Communication

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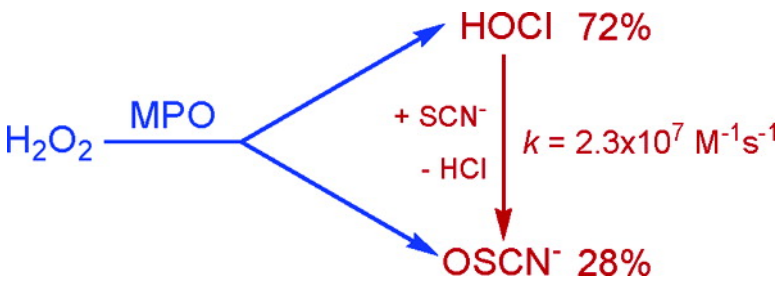
J. Am. Chem. Soc., **2004**, 126 (49), 15976-15977• DOI: 10.1021/ja0438361 • Publication Date (Web): 19 November 2004



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Published on Web 11/19/2004

Redox Buffering of Hypochlorous Acid by Thiocyanate in Physiologic Fluids

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Neutrophils, which comprise 33−75% of all leukocytes in humans, possess an impressive armory of oxidative and nonoxidative mechanisms for combating microorganisms. The oxidative defense mechanism of neutrophils is based upon NADPH oxidase and myeloperoxidase (MPO). The NADPH oxidase system reduces molecular oxygen to generate reactive oxygen species (ROS), including H_2O_2. In the presence of H_2O_2, MPO is capable of oxidizing all of the halides (except F^-), as well as the pseudohalide thiocyanate (SCN^-), to produce hypohalites that are many orders of magnitude more cytotoxic than H_2O_2 itself. Hypohalites, and in particular hypochlorous acid (HOCl), appear to play a pivotal role in inflammation.[1] When unchecked, neutrophil-induced inflammation results in local tissue damage, and MPO has been linked to numerous chronic diseases, such as atherosclerosis,[2] cystic fibrosis,[3] and periodontitis.[4] Since HOCl is an indiscriminant oxidant, whereas hypothiocyanite ($OSCN^-$) is not lethal to mammalian cells,[5−9] considerable attention has focused on the substrate selectivity of MPO.[10,11] We demonstrate here that HOCl is capable of rapidly oxidizing SCN^- to give $OSCN^-$. Thus, the nonenzymic transfer of oxidizing equivalents from HOCl to SCN^- substantiates the hypothesis that SCN^- can serve the role of a redox buffer, thereby governing the lifetime of the more powerful oxidant HOCl and its potential for host self-destruction.

MPO is in the ferric form in its resting state. Rapid reaction with H_2O_2 produces compound I (MPO−I), which is two oxidizing equivalents above MPO:

$$\mathrm{MPO} + \mathrm{H_2O_2} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{MPO{-}I} \qquad (1)$$

$$\mathrm{MPO{-}I} + \mathrm{X^-} \xrightarrow{k_2} \mathrm{MPO} + \mathrm{OX^-} \qquad (2)$$

The substrate specificities of MPO are determined by the rates of reaction of MPO−I with X^-.[12] Because of the relative abundance of halides, it is often suggested that the chief substrate of MPO in blood plasma is Cl^-, and that the HOCl which is produced is responsible for the cytocidal capacity of the MPO system.[13] However, we observe that the nonenzymic rate of oxidation of SCN^- by HOCl to give hypothiocyanite ($OSCN^-$) is nearly diffusion-controlled,[14] thus suggesting that the major hypohalite produced by MPO in the absence of efficient reductants is $OSCN^-$:

$$\mathrm{OCl^-} + \mathrm{H^+} \underset{k_a}{\overset{k_{-a}}{\rightleftharpoons}} \mathrm{HOCl} \qquad (3)$$

$$\mathrm{HOCl} + \mathrm{SCN^-} \xrightarrow{k_3} \mathrm{Cl^-} + \mathrm{OSCN^-} + \mathrm{H^+} \qquad (4)$$

