Nitrosation of *N*-methylhydroxylamine by nitroprusside. A kinetic and mechanistic study†

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The kinetics of the reaction between aqueous solutions of $Na_2[Fe(CN)_5NO]\cdot 2H_2O$ (sodium pentacyanonitrosylferrate(II), nitroprusside, SNP) and MeN(H)OH (*N*-methylhydroxylamine, MeHA) has been studied by means of UV-vis spectroscopy, using complementary solution techniques: FTIR/ATR, EPR, mass spectrometry and isotopic labeling (^{15}NO), in the pH range 7.1–9.3, $I = 1$ M (NaCl). The main products were *N*-methyl-*N*-nitrosohydroxylamine (MeN(NO)OH) and $[Fe(CN)_5H_2O]^{3-}$, characterized as the $[Fe(CN)_5(pyCONH_2)]^{3-}$ complex ($pyCONH_2$ = isonicotinamide). No reaction occurred with Me_2NOH (*N*,*N*-dimethylhydroxylamine, Me_2HA) as nucleophile. The rate law was: $R = k_{exp}\,[Fe(CN)_5NO^{2-}] \times [MeN(H)OH] \times [OH^-]$, with $k_{exp} = 1.6 \pm 0.2 \times 10^5\ M^{-2}\ s^{-1}$, at 25.0 °C, and $\Delta H^{\#} = 34 \pm 3$ kJ mol^{-1}, $\Delta S^{\#} = -32 \pm 11$ J K^{-1} mol^{-1}, at pH 8.0. The proposed mechanism involves the formation of a precursor associative complex between SNP and MeHA, followed by an OH^--assisted reversible formation of a deprotonated adduct, $[Fe(CN)_5(N(O)NMeOH)]^{3-}$, and rapid dissociation of MeN(NO)OH. In excess SNP, the precursor complex reacts through a competitive one-electron-transfer path, forming the $[Fe(CN)_5NO]^{3-}$ ion with slow production of small quantities of N_2O. The stoichiometry and mechanism of the main adduct-formation path are similar to those previously reported for hydroxylamine (HA) and related nucleophiles. The nitrosated product, MeN(NO)OH, decomposes thermally at physiological temperatures, slowly yielding NO.

Introduction

The reactivity of the nitrosyl group bound to transition metal centers is a matter of increasing interest[1,2] in the context of studies on the chemistry and biochemistry of NO.[3] Addition reactions of nucleophiles (B) to nitrosonium (NO^+)-complexes have been considered for a long time.[4] Although a general stoichiometric picture is well established, detailed kinetic and mechanistic studies are scarce, mainly performed with sodium nitroprusside, $Na_2[Fe(CN)_5NO]\cdot 2H_2O$ (SNP).[5] The latter emphasis may be understood in terms of the well known hypotensive ability of SNP.[6]

Some biorelevant nucleophiles are OH^-,[7] nitrogenated species, *viz.*, NH_3,[8] amines and amino acids,[9] hydroxylamine (HA),[10] nitrite,[11] and thiolates, RS^- (cysteine, glutathione, *etc*).[12] The underlying mechanisms comprise an initial reversible addition of B to the NO^+ containing complex, of general formula $[MX_5(NO^+)]^x$ (M^{II} = Fe, Ru, Os; X = amines, polypyridines, porphyrins, *etc.*). The adducts, $\{[MX_5(N(O)B\}^x$, may be subsequently reactive through some type of reorganization, most commonly involving deprotonations and/or redox transformations, with oxidation of B and reduction of NO^+. Usually, the reactions comprise the nitrosation of the B substrates.[4,5] In addition to interest in the influence of the MX_5 fragment on the electrophilic ability of the bound NO^+-groups,[13] there is concern regarding the specific properties of the nucleophiles in determining the stoichiometry and mechanism of the addition reactions.[14]

