Nitroxyl Complexes

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Three Redox States of Nitrosyl: NO^+, $NO^{\bullet}$, and NO^-/HNO Interconvert Reversibly on the Same Pentacyanoferrate(II) Platform**

Andrea C. Montenegro, Valentín T. Amorebieta, Leonardo D. Slep, Diego F. Martín, Federico Roncaroli, Daniel H. Murgida, Sara E. Bari, and José A. Olabe**

One-electron redox changes of bound nitrosyl species[1] are at the heart of the enzymatic processes involving reversible conversions of NO_2^- into NH_3, which occur either in bacterial processes in soils[2] or in the biosynthesis/degradation events of nitrogen monoxide (NO) in the biological fluids of mammals.[3] This explains the interest in the coordination chemistry of NO and the very reactive redox-active partners, the nitrosonium cation (NO^+) and the nitroxyl anion (NO^-).[4] The three diatomic entities have been identified when bound to transition metals. Although a fragment model considering covalent interactions between the metal and nitrosyl is currently employed,[5] detailed spectroscopic measurements and/or theoretical calculations frequently allow the use of a limiting description comprising NO^+, $NO^{\bullet}$, and NO^- ligands bound to metals with the corresponding formal charges.[4,5]

The chemistry of NO^+ and $NO^{\bullet}$ complexes is reasonably well understood.[1,4] Structural, spectroscopic, and/or kinetic studies have been reported for NO^- complexes, mainly with five- and six-coordinated Co^{III},[6,7a–e] Cr^{III},[7f–h], Fe^{III},[7i,j] and Pt^{IV} compounds.[7k] Recently, NO^- has been characterized in an iron(II) cyclam derivative, as a product of the successive (reversible) reductions of the corresponding NO^+ complex in CH_3CN.[1b] Complexes containing HNO, the acid conjugate of NO^-, have been obtained with low-spin d^6 metals of the second- and third-row transition series (Ru^{II}, Os^{II}, Ir^{III}, Re^{I}), and are reported to be short-lived in solution.[8] X-ray structures of derivatives of the first three metals have been reported after isolation from non-aqueous media.[9] The insolubility in aqueous solutions limits their bioinorganic relevance; only a water-soluble adduct of HNO with myoglobin, $Mb^{II}(HNO)$, has been characterized, with an unusual stability attained through interaction of the ligand with distal amino acids.[10]

The nitroprusside ion, $[Fe^{II}(CN)_5(NO)]^{2-}$ (**1**), is a widely used hypotensive agent.[11,12] Given the great inertness of bound NO^+ toward dissociation, the NO-releasing ability of **1** in biological fluids has been ascribed to the generation of the one-electron-reduced product $[Fe^{II}(CN)_5(NO)]^{3-}$ (**2**), probably upon reaction of **1** with the thiolates.[11–13] The possibility of further reduction of **2** in these media is uncertain, and raises an additional question on the detailed mechanisms of reactions operating upon injection of **1**. The consideration of possible $Fe^{II}(HNO)$ intermediates is also pertinent to the ongoing pharmacological assays of nitroxyl donors,[14] and to several heme-catalyzed instances of nitrogen metabolism.[15–17] In this track, the two-electron reduction of **1** has been previously achieved at a Hg cathode at approximately −1.0 V (normal hydrogen electrode), which leads to an ill-defined product,[18] and theoretical predictions have been advanced on the structural and spectroscopic parameters for $[Fe^{II}(CN)_5(HNO)]^{3-}$.[19] Herein, our main objective is the full characterization of iron-bound nitroxyl in aqueous solution, which is of fundamental concern.

Figure 1 shows the changes in the UV/Vis spectra upon sequential addition of two equivalents of the two-electron reductant dithionite ($S_2O_4^{2-}$)[20] to a solution of **1** at pH 10. With the first equivalent **1** transforms into **2** almost quantitatively (ca. 90%), as evidenced by the characteristic new bands at 348 and 440 nm [$\varepsilon = 3.5\times10^3$ and $5.5\times10^2\,M^{-1}cm^{-1}$, respectively; Eq. (1)].[21] Addition of the second equivalent converts **2** into a red species **3**, with $\lambda_{max} = 445$ nm, $\varepsilon = (4.2\pm$

