# Thiocyanate Is an Efficient Endogenous Scavenger of the Phagocytic Killing Agent Hypobromous Acid

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# Thiocyanate Is an Efficient Endogenous Scavenger of the Phagocytic Killing Agent Hypobromous Acid

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Second-order rate constants for the reaction of HOBr/$OBr^-$ (a putative killing agent of eosinophils and a reactive oxygen species that is implicated in mutagenesis and in human inflammatory diseases) with $SCN^-$ (an endogenous species in human physiologic fluids) are determined by stopped-flow spectroscopy. The proposed mechanism includes parallel pathways with $Br^+$ transfer to $SCN^-$ by general acid catalysis and by direct reaction with HOBr. HOBr reacts with $SCN^-$ with a second-order rate constant ($2.3 \times 10^9$ $M^{-1}$ $s^{-1}$) that is 2 orders of magnitude larger than that previously measured for the reaction of HOCl with $SCN^-$ ($2.3 \times 10^7$ $M^{-1}$ $s^{-1}$), and very close to the diffusion limit. In contrast to $OCl^-$, $OBr^-$ exhibits a measurable rate of reaction with $SCN^-$ ($3.8 \times 10^4$ $M^{-1}$ $s^{-1}$). On a molar basis, $SCN^-$ is the most effective scavenger of HOBr to be reported to date (200 times more effective than cysteine and 650 times more effective than methionine). Computational models suggest that $SCN^-$ is competitive with respect to other scavengers at physiologically relevant concentrations, which leads us to propose it may limit the lifetime of HOBr and its propensity to inflict host tissue damage during inflammatory response, especially during eosinophilia. Furthermore, the product of the nonenzymatic reaction of HOBr and $SCN^-$, hypothiocyanite ($OSCN^-$), is an effective antimicrobial that is relatively innocuous toward mammalian cell lines. Since one of the principal charges of eosinophil cells is to clear extracellular parasites via nonphagocytic mechanisms that involve degranulation of eosinophil peroxidase (EPO, the principal mammalian enzyme that produces HOBr), a larger role for $OSCN^-$ is suggested for parasitic infection.

## Introduction

Reactive oxygen species (ROS)[1] that are produced by professional phagocytes are implicated in host tissue damage that occurs during acute and chronic inflammation (*1*). A cascade of ROS begins with the production of superoxide ($O_2^{-\bullet}$) by NADPH oxidase (*2*), which is normally latent in phagocytes but is activated during a respiratory burst. Superoxide, a modest oxidizing agent (*3*) and somewhat selective free radical, can couple with nitric oxide synthase (NOS) to produce a cascade of reactive nitrogen species (RNS) (*4*), but the majority disproportionates to yield hydrogen peroxide ($H_2O_2$) in a reaction that is efficiently catalyzed by superoxide dismutase (SOD) (*5*). Hydrogen peroxide is a powerful, albeit kinetically inert, oxidant which is exploited by professional phagocytes, with the assistance of peroxidases (*6*), to produce more effective killing agents (*7*). The hypohalous acids HOX (X = Cl, Br, SCN) are important human defense factors that are produced by the peroxidase-catalyzed reaction of $H_2O_2$ with the halides $Cl^-$ and $Br^-$ and the pseudohalide thiocyanate ($SCN^-$). Hypochlorous acid (HOCl) and hypobromous acid (HOBr) are both substantially more cytotoxic than $H_2O_2$ (*8*). The relative (pseudo)halide substrate selectivities of the defensive peroxidases depend on two factors, bioavailability of the (pseudo)halide and the redox potential of the peroxidase. Thus, only myeloperoxidase (MPO) exhibits a sufficiently high redox potential to oxidize $Cl^-$ under typical physiologic conditions ($\epsilon^{\circ}_{Cl^-} > \epsilon^{\circ}_{Br^-} > \epsilon^{\circ}_{SCN^-}$) (*9*), whereas $Br^-$ is believed to be the preferred halide substrate for eosinophil peroxidase (EPO) (*10*, *11*). In addition to $Cl^-$ and $Br^-$, both enzymes catalyze the oxidation of $SCN^-$ to give hypothiocyanite ($OSCN^-$), and $OSCN^-$ is, in general, a major product of all human defensive peroxidases (*9*, *12*, *13*).