The rate of the reaction of HOCl and SCN^- (eq 4) is too fast at physiologic pH to measure by stopped-flow. However, the equilibrium of eq 3 is driven to OCl^- under basic conditions (pK_a(HOCl) = 7.4), thus sufficiently slowing reaction 4 to facilitate measurement. Single-mixing stopped-flow reactions of HOCl with excess SCN^- (i.e., pseudo-first-order conditions) yield traces at 300 nm (near λ_{max} for OCl^-) that fit single-exponential kinetics models between 500 μM < $[OH^-]$ < 1.03 M, thus suggesting first-order dependence on $[OCl^-]$. The reaction rates were also first-order with respect to $1/[OH^-]_0$ (i.e., first-order with respect to $[H^+]$) over three decades of change in $[OH^-]$ (Figure 1) and first-order with respect to $[SCN^-]$ (Supporting Information). Production of $OSCN^-$ as the primary oxidation product was indicated by an increase in absorbance at 240 nm and further confirmed by employing a double-mixing stopped-flow sequence to convert the product to $(SCN)_2$ via comproportionation of $OSCN^-$ and SCN^- (Supporting Information).[15] These data are consistent with a facile proton equilibrium to generate HOCl (eq 3), followed by a rate-limiting reaction (eq 4) of HOCl with SCN^- to yield $OSCN^-$ ($k_3 = 2.34(9) \times 10^7\ M^{-1}\ s^{-1}$). Considering only this reaction, we estimate at mean concentrations of SCN^- that are found in saliva (1−3 mM) and plasma (20−120 μM), the half-life of HOCl cannot be more than ca. 15 μs and 400 μs, respectively.

While the preceding discussion of the half-lives of HOCl in a medium that contains SCN^- is illuminative, it does not address the issue of steady-state concentrations of HOCl in physiologic fluids, nor the potential physiological consequences of eq 4. To broach these topics, it is necessary to consider dynamic models. Such models can be developed because all of the rate constants[11] for eqs 1−4 have been measured by us and others.[16,17] Although it is not our intention in this Communication to provide a complete model for the redox buffering of HOCl by SCN^-, Figure 2 illustrates the consequence of the k_3 pathway for a relatively low concentration of MPO (1 μM) and a high concentration of H_2O_2 (1 mM), conditions that are conceivable for extracellular MPO during a respiratory burst. The initial rates of reaction of MPO−I with X^- determine the relative amount of the two hypohalites that are initially formed (not illustrated on the time-scale of Figure 2):

$$\%\mathrm{X^-} = \frac{k_2^{\mathrm{X^-}}[\mathrm{X^-}]}{k_2^{\mathrm{Cl^-}}[\mathrm{Cl^-}] + k_2^{\mathrm{SCN^-}}[\mathrm{SCN^-}]} \qquad (5)$$

which equals 72% OCl^- and 28% SCN^- for typical blood plasma concentrations ($[Cl^-]_0$ = 100 mM and $[SCN^-]_0$ = 100 μM).[18] However, the relative amounts of these hypo(pseudo)halites change as the HOCl that is produced in eq 2 reacts with SCN^- via eq 4. The concentration−time traces of Figure 2 were computed using rate equations that were programmed into Mathematica. The simultaneous differential equations were solved by numerical methods since the complexity of the equations forbids the development of closed solutions (Supporting Information). No assumptions were made to simplify the rate laws except that the Brønsted acid−base chemistry is facile with respect to all other kinetic processes, and that the concentration of Cl^- was assumed to remain constant by virtue of its high concentration. Dynamic changes in concentration were taken into consideration for all other species. Note that

10.1021/ja0438361 CCC: $27.50

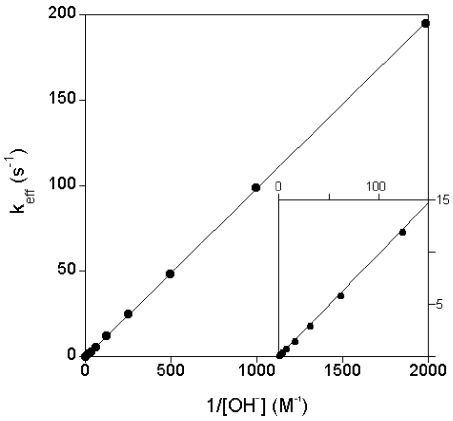

Figure 1. Plot of k_{eff} ($[SCN^-]_0 = 10$ mM, $[OCl^-]_0 = 0.1$ mM, $\mu = 1$) as a function of $[OH^-]^{-1}$ illustrating first-order dependence. The data are fit to the linear function: $k_{eff} = (9.85 \times 10^{-2}\ M\ s^{-1})/[OH^-] + 1.27 \times 10^{-2}\ s^{-1}$.

