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FTIR Investigation of the Intermediates Formed in the Reaction of Nitroprusside and Thiolates

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Nitroprusside, $[Fe(CN)_5(NO)]^{2-}$ (NP), was first synthesized in the middle of the 19th century, and it has been used as a qualitative indicator of sulfhydryl groups since its discovery.[1] Surprisingly, there exists no evidence for the structure of the transient "red products" (RPs) that are formed when NP reacts with thiolates, although they are widely postulated to be adducts of organothionitrites (RSNO). The hypotensive action of NP was first demonstrated 70 years ago,[2] and NP came into use as a drug more than 40 years ago.[3] The action of NO upon smooth muscle takes place intercellularly by binding to the active site of guanylate cyclase (GC).[4] It has long been recognized that NP is activated in vivo by one-electron reduction with ensuing release of cyanide.[5] It has been suggested that endogenous thiols are obligatory (as reductants or *vis-à-vis* RSNO adducts) for the initiation of the hypotensive actions of NP.[6] We describe in vitro time-resolved IR spectra that for the first time evidence the molecular structures of species formed by one-electron reduction of NP and the RP in aqueous media.

NP can be reduced by one electron to $[Fe(CN)_5(NO)]^{3-}$ (NP_1^-).[7] Earlier pulse radiolysis studies of the aqueous reduction of NP suggest NP_1^- rapidly loses CN^- to give $[Fe(CN)_4(NO)]^{2-}$ (NP_2^-).[8] Both NP_1^- and NP_2^- have been previously isolated by Na reduction of NP in anhydrous NH_3 and their IR spectra have been measured as Nujol mulls.[9] The crystal structure of $(NBu_4^+)_2$ NP_2^- has been determined.[10] We have studied the outer-sphere reduction of NP by several reagents including dithionite[11] and have for the first time measured the IR spectra of NP_1^- and NP_2^- in aqueous media. Equilibrium mixtures[12] of NP_1^- and NP_2^- can be prepared as a function of pH.[7] Since eq 2 involves the protonation of a strong base ($K_2 = 2.0 \times 10^9$), the ratio of NP_1^-:NP_2^- (eq 1, $K_1 = 6.6 \times 10^{-5}$) is determined by the $[CN^-]$.[13] Thus, NP_1^- and NP_2^- are both observed for $[NP]_{total} = 1-10$ mM at neutral pH in the absence of added CN^-. The observed ν_{NO} of 1648 and 1800 cm^{-1} for NP_2^- and NP_2^- (Figure 1)[14] is expected for a bent six-coordinate $\{MNO\}^7$ and linear five-coordinate $\{MNO\}$,[7] respectively.[15]

$$[Fe(CN)_5(NO)]^{3-} \rightleftharpoons [Fe(CN)_4(NO)]^{2-} + CN^- \qquad (1)$$

$$CN^- \ H^+ \rightleftharpoons HCN \qquad (2)$$

NP reacts rapidly with thiolate (not thiol)[16] to give an intense red solution. In the 150 years since this reaction was first discovered, there have been many suggestions regarding the structure of the chromophore,[17] but it is generally assumed that the RP is $[Fe(CN)_5(RSNO)]^{3-}$ (NP_{SR}^-), the result of nucleophilic attack of RS^- on the NO-based LUMO of NP.[18] We have measured the UV-visible and IR spectra that evolve after NP is reacted with EtS^-. These studies have identified a band at 1380 cm^{-1} (Figure 2, bottom) that correlates with loss of the red color (520 nm).[19,20] The band shifts to 1350 cm^{-1} when ^{15}NO-labeled NP (^{15}NP) is employed (Supporting Information).[21] Free RSNO compounds exhibit ν_{NO} stretches about 1500 cm^{-1}.[22] Assuming RSNO compounds are π-acid ligands, the ν_{NO} stretch of RSNO might be expected to decrease upon binding to an electron-rich transition-metal moiety such as $[Fe(CN)_5]^{3-}$.[23] In addition to the ν_{NO} stretch, ν_{CN} stretches at 2102, 2085 (sh), and 2072 cm^{-1} confirm the $Fe(CN)_5$ formulation.[24] Although there are no crystal structures of metal complexes of RSNO ligands, we note that related ligands that exhibit dominant O=N−X resonance structures tend to be N-bound,[25] whereas ligands with dominant O−N=X resonance structures are usually O-bound.[26] Given this precedent and the observed vibrational spectrum, we assign the molecular structure of the RP as $[Fe(CN)_5(\eta^1\text{-N-RSNO})]^{3-}$.