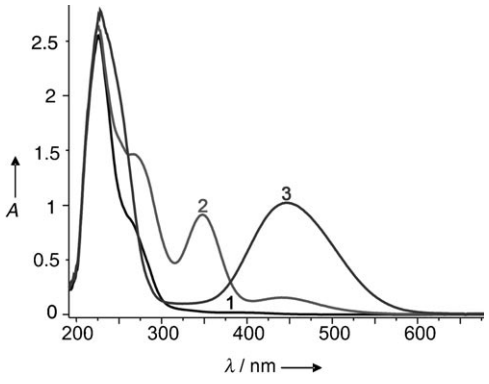

Figure 1. Spectra of the reduced complexes **2** and **3** obtained by two sequential one-equivalent additions of $S_2O_4^{2-}$ to 3×10^{-4} M $[Fe^{II}(CN)_5(NO)]^{2-}$ (**1**) at pH 10, $T = 25.0$ °C.

[*] A. C. Montenegro, Prof. Dr. L. D. Slep, D. F. Martín, Dr. F. Roncaroli, Prof. Dr. D. H. Murgida, Dr. S. E. Bari, Prof. Dr. J. A. Olabe
Departamento de Química Inorgánica, Analítica y Química Física
Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires and INQUIMAE, CONICET
Intendente Güiraldes 2160, Ciudad Universitaria, Pabellón 2, Buenos Aires, C1428EHA (Argentina)
Fax: (+54) 11 4576-3341
E-mail: bari@qi.fcen.uba.ar
olabe@qi.fcen.uba.ar
Homepage: http://www.qi.fcen.uba.ar
Prof. Dr. V. T. Amorebieta
Departamento de Química, Facultad de Ciencias Exactas
Universidad Nacional de Mar del Plata
Funes y Roca, Mar del Plata, B7602AYL (Argentina)

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0.3) × 10^3 M^{-1} cm^{-1}, and a shoulder at 236 nm. These absorptions decay under excess reductant conditions, which suggests further reduction of the ligand.

$$[Fe^{II}(CN)_5(NO)]^{2-} + e^- \rightleftharpoons [Fe^{II}(CN)_5(NO)]^{3-} \quad (1)$$

Compound **3** is unstable at pH 10 and decomposes to form **2** (70–85% yield) with a half-life of about 50 min (see the Supporting Information, SI 1). However, if the pH is rapidly adjusted to 6–7, fresh solutions of **3** become much more stable, even toward the addition of one equivalent of $[Fe^{III}(CN)_6]^{3-}$, aqueous iron(III), or methyl viologen. Conversely, one-equivalent additions of the same oxidants to **3** at pH 10 regenerate almost quantitatively **2** and **1**. Moreover, by adding one additional equivalent of **1** to **3**, the original absorbance of **2** is duplicated, thus revealing comproportionation.

In the UV/Vis spectrum of **3** we assign the main band at 445 nm to a metal-to-ligand charge-transfer (MLCT) transition in the $[Fe^{II}(CN)_5(L)]^{3-}$ ion [Eq. (2)], close to the theoretically predicted value at 460 nm for L = HNO.[19] Intense bands in the visible region have been reported for related $[Fe^{II}(CN)_5NOR]^{3-}$ ions, with R = thiolate,[22] phenyl,[23] and an alkyl derivative.[21]

$$[Fe^{II}(CN)_5(NO)]^{3-} + e^- \rightleftharpoons [Fe^{II}(CN)_5(L)]^{n-} \quad L = NO^-/HNO \quad (2)$$

Upon the two-electron reduction of **1**, the attenuated total reflection (ATR)/FTIR spectrum of **3** reveals the disappearance of the stretching frequencies of **1** (ν_{NO}, 1938 cm^{-1}; ν_{CN}, 2142 cm^{-1})[22] and the buildup of new bands at 1384 and 2088/2040 cm^{-1}. The first band shifts to 1352 cm^{-1} upon ^{15}N labeling of the FeNO moiety (see the Supporting Information, SI 2), which supports an assignment to ν_{NO}, in good accordance with other $[M^{II}X_5(HNO)]^x$ complexes.[8–10] We assign the second two bands to axial and equatorial ν_{CN} modes, as they remain unchanged upon ^{15}N labeling. The trends of ν_{NO} from **1** to **2** (1648 cm^{-1})[22] and **3** reflect predominantly nitrosyl-centered reductions, with added electrons populating the antibonding FeNO moiety.[4]

