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Hypothesis

Thermodynamic considerations on the formation of reactive species from hypochlorite, superoxide and nitrogen monoxide

Could nitrosyl chloride be produced by neutrophils and macrophages?

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Abstract

Hypohalous acids are poor one-electron oxidizing agents, such that reactions with hydrogen peroxide to yield radical species are not feasible. However, the oxidation of superoxide by hypohalous acids can be a source of hydroxyl or haline radicals. The oxidation of nitrogen monoxide by hypochlorous acid is favourable, but in all likelihood cannot compete with the diffusion-controlled reaction with superoxide to yield peroxynitrite. The reaction of the latter with hypochlorous acid may lead to nitrosyl chloride, a strongly oxidizing agent [$E^{\circ\prime}(\mathrm{NOCl/NO^{\bullet}, Cl}) = 1.0$ V] that is capable of nitrosylating organic compounds and thereby generating mutagens or promutagens.

Key words: Hypochlorite; Nitric oxide; Peroxynitrite; Nitrosyl chloride; Neutrophil; Macrophage

1. Introduction

The main microbial agent produced by activated neutrophils is hypochlorite, which is formed from chloride and hydrogen peroxide by the enzyme myeloperoxidase. Neutrophils also produce superoxide and nitrogen monoxide**. These radical species react at at a diffusion-controlled rate [2] to form peroxynitrite [$\mathrm{O{=}NOO^-}$, oxoperoxonitrate(1−)], a strongly oxidizing agent [3]. Evidence for formation of oxoperoxonitrate(1−) by macrophages [4] and neutrophils [5] has been presented. Gibbs energy changes for reactions between superoxide, hypochlorous acid and hydrogen peroxide were published [6]. There are two reasons for re-examining this study: (i) the discovery that nitrogen monoxide is formed in vivo [7,8] necessitates the inclusion of nitrogen containing compounds, and (ii) more accurate thermodynamic data are now available for inorganic radicals. I will show here that formation of nitrosyl chloride is energetically feasible, and that this compound is strongly oxidizing. Aside from this, nitrosyl chloride is known to be a nitrosylating agent [9]. Currently, the only physiologically relevant mechanism to arrive at a nitrosylating species is via the autoxidation of nitrogen monoxide [10].

2. Thermodynamics

Standard Gibbs energies of formation were obtained from the literature [3,11–13], and Gibbs energies of solvation were added where appopiate such that all values, including reduction potentials, refer to 1 molal solutions. For this reason many of the reported values that pertain to gases are not standard values, and this is indicated by an apostrophe, as in $E^{\circ\prime}$. One- and two-electron potentials of the hypohalous acids are shown in Table 1. Three schemes have been constructed which show the energetics of hypohalous acids with superoxide and hydrogen peroxide. Nitrogen monoxide could react with a number of these compounds, and the Gibbs energy changes of these reaction have been collected in Table 2.

3. Discussion

Like hydrogen peroxide, the hypohalous acids are poor one-electron and strong two-electron oxidizing agents, as shown in Table 1. This explains why the oxidation of hydrogen peroxide to superoxide by the hypohalous acids is quite unfavourable, in contrast to the two-electron oxidation to dioxygen. Indeed, there is ex-

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**Allowed names for chemical compounds are used. However, when the spelling of a name is uncertain (peroxynitrite or peroxonitrite) or when such a name is not recommended (nitric oxide), systematic names are used [1].

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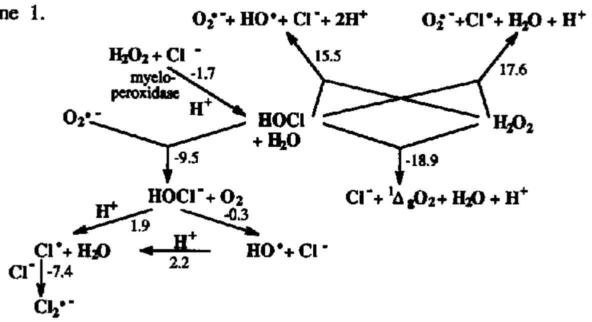
perimental evidence that the one-electron oxidation of hydrogen peroxide by hypochlorous acid does not take place [14]. On the other hand, the two-electron oxidation is so favourable that, in the case of ClO^-, and BrO^-, but not IO^-, formation of singlet $(^1\Delta_g)$ dioxygen is thermodynamically possible (Schemes 1–3), in agreement with experimental observations [14,15]. Reduction of hypochlorous acid by superoxide is possible followed by formation of the hydroxyl radical, and has been described [16–18]. The formation of hydroxyl radical and chloride from $HOCl^•$, the hydroxochlorate(1−) radical, is only slightly favourable [19]. In the presence of chloride and at low pH, formation of $Cl_2^{•-}$ via $Cl^•$ is possible. In addition, the dihydrogen phosphate anion reacts at neutral pH with $HOCl^•$ to form the chlorine radical [20].

