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Nitric oxide sensors based on copper(II) complexes of N-donor ligands

Pankaj Kumar, Apurba Kalita, Biplab Mondal *

Department of Chemistry, Indian Institute of Technology Guwahati, Assam 781039, India

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ABSTRACT

Two Cu(II) complexes, **1** and **2**, were synthesized with N-donor ligands $\mathbf{L_1}$ and $\mathbf{L_2}$ [$\mathbf{L_1}$ = N^1,N^1-dimethylethane-1,2-diamine, $\mathbf{L_2}$ = N^1,N^1-dimethyl-N^2-{(pyridin-2-yl)methyl}ethane-1,2-diamine], respectively, and were characterized. Complexes **1** and **2** upon reaction with NO, found to show reduction of Cu(II) to Cu(I) in various solvents. Ligands $\mathbf{L_3}$, $\mathbf{L_4}$ and $\mathbf{L_5}$ are the dansyl derivatives of $\mathbf{L_1}$, 2-(pyridin-2-yl)ethanamine and $\mathbf{L_2}$, respectively. Three Cu(II) complexes, **3**, **4** and **5** were synthesized with ligands $\mathbf{L_3}$, $\mathbf{L_4}$ and $\mathbf{L_5}$ respectively. The fluorescence intensity of these ligands were found to be quenched significantly on complexation due to the paramagnetism of the Cu(II) center. However, when exposed to NO in methanol or aqueous medium, the fluorescence intensity of the fluorophores has been found to be restored because of reduction of paramagnetic Cu(II) to diamagnetic Cu(I).

1. Introduction

Since the discovery as a signaling molecule in humans, nitric oxide (NO) has attracted enormous interest from chemists and biochemists [1–7]. It is also known that NO plays diverse roles in biological processes; e.g. it regulates vasodialation, defense against pathogens, enhances the signal transmission etc. [1–3]. These essentially inspire a wide range of research to identify the precise roles of NO in biology and to understand the various biological reaction pathways of NO *in vivo*. Since NO is a reactive free radical and easily diffuses through most of the cells and tissues, it is difficult to follow NO immediately after production. Hence, a selective probe to detect the formation and migration of NO with spatiotemporal resolution directly from living cells is highly desirable compare to other methods such as spectroscopy, chemoluminscence, EPR and amperometry [8].

The fluorescence-based detection technique is found to satisfy almost all the requirements, like biocompatibility, non toxic, specific, fast and direct NO detection [8,9]. Starting from the early examples of fluorescence-based sensors such as *o*-diaminonaphthalene (DAN) and *o*-diaminofluoresceins (DAFs), a number of fluorescent probes have been exemplified in literature [10–17]. However, some of them are unable to detect or monitor NO itself, as their fluorescent response depends on the formation of a triazole species by oxidized NO products such as N_2O_3 and thus the NO related bio-events would not be detected in real time. Recently, a highly selective fluorescent imaging agent, for NO has been reported which displays a rapid and linear response [18].

A number of iron complexes were reported to sense NO in aqueous medium; but exhibit diminished fluorescence, which is not desirable for biological applications [19]. In addition, iron complexes which show fluorescence enhancement, are air sensitive and exhibit only modest turn-on emission with NO [20]. These difficulties essentially prevent the applications of iron complexes as NO sensor.

In recent times, a number of metal complexes have been exemplified based on fluorophore displacement strategy as direct fluorescent sensors for NO, however, most of them are found to be compatible only with organic solvents [20–30]. In aqueous environments, fluorescence turn-on/enhancement may arise from the replacement of the fluorophore ligand from the metal center by water itself. Thus, the strategy of reduction of a metal center by NO with a concomitant fluorescence enhancement has been adopted. The fluorescence intensity of fluorescent ligand is known to be quenched on coordination to Cu(II) center. The reduction of Cu(II) by NO to Cu(I) is expected to restore the quenched fluorescence of a ligand fluorophore [31]. The reduction of Cu(II) center to Cu(I) by NO has been reported earlier in Cu(II)-dithiocarbamate, Cu(II)-phen or dmp (phen = 1,10-phenanthroline; dmp = 2,9-dimethyl-1,10-phenanthroline) complexes [32,33]. Recently, Cu(II) center in complexes of various amine ligands have also been reported to undergo reduction by NO [34–38].