We report a kinetic and mechanistic study of the reactions of *N*-methylhydroxylamine (MeHA) and *N*,*N*-dimethylhydroxylamine (Me_2HA) with SNP. This is complementary to the previous work with HA.[10] Recently, a diverse stoichiometric and mechanistic scenario was found for the nucleophilic additions of hydrazine (N_2H_4) and substituted derivatives: Me-hydrazine, 1,1-$(Me)_2$hydrazine and 1,2-$(Me)_2$hydrazine, toward SNP.[14] In contrast to the general common path leading to N_2O as a main reduction product of NO^+, the 1,2-$(Me)_2$hydrazine reactant led to the full six-electron reduction of NO^+ to NH_3, associated with the additional formation of a very stable product, azomethane (MeN=NMe). Such profound changes in the reactivity might have a significant biochemical relevance for HA-derivatives. HA is endogenously synthesized in the body and has a metabolic role in diverse processes.[3] Although the three compounds: HA, MeHA and Me_2HA form nitroxide radicals, only HA is able to generate free NO,[15] and it has been reported that the vasorelaxing activity of HA decreases with alkylation.[16] The reactions of HA and derivatives generating nitrosyl-compounds,[17] or reacting with them with formation of molecules with intermediate redox states (N_2, NO, N_2O) are an example of the rich chemistry associated with the natural nitrogen-redox cycles, including bacterial transformations in soils (nitrification and denitrification).[18]

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† Electronic supplementary information (ESI) available: ESR spectrum in aqueous solution of the $[Fe(CN)_4NO]^{2-}$ radical (Fig. 1) formed through cyanide labilization of the $[Fe(CN)_5NO]^{3-}$ intermediate, and Eyring plot for the calculation of activation parameters, including a Table with experimental rate constants at different temperatures, pH 8.0 (Fig. 2). See DOI: 10.1039/b805329d

Experimental

N-Methylhydroxylamine hydrochloride, MeN(H)OH·HCl (>98%) and *N*,*N*-dimethylhydroxylamine hydrochloride, $(Me)_2NOH$·HCl (>98%) were from Fluka, $Na_2[Fe(CN)_5NO]\cdot 2H_2O$ was from Merck, and isonicotinamide ($pyCONH_2$) was from Aldrich. All were analytical or reagent grade, and were used without further purification. $Na_2[Fe(CN)_5{}^{15}NO]\cdot 2H_2O$ was prepared as previously described,[19] by using $Na^{15}NO_2$ (99 atom% ^{15}N) from MSD Isotopes. *N*-Methyl-*N*-nitrosohydroxylamine as the sodium salt (Na[MeN(NO)O]) was synthesized according to literature procedures, see below.[20] Buffer solutions based on phosphates or borax and NaCl were prepared in the range $7.1 \pm 0.2 < pH < 9.3 \pm 0.2$, by using de-ionized water. Ionic strength was adjusted to 1 M with NaCl. A calibrated pH-meter (Hanna HI 9231) was employed, using Merck standard buffers.

UV-vis spectra and kinetics were measured on an Ocean Optics HR 2000, CG-UV-NIR diode array spectrophotometer, using quartz cuvettes. Some kinetic studies were carried out with a Hi-Tech Scientific SFA-20 stopped-flow accessory. The EPR spectra were measured with a Bruker ER 200D X-band spectrometer, previously calibrated with the reference spectrum of 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO, $a_N(NO) = 1.72$ mT, $g = 2.0051$) in water (1–5 μM).[21] FTIR/ATR spectra were recorded with a Perkin Elmer Spectrum BX spectrophotometer, equipped with a standard pre-mounted horizontal ATR accessory with a flat sampling plate of ZnSe. Argon saturated buffered solutions of the reactants; contained in 10 ml glass-syringes were mixed in a 500 μl homemade flow-cell mounted on the ZnSe crystal. The samples were transferred into the cell by aspiration with a syringe. The spectra were obtained in buffered solutions as differences from the SNP or MeHA solutions plus isonicotinamide as the used scavenger. An Extrel Emba II quadrupolar mass spectrometer was used for identifying gaseous products, as described elsewhere.[14,22]