From the perspective of inflammatory disease, the reactivity patterns of the phagocytic ROS are important. Thus, according to the oxidative damage hypothesis for atherogenesis (*14*), ROS oxidize protein and lipid components of low-density lipoprotein (LDL). Superoxide is relatively unreactive with respect to proteinaceous components, but instead exhibits higher reactivity toward lipids (*15*). In contrast, HOCl and HOBr are more reactive toward proteins and less reactive toward lipids (*16*, *17*). Chronic inflammation is also associated with an increased risk of cancer (*18*), and HOBr is implicated in mutagenesis (*19*). Although these two hypohalous acids exhibit promiscuous reaction chemistry, when faced with a variety of potential reactants, they demonstrate somewhat different reactivity trends (*17*). Finally, and in contrast to these other ROS, $OSCN^-$ is a mild oxidant that appears to react selectively with cysteine derivatives (*20*−*23*). Furthermore, given the fact that $OSCN^-$ is apparently not cytotoxic to mammalian cells (*24*−*28*), any host cell damage it inflicts must be repairable.

Given the cytotoxic nature of many of the aforementioned ROS, it is critical that scavengers be available to govern their reactivities (*3*). In general, the most effective scavengers of HOCl and HOBr are organosulfur compounds and, in particular, cysteine (Cys) (*17*, *29*, *30*) and methionine (Met) (*31*) derivatives, including the small tripeptide, glutathione (GSH), which exhibits millimolar cytosolic concentrations in human cells (e.g., 2 mM in erythrocytes) (*32*) and macromolecules, such as human

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[1] Abbreviations: Cys, cysteine; EPO, eosinophil peroxidase; GSH, glutathione, reduced; HSA, human serum albumin; LDL, low-density lipoprotein; Met, methionine; MPO, myeloperoxidase; NADPH, nicotinamide adenine dinucleotide phosphate, reduced; NOS, nitric oxide synthase; RNS, reactive nitrogen species; ROS, reactive oxygen species; RSS, reactive sulfur species; SOD, superoxide dismutase; Tyr, tyrosine.

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serum albumin (HSA), which bears a single reduced Cys group on its surface, six Met groups, and is abundant in plasma (ca. 600 $\mu$M) (*33*). In addition to these more widely recognized scavengers, we have recently demonstrated that HOCl rapidly oxidizes $SCN^-$ in a nonenzymatic reaction to produce $OSCN^-$ ($k = 2.3 \times 10^7$ $M^{-1}$ $s^{-1}$) (*34*). Since $SCN^-$ is an endogenous inorganic anion in human physiologic fluids with concentrations in the micromolar range in plasma (*35*) to millimolar in saliva (*36*), we have suggested that $SCN^-$ may limit host tissue damage by restricting the lifetime of the less discriminate oxidant HOCl (*34*). In the present paper, we report our investigation of the kinetics and mechanism of the reaction of HOBr with $SCN^-$.

## Experimental Procedures

**Reagents.** All chemicals were ACS certified grade or better. Water was doubly distilled in glass. When phenolphthalein was used as an indicator, sodium hydroxide solutions that were mostly free of $CO_2$ contamination were quantified by titration with a standardized hydrochloric acid solution. When methyl orange was used as an indicator, hydrochloric acid solutions were standardized with sodium bicarbonate. The buffer solutions were prepared from solid $Na_3PO_4 \cdot 12H_2O$, the ionic strength was adjusted with $NaClO_4$, and the pH was adjusted with NaOH or $HClO_4$. Thiocyanate stock solutions were prepared from solid NaSCN that was dried in a 150 °C oven to constant weight. Hypobromite ($OBr^-$) solutions were prepared by adding $Br_2$ to ice-cold solutions of NaOH (*37*). Hypobromite solutions were standardized spectrophotometrically at 329 nm and were used within 2 h to minimize errors due to decomposition. A molar absorptivity of $\epsilon_{329} = 300$ $M^{-1}$ $cm^{-1}$ was determined by a calibration curve of solutions of $OBr^-$ that were titrated iodometrically.