Resonance Raman results, obtained after irradiation close to the MLCT band for compound **3**, are displayed in SI 3 in the Supporting Information. As for the IR measurements, the Raman bands of **1** at about 2150 cm^{-1} (ν_{CN})[24] disappear completely upon reduction. We assign the new bands at 2100 and 565 cm^{-1}, not shifted upon ^{15}N labeling, to ν_{CN} and probably mixed ν_{Fe-C}/δ_{FeCN}, respectively, by comparison with related modes for $[Fe^{II}(CN)_5L]^{n-}$ complexes (L = pyridine, 4-cyanopyridine, pyrazine, etc.).[25] For the shifted bands at 1380, 1304, 1214, 662 cm^{-1}→1350, 1286, 1204, 649 cm^{-1}, we assign the first two as asymmetric and symmetric stretching modes, predominantly ν_{NO}, in the $Fe^{II}(HNO)$ moiety (see the IR assignment).[8–10] The band at 662 cm^{-1} is traced to a mixed ν_{Fe-N}/δ_{FeNO} mode.[10b,26] The assignment of the 1214 cm^{-1} band remains uncertain.[27]

Figure 2 shows the results of ^1H NMR experiments, which provide conclusive evidence on the HNO ligand. At pH 6, a singlet signal at $\delta = 20.02$ ppm splits into a doublet upon ^{15}N labeling, with $J_{NH} = 71.14$ Hz. These signatures are diagnostic

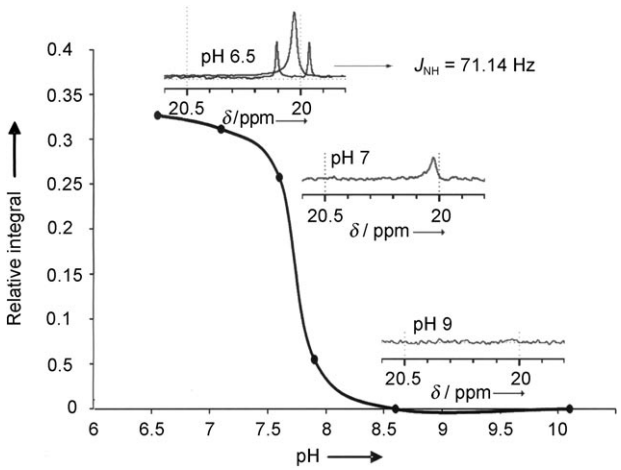

Figure 2. ^1H NMR titration of complex **3**. Top left insert: signal splitting upon ^{15}N labeling of the $Fe^{II}(HNO)$ moiety.

of bound HNO.[8–10] Most importantly, the integrated intensity of the H signal decreases with increasing pH, is not detected at pH 10, and is partly recovered when the pH is returned to 6–7. These results strongly suggest the onset of the equilibrium in Equation (3).

$$[Fe^{II}(CN)_5(HNO)]^{3-} \rightleftharpoons [Fe^{II}(CN)_5(NO)]^{4-} + H^+ \quad K_a \quad (3)$$

From Figure 2, a pK_a of 7.7 is determined, expectedly lower than the reported value for free ^1HNO of 11.6.[28] As far as we know, this is the first report on the pK_a of bound HNO on a given metal fragment in aqueous solutions.

In contrast to the results at pH 10, a very slow decay of the main absorption band was monitored at pH 6 (see the Supporting Information, SI 4). The stoichiometry obeys Equation (4), and reveals a nonredox dissociation of HNO ($k_4 = 1.4 \times 10^{-5}$ s^{-1}, 25.0 °C) followed by a fast dehydrative dimerization to N_2O.[28]

$$[Fe^{II}(CN)_5(HNO)]^{3-} + CN^- \rightarrow [Fe^{II}(CN)_6]^{4-} + {}^1/_2\,N_2O + {}^1/_2\,H_2O \quad (4)$$

The small value of k_4 is of the same order of magnitude as that found for the release of NO[13] and other strongly binding ligands in the series of $[Fe^{II}(CN)_5(L)]^{n-}$ complexes,[12] thus providing firm evidence on the proposed role of $Fe^{II}(HNO)$ as a reactive intermediate in diverse redox processes, such as the disproportionation of NH_2OH catalyzed by pentacyanoferrates[29] and the full six-electron reduction of **1** to NH_3 with 1,2-dimethylhydrazine.[30] This is also the case for the enzymatic conversions afforded by syroheme[15] and cytochrome *c*[16] NO_2^- reductases, as well as for the oxidation of NH_2OH to NO_2^- catalyzed by the heme-based hydroxylamine oxidoreductase.[17]

