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Nitrosation, Thiols, and Hemoglobin: Energetics and Kinetics

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ABSTRACT: Nitrosothiols are powerful vasodilators. Although the mechanism of their formation near neutral pH is an area of intense research, neither the energetics nor the kinetics of this reaction or of subsequent reactions have been addressed. The following considerations may help to guide experiments. (1) The standard Gibbs energy for the homolysis reaction RSNO → RS$^{\bullet}$ + NO$^{\bullet}$(aq) is +110 ± 5 kJ mol^{-1}. (2) The electrode potential of the RSNO, H^{+}/RSH, NO$^{\bullet}$(aq) couple is −0.20 ± 0.06 V at pH 7. (3) Thiol nitrosation by NO_2^- is favorable by 37 ± 5 kJ mol^{-1} at pH 7. (4) N_2O_3 is not involved in in vivo nitrosation mechanisms for thermodynamic — its formation from NO_2^- costs 59 kJ mol^{-1} — or kinetic — the reaction being second-order in NO_2^- — reasons. (5) Hemoglobin (Hb) cannot catalyze formation of N_2O_3, be it via the intermediacy of the reaction of Hb[FeNO$_2$]$^{2+}$ with NO$^{\bullet}$ (+81 kJ mol^{-1}) or reaction of Hb[FeNO]$^{3+}$ with NO_2^- (+88 kJ mol^{-1}). (6) Energetically and kinetically viable are nitrosations that involve HNO_2 or NO$^{\bullet}$ in the presence of an electron acceptor with an electrode potential higher than −0.20 V. These considerations are derived from existing thermochemical and kinetics data.

$$\Delta_{BDE}H^{\circ}\ RS-NO_g \rightarrow \left| \begin{array}{l} \Delta_{BDE}G^{\circ}\ RS-NO_{aq} \\ E^{\circ\prime}\ (RSNO, H^+/RSH, NO^{\bullet}_{aq}) \\ \Delta_{rxn}G^{\circ\prime}\ (RSH + HNO_2 \rightarrow RSNO + H_2O) \end{array} \right.$$

INTRODUCTION

How nitrogen monoxide can escape from blood to contribute to relaxation of blood vessels is an unsolved mystery. NO$^{\bullet}$ in blood is rapidly consumed by binding to deoxyhemoglobin[1,2] and reaction with oxyhemoglobin.[3−5] Nitrosation of a thiol or formation of dinitrosyl iron complexes may be a way to preserve NO$^{\bullet}$, although reduction by one electron is necessary to set NO$^{\bullet}$ free from a nitrosothiol. The energetics of these reactions have not been addressed. I show here that standard Gibbs energies and electrode potentials and the rate constants derived from these are easily calculated. The results allow one to eliminate reaction mechanisms and thereby to focus on possible pathways.

NITROSATION BY NO_2^-

Standard Gibbs Bond Dissociation Energy of RS−NO. The energetics of nitrosation of thiols require knowledge of the Gibbs energy of reaction 1, in which RSNO represents a nitrosated cysteine as in *S*-nitrosoglutathione

$$RSNO(aq) \rightarrow RS^{\bullet}(aq) + NO^{\bullet}(aq) \quad (1)$$

For CH_3CH_2SNO, Bartberger et al. calculated a bond dissociation energy of 134 kJ mol^{-1} and a Gibbs dissociation energy of 89.5 kJ mol^{-1} in the gas phase.[6] With the same ab initio technique, Baciu and Gauld reproduced this value and calculated a slightly higher bond dissociation energy of 139 kJ mol^{-1} for nitrosocysteine.[7] Assuming that the $-T\Delta S$ terms for both nitroso compounds are the same, −44.5 kJ mol^{-1}, one arrives at a gas-phase Gibbs bond dissociation energy of nitrosocysteine of 94.5 kJ mol^{-1}. To derive a Gibbs bond dissociation energy that is valid in water, we must dissolve nitrosocysteine, cysteine, and NO$^{\bullet}$. To a first approximation, the hydration energies of cysteine and nitrosocysteine are assumed to be the same. Additionally, NO$^{\bullet}$ needs to be dissolved, which costs 15 kJ mol^{-1} (Table 1). Thus, in water, $\Delta_{rxn}G^{\circ}{}_1$ = +110 kJ mol^{-1} with an estimated error of 5 kJ mol^{-1}, which reflects the uncertainty in the ab initio calculations and the fact the hydration energies do not fully cancel because R−SNO is more polar than R−SH.[8,9]