**UV/Visible Spectroscopy.** Electronic spectra were measured using a HP 8452A diode array spectrophotometer or the monochromator of the HI-TECH SF-61 DX2 stopped-flow instrument.

**pH Measurements.** The $[OH^-]$ for the unbuffered solutions was determined by acid−base titration against standardized HCl solutions. The $[H^+]$ of the buffered solutions was determined with an Orion Ion Analyzer EA920 using an Ag/AgCl combination pH electrode. The electrode response was corrected for measurements under alkaline conditions with a gradient of standard solutions that were individually titrated. The ionic strength was kept constant at 1 M for all solutions ($NaClO_4$ + NaOH + NaSCN + $Na_3PO_4$ + $Na_2HPO_4$). The value of $pK_w = 13.79$ was used for the $[OH^-]$ or $[H^+]$ calculations according to Martell and Smith (*38*).

**Stopped-Flow Studies.** Kinetic measurements were made with a HI-TECH SF-61 DX2 stopped-flow spectrophotometer using a Xe arc lamp and a PMT detector. Temperature control of the observation cell was maintained with a Lauda RC-20 circulator. The $[SCN^-]$, pH, [phosphate], and ionic strength dependencies of the rate law were determined under pseudo-first-order conditions at $\lambda = 329$ nm. All experiments were carried out at $T = 291$ K. Because of the low concentrations of $OBr^-$ (0.02−0.04 mM) that were necessary to follow the kinetics, a small change in the absorbance had to be monitored. To improve the signal-to-noise, each stopped-flow measurement was repeated 27 times (Figure 1). The monochromatic kinetic traces were fit with HI-TECH KinetAsyst 3.14 software (Hi-Tech, U.K.).

## Results

The reaction of $SCN^-$ with HOBr is very fast, but it could be monitored by the stopped-flow method using high $OH^-$ concentrations and low reactant concentrations. The reaction was studied by following the loss of $OBr^-$ at $\lambda = 329$ nm ($\epsilon = 300$ $M^{-1}$ $cm^{-1}$). Neither $SCN^-$ nor $Br^-$ has significant absorbance at this wavelength. Pseudo-first-order conditions were employed for all of the stopped-flow experiments by using excess $[SCN^-]$ relative to the $[OBr^-]$. Single-exponential kinetic traces and the linear dependence of $k_{obs}$ on $[SCN^-]$ demonstrate

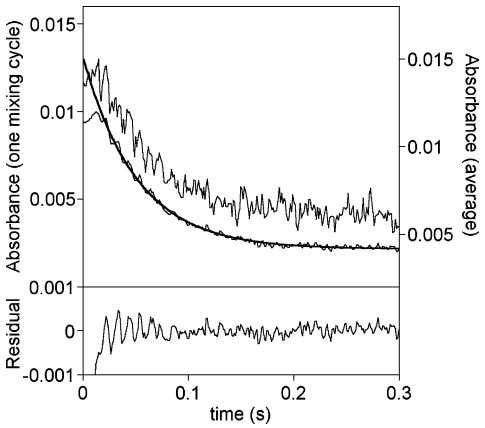


**Figure 1.** Change in absorbance at 329 nm upon mixing of NaSCN (0.40 mM) with NaOBr (0.04 mM) in the presence of NaOH (1.0 M) at 291 K. The ordinate scale on the left is for a representative single-mixing cycle, and the ordinate scale on the right is for an average of 27 mixing cycles. Note that the two ordinate scales are displaced by 0.002 AU with respect to one another. Also, the abscissa scale includes approximately 20 ms of pretrigger time, which was accounted for when fitting the data. The pretrigger time was independently determined through the development of a calibration curve for the reaction of ascorbic acid and dichloroindophenol (Tonomura, B., Nakatani, H., Ohnishi, M., Yamaguchi-Ito, J., Hiromi, K. (1978) *84*, 370−383) using standard methods (Dunn, B. C., Meagher, N. E., Rorabacher, D. B. (1996) *J. Phys. Chem. 100*, 16925−16933). A single-exponential least-squares fit of the averaged data is illustrated (bold line, with the residuals depicted at the bottom of the graph).