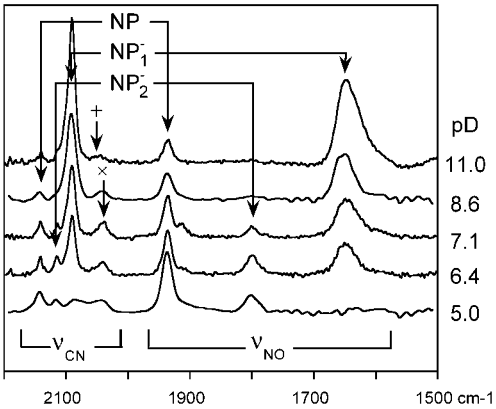

Figure 1. Initial IR spectra obtained of the reaction products of NP (10 mM) and $Na_2S_2O_4$ (5 mM) in 0.8 M K_3PO_4/DCl buffer in D_2O (ca. 100 ms after mixing) as a function of pH. A background consisting of the solvent system (buffer in D_2O) has been subtracted. Note the presence of unreacted NP and the different NP_1^- and NP_2^- ν_{NO} molar absorptivities. NP 2142 (ν_{CN}), 1936 (ν_{NO}); NP_1^- 2088 (ν_{CN}), 1648 (ν_{NO}); NP_2^- 2115 (ν_{CN}), 1800 (ν_{NO}); $[Fe(CN)_5(NO_2)]^{4-}$ (+) 2048 (ν_{CN}); $[Fe(CN)_6]^{4-}$ (×) 2038 (ν_{CN}). The low-frequency shoulder on the 1936 cm^{-1} band remains unidentified.

Figure 2 illustrates the time-dependent IR spectra that are measured when the RP of NP and EtSH decomposes at pD = 11. The initial spectrum shows ν_{CN} (2102, 2085 (sh), and 2072 cm^{-1}) and ν_{NO} (1380 cm^{-1}) bands that are assigned to the RP. Bands at 2088 and 1648 cm^{-1} due to NP_1^- and 2038 cm^{-1} due to ferrocyanide, $[Fe(CN)_6]$,$^{4-}$ are observed as the RP decomposes (see ref 7). The ferrocyanide ν_{CN} band rapidly obliterates the weak band at 2048 cm^{-1} present in the initial spectrum (labeled in Figure 1 but not in Figure 2) that corresponds to the nitro species $[Fe(CN)_5(NO_2)]$,$^{4-}$ the product of competitive nucleophilic attack by OH^-.[27] Note the principal reaction that is illustrated in Figure 2 is RP →NP_1^- (the molar absorptivity of ferrocyanide is an order

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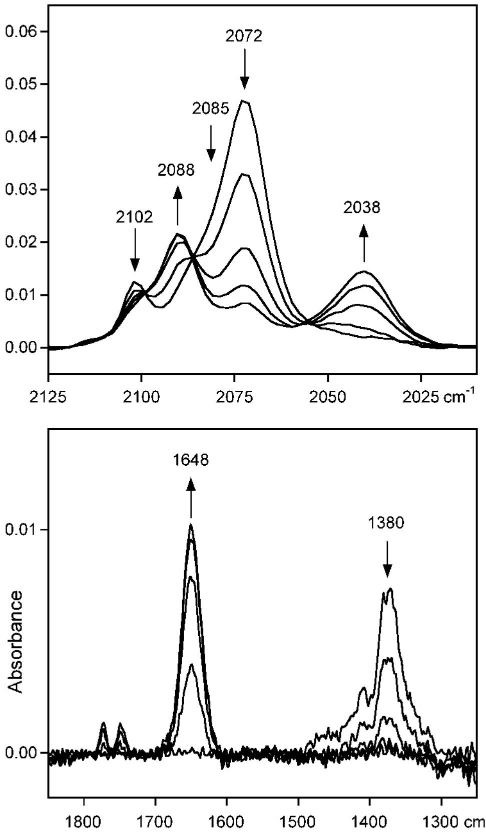

