **1** and **2** in CH_3CN for the Cu(II)/Cu(I) couple revealed reduction potentials vs Fc/Fc^+ of −1.524 (irrev) and −0.816 V (rev), respectively, confirming that **2** is indeed more easily reduced than **1** (Figure S1).

Fluorescence experiments at 25 °C indicated significant copper-induced ligand quenching in 20 μM, 4:1 CH_3OH:CH_2Cl_2, solutions of **1** and **2**, with a 31(±2)- and 23(±0.5)-fold reduction in intensity relative to free Ds-Hen and Ds-HAMP (40 μM), respectively. Addition of 100 equiv of NO to either solution immediately restored significant emission intensity under anaerobic conditions, the respective enhancements in integrated fluorescence being 6.1(±0.2)- and 8.8(±0.1)-fold for **1** and **2**, respectively (Figure 2). This NO-induced turn-on fluorescence does not occur in pure CH_2Cl_2 solutions of the copper complexes (Figure S2), which proves that methanol or a protic solvent is required (see Scheme 1). The NO detection limit of these copper sensors is 10 nM, which is nearly 3 orders of magnitude greater sensitivity than our dirhodium complex.[19]

Since copper complexes **1** and **2** are water-soluble, their reactivity with NO was further investigated in aqueous buffer (50 mM CHES, pH 9, 100 mM KCl). In this medium, copper remains coordinated by the two bidentate Ds-en ligands in **1**.[26] A 4.3(±0.5)- or 4.5-(±0.6)-fold quenching was observed at pH 9 for **1** and **2** (10 μM), respectively, at 37 °C. Addition of 100 equiv of NO into these solutions caused a fluorescence increase of 2.3(±0.2)- or 2.0(±0.2)-fold, respectively, within 30 min (Figure 2). Although this pH is not quite in the typical physiological range, these complexes allow

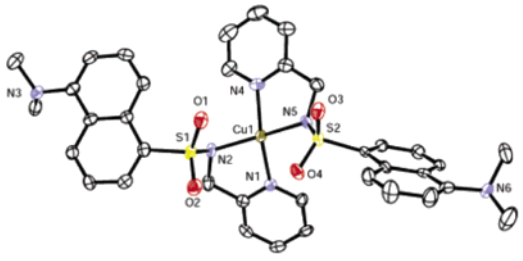


Figure 1. ORTEP diagram of [Cu(Ds-AMP)$_2$] (**2**) showing 50% probability thermal ellipsoids. Selected bond distances (Å) and angles (deg): Cu1−N1 = 1.992(4), Cu1−N2 = 1.979(4), Cu1−N4 = 2.003(4), Cu1−N5 = 1.949(4), N1−Cu1−N2 = 83.94(17), N1−Cu1−N4 = 150.41(17), N1−Cu1−N5 = 101.74(17), N2−Cu1−N4 = 100.92(18), N2−Cu1−N5 = 162.13(17), N4−Cu1−N5 = 82.60(17). X-ray data are provided in Table S1 of Supporting Information.

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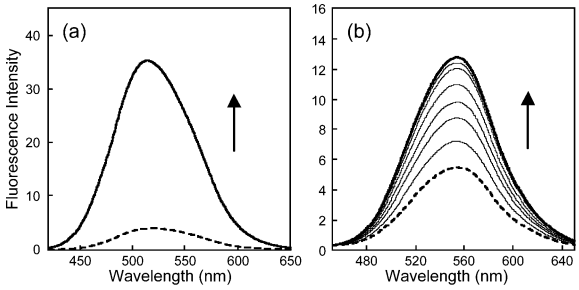

Figure 2. Fluorescence responses (λ_{ex} = 342 nm) for a deoxygenated (a) 20 μM solution of **2** in 4:1 CH_3OH:CH_2Cl_2 before (dashed line) and after (solid line) admission of 100 equiv of NO at 25 °C; and (b) 10 μM solution of **2** in CHES buffer at pH 9 (dashed line) and following the addition of 100 equiv of NO at 3, 6, 10, 15, 20, 25, and 30 min at 37 °C (solid lines).

the detection of nitric oxide in purely aqueous media with significant turn-on emission via restoration of metal-quenched fluorescence.

On the basis of the anticipated mechanism depicted in Scheme 1, fluorescence enhancement would occur by formation of a diamagnetic Cu(I) species,[28] dissociation of the sulfonamide functionality following protonation by H^+ formed in the reaction, or both. Conclusive evidence for both was obtained. Proof that a Cu(I) species forms in the reaction of **2** with 1 equiv of NO in 4:1 CH_3OH:CH_2Cl_2 was provided by EPR spectroscopy at 50 K, which displayed an ~15% decrease in the intensity of the initial Cu^{II} EPR signal (Figure S3). One equivalent was used to avoid contamination of the Cu(II) EPR signal by that of free NO. Addition of $[Cu(CH_3CN)_4](BF_4)$ to the ligand Ds-HAMP in the presence of base in 4:1 CH_3OH:CH_2Cl_2 reduced the fluorescence intensity by ~40% (Figure S4). These results indicate that reduction of the Cu(II) centers is, at best, only partially responsible for the turn-on fluorescence of **1** and **2** by NO. Evidence for a sulfonamide functionality (ν_{N-H} = 3083 cm^{-1} in KBr, Figure S5) in the IR spectrum of the reaction product of NO with **2** in 4:1 CH_3OH:CH_2Cl_2 following solvent removal reveals that ligand protonation also occurs. The protonated sulfonamide group may be weakly coordinated to, or dangling from, the coordination sphere following the NO reaction, contributing to the change in fluorescence intensity. The ^1H NMR spectrum indicated that complete release of Ds-HAMP from the Cu center does not occur (Figure S6). In addition, $NOBF_4$ was allowed to react with **2**. The fluorescence was enhanced by half that obtained in the NO-induced turn-on (Figure S7). There was no IR band in the 1400−1600 cm^{-1} range corresponding to ν_{NN-O},[29] and no feature in the 1600−1900 cm^{-1} region due to a copper nitrosyl (in KBr).[30,31] A band at 3083 cm^{-1} (in KBr), arising from ν_{N-H}, did appear, however (Figure S5). These results are best interpreted by reaction of NO^+ with methanol solvent to form CH_3ONO and H^+, the latter of which can protonate and partially dissociate the sulfonamide functionality to turn-on fluorescence.

In summary, two Cu(II) complexes were obtained that function as fluorescence-based NO sensors. Addition of NO to CH_3OH:CH_2Cl_2 or pH 9 CHES-buffered aqueous solutions of the Cu(II) probes afforded a substantial increase in fluorescence intensity. These observations indicate that Cu(II) complexes can be used to sense nanomolar quantities of NO in both organic and aqueous environments. Moreover, they provide an important demonstration that fluorescent cupric complexes can function as NO sensors for use in biological systems with turn-on emission.

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Supporting Information Available: Table S1, Figures S1−S7, and X-ray crystallographic file (CIF) of **2**. The material is available free of charge via the Internet at http://pubs.acs.org.

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