The kinetic studies on the reaction between SNP and MeHA were mainly performed at 25.0 ± 0.2 °C, on buffered, argon saturated solutions, under pseudo-order conditions in SNP or MeHA, at different pHs. In excess MeHA conditions, the temperature range was extended to 12–40 °C for obtaining activation parameters. A huge excess of isonicotinamide was used as a scavenger for the $[Fe(CN)_5H_2O]^{3-}$ product (λ_{max} 440 nm, $\varepsilon = 640$ M^{-1} cm^{-1}),[23] forming the stable and intensely colored $[Fe(CN)_5(pyCONH_2)]^{3-}$ complex (λ_{max} 435 nm, $\varepsilon = 4570$ M^{-1} cm^{-1}).[24] This trapping procedure was useful for sensitivity purposes, and also allowed the exclusion of the main product $[Fe(CN)_5H_2O]^{3-}$ from the reaction medium, precluding its further reactivity with MeHA.[25] In a typical run, freshly prepared buffered solutions of 0.1–50 mM SNP were diluted in the spectrophotometer cell (1 or 0.1 cm path) containing an aliquot of 0.1–70 mM MeHA and 50–250 mM isonicotinamide, with final $I = 1$ M (NaCl).

For the synthesis of Na[MeN(NO)O], one gram of MeN(H)OH.HCl was dissolved in a minimum amount of ethanol in a 0.025 dm^3 round-bottom flask, equipped with a mechanical stirrer. The solution was neutralized with the theoretical amount of solid NaOH (0.48 g), and cooled below 0 °C in an ice–salt bath. Cooled *N*-butylnitrite (theoretical amount, 2.4 mL) was added very slowly through a funnel, maintaining the temperature of the reaction mixture below 10 °C. After stirring for 10 minutes, the mixture was concentrated under vacuum. The product, containing some NaCl, was filtered off and washed several times with small portions of cold diethyl ether.

Results

Fig. 1 shows the monoexponential increase in the absorbance of the final product, $[Fe(CN)_5(pyCONH_2)]^{3-}$, either in excess conditions of MeHA or of SNP, at 25.0 °C. No reaction at all was observed by using Me_2HA as the reactant. With excess MeHA (Fig. 1a), a nearly full conversion (95%) of initial SNP to $[Fe(CN)_5(pyCONH_2)]^{3-}$ was obtained, with no evolution of gaseous products. The degree of advancement of the reaction was also monitored by FTIR-ATR. Fig. 2 (top) shows the disappearance of the initial stretching modes of cyanide (ν_{CN}, 2142 cm^{-1}), and nitrosyl (ν_{NO}, 1935 cm^{-1}) in SNP.[26] The new band at 2042 cm^{-1} (ν_{CN}) reflects the formation of $[Fe(CN)_5(pyCONH_2)]^{3-}$.[27] In Fig. 2 (bottom), other bands at 953, 1057, 1217, 1278 and 1370 cm^{-1} can be appreciated, corresponding to the diazeniumdiolate functional group $[-N(NO)O]^-$, a structure that is present in *N*-methyl-*N*-nitrosohydroxylamine, MeN(NO)OH.[28,29] The overall stoichiometry may be described by eqn (1) and (2):

$$[Fe(CN)_5NO]^{2-} + MeN(H)OH + OH^- \rightarrow [Fe(CN)_5H_2O]^{3-} + MeN(NO)OH \quad (1)$$

$$[Fe(CN)_5H_2O]^{3-} + pyCONH_2 \rightarrow [Fe(CN)_5(pyCONH_2)]^{3-} + H_2O \quad (2)$$