How easily is RSNO reduced by one electron to liberate NO$^{\bullet}$? The electrode potential of the RSNO, H^{+}/RSH, NO$^{\bullet}$(aq) couple, reaction 2, follows from addition of reactions 1 and 3 (Table 1) and is −0.20 ± 0.06 V at pH 7 vs the normal hydrogen electrode.

$$RSNO + H^+ + e^- \rightarrow RSH + NO^{\bullet} \quad (2)$$

$$RS^{\bullet} + e^- + H^+ \rightarrow RSH \quad (3)$$

Monohydrogenascorbate, with $E^{\circ\prime}$(asc$^{\bullet-}$, H^{+}/Hasc^{-}) = +0.28 V,[10] should thus not reduce RSNO, as observed. On the basis of this observation and that dithionite did reduce RSNO, Bohle and co-workers concluded that the electrode potential was less than 0 V,[11] in agreement with the present estimate. A value of −0.20 V implies that, in the presence of redox couples with electrode potentials larger than that value, generation of NO$^{\bullet}$(aq) is uphill. On the other hand, redox couples with such potentials would help formation of RSNO from RSH and NO$^{\bullet}$. Indeed, iron is known to help in formation of nitrosothiols.[12]

Energetics of Nitrosation. The energetics of nitrosation by HNO_2 are now calculated by addition of reactions −1, −3, and 4 (Table 1), in which RSH stands for glutathione and represents thiols in general. Here and below frequent use is made of the equalities $\Delta_{rxn}G^{\circ} = -RT\ln K = -n\mathrm{F}\Delta E^{\circ}$ in which R is the gas constant, *n* the number of electrons in the reaction equation, and F the Faraday constant. Nitrosation of RSH by

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Table 1. Thermodynamic and Kinetic Quantities[a]

property	molecule, reaction	value at 25 °C	ref
$\Delta_f G°$		kJ·mol^{-1}	
	NO_2^-	−38	41
	H_2O (l)	−237.13	42
	$NO^•$(g)	+86.5	42
	$NO_2^•$(g)	+51.3	42
	N_2O_3(g)	+139.5	42
Henry constant		M/100 kPa	
	$NO^•$	1.93×10^{-3}	43,44
	$NO_2^•$	1.1×10^{-2}	45,46
	N_2O_3	0.70	47
$\Delta_f G°$		kJ·mol^{-1}	
	$NO^•$(aq)	+102	*b*
	$ONOO^•$(aq)	+117 ± 10	32
	$NO_2^•$(aq)	+62.5	*b*
	N_2O_3(aq)	+140	*b*
$E°$		V vs NHE	
$E°'_2$	RSNO, H^+/RSH, $NO^•$(aq)	−0.20 ± 0.06 (pH 7)	*c*
$E°'_3$	$RS^•$, H^+/RSH	+0.94 ± 0.03 (pH 7)	15
$E°_4$	HNO_2, H^+/$NO^•$(aq), H_2O	+0.81 (pH 0)	*b*
$E°'_4$		+0.18 (pH 7)	
$E°_8$	$NO_2^•$(aq)/NO_2^-	+1.04	41
$E°'_{-15}$	$HbFe^{3+}$/$HbFe^{2+}$	+0.122 (pH 7.1, 0.2 M Cl^-)	51
$E°'_{18}$	$CytcFe^{3+}$/$CytcFe^{2+}$	+0.26 V (pH 7)	52
$E°$	$HbFe^{3+}$, $NO^•$(aq) /$Hb[FeNO]^{2+}$	+0.71 (T state) +0.80 (R state)	*c* *c*
$E°$	$Hb[FeNO]^{3+}$/ $Hb[FeNO]^{2+}$	+0.47 (T state) +0.54 (R state)	*c* *c*
$E°'$	$S^{•-}$, H^+/HS^-	+0.92 V (pH 7)	16
	$S^{•-}$, $2H^+$, H_2S	+0.92 V (pH 7)	*c*