This strategy has been adopted by Ford et al. in their report of Cu(II) complexes of *bis*{2-(3,5-dimethyl-1-pyrazolyl)ethyl)-ammine} (pza) with appended Ru(II) and Re(I) luminophores as possible luminescent sensor for NO; though these were found to be too labile to be a practical sensor [39]. On the other hand, this strategy indeed found to work in the reaction of NO with $[Cu^{II}(DAC)]^{2+}$ [DAC is the N-derivatized cyclam-1,8-*bis*(anthracen-9-ylmethyl)-1,4,8,11-tetraazacyclotetradecane] [40,41].

* Corresponding author. Tel.: +91 361 258 2317; fax: +91 361 258 2339.
E-mail address: biplab@iitg.ernet.in (B. Mondal).

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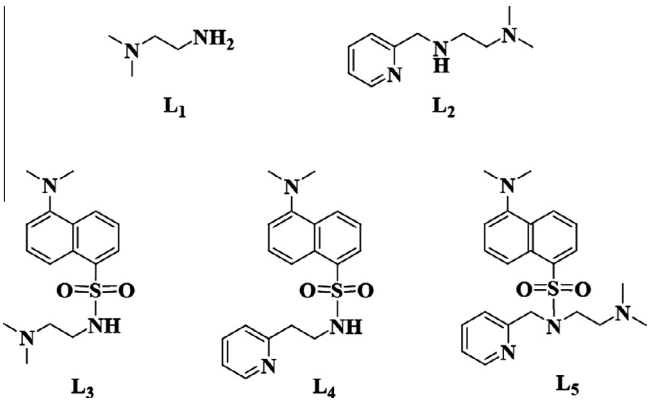


Fig. 1. Ligands used for the present study.

Lippard's group reported Cu(II) complexes of anthracenyl and dansyl fluorophore ligands as NO sensors [22,23,27,28]. The quenched fluorescence intensity of the ligand fluorophore was observed to restore in presence of NO in methanol/dichloromethane solutions of the complexes. In addition, $[Cu(Ds\text{-}en)_2]$ and $[Cu(Ds\text{-}AMP)_2]$, where Ds-en and Ds-AMP are the conjugate bases of dansylethylenediamine (Ds-Hen) and dansyl aminomethylpyridine (Ds-HAMP), respectively, have found to detect NO in aqueous solution, also [28,42]. However, these compounds were unable to sense NO at a physiologically more relevant pH [28].

From our laboratory, recently we have reported the two examples of Cu(II) complex-based fluorescent NO sensor for methanol and water medium buffered at pH ~7.2 [43,44]. However, these have not been found to behave as turn on/off sensors; perhaps, because of the demetallation of the ligand after reduction followed by undefined product formation during air oxidation of the reduced Cu(I) [45].

In this direction we have studied the NO reactivity of two Cu(II) complexes of ligands $\mathbf{L_1}$ and $\mathbf{L_2}$, respectively. Successively, three fluorophore ligands with pendant dansyl group have been synthesized and studied as fluorescence sensors for NO (Fig. 1).

2. Experimental

2.1. Materials and methods

All reagents and solvents were purchased from commercial sources and were of reagent grade. Acetonitrile was distilled from calcium hydride. Deoxygenation of the solvent and solutions were effected by repeated vacuum/purge cycles or bubbling with nitrogen for 30 min. NO gas was purified by passing through KOH and P_2O_5 column. UV–Vis spectra were recorded on a Perkin Elmer Lambda 25 spectrophotometer. FT-IR spectra of the solid samples were taken on a Perkin Elmer spectrophotometer with samples prepared as KBr pellets. The fluorescence spectra were recorded in solution in VARIAN Cary Eclipse Fluorescence Spectrophotometer at room temperature. Quinine sulfate in acidic medium was used as the reference compound for the determination of fluorescence quantum yield. Solution electrical conductivity was checked using a Systronic 305 conductivity bridge. ^{1}H NMR spectra were obtained with a 400 MHz Varian FT spectrometer. Chemical shifts (ppm) were referenced either with an internal standard (Me_4Si) or to the residual solvent peaks. The X-band Electron Paramagnetic Resonance (EPR) spectra were recorded on a JES-FA200 ESR spectrometer, at room temperature. Elemental analyses were obtained from a Perkin Elmer Series II Analyzer. The magnetic moment of complexes is measured on a Cambridge Magnetic Balance.