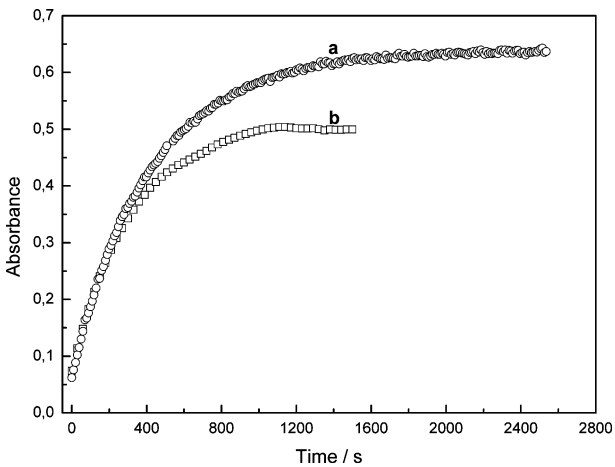


Fig. 1 Absorbance of $[Fe(CN)_5(pyCONH_2)]^{3-}$, measured at 440 nm, *vs* time for the reaction of SNP with MeHA in the presence of 140 mM $pyCONH_2$, pH 8.7, $I = 1$M (NaCl), $T = 25.0$ °C. (a) SNP 0.14 mM, MeHA 3.1 mM; (b) SNP 3.5 mM, MeHA 0.154 mM.

Reaction (2) has been independently measured by stopped-flow techniques, with $k_2 = 296$ M^{-1} s^{-1} at 25.0 °C.[30] Under the selected working conditions (Table 1), reaction (2) evolves several orders of magnitude faster than (1). The MeN(NO)OH product was additionally characterized in the clarified reaction solutions by UV-vis spectroscopy, after room-temperature vacuum distillation of the exhausted reaction mixtures.[28,29] The spectra and other properties of this compound were identical to those from synthetic Na[MeN(NO)O] (see Experimental section). The protonated structure MeN(NO)OH absorbs at *ca.* 230 nm

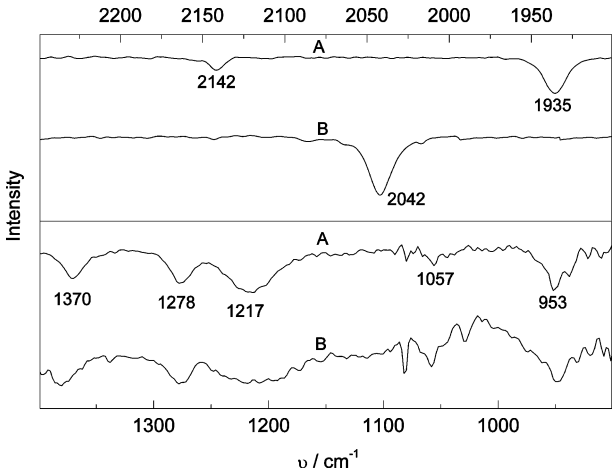


Fig. 2 HATR (horizontal attenuated total reflectance) spectra for the addition reaction, with 200 mM MeHA and 14 mM SNP, in the presence of 200 mM $pyCONH_2$, pH 9.3, I = 1M (NaCl), room temperature. Top: (A) Spectrum of the reactant, SNP. (B) ν_{CN} for the $[Fe(CN)_5(pyCONH_2)]^{3-}$ product. Bottom: (A) Spectrum of independently synthesized $Na[CH_3N(NO)O]$ in aqueous solution. The characteristic frequencies of the [–N(NO)O$^-$] group are indicated. (B) Spectrum of the reaction product.

Table 1 Rate constants for the reaction of SNP with MeHA at different concentrations of reactants and pH[a,b]

SNP/mM	MeHA/mM	pH	$10^3\ k_{obs}/s^{-1}$	$10^{-5}\ k_{exp}/M^{-2}\ s^{-1}$
82.0	1.9	7.1	2.30	2.0
39.0	3.7	7.1	0.74	1.5
3.2	6.0	7.1	0.10	1.4
0.63	11.0	7.1	0.22	1.6
1.2	16.4	7.1	0.37	1.8
0.23	25	7.1	0.50	1.6
2.2	35.3	7.5	1.98	1.8
2.2	54.5	7.5	2.83	1.6
0.18	3.6	8.2	0.82	1.4
0.19	7.0	8.2	1.53	1.4
3.5	0.07	8.7	3.20	1.8
3.1	0.14	8.7	2.30	1.5
0.13	1.4	8.8	1.32	1.5
0.13	2.8	8.8	2.80	1.6
0.25	6.9	8.8	6.71	1.6
0.36	3.1	9.3	10.0	1.6
0.08	3.2	9.3	10.3	1.6
0.08	6.5	9.3	21.9	1.7