property	molecule, reaction	value at 25 °C	ref
	$\Delta_{rxn}G°$, *K*, *k*		
$\Delta_{rxn}G°_1$	$RSNO \rightarrow RS^• + NO^•$	+110 ± 5 kJ·mol^{-1}	*c*
$\Delta_{rxn}G°_5$	$RSH + NO_2^- + 2H^+ \rightarrow RSNO + H_2O$	−37 ± 5 kJ·mol^{-1}	*c*
$\Delta_{rxn}G°_6$	$2NO_2^- + 2H^+ \rightarrow N_2O_3$(aq) + H_2O	−21 ± 3 kJ·mol^{-1}	*b*
$\Delta_{rxn}G°'_6$ (pH 7)		+59 ± 3 kJ·mol^{-1}	*b*
K_6 (pH 7)		4.5×10^{-11} M^{-1} [d]	*b*
k_6, k_{-6} (pH 7)		$k_6 = 2.4 \times 10^{-8}$ M^{-1} s^{-1}, $k_{-6} = 5.3 \times 10^2$ s^{-1}	48
$\Delta_{rxn}G°_7$	N_2O_3(aq) → $NO^•$(aq) + $NO_2^•$(aq)	+24.0 kJ/mol	*b*
K_7		6.1×10^{-5} M^{-1}	48,49
k_7, k_{-7}		$k_7 = 8.0 \times 10^4$ s^{-1}, $k_{-7} = 1.1 \times 10^9$ M^{-1}s^{-1}	48
$\Delta_{rxn}G°'_{10}$	$HbFe^{2+} + NO_2^- + 2H^+ \rightarrow HbFe^{3+} + NO^•$(aq) + H_2O	−6 kJ mol^{-1} (pH 7)	*c*
k_{10}		1.0 M^{-1} s^{-1}	31
$\Delta_{rxn}G°_{11}$	$HbFe^{3+} + NO_2^- \rightarrow Hb[FeNO_2]^{2+}$	−16 to −19 kJ mol^{-1}	21,50
K_{11}	Hb-$Fe^{3+} + NO_2^- \rightarrow Hb[FeNO_2]^{2+}$	2.0×10^3 M^{-1} (37 °C, pH 7.4), 0.56×10^3 M^{-1} (pH 7.4)	21,50
$\Delta_{rxn}G°_{12}$	$Hb[FeNO_2]^{2+} + NO^•$(aq) → $HbFe^{2+} + N_2O_3$(aq)	+81 kJ mol^{-1}	*c*
K_{13}	$HbFe^{3+} + NO^•$(aq) → $Hb[FeNO]^{3+}$	1.3×10^4 M^{-1}	25
$\Delta_{rxn}G°_{14}$	$Hb[FeNO]^{3+} + NO_2^- \rightarrow HbFe^{2+} + N_2O_3$	+88 kJ mol^{-1}	*c*
$\Delta_{rxn}G°'_{16}$	$Hb[FeNO]^{3+} + H_2O \rightarrow HbFe^{2+} + NO_2^- + 2H^+$	+22 kJ mol^{-1} (pH 7)	*c*
	$HbFe^{2+} + NO^•$(aq) → $Hb[FeNO]^{2+}$	8.7×10^9 M^{-1} (T state), 1.7×10^{11} M^{-1} (R state)	25

[a]Errors, where indicated, are estimates. Numerical subscripts refer to equations in the text. [b]The value follows directly from the literature values quoted in the table. [c]The value is derived in the text. [d]Value corrected for pH and the standard state of water.

$$RS^• + NO^•(aq) \rightarrow RSNO \quad (-1)$$
$$NO_2^- + 2H^+ + e^- \rightarrow NO^•(aq) + H_2O \quad (4)$$
$$\underline{RSH \rightarrow RS^• + e^- + H^+ \quad (-3)}$$
$$RSH + NO_2^- + 2H^+ \rightarrow RSNO + H_2O \quad -37 \pm 5 \text{ kJ mol}^{-1} \quad (5)$$

HNO_2 is thus favorable by -37 ± 5 kJ mol^{-1} at pH 7, which compares well with the −33 kJ mol^{-1} derived from the equilibrium constant of 6×10^5 M^{-1} listed by Williams[13] for cysteine. What do these Gibbs energies mean? Given a concentration of 0.5 μM nitrite, the ratio of RSNO to RSH should be between 0.5:1 and 1:1. Thus, given millimolar concentrations of thiols, one would also expect millimolar concentrations of nitrosothiols. This is not found, which may show that production is rate limiting.