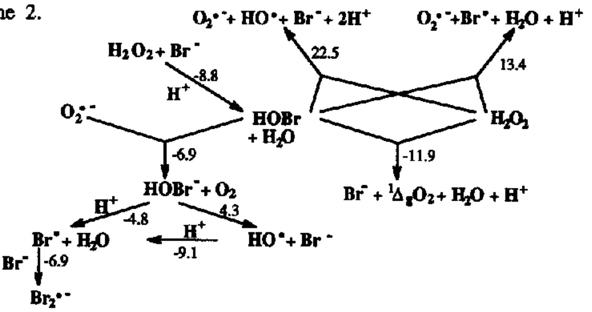
The presence of nitrogen monoxide makes it necessary to consider the reactions collected in Table 2. The hydroxyl radical adds to nitrogen monoxide in a diffusion-controlled reaction [21], forming nitrite [dioxonitrate(1−)], rather than the nitrosyl cation, NO^+. Similarly, a chlorine radical could add to nitrogen monoxide and form nitrosyl chloride. Given the reactivities of chlorine and hydroxyl radicals, it is far more likely that a reaction will occur between nitrogen monoxide and superoxide. Superoxide is less reactive than the chlorine or hydroxyl radical, but the reaction with nitrogen monoxide is close to, if not diffusion controlled, 6.7×10^9 $M^{-1} \cdot s^{-1}$ [2]. The oxidation of nitrogen monoxide to nitrogen dioxide by hydrogen peroxide is a favourable process. We found no evidence for such a reaction when the nitrogen monoxide concentration was monitored (Luca, Beckman and Koppenol, unpublished observation), although it has been reported that singlet dioxygen is a product [22]. We have currently no explanation for this observation. Because hypochlorous acid is a poor one-electron oxidizing agent, the oxidation of nitrogen monoxide to the nitrosyl cation, chlorine atom and water, *or* to the nitrosyl cation, hydroxyl radical and chloride, is very unfavourable, and these reactions are not expected to occur. In contrast, the oxidation of nitrogen monoxide to nitrogen dioxide by hypochlorous acid is very favourable. No rate data has been provided for this reaction.

It has been shown that hydrogen oxoperoxonitrate, O=NOOH, reacts with iodide to form IO^-, oxoiodate(1−), but not with bromide or chloride to form the corresponding hypohalites [23]. These reactions are favourable for iodide (−20 kcal), but less so for bromide (−4 kcal) and unfavourable for chloride (+4 kcal). Thus, formation of additional hypochlorite via this route is unlikely.

Scheme 1.



Scheme 2.



Scheme 3.

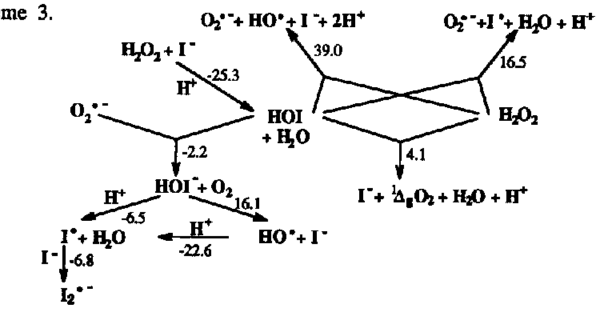


Table 1
One- and two-electron reduction potentials of couples involving the hypohalous acids

Redox couple	$E^{o\prime}$ (V) at pH 7			
	Cl	Br	I	OH
One-electron reduction potentials				
HOX/HOX^- [12]	+0.25	+0.14	+0.03	−0.5[a]
HOX/HO•, X^-	+0.26	−0.05	−0.73	+0.32
HOX, H^+/H_2O, $X^•$	+0.17	+0.35	+0.25	
Two-electron reduction potentials				
HOX/H_2O, X^-	+1.08	+0.93	+0.57	+1.32

Reduction potentials apply to 1 molal concentrations.

[a] This potential is an estimate based on an electron affinity of 0 eV for hydrogen peroxide and a hydration energy of −98 kcal/mol for $H_2O_2^-$, identical to that of HO_2^- [13].