[a] Monitored at 440 nm, 25 °C, I = 1 M (NaCl). The concentration of OH$^-$ was computed from the pH, with $K_w = 1.0 \times 10^{-14}$. [b] Triplicate runs were averaged for each entry.

($\varepsilon \approx 7000\ M^{-1}\ cm^{-1}$), and the [MeN(NO)O]$^-$ form shifts to *ca.* 250 nm ($\varepsilon \approx 8000\ M^{-1}\ cm^{-1}$).

The stoichiometric and kinetic picture is shown to be significantly different when using excess SNP over MeHA. A yield of only *ca.* 75% of $[Fe(CN)_5(pyCONH_2)]^{3-}$ is achieved in the minutes time scale (Fig. 1b). More $[Fe(CN)_5(pyCONH_2)]^{3-}$ is formed through a very slow process, which also reveals the formation of N_2O.

Additional, direct evidence for the presence of a possible competitive path to the main process described by eqn (1) and (2) comes from FTIR and EPR measurements.

In addition to the markers for SNP consumption and for the formation of $[Fe(CN)_5(pyCONH_2)]^{3-}$, Fig. 3 shows a new cyanide absorption at 2086 cm^{-1}. Fig. 1 ESI † shows an EPR spectrum containing a 1 : 1 : 1 triplet characterized by $a_N(NO) = 1.47$ mT and $g = 2.0293$. Both IR and EPR new features support the formation of the $[Fe(CN)_5NO]^{3-}$ radical.[26,31]

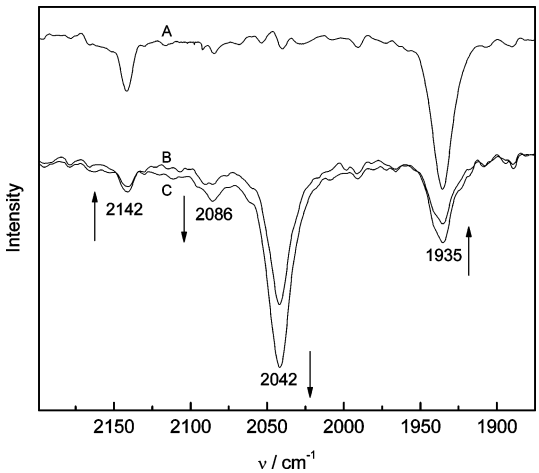


Fig. 3 HATR spectra for the addition reaction, with 600 mM SNP, 23 mM MeHA, 200 mM $pyCONH_2$, pH 9.3, I = 1 M (NaCl), room temperature. (A) Initial spectrum of SNP. (B and C) Spectra during reaction progress.

As seen in Fig. 1a, the individual kinetic runs for the buildup of $[Fe(CN)_5(pyCONH_2)]^{3-}$ gave good single exponential behavior ($r^2 \geq 0.999$), yielding the pseudo-first order rate constants, k_{obs} (Table 1), which display a first-order dependence on the analytical concentrations of MeHA and OH$^-$.

Activation parameters obtained through an Eyring plot lead to $\Delta H^{\#} = 34 \pm 3$ kJ mol^{-1} and $\Delta S^{\#} = -32 \pm 11$ J K^{-1} mol^{-1}, at pH 8.0 (Fig. 2 ESI†). Given the specific stoichiometric features appearing when SNP is in excess over MeHA (Fig. 1b), we used the initial rate method for the kinetic analysis of the exponential increase.[32] The resulting rates were proportional to the initial concentration of MeHA, yielding k_{obs} values (Table 1), which also correlated linearly with the concentrations of SNP and OH$^-$.

Fig. 4 summarizes the overall kinetic results through a logarithmic plot. We conclude that the same third-order rate law holds for the nucleophilic addition process, either in excess conditions of MeHA or SNP, eqn (3):

$$R = k_3 \times [Fe(CN)_5NO^{2-}] \times [MeN(H)OH] \times [OH^-] \qquad (3)$$

A linear fit of the data yields $k_3 = k_{exp} = (1.6 \pm 0.2) \times 10^5\ M^{-2}\ s^{-1}$ (T = 25.0 °C). The unreactivity of the MeN(NO)OH product toward $[Fe(CN)_5NO]^{2-}$ or $[Fe(CN)_5H_2O]^{3-}$ was monitored by performing independent experiments. No radical formation could be detected in these assays.

A final, independent experiment, showed the formation of ^{15}NO (m/z 31) and $^{15}N_2O$ (m/z 46) when the exhausted solutions were warmed at 35–40 °C.

Discussion

The value of $k_{exp} = (1.6 \pm 0.2) \times 10^5\ M^{-2}\ s^{-1}$ for the nucleophilic addition reaction is only slightly smaller than that found in

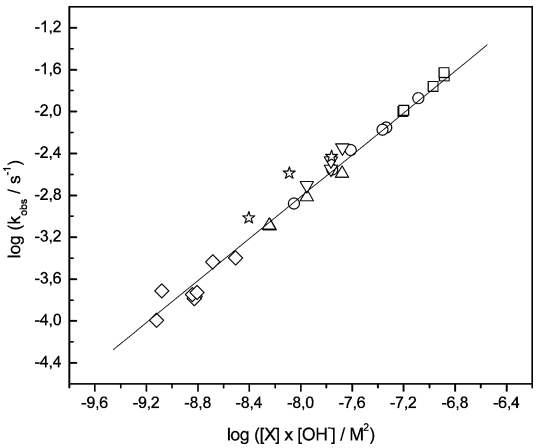


Fig. 4 Logarithmic plot of the pseudo-first-order rate constants (k_{obs}, s^{-1}) *vs* ([X] × [OH^-]/M^2) with [X] = MeHA or SNP, at I = 1 M (NaCl), 25.0 °C. Excess of MeHA: □, pH 9.3; ○, pH 8.8; △, pH 8.2; ▽, pH 7.5; ◇, pH 7.1. Excess of SNP: ☆, pH 8.7 and 7.1.

the reaction of SNP with HA, k_{exp} = 4.5 × 10^5 M^{-2} s^{-1}, at 25.0 ± 0.2 °C.[10] We propose a three-step mechanism involving an associative pre-equilibrium between the two main reactants (eqn (4)), followed by an OH^--assisted process forming an addition intermediate (eqn (5)), in which the N-atom of MeHA forms a covalent bond with the N-atom of the NO^+ ligand in SNP. Reaction (6) describes the dissociation of the adduct through the cleavage of the Fe–N bond, releasing a nitrosation product, *N*-methyl-*N*-nitrosohydroxylamine.

$$[Fe(CN)_5NO]^{2-} + MeN(H)OH \rightleftarrows \{[Fe(CN)_5NO]^{2-}\cdot[MeN(H)OH]\} \quad (4)$$

$$\{[Fe(CN)_5NO]^{2-}\cdot[MeN(H)OH]\} + OH^- \rightleftarrows [Fe(CN)_5(N(O)NMeOH)]^{3-} + H_2O \quad (5)$$

$$[Fe(CN)_5(N(O)NMeOH)]^{3-} + H_2O \rightarrow [Fe(CN)_5(H_2O)]^{3-} + MeN(NO)OH \quad (6)$$