Nitrosation may also involve two NO_2^- and N_2O_3 as an intermediate according to the following set of reactions (see Table 1),

$$2NO_2^- + 2H^+ \rightarrow N_2O_3(aq) + H_2O \quad (6)$$
$$N_2O_3(aq) \rightarrow NO^•(aq) + NO_2^•(aq) \quad (7)$$
$$NO_2^•(aq) + e^- \rightarrow NO_2^{•-} \quad (8)$$
$$RSH \rightarrow RS^• + e^- + H^+ \quad (3)$$
$$\underline{RS^• + NO^• \rightarrow RSNO \quad (-1)}$$
$$RSH + 2NO_2^- + 2H^+ \rightarrow RSNO + H_2O + NO_2^- \quad (9)$$

but the overall energetics are those of reaction 5, −37 kJ mol^{-1}. Nitrosation by the simplest of nitrosothiols, HSNO, is ca. 10 kJ mol^{-1} less favorable, as is easily calculated from the minor difference between the bond strengths, that of RS−NO being ca. 12 kJ stronger[6] than that of HS−NO,[14] and between the electrode potentials, $E°'_3(RS^•,H^+/RSH) = +0.94$ V[15] and $E°'(S^{•-},H^+/HS^-) = +0.92$ V,[16] at pH 7. Given a pK_1 of H_2S of 7.1, this value also applies to $E°'(S^{•-},2H^+/H_2S)$ at pH 7. Consequently, transnitrosation of RSH by HSNO is downhill by the same amount.

NITROSATION BY N_2O_3

One or Two NO_2^-? Given that nitrosation is possible with one or two NO_2^-, we now ask which reaction is more likely. An interesting observation, published in 2003,[17] is that a concentration of ca. 2.5 μM in blood causes some vasodilation with a much larger effect observed at about 200 μM. How can one produce $NO^•$, or RSNO, from NO_2^- and deliver the former to the endothelial cell from where it can diffuse into the muscle layer surrounding the blood vessel? Basu et al.[18] followed up on a proposal by Robinson and Lancaster[19] that deoxyhemoglobin catalyze formation of N_2O_3 from NO_2^- according to reactions 10−12. The advantage of N_2O_3 is that it does not interact with Fe^{2+} in hemoglobin.

$$HbFe^{2+} + NO_2^- + 2H^+ \rightarrow HbFe^{3+} + NO^•(aq) + H_2O \quad (10)$$

$$HbFe^{3+} + NO_2^- \rightarrow Hb[FeNO_2]^{2+} \quad (11)$$

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$$Hb[FeNO_2]^{2+} + NO^{\bullet}(aq) \rightarrow HbFe^{2+} + N_2O_3(aq) \quad (12)$$

Addition of Reactions 10−12 results in reaction 6; thus, hemoglobin is thought to act as a catalyst. As shown in Table 1, $\Delta_{rxn}G^{\circ\prime}{}_6$ = +59 kJ mol^{-1} at pH 7, which, given a plasma concentration of 2.5 μM NO_2^-,[17] results in an equilibrium concentration of 2.8 × 10^{-22} M N_2O_3. Given that the rate of hydrolysis, k_{-6}, is known (Table 1), k_6 is 2.4 × 10^{-8} M^{-1} s^{-1}. Half-lives of Reactions 6 and −6 can now be calculated. The $t_{1/2}$ of hydrolysis of N_2O_3 (reaction −6) is (ln 2)/k_{-6} or 0.693/530 s^{-1} = 1.3 ms. The $t_{1/2}$ of reaction 6 is given by 1/(k_6[NO_2^-]) or 1.7 × 10^{13} s or slightly more than 500 000 years. At a concentration of 200 μM NO_2^-, these numbers are different but still do not support formation of N_2O_3.

Another reaction that ought to be taken into account is reaction 7, dissociation of N_2O_3. If N_2O_3 were formed it would, at that dilute concentration, completely dissociate into NO$^{\bullet}$ and $NO_2^{\bullet}$ before it hydrolyzes: the $t_{1/2}$ of reaction 7 (Table 1) is 0.693/8.0 × 10^4 s^{-1} = 8.7 μs. Thus, formation of N_2O_3 is thermodynamically and kinetically unlikely.