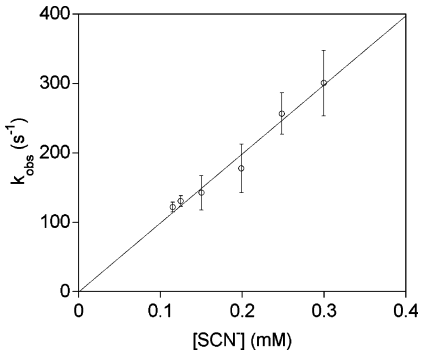


**Figure 2.** Thiocyanate concentration dependency of the rate law under pseudo-first-order conditions: $[OBr^-] = 0.026$ mM; phosphate buffer = 0.10 M; $[SCN^-] = 0.12-0.30$ mM; pH = 12.3; $I = 0.60$ M ($Na_2HPO_4$ + $Na_3PO_4$); $T = 291$ K. Kinetic traces were evaluated at 329 nm.

that the reaction is first-order in both reactants (Figures 1 and 2). The observed pseudo-first-order rate constants (at constant $[OBr^-]$, $[SCN^-]$, and ionic strength) exhibit inverse dependency on the $[OH^-]$, indicating that HOBr is more reactive than $OBr^-$ (Figure 3, where $k_{eff} = (k_1 + k_3K_2/[OH^-])$, cf. eq 5, vide infra). The mechanism of eqs 1−3 is consistent with these observations, and the rate law of eq 4 results from assuming steady-state [HOBr] from eqs 1−3:

$$OBr^- + SCN^- \rightarrow \text{products} \quad (1)$$

$$H_2O + OBr^- \rightleftharpoons HOBr + OH^- \quad (2)$$

$$HOBr + SCN^- \rightarrow \text{products} \quad (3)$$

$$\frac{-d[OBr^-]}{dt} = \left(k_1 + \frac{k_2k_3}{k_{-2}[OH^-] + k_3[SCN^-]}\right)[OBr^-][SCN^-] \quad (4)$$

A value of $k_{-2} \approx 3 \times 10^9$ $M^{-1}$ $s^{-1}$ has been estimated from

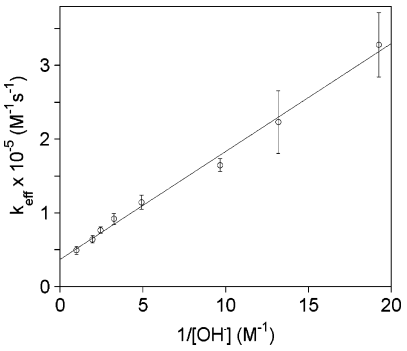


**Figure 3.** Hydroxide ion concentration dependency of the rate law under pseudo-first-order conditions: $[OBr^-] = 0.040$ mM; $[SCN^-] = 0.30$ mM; $[OH^-] = 0.052-1.02$ M; $I = 1.00$ M ($NaClO_4$ + NaOH); $T = 291$ K. Kinetic traces were evaluated at 329 nm.

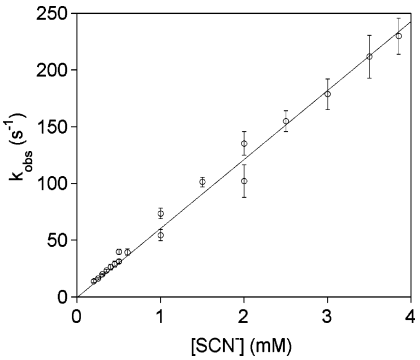


**Figure 4.** Thiocyanate concentration dependency of the rate law under pseudo-first-order conditions: $[OBr^-] = 0.026$ mM; $[SCN^-] = 0.20-0.39$ mM; $[OH^-] = 0.58$ M; $I = 1.00$ M ($NaClO_4$ + NaOH); $T = 291$ K. Kinetic traces were evaluated at 329 nm.