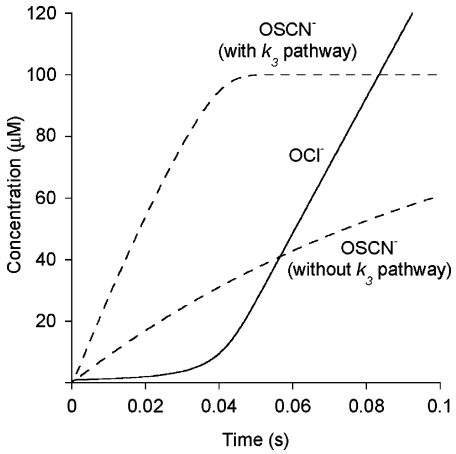

Figure 2. Concentration−time curves for HOCl (solid line) and $OSCN^-$ (dashed line), with and without consideration of the k_3 pathway.

in reality, such high concentrations of H_2O_2 would deactivate MPO on the millisecond time scale by formation of halide-inactive compound II.[19] Nonetheless, Figure 2 clearly illustrates the redox-buffering effect of SCN^-; substantial quantities of HOCl are not produced until the complete oxidation of SCN^-. Figure 2 also illustrates another significant aspect of the k_3 pathway, an increase in the rate of production of $OSCN^-$ over that from the k_2 pathway alone. Whether this increased rate via the k_3 pathway has physiological consequence remains to be demonstrated.

We propose that the relative amounts of HOCl and $OSCN^-$ that are produced in vivo are not dictated by enzyme substrate selectivities alone but rather by kinetic competition between the rate of reaction of HOCl with SCN^- and other reductants. While physiologic fluids are very complicated and we cannot expect to accurately predict a priori whether the reaction of SCN^- with HOCl is competitive in vivo, we can begin to probe this issue because normal reference values are known for plasma components,[20] and the rate constants for reaction of many of these species with HOCl are available.[21] When the low molecular weight components of human blood plasma are taken into consideration, assuming these species are in excess with respect to HOCl and partitioning is dictated by initial rates, we arrive at the remarkable conclusion that SCN^- would consume nearly all available HOCl (Table 1 and Supporting Information). However, there are many macromolecular components of plasma that are likely to compete effectively for HOCl. For example, human serum albumin (HSA) is the main protein component of plasma with a normal concentration range of 35−55 g/L (about 750 μM). HSA bears HOCl reactive groups (e.g., 12 Met, 2 Cys, 67 S-S).

Table 1. . Predicted Partitioning of HOCl in Human Blood Plasma by the Components of the Low MW Fraction that are Expected to Exhibit a Significant Rate of Reaction

component	concn (M)	k_3' ($M^{-1}\,s^{-1}$)[a]	% HOCl
Cys	3×10^{-7}	3×10^{7}	0.05%
Met	5×10^{-8}	4×10^{7}	0.01%
His	2×10^{-5}	1×10^{5}	0.01%
Lys	4×10^{-5}	5×10^{3}	<0.01%
Trp	2×10^{-5}	1×10^{4}	<0.01%
AA	7×10^{-8}	2×10^{6}	<0.01%
taurine	7×10^{-8}	5×10^{5}	<0.01%
SCN^-	1×10^{-3}	2×10^{7}	99.93%

[a] Value k_3' is defined in eq 4 where SCN^- is replaced with the component.

Experiments are underway to explore the competitiveness of the various components of blood plasma with respect to SCN^-.

It has been previously suggested that the SCN^- reaction pathway of MPO might serve as a means of ameliorating self-destruction by the more powerful reactant HOCl by acting as a redox buffer, but no specific theories were advanced that explained how the enzyme might regulate the two substrate pathways.[16] The nonenzymic transfer of the oxidizing equivalents of HOCl to SCN^- that are described here serves such a role by governing the lifetime of the more powerful oxidant, thereby moderating the potential autotoxicity of HOCl. Furthermore, the oxidizing equivalents of HOCl are preserved in $OSCN^-$, a more discriminate[22] biocide that is not lethal to mammalian cells.

Acknowledgment. We are grateful to the PRF (35088-AC3) and the OCAST (HR02-019) for their financial support.

Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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(1) Hereafter, "HOCl" refers to the equilibrium mixture of HOCl and OCl^-, about 1:1 at pH = 7.4.
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(11) $k_1 = 2.3 \times 10^7\ M^{-1}\ s^{-1}$, $k^{-1} = 58\ s^{-1}$, $k_2(Cl^-) = 2.5 \times 10^4\ M^{-1}\ s^{-1}$, $k_2(SCN^-) = 9.7 \times 10^6\ M^{-1}\ s^{-1}$, $K_a = 4.0 \times 10^{-8}$ M, $k_3 = 2.3 \times 10^7\ M^{-1}\ s^{-1}$.
(12) Rate = $k_2[MPO-I][X^-]$.
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JA0438361