Catalysis by Hemoglobin. Can hemoglobin act as a catalyst as proposed?[18] Formation of N_2O_3 must be fast or NO$^{\bullet}$ disappears by binding to hemoglobin or reaction with oxyhemoglobin. Thus, given a $t_{1/2}$ of 1.7 × 10^{13} s, N_2O_3 needs to be produced on the second time scale, ca. 10^{13} times faster. If that were feasible, it would not help because the rate of hydrolysis would increase by the same factor. Are these results very sensitive to the precise values of the Gibbs energies? The answer is no: to be physiologically relevant, nanomolar concentrations of N_2O_3 need to be produced. To achieve a 1 nM concentration of N_2O_3 at equilibrium, the Gibbs energy of reaction 6 has to change by 69 kJ/mol to become −10 kJ mol^{-1}. Selective use of the R and T states of hemoglobin, if possible, may change the energetics favorably by ca. 10 kJ/mol,[20] which still does not make formation of N_2O_3 possible. It needs to be pointed out, pro forma, that if R and T states are involved then hemoglobin is not acting as a true catalyst.

The energetics of reactions 10 and 11 are easily calculated from the data collected in Table 1: these are −6 and −16 kJ/mol, respectively. Given that $\Delta_{rxn}G^{\circ\prime}{}_6$ = +59 kJ/mol at pH 7, the Gibbs energy change of reaction 12 at pH 7 is +81 kJ/mol. Were one to use the binding constant of the methemoglobin−nitrite complex determined by Goetz et al.,[21] then the Gibbs energy change of reaction 12 is +84 kJ mol^{-1}. This shows that tighter binding of NO_2^- to methemoglobin does not help the formation of N_2O_3.

Following a study by Fernandez and Ford,[22] Hopmann et al.[23] also considered an alternative mechanism for formation of N_2O_3

$$HbFe^{3+} + NO^{\bullet} \rightarrow Hb[FeNO]^{3+} \quad (13)$$

$$Hb[FeNO]^{3+} + NO_2^- \rightarrow HbFe^{2+} + N_2O_3 \quad (14)$$

$$HbFe^{2+} \rightarrow HbFe^{3+} + e^- \quad (15)$$

$$NO_2^- + 2H^+ + e^- \rightarrow NO^{\bullet} + H_2O \quad (4)$$

Reactions 4 and 13−15 also add up to reaction 6; thus, $\Delta_{rxn}G^{\circ\prime}{}_{14}$ = +88 kJ mol^{-1} at pH 7.

Comparison with DFT Calculations. The energetics of reactions 12 and 14 have been estimated by density functional theory calculations. Hopmann et al.[23] report that reaction 12 is uphill by 71−84 kJ mol^{-1}, in good agreement with the value of +81 kJ mol^{-1}. However, Berto and Lehnert,[24] using a more refined model of the active site, claim that reaction 12 is slightly exothermic, between −4 and −13 kJ mol^{-1}, which is incorrect by 85−95 kJ mol^{-1}. For reaction 14, Hopmann et al.[23] conclude that electron transfer from NO_2^- to $Hb[FeNO]^{3+}$ is favorable by 29 kJ mol^{-1}, which is off by 117 kJ mol^{-1}. In spite of the large difference in Gibbs energies for reactions 12 and 14, Hopmann et al.[23] conclude that both reactions are energetically reasonable. However, electron transfer from NO_2^- to $Hb[FeNO]^{3+}$ is not exothermic: from the equilibria between NO$^{\bullet}$ and $HbFe^{2+}$ and NO$^{\bullet}$ and $HbFe^{3+}$ [25] one calculates (Table 1) that $E^{\circ}(Hb[FeNO]^{3+}/Hb[FeNO]^{2+})$ is +0.47 V for T-state hemoglobin and +0.54 V for R-state hemoglobin. Combined with the electrode potential of the $NO_2^{\bullet}/NO_2^-$ couple, 1.04 V (Table 1), electron transfer is unfavorable by at least 48 kJ/mol. It is truly regrettable that these ab initio calculations provide neither consistent nor proper estimates of Gibbs energies because it implies that any proposed intermediates and transition states are similarly compromised. Gibbs energies can be correctly and rapidly calculated per manum.