The remarkably inert $[Fe^{II}(CN)_5(HNO)]^{3-}$ ion is the first non-heme iron–nitroxyl complex prepared and characterized in aqueous solution, with accessible, reversible redox interconversions of the nitrosyl group on the same fragment. We provide the first pK_a value for a bound HNO/NO^- equilib-

rium under biologically relevant conditions. The decompositions of $[Fe^{II}(CN)_5(HNO)]^{3-}$ and $[Fe^{II}(CN)_5(NO)]^{4-}$ are different under our reaction conditions: the first generates $[Fe^{II}(CN)_6]^{4-}$ and N_2O, whereas the conjugate base behaves as a strong reductant, forming $[Fe^{II}(CN)_5(NO)]^{3-}$. Preliminary experiments indicate that $[Fe^{II}(CN)_5(HNO)]^{3-}$ is poorly reactive even toward oxygen, with slow production of **1** in the hours timescale. A systematic study of the reactivity of bound HNO/NO^- toward biologically relevant substrates is under way.

Experimental Section

A freshly prepared solution of 0.19 M $Na_2S_2O_4$, previously standardized with a solution of $K_3[Fe(CN)_6]$[20] in degassed 0.1 M NaOH, was added to a 3×10^{-4} M solution of $Na_2[Fe(CN)_5NO]\cdot2H_2O$ (pH 10; 0.01 M phosphate buffer, 5×10^{-4} M ethylenediaminetetraacetate (EDTA), 6×10^{-3} M NaCN, $I=0.1$ M (NaCl)) in a 10-mm quartz cuvette under a nitrogen atmosphere and with continuous stirring. The reduction was performed in two sequential steps. The first comprised the addition of one equivalent of reductant to generate compound **2**. In the second step, the addition of a second equivalent led to the total consumption of **2**, with concomitant formation of **3**. Given the back-oxidation of **3** to **2** (see the Supporting Information, SI 1), and to guarantee an optimum yield of only **3** at pH 6, a 5% excess of dithionite was added at this point. Then, the pH was immediately adjusted to 6 with 1.2 M HCl. A comprehensive description of the reduction process is provided in the Supporting Information (SI 5). The pH was controlled with a Hanna pH211 instrument, calibrated against commercial standard buffers. UV/Vis spectra were recorded on a Hewlett–Packard 8453 diode-array spectrophotometer in the range 200–900 nm. $Na_2[Fe(CN)_5{}^{15}NO]\cdot2H_2O$ was obtained according to literature procedures.[31] Qualitative and quantitative gas production were conducted using a thermostatic homemade flow reactor, as described elsewhere.[29] GC–MS measurements for N_2O and UV/Vis spectral results for $[Fe(CN)_6]^{4-}$ allowed a rigorous assessment of the stoichiometry in Equation (4). For ATR/FTIR, resonance Raman, and NMR experiments, the preparations were performed in a 10-mm quartz cuvette as indicated above, but starting from a 3×10^{-3} M solution of $Na_2[Fe(CN)_5NO]\cdot2H_2O$. Monitoring in the complete UV/Vis range was performed on aliquots in a 1-mm quartz cuvette. ATR/FTIR spectra were obtained with a Thermo Mattson (model Genesis II) instrument. Resonance Raman spectra were measured with the 457.9 nm excitation line of an Ar^+ laser (Coherent Innova 70C) using a Jobin Yvon XY-800 spectrograph equipped with a liquid-nitrogen-cooled CCD camera. Accumulation times were typically 20 s, the increment per data point was 2 cm^{-1}, and laser power at the sample was 12 mW. All measurements were performed under constant stirring to avoid laser-induced damage. The ^1H NMR studies were performed on a Bruker 500 MHz instrument, with deoxygenated solutions of the reduced complex, at pH 6, containing 25% D_2O. The pH-independent ($pH>6.5$) singlet signal of the acetate-methylene group of CaEDTA ($\delta=2.45$ ppm) was used as integration reference in the titration experiments. The pH was measured outside the NMR tube.

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