Table 2
Gibbs energies of reactions involving nitrogen monoxide at pH 7 and for 1 molal solutions

Reaction		$\Delta G^{o\prime}$(kcal/mol)
$O_2^{•-}$ + NO•	→ $ONOO^-$	−22 [2]
Cl• + NO•	→ ClNO	−35
HO• + NO•	→ NO_2^- + H^+	−48
HOCl + NO•	→ $NO_2^•$ + Cl^- + H^+	−31
HOCl + NO• + H^+	→ NO^+ + Cl• + H_2O	+24
HOCl + NO•	→ ClNO + HO•	+17
H_2O_2 + NO•	→ $NO_2^•$ + H_2O	−33

Compared to the various radicals that may be formed (see Schemes 1–3), hydrogen oxoperoxonitrate and hypochlorous acid are relatively stable compounds. One should consider, therefore, whether a reaction between these two species can occur that is analogous to the reaction between hydrogen peroxide and hypochlorite. The energetics indicate that singlet dioxygen is energetically not feasible, but, interestingly, nitrosyl chloride may be formed, as shown in Reaction 1:

$$\mathrm{HOCl + O{=}NOOH \rightarrow ClNO + O_2 + H_2O} \quad \Delta G^{\circ\prime}(\mathrm{pH}\ 7) = -19\ \mathrm{kcal} \qquad (1)$$

From the Gibbs energies of formation of nitrosyl chloride, the nitrosyl cation and chloride, one calculates that nitrosyl chloride is a strong oxidant:

$$\mathrm{ClNO + e^- \rightarrow NO^\bullet + Cl^-} \quad E^{\circ\prime} = 1.0\ \mathrm{V} \qquad (2)$$

This reduction potential is similar to that of ozone [13], nitrogen dioxide [12,24], and chlorine dioxide [25,26]. bSince $E^\circ(\mathrm{NO^+/NO^\bullet})$ equals 1.21 V [12], dissociation is unfavourable (Reaction 3):

$$\mathrm{ClNO \rightarrow NO^+ + Cl^-} \quad \Delta G^{\circ\prime} = +5\ \mathrm{kcal} \qquad (3)$$

However, the subsequent hydrolysis, Reaction 4, is favourable, which may make Reaction 3 proceed in the direction indicated.

$$\mathrm{NO^+ + H_2O \rightarrow NO_2^- + 2H^+} \quad \Delta G^{\circ\prime}(\mathrm{pH}\ 7) = -22\ \mathrm{kcal} \qquad (4)$$

A reaction that results directly in dioxochlorate(1−) and the nitrosyl cation is unfavourable:

$$\mathrm{HOCl + O{=}NOOH \rightarrow ClO_2^- + NO^+ + H_2O} \quad \Delta G^{\circ} = +21\ \mathrm{kcal} \qquad (5)$$

Similarly, the one electron oxidation of hypochlorous acid by hydrogen oxoperoxonitrate (Reaction 6) has a positive Gibbs energy change:

$$\mathrm{HOCl + O{=}NOOH \rightarrow ClO^\bullet + NO_2^\bullet + H_2O} \quad \Delta G^{\circ\prime} = +4\ \mathrm{kcal} \qquad (6)$$

The small positive Gibbs energy is not likely to prevent this reaction from occuring, because the products, chlorine monoxide and nitrogen dioxide, dissappear rapidly in subsequent reactions. Quantum mechanic studies appear to indicate that chlorine monoxide and nitrogen dioxide do not react to form O=NOOCl [27]. While Reaction 6 cannot be excluded on thermodynamic grounds, it does not yield a nitrosylating species.

Nitrosyl chloride can nitrosylate organic compounds directly [9], and therefore its presence poses two dangers: first, it is a strong oxidant, and second, nitrosylation leads to compounds that are often mutagenic or promutagenic. N-Nitrosylation by activated neutrophils has been observed [28]. Hydrogen oxoperoxonitrate does not nitrosylate, but nitrates, aromatic compounds [29,30], and is therefore not directly responsible for the formation of nitroso compounds. Recently, we observed a rapid reaction, comparable to that between hypochlorite and hydrogen peroxide [14], between hypochlorite and hydrogen oxoperoxonitrate with the help of the stopped-flow technique. We also obtained preliminary evidence for the formation of *p*-nitrosophenol from phenol, hypochlorite and oxoperoxonitrate(1−) (Bartlett, Ramezanian and Koppenol, unpublished observations).

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