Are reactions 12 and 14 possible if we let N_2O_3 hydrolyze? We deduct the Gibbs energy of reaction 6 and obtain +22 and +29 kJ mol^{-1}, respectively. These numbers are small enough to let the reactions proceed if products are removed. The Gibbs energy of +29 kJ mol^{-1} also applies to reaction 16

$$Hb[FeNO]^{3+} + H_2O \rightarrow HbFe^{2+} + NO_2^- + 2H^+ \quad (16)$$

Reaction 11 results in $Hb[FeNO_2]^{2+}$, in which NO_2^- is thought[18] to be partially oxidized by Fe^{3+}. Given a binding energy of only 16 kJ mol^{-1} (reaction 10) and the difference in electrode potential between the couples $HbFe^{3+}/HbFe^{2+}$ and $NO_2^{\bullet}(aq)/NO_2^-$ of 0.9 V (Table 1), such a partial electron transfer is unlikely, as was recognized by Berto and Lehnert.[24]

The conclusion is that N_2O_3 cannot play a role in the preservation of NO$^{\bullet}$. Furthermore, given the low physiological concentration of NO_2^-, any mechanism that relies on two NO_2^- to occur on a second time scale is kinetically doomed.

Nitrosation by HNO_2, by NO$^{\bullet}$ and an Electron Acceptor, and by ONOO$^{\bullet}$. Returning to the original observation, which is that injection of 0.40 mM NO_2^- into the brachial artery of the upper arm resulted in a final concentration of ca. 2.5 μM as measured in the ipsolateral antecubital vein, led to noticeable vasodilation[17] and having shown that the N_2O_3 pathway is most unlikely, one can ask whether HNO_2, present under these conditions at a concentration of ca. 0.25 nM, is the agent responsible. Like N_2O_3, HNO_2 is neutral and could penetrate endothelial cells. Nitrosation is thermodynamically possible, but is it fast enough? The rate of nitrosation is given by

$$\text{rate} = k[H^+][HNO_2][RSH] \quad (17)$$

in which k = 4.6 × 10^5 M^{-2} s^{-1}.[13] It is of course not correct to use eq 17 if the thiol of interest, for instance, hemoglobin β-chain cysteine 93, is not homogeneously distributed. The following considerations, therefore, result in only a rough estimate. Given a concentration inside the red blood cell of 5 mM hemoglobin, and thus of 10 mM β-chain cysteine 93, and of a HNO_2 concentration of 0.25 × 10^{-9} M, then the rate of nitrosothiol formation is 1× 10^{-13} M s^{-1}, which would appear to be too slow. However, the concentration of NO_2^- at the site of injection was much higher. It may thus be possible that the small extent of vasodilation was caused by HNO_2.

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If nitrosation does not involve NO_2^- but $NO^\bullet$ then an electron acceptor with an electrode potential larger than −0.20 V (reaction 2) is required. Iron(III) cytochrome *c* is such an electron acceptor for the nitrosation of glutathione,[26] and the overall reaction is favorable: Mechanistically, reaction 19

$$\mathrm{CytcFe^{3+} + e^- \rightarrow CytcFe^{2+}} \quad (18)$$
$$\underline{\mathrm{RSH + NO^\bullet \rightarrow RSNO + H^+ + e^-} \quad (-2)}$$
$$\mathrm{CytcFe^{3+} + NO^\bullet + RSH \rightarrow CytcFe^{2+} + RSNO + H^+} \quad -44\ \mathrm{kJ\ mol^{-1}} \quad (19)$$

requires three reactants to be present at the same time in close proximity, which makes it kinetically unlikely. However, this problem is obviated by binding of glutathione to cytochrome *c* prior to reaction with $NO^\bullet$. A similar mechanism can be written for methemoglobin with an overall Gibbs energy change of −26 kJ mol^{-1}. Dioxygen may also act as an electron acceptor;[27] given an $E°(O_2/O_2^{\bullet-})$ of −0.35 V,[28] the reaction is uphill but pulled through by the diffusion-controlled reaction of the product, $O_2^{\bullet-}$, with another $NO^\bullet$.[29] As experimentally observed, this nitrosation reaction is second order in $NO^\bullet$.[27]