Figure 2. Reaction of 25 mM NP with 25 mM EtSH in 0.8 M pD = 11 K_3PO_4/DCl buffer. A background consisting of the solvent system (buffer in D_2O) has been subtracted. The time intervals shown were 0, 2, 5, 9, and 12 min. The CN stretches are shown at the top, and the NO stretches, at the bottom. Not shown, relatively small bands at 2142 and 1936 cm^{-1} corresponding to unreacted NP.

of magnitude larger than that of NP_1^-). The weak bands at 1773 and 1747 cm^{-1} are due to the ethyl ester of Roussin's red salt, $Fe_2(SEt)_2(NO)_4$,[28] which we sometimes observed as a minor product during the reduction of NP under anaerobic conditions. Importantly, there is a 1:1 correspondence between the loss of the RP and the formation of NP_1^-.

Under aerobic conditions (anaerobic conditions were employed in our study), NP is known to be a catalyst for the oxidation of thiols to disulfides.[29] Furthermore, NP reacts with enzymes (e.g., papain and glyceraldehyde phosphate dehydrogenase) that possess SH groups at their active sites to form disulfide bonds that lead to enzyme inhibition.[30] Our observations concerning the structure and fate of the RP (apparently homolytic cleavage of the N−S bond of the RP to yield NP_1^- and thiyl radical) are consistent with a mechanism of NP-catalyzed oxidation of thiols to disulfides that has been proposed previously.[31]

We point out that the reactions described here have been studied previously using primarily electronic and EPR spectroscopy. The present study using IR spectroscopy affords the first evidence of the molecular structures of intermediates. We also note that IR spectroscopy is superior to UV−visible and Raman spectroscopy for the study of NP and the RP since these species are subject to photolytic decomposition.[7,32−33] Since we have not probed the electronic spectrum when obtaining the data of Figure 2, we conclude the chemical transformations described herein (e.g., homolytic N−S bond cleavage) are dark reactions.

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Supporting Information Available: Experimental details and additional spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) This literature value for K_1, determined by pulse radiolysis,[8] is consistent with the spectra of Figure 1 and the observation (Supporting Information) that equimolar amounts of NP_1^- and NP_2^- are observed at pH = 7.22 (pD = 7.40) for. $[NP_1^-] + [NP_2^-] = 600\ \mu M$ (from which we calculate $K_1(pH = 7.22) = [CN^-]/(1 + K_2[H^+]) = 2.5 \times 10^{-6}$).
(14) In Nujol, $(NEt_4)_3[Fe(CN)_5(NO)]$: 2075 (ν_{CN}), 1568 (ν_{NO}); $(NEt_4)_2[Fe(CN)_4(NO)]$: 2110 (ν_{CN}, sh), 2100 (ν_{CN}), 1746 (ν_{NO}). See also the DFT study: Wanner, M.; Scheiring, T.; Kaim, W.; Slep, L. D.; Baraldo, L. M.; Olabe, J. A.; Záliš; Baerends, E. J. *Inorg. Chem.* **2001**, *40*, 5704.
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(20) The ν_{NO} stretch of NP_{SR}^- may be compared with the range of 1338−1394 cm^{-1} that has been computed for $[Fe(CN)_5(HNO)]^{3-}$ using DFT (González Lebrero, M. C.; Scherlis, D. A.; Estiú, G. L.; Olabe, J. A.; Estrin, D. A. *Inorg. Chem.* **2001**, *40*, 4127) and the value of 1385 cm^{-1} that has been measured for MbHNO (Farmer, P. J., Immoos, C. E.; Bocian, D. F.; Czarnecki, K. Personal communication.).
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(25) See for example the CSD refcodes: BANWUI, CMPBPU, CNSBZP, FAVCIO, FAVFIR, HISDUI, HISFAQ, HISFEU, HISFIY, HISFOE, KEHPOC, KEHPUI, MNSPPT, SASSEK, SOYZEL, ZONXEF.
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