A steady-state mechanistic treatment for the adduct-intermediate leads to a rate law which is consistent with the experimentally found one. Following the fast prior equilibrium (eqn (4), with K_4 = *ca.* 0.5 M^{-1}),[13] the value of k_{exp} in eqn (3) can be equalized to $k_{exp} = K_4 \times k_5 \times k_6/(k_{-5} + k_6)$. Two limiting cases may be considered: (a) If $k_{-5} >> k_6$, then $k_{exp} = K_4 \times K_5 \times k_6$, and the rate law would represent a process controlled by the slow adduct decomposition reaction (6). (b) If $k_{-5} << k_6$, then $k_{exp} = K_4 \times k_5$. As discussed below, the latter situation is more plausible, and means that the intermediate formation step is rate-controlling. A significant reorganization energy has been calculated for the conversion of linear Fe–NO^+ to bent Fe–N(O)B structures for adducts with B = OH^- and nitrogen hydrides.[13,14,33] Different mechanistic interpretations have been given in the literature to the values of the experimental rate constants.[7–10,12–14] For nucleophiles forming fairly stable and detectable adducts (B = OH^-, SR^-), the reactions have been studied by monitoring the absorbance increase of the deprotonated intermediates.[7,12,13] Thus, the mechanism can be described by reactions like (4) and (5), with (6) being irrelevant under these conditions. In contrast, the adducts become undetectable for most of the nitrogen hydrides, suggesting a fast decomposition, leading to $[Fe(CN)_5(H_2O)]^{3-}$ as the final product. Exceptionally, an intermediate could be detected in the reaction of azide with SNP, under similar conditions to those in the present study, showing a decomposition rate of 0.2 s^{-1}, independent of azide concentration.[10,34] It is highly feasible that other N-binding adducts may reorganize and dissociate with rates of the same order of magnitude, given that similar Fe–N bonds must be broken.

As reviewed recently,[5] a generalized approach to nucleophilic reactivity gives a strong support to mechanistic pathways involving a comparatively slow formation of the adduct-intermediates. Further evidence in favour of the sequence (4)–(6) with a slow k_5 step is given by the requirement that a removable H-atom must be placed at the adjacent position of the nucleophilic N-atom of HA or MeHA. For this reason, Me_2HA was shown to be unreactive, as was the case with NMe_3.[8] The modest decrease of k_{exp} for MeHA *vs* HA is of the same order of magnitude as found for hydrazine and its methylsubstituted derivative,[14] probably related to steric effects of the Me group influencing either the slower formation of the adduct or its faster back dissociation (eqn (5)). Finally, the negative values of the activation entropies found by us and others[5,7,10,12] are also consistent with an associative mechanism for the nucleophilic additions, as well as with k_5 behaving as rate-controlling.[35]

The formation of NO at physiological temperatures may be traced to reaction (7), namely a secondary process involving bimolecular decomposition of *N*-methyl-*N*-nitrosohydroxylamine, also producing azoxymethane.[28] In this way, MeHA might behave as a slow NO-donor,[36] in contrast with HA, which rapidly generates N_2O.

$$2\ MeN(^{15}NO)OH \rightarrow MeN{=}N(O)Me + H_2O + 2\ ^{15}NO \quad (7)$$

Finally, the complex stoichiometry described above in excess SNP conditions (Fig. 1b) must be accounted for. By considering the previously analyzed mechanistic framework, we propose that the precursor complex (eqn (4)) is competitively reactive with respect to the adduct-intermediate production (eqn (5)), through a new electron-transfer channel. The indirect (decreased initial SNP consumption) and direct experimental evidence (IR, EPR) allow us to propose reaction (8), accordingly with the formation of $[Fe(CN)_5NO]^{3-}$ and undetermined oxidized products of MeHA.[37,38]

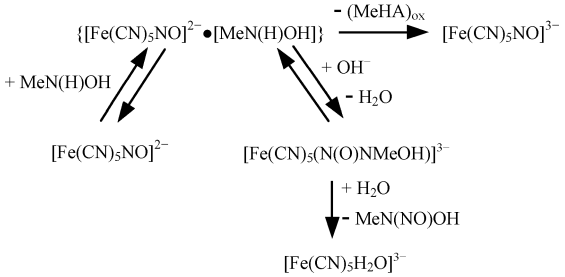
$$\{[Fe(CN)_5NO]^{2-}\cdot[MeN(H)OH]\} \rightarrow [Fe(CN)_5NO]^{3-} + (MeHA)_{ox} \quad (8)$$

Alternative paths for SNP reductions, involving either direct one-electron transfer leading to $[Fe(CN)_5NO]^{3-}$ or nucleophilic additions (formal two-electron transfer) have also been described for the reactions of thiolates with SNP, without clear kinetic discrimination.[39] Finally, the very slow conversion of $[Fe(CN)_5NO]^{3-}$ into $[Fe(CN)_5(pyCONH_2)]^{3-}$ may be traced to the small dissociation rates of NO from $[Fe(CN)_5NO]^{3-}$ or $[Fe(CN)_4NO]^{2-}$ (the latter is formed through cyanide labilization at pHs < 9), with k_{-NO} = *ca.* 10^{-5} s^{-1} at 25 °C.[40] It has been shown that the release of NO leads to an intermediate dinitrosyl species, proposed to be $[Fe(CN)_4(NO)_2]^{2-}$, which further disproportionates into SNP and N_2O. We believe that in the hours-time scale of the reactions occurring subsequently to eqn (8), this is a feasible path for N_2O formation.[40] The evidence comes from the

unique $^{15}N_2O$ product, free of mixed species with ^{14}N. Thus, all the N_2O comes from ^{15}N originally present in labeled SNP. We must discard a reduction event at the nitrosohydroxylamine moiety.

Conclusions

The reaction of MeHA with SNP evolves as described in Scheme 1. An initial association of reactants is followed by a predominant path with reversible adduct-formation, implying a covalent attachment of the nucleophilic N-atom of MeHA to the N-atom of the nitrosonium ligand in SNP, with OH^--assisted deprotonation. Further fast cleavage of the Fe–N bond gives $[Fe(CN)_5H_2O]^{3-}$ and a soluble nitrosation product, *N*-methyl-*N*-nitrosohydroxylamine (MeN(NO)OH). With excess SNP, a competitive electron-transfer path is available for the initial association complex, leading to $[Fe(CN)_5NO]^{3-}$, which subsequently decomposes very slowly forming N_2O. Although the third-order rate laws in the concentrations of complex, nucleophile and OH^- are the same for the reactions of SNP with HA and MeHA, with closely similar rate constants, facile deprotonation processes allow the formation and rapid release of N_2O in the case of HA. In contrast, the presence of the Me group favors the dissociation of MeN(NO)OH. This is a toxic chemical, behaving as a slow NO-donor, and could react similarly to the *N*-nitrosamines, which are well-known chemical carcinogens that are metabolized to strongly alkylating electrophiles reacting with DNA at several nucleophilic sites.[41]



Scheme 1

Acknowledgements

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35 The values calculated by us through an Eyring plot of the data for the addition of HA[10] are: $\Delta H^{\#} = 40 \pm 3$ kJ mol^{-1} and $\Delta S^{\#} = -13 \pm 2$ J K^{-1} mol^{-1}, in expected close agreement with the presently reported ones for MeHA. As the measured values reflect composite quantities (reactions (4) and (5)), we estimated the activation parameters for reaction (4) as *ca.* 1.5 kJ mol^{-1} and 8 J K^{-1} mol^{-1} for $\Delta H^{\#}$ and $\Delta S^{\#}$, respectively,[13] revealing a minor contribution.
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path under excess SNP conditions, given that NO^+ maintains its oxidizing ability in the precursor complex. On the other hand, with an excess of MeHA, the adduct-formation path becomes much favored because of the H-bonding stabilization of the adduct, associated with the specific interactions between MeHA and the donor cyano-ligands[38].

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