Alternatively, $NO^\bullet$ may first bind to iron(III) followed by the nitrosation reaction. In the case of methemoglobin this process is less favorable but still possible: Experimental evidence for this

$$\mathrm{Hb[FeNO]^{3+} \rightarrow HbFe^{3+} + NO^\bullet} \quad (-13)$$
$$\mathrm{HbFe^{3+} + e^- \rightarrow HbFe^{2+}} \quad (-15)$$
$$\mathrm{RSH \rightarrow RS^\bullet + H^+ + e^-} \quad (-3)$$
$$\underline{\mathrm{RS^\bullet + NO^\bullet \rightarrow RSNO} \quad (-1)}$$
$$\mathrm{Hb[FeNO]^{3+} + RSH \rightarrow HbFe^{2+} + RSNO + H^+} \quad -8\ \mathrm{kJ\ mol^{-1}} \quad (20)$$

pathway exists.[30] However, in vivo, this reaction pathway seems unlikely as the concentration of methemoglobin is small and because $NO^\bullet$ is more likely to react with oxy- and deoxy-hemoglobin.

A modification that involves NO_2^- and hemoglobin as a catalyst allows the following kinetically and thermodynamically feasible reactions: The only assumption made is that reaction of $Hb[FeNO]^{3+}$ with RSH takes place before $NO^\bullet$ relocates to $HbFe^{2+}$ or reacts with oxyhemoglobin. Indeed, if dissociation of $NO^\bullet$ from $HbFe^{3+}$ takes place then we have reaction 10, which is slow, 1 M^{-1} s^{-1} at pH 7.5.[31]

$$\mathrm{HbFe^{2+} + NO_2^- + 2H^+ \rightarrow Hb[FeNO]^{3+} + H_2O} \quad -29\ \mathrm{kJ\ mol^{-1}} \quad (-16)$$
$$\underline{\mathrm{Hb[FeNO]^{3+} + RSH \rightarrow HbFe^{2+} + RSNO + H^+} \quad -8\ \mathrm{kJ\ mol^{-1}} \quad (20)}$$
$$\mathrm{RSH + NO_2^- + H^+ \rightarrow RSNO + H_2O} \quad -37\ \mathrm{kJ\ mol^{-1}} \quad (5)$$

We found recently evidence for $ONOO^\bullet$ as an intermediate in the oxidation of $NO^\bullet$ to $NO_2^\bullet$.[32] Can it play a role in nitrosation? Given an electrode potential of +0.51 V[33] for $E°(ONOO^\bullet/ONOO^-)$, oxidation of RSH ($E°'_3$ = +0.94 V, Table 1) is uphill, but overall the reaction would be favorable, as the oxidation is followed by reaction of $NO^\bullet$ with $RS^\bullet$, reaction −1. Now there are several possibilities in theory, but none of them is practical: Dissociation of $ONOO^-$ could yield the necessary $NO^\bullet$, but this reaction is slow, 0.02 s^{-1}.[34] More likely is protonation of $ONOO^-$ to yield ONOOH, which oxidizes or nitrates other molecules in the vicinity. Formation of RSNO with a second $NO^\bullet$ is kinetically unlikely. Oxidation of RSH by $NO_2^\bullet$ is similarly unrealistic, not in the least because formation of the latter from $NO^\bullet$ under in vivo conditions is extremely slow.[35]

CONCLUSION

Nitrosation by N_2O_3 and $ONOO^\bullet$ can be excluded, by HNO_2 may be possible, and reactions that involve $NO^\bullet$ require a suitable electron acceptor. The mechanism proposed in reactions −16 and 20 needs to be investigated further.

The equations and energetics provided here can be used as LEGO blocks to build a reaction mechanism. Once an energetically favorable mechanism has been established, one must ask the question whether the kinetics are fast enough. It is important to keep in mind that the reactions used to calculate a Gibbs energy, such as reactions −1, 3, −13, and −15 above, do not necessarily take place: they serve to produce the Gibbs energy of reaction 20. In particular, given that the $RS^\bullet$ radical is in equilibrium with $R^\bullet SH$, where $R^\bullet$ stands for a carbon-centered radical elsewhere in the molecule,[36,37] one would do well to avoid $RS^\bullet$ in mechanisms of nitrosation.

The approach used here is not new,[38−40] requires only pencil and paper, and may help in defining the reaction one has an interest in prior to embarking on possibly elaborate, expensive, and technically difficult laboratory experiments or in silico calculations.

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Notes

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