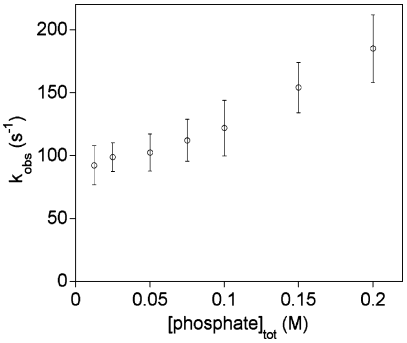


**Figure 5.** Phosphate concentration dependency of the rate law under pseudo-first-order conditions: $[OBr^-] = 0.026$ mM; $[SCN^-] = 0.20$ mM; pH = 12.4; $[phosphate]_{total} = 0.013-0.20$; $I = 1.00$ M ($NaClO_4$ + $Na_2HPO_4$ + $Na_3PO_4$); $T = 291$ K. Kinetic traces were evaluated at 329 nm.

p$K_a$ values by Gerristen et al. (*39*), and $k_3$ must be less than the diffusion-controlled limit ($10^9-10^{10}$ $M^{-1}$ $s^{-1}$); therefore, $k_{-2}[OH^-] \gg k_3[SCN^-]$ for all of the reaction conditions that were employed ($[OH^-] \geq 52$ mM and $[SCN^-] \leq 390$ $\mu$M) and eq 4 may be simplified to eq 5.

$$\frac{-d[OBr^-]}{dt} = (k_1 + k_3k_2[OH^-]^{-1})[OBr^-][SCN^-] \quad (5)$$

Figure 4 illustrates the $[SCN^-]$ dependency of the observed pseudo-first-order rate constant, where due to the high $[OH^-]$, the contribution of the eqs 2−3 pathway, as determined from the $1/[OH^-]$ dependency, is less than 40%. Phosphate buffer is found to accelerate the reaction of $OBr^-$ and $SCN^-$ (Figure 5).

## Discussion

**Mechanism of the Reaction of HOBr and $SCN^-$.** Using a p$K_a$ value for HOBr of 8.59 at an ionic strength of 1 (*39*), we compute a second-order rate constant for the reaction of HOBr and $SCN^-$ ($k_3 = 2.3 \times 10^9$ $M^{-1}$ $s^{-1}$) that is remarkably larger than that observed for HOCl and $SCN^-$ ($2.3 \times 10^7$ $M^{-1}$ $s^{-1}$) (*34*). Furthermore, in contrast to the fact that $OCl^-$ does not react with a measurable rate constant, $OBr^-$ reacts with $SCN^-$ with a sizable rate constant ($3.8 \times 10^4$ $M^{-1}$ $s^{-1}$), as determined from the nonzero intercept of Figure 3.

It has been previously observed by Margerum et al. that the reactions of HOBr with $ClO_2^-$ are general acid-catalyzed by $H_2PO_4^-$ (*40*). General acid catalysis has also been recognized for the reactions of HOCl with $ClO_2^-$, $SO_3^{2-}$, and $I^-$ (*41*−*43*). General acid catalysis can be explained by two mechanisms (or a combination thereof). The first mechanism consists of the acid-assisted protonation of $OBr^-$, resulting in the formation of the more reactive HOBr (eq 2 and eqs 6−8):

$$OBr^- + red \rightarrow products \quad (6)$$

$$OBr^- + HPO_4^{2-} \rightleftharpoons HOBr + PO_4^{3-} \quad (7)$$

$$HOBr + red \rightarrow products \quad (8)$$

The second mechanism involves acceleration of the HOBr + reductant = products reactions. The latter model assumes, in addition to the aforementioned pathway (eqs 6−8), a formation of an associative intermediate, which subsequently decomposes to give the products (eqs 9−11):

$$HOBr + red \rightleftharpoons \{BrOH\text{--}red\} \quad (9)$$

$$\{BrOH\text{--}red\} \rightarrow products \quad (10)$$

$$\{BrOH\text{--}red\} + HPO_4^{2-} \rightarrow products \quad (11)$$

The rate laws for the first and second model for general acid catalysis are given by eqs 12 and 13, respectively. Because of

$$\frac{-d[OBr^-]}{dt} = \left(k_1 + