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A Ruthenium Nitrosyl That Rapidly Delivers NO to Proteins in Aqueous Solution upon Short Exposure to UV Light

Apurba K. Patra and Pradip K. Mascharak*

Department of Chemistry and Biochemistry, University of California, Santa Cruz, California 95064

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Two Ru(III) complexes, $[Ru(PaPy_3)(Cl)](BF_4)$ (**2**) and $[Ru(PaPy_3)(NO)](BF_4)_2$ (**3**) ($PaPy_3H$ = *N*,*N*′-bis(2-pyridylmethyl)amine-*N*-ethyl-2-pyridine-2-carboxamide), have been synthesized and characterized by spectroscopy and X-ray diffraction. Nitrosyl complex **3** has been prepared by passage of purified NO gas to the hot methanolic solution of the chloro derivative **2**. Complex **3** exhibits ν_{NO} stretching frequency at 1899 cm^{-1} indicating a $\{Ru–NO\}^6$ configuration. Clean ^1H NMR spectra of **3** in D_2O and CD_3CN confirm the $S = 0$ ground state. When an aqueous solution of $[Ru(PaPy_3)(NO)](BF_4)_2$ is exposed to low intensity UV light, it rapidly loses NO and forms $[Ru(PaPy_3)(H_2O)]^{2+}$. This reaction can be conveniently used to transfer NO to proteins such as myoglobin (Mb) and cytochrome *c* oxidase. The NO transfer reaction is clean and occurs upon short exposure to light.

Following the discovery of the role of nitric oxide (NO) in neurotransmission, control of blood pressure, and inhibition of tumor growth,[1,2] the desire to deliver NO at biological targets under physiological conditions has inspired research in the area of designed molecules that release NO upon demand.[3] Intense effort has been directed toward metal nitrosyl complexes[4] that release NO upon illumination since such species could be used as antitumor agents in photodynamic therapy (PDT).[5] Several groups have utilized sodium nitroprusside ($Na_2[Fe(CN)_5(NO)]$) and Roussin's salts ($(NH_4)[Fe_4S_3(NO)_7]$ and $Na_2[Fe_2S_2(NO)_4]$) in their light-driven NO release studies.[4] Very recently, two groups have reported the photochemical behavior of the non-porphyrin ruthenium nitrosyls [Ru(salen)(Cl)(NO)], $[Ru(salen)(NO)(H_2O)]^+$ [H$_2$-salen = *N*,*N*′- bis(salicylaldehyde)ethylenediimine], and related complexes.[6,7] These species afford NO upon irradiation with 355 nm light (Nd:YAG laser)[6] or 200 W mercury lamp[7] in different solvents. The NO release is slow, and the complexes are eventually converted into the corresponding solvated products.

Recently, we have reported the Fe(III) complex $[Fe(PaPy_3)(NO)](ClO_4)_2$ (**1**) derived from the designed ligand *N*,*N*-bis(2-pyridylmethyl)amine-*N*-ethyl-2-pyridine-2-carboxamide ($PaPy_3H$, H is the dissociable carboxamide proton). This diamagnetic $\{Fe–NO\}^6$ complex rapidly loses NO in CH_3CN upon illumination. The photorelease of NO occurs upon exposure to visible light of low intensity (50 W incandescent lamp), and the reaction is reversible.[8] In this paper, we report the low spin Ru(III) complex of the same ligand, namely $[Ru(PaPy_3)(Cl)](BF_4)$ (**2**). When NO is purged into the methanolic solution of **2**, the nitrosyl complex $[Ru(PaPy_3)(NO)](BF_4)_2$ (**3**) is formed. Unlike $[Fe(PaPy_3)(NO)](ClO_4)_2$, complex **3** does not lose NO upon illumination to visible light. However, when an aqueous solution of **3** is exposed to low intensity UV light, it rapidly loses NO and forms $[Ru(PaPy_3)(H_2O)]^{2+}$. We also report here that this reaction can be conveniently used to transfer NO to proteins such as myoglobin (Mb) and cytochrome *c* oxidase.

A purified batch of $RuCl_3 \cdot 3H_2O$ was heated to reflux for 3 h in absolute ethanol to generate a deep green solution.

* To whom correspondence should be addressed. E-mail: pradip@chemistry.ucsc.edu.

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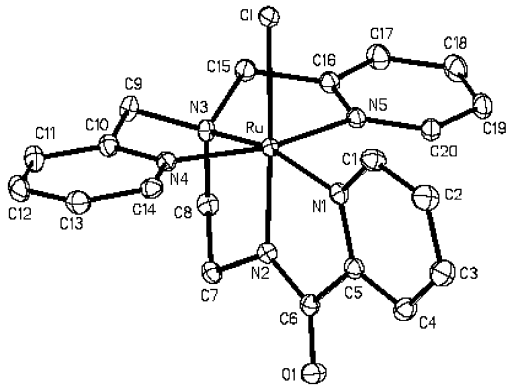

Figure 1. ORTEP diagram of $[Ru(PaPy_3)(Cl)]^+$, the cation of **2**, showing the atom labeling scheme. All H atoms and the H_2O molecule of crystallization have been omitted for clarity. Selected bond lengths [Å] and bond angles [deg]: Ru−N1 2.0917(14), Ru−N2 1.9306(13), Ru−N3 2.0689(13), Ru−N4 2.0627(14), Ru−N5 2.0725(14), Ru−Cl 2.4139(4), Cl−Ru−N2 177.03(4), N1−Ru−N2 80.61(5), N1−Ru−N3 164.31(5), N1−Ru−N4 99.54(5), N1−Ru−N5 96.72(5), N2−Ru−N3 84.08(6), N2−Ru−N4 88.79(5), N2−Ru−N5 95.11(6), N3−Ru−N4 83.49(5), N3−Ru−N5 81.18(5), N3−Ru−Cl 96.43(4), N4−Ru−N5 163.70(5), N4−Ru−Cl 88.37(4), N5−Ru−Cl 87.85(4).

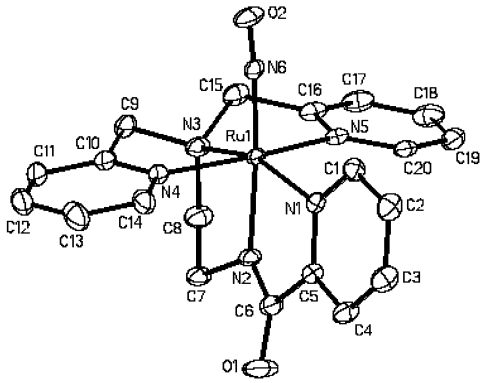

Figure 2. ORTEP diagram of $[Ru(PaPy_3)(NO)]^{2+}$, the cation of **3**, showing the atom labeling scheme. All H atoms and the solvent molecules of crystallization have been omitted for clarity. Selected bond lengths [Å] and bond angles [deg]: Ru−N1 2.097(2), Ru−N2 1.997(2), Ru−N3 2.073(3), Ru−N4 2.074(2), Ru−N5 2.086(2), Ru−N6 1.779(2), N6−O2 1.142(3), Ru−N6−O2 170.9(2), N6−Ru−N2 172.23(11), N1−Ru−N2 78.22(10), N1−Ru−N3 159.40(9), N1−Ru−N4 97.50(10), N1−Ru−N5 97.82(9), N2−Ru−N3 81.35(10), N2−Ru−N4 83.51(10), N2−Ru−N5 93.41(10), N3−Ru−N4 83.76(11), N3−Ru−N5 80.66(10), N3−Ru−N6 101.97(10), N4−Ru−N5 163.41(10), N4−Ru−N6 89.91(10), N5−Ru−N6 94.06(10).

Addition of an ethanolic solution of the deprotonated ligand to this green solution caused a rapid change of color to red. Further heating of the red reaction mixture followed by filtration and addition of $NaBF_4$ afforded the dark red complex **2** in high yield. When purified NO gas was passed through the degassed methanolic solution of **2** and $NaBF_4$ at 60 °C, its red color changed slowly to a clear orange showing the formation of complex **3**. Cooling of this solution at −20 °C afforded orange needles of **3** in 60% yield. The IR spectrum of **3** (in KBr disk) displays a strong NO stretch (ν_{NO}) at 1899 cm^{-1}, and its ^1H NMR spectrum in D_2O (or in CD_3CN) clearly reveals its $S = 0$ ground state (Supporting Information, Figure S1). The structure of the cations of **2** and **3** are shown in Figures 1 and 2, respectively.[9,10] In both complexes, the *tert*-amine nitrogen and the three pyridine nitrogen atoms occupy the equatorial coordination positions. The carboxamido nitrogen atom is *trans* to Cl^- and NO in **2** and **3**, respectively. This kind of coordination has been observed in $[Fe(PaPy_3)(Cl)](ClO_4)$[11] and **1**.[8] The Ru−Cl distance of **2** (2.4139(4) Å) is longer than that noted for [Ru(salen)(Cl)(NO]) (2.3537(16) Å)[7] and [Ru(cyclam)(Cl)(NO)]-$(ClO_4)_2$ (2.327(1) Å).[12] Strong *trans* effect of the carboxamido nitrogen[13] is presumably responsible for this lengthening of the Ru−Cl bond in **2**. The average Ru−N(O) distance of **3** (1.780(2) Å) is close to the corresponding distance in the salen complexes,[6,7] and the Ru−N−O bond is almost linear (av angle = 173.2(2)°). Complexes **2** and **3** exhibit reversible cyclic voltammograms with $E_{1/2}$ at −0.135 and −0.225 V (versus aqueous saturated calomel electrode) respectively in CH_3CN. In both cases, the initial electrode process is cathodic upon scan in the negative potential direction starting from 0.4 V (Supporting Information, Figure S2). This behavior indicates the presence of Ru(III) in **3**. Ligands with carboxamido nitrogen donor(s) are known to stabilize high oxidation state of metals,[13] and the behavior of $PaPy_3^-$ in **2** and **3** is no exception.

Previously, we have reported that a solution of complex **1** in CH_3CN rapidly loses NO when exposed to ordinary light (50 W tungsten lamp).[8] The present complex **3** however behaves quite differently. It is soluble in water as well as in organic solvents such as CH_3CN, acetone, MeOH, and DMF. The solutions of **3** are completely stable under ordinary light. For example, when an aqueous solution of **3** is illuminated with a 100 W tungsten lamp for hours, no change in the electronic spectrum is observed. Even upon heating at 100 °C, the aqueous solution exhibits no change. Quite in contrast, when the aqueous solution is illuminated with a low-intensity UV lamp (7 mW/cm^2, λ_{max} = 302 nm), the yellow solution rapidly turns orange. Successive scanning of the electronic absorption spectrum (Figure 3) shows that,

(9) Crystal data for $[(PaPy_3)Ru(Cl)](BF_4)\cdot H_2O$ (**2**·H_2O): red plates of dimensions 0.55 × 0.16 × 0.06 mm^3; monoclinic, space group $P2_1/c$, a = 8.5012(11) Å, b = 18.408(2) Å, c = 14.3838(17) Å, β = 95.476(6)°, V = 2240.7(5) $Å^3$, Z = 4, ρ_{calcd} = 1.742 Mg m^{-3}, $2\theta_{max}$ = 60°, μ(Mo Kα) = 0.881 mm^{-1}, ω scans, λ = 0.71073 Å; the data were collected at 90(2) K on a Bruker SMART 1000 diffractometer; a total of 29353 reflections were measured, of which 6495 were independent (R_{int} = 0.0188) and included in the refinement; min/max transmission = 0.643/0.949; solution by direct methods (SHELXL-97, Sheldrick, 1990); refinement by full-matrix least-squares based on F^2 (SHELXL-97, Sheldrick, 1997); 313 parameters, R1 = 0.0283, wR2 = 0.0597 for all data; R1 = 0.0251 computed for 6006 observed data ($I > 2\sigma(I)$).

(10) (a) Crystal data for $[(PaPy_3)Ru(NO)](BF_4)_2\cdot MeCN\cdot 0.25Et_2O$ (**3**·MeCN·1/4Et_2O): Yellow-orange dichroic needlelike crystal of dimensions 0.26 × 0.12 × 0.10 mm^3; triclinic, space group $P\bar{1}$, a = 10.1081(12) Å, b = 16.5739(18) Å, c = 17.3238(19) Å, α = 75.761, β = 86.966(4), γ = 84.073(7)°, V = 2796.9(5) $Å^3$, Z = 4, ρ_{calcd} = 1.688 Mg m^{-3}, $2\theta_{max}$ = 60.06°, μ(Mo Kα) = 0.652 mm^{-1}, ω scans, λ = 0.71073 Å; the data were collected at 90(2) K on a Bruker SMART 1000 diffractometer; a total of 36676 reflections were measured, of which 16057 were independent (R_{int} = 0.0348) and included in the refinement; min/max transmission = 0.849/0.938; solution by direct methods (SHELXL-97, Sheldrick, 1990); refinement by full-matrix least-squares based on F^2 (SHELXL-97, Sheldrick, 1997); 777 parameters, R1 = 0.0692, wR2 = 0.1116 for all data; R1 = 0.047 computed for 12279 observed data ($I > 2\sigma(I)$).

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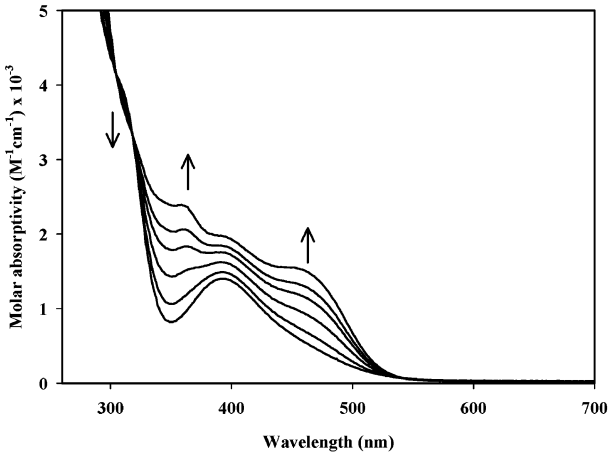

Figure 3. Conversion of $[Ru(PaPy_3)(NO)]^{2+}$ (**3**) into $[Ru(PaPy_3)(H_2O)]^2$ in aqueous solution under illumination with a low-intensity UV light ($t_{1/2}$ = 3 min).

upon exposure to UV light, a new band with λ_{max} at 455 nm appears, and the original 395 nm band of **3**[14] is replaced with a stronger double-humped absorption with λ_{max} at 400 and 355 nm. Since the final electronic absorption spectrum is identical to that obtained from the filtrate of a reaction mixture of **2** and $AgClO_4$ in water, it is clear that **3** is converted into $[Ru(PaPy_3)(H_2O)]^{2+}$ following the release of NO upon UV illumination. The $[Ru(PaPy_3)(NO)]^{2+} \rightarrow [Ru(PaPy_3)(H_2O)]^{2+}$ transformation is clean and shows isosbestic points (Figure 3). The NO release reaction is quite rapid under this condition. For example, the sample of Figure 3 (0.4 mM of **3**) was converted completely into $[Ru(PaPy_3)(H_2O)]^{2+}$ within 6 min. Although the reported ruthenium nitrosyls derived from salenH_2 release NO upon exposure to light, the rate of NO release has been reported to be quite slow (200 W mercury lamp, 2 h).[7] In our previous report, we suggested that the rapid NO release from **1** is related to the *trans* effect of the carboxamido nitrogen. In the present study, similar rapid NO release from **3** supports this notion.

Although the possibility of NO delivery to biological targets has always been the impetus for the photochemical studies on metal nitrosyls, problems related to solubility, back-reactions, and slow NO release have disturbed such attempts. Complex **3** however is a very convenient NO donor to proteins in aqueous solutions under controlled conditions. For example, when **3** is added to an aqueous solution of

(14) We assign this band of **3** to a metal-to-ligand charge transfer transition (MLCT). Very similar absorptions have been noted with [Ru(salen)(Cl)(NO)] (λ_{max} = 384 nm) and related complexes.[6] Complex **1** exhibits this band at 500 nm.[8] Collectively, the photolability of NO noted with **1** and **3** under lights of different energy (vis and UV, respectively) indicates that NO labilization may result from population of a dissociative excited state as suggested by Ford and co-workers.[6]

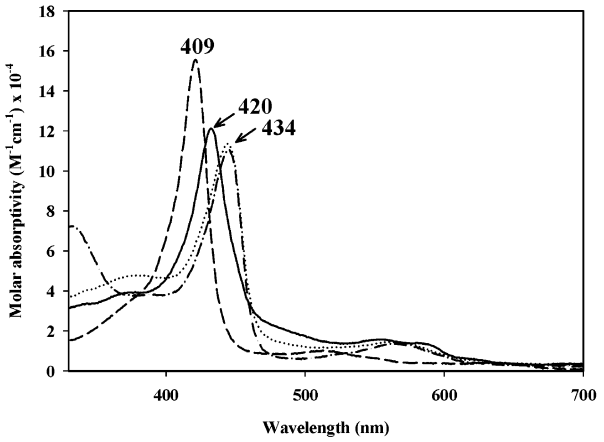

Figure 4. Conversion of reduced Mb to Mb−NO adduct upon reaction with **3** in aqueous solution under exposure to low-intensity UV light: dashed line, Met-Mb; dash−dot line, reduced Mb (with slight excess of dithionite); dotted line, a mixture of solutions of reduced Mb (with slight excess of dithionite) and **3** (1:5) under ordinary light (kept for 20 min); solid line, same Mb + **3** solution exposed to low-intensity UV light for 30 s.

reduced horse heart muscle Mb (Figure 4, dash-dot line) under ordinary light, no reaction is observed (Figure 4, dotted line). However, when the mixture is exposed to the same low-intensity UV lamp, the 420 nm Soret band of the Mb−NO adduct is rapidly generated (Figure 4, solid line). The reaction is clean and is complete within 30 s. Similarly, when an aqueous solution of reduced cytochrome *c* oxidase and **3** is exposed to the UV light, the Soret band of the NO-adduct at 428 nm is obtained within 60 s (Supporting Information, Figure S3).

In conclusion, $[Ru(PaPy_3)(NO)](BF_4)_2$ (**3**) is a ruthenium−nitrosyl that rapidly releases NO upon illumination with low-intensity UV light. Since this complex is soluble in water and transfers NO to proteins only upon short exposure to UV light, it can be used as a convenient NO donor to proteins under controlled conditions. Experiments are under way in this laboratory to extend such utility of **3** and related complexes to various other biological targets.

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Supporting Information Available: Experimental procedures, analytical and spectroscopic data for **2** and **3**, ^1H NMR spectrum of **3** in D_2O (Figure S1), cyclic voltammograms of **2** and **3** in CH_3CN (Figure S2), NO transfer reaction by **3** to cytochrome *c* oxidase (Figure S3), X-ray structural data, and tables of atomic coordinates, bond lengths and angles, anisotropic displacements, and hydrogen coordinates. This material is available free of charge via the Internet at http://pubs.acs.org.

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