Single crystals were grown by slow diffusion followed by slow evaporation technique. The intensity data were collected using a Bruker SMART APEX-II CCD diffractometer, equipped with a fine focus 1.75 kW sealed tube Mo Kα radiation (λ = 0.71073 Å) at 276(3) K, with increasing ω (width of 0.3° per frame) at a scan speed of 3 s/frame. The SMART software was used for data acquisition. Data integration and reduction were undertaken with SAINT and XPREP software [46]. Multi-scan empirical absorption corrections were applied to the data using the program SADABS [47]. Structures were solved by direct methods using SHELXS-97 and refined with full-matrix least squares on F^2 using SHELXL-97 [48]. All non-hydrogen atoms were refined anisotropically. Structural illustrations have been drawn with ORTEP-3 for Windows [49].

2.2. Synthesis

2.2.1. Synthesis of $\mathbf{L_2}$

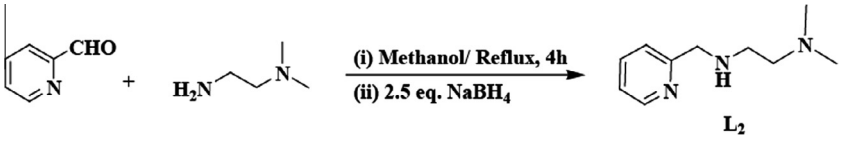
Pyridine-2-carbaldehyde (1.07 g, 10 mmol) and N,N-dimethylethylenediamine (0.880 g, 10 mmol) in 20 ml methanol was refluxed at 60 °C for 4 h (Scheme 1). The resulting solution was dried under reduced pressure and the dark oil thus obtained was subjected to chromatographic purification using silica gel column to yield the pure Schiff base. The Schiff base was then reduced to the corresponding ligand, $\mathbf{L_2}$, using 2.5 equivalent $NaBH_4$ in methanol solution. The pure $\mathbf{L_2}$ was obtained after chromatographic purification using silica gel column and characterized using various spectroscopic techniques.

Yield: 1.21 g, ~69%. FT-IR in KBr: 2793, 1590, 1429, 1341, 1170, 766 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ_{ppm}: 2.21(6H), 2.44–2.47(2H), 2.72–2.75(2H), 3.93(2H), 7.13–7.16(1H), 7.32–7.34(1H), 7.61–7.65(1H), 8.54–8.55(1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_{ppm}: 45.1, 46.4, 54.8, 58.7, 121.4, 121.8, 136.0, 148.7, and 159.4. ESI-Mass (m + 1): Calc. 180.14; Found, 180.09.

2.2.2. Synthesis of $\mathbf{L_3}$

The fluorescent ligand $\mathbf{L_3}$ was prepared by the introducing of the dansyl group in the ligand N,N-dimethylethane-1,2-diamine, $\mathbf{L_1}$. This was done by stirring an equimolar mixture of N,N-dimethylethane-1,2-diamine (0.176 g, 2 mmol) and dansyl chloride (538 g, 2 mmol) in presence of triethylamine in distilled chloroform for 5 h at room temperature (Scheme 2).

The volume of the resulting solution was dried under reduced pressure and fluorescent mass was subjected to column chromatographic purification to result the pure ligand $\mathbf{L_3}$. Yield: 0.526 g, ~82%. FT-IR in KBr: 2944, 2863, 1457, 1320, 1144, 791, 625 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ_{ppm}: 1.90 (6H), 2.16–1.19 (2H), 2.76–2.86 (8H), 7.08–7.10 (1H), 7.43–7.47 (2H), 8.16–8.19 (1H), 8.24–8.26 (1H) 8.44–8.47 (1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_{ppm}: 39.9, 44.3, 45.2, 56.7, 114.9, 118.6, 122.9, 128.2, 129.4, 129.6, 130.1, 134.3 and 151.7. ESI-Mass (m + 1): Calcd. 322.15; Found, 322.16.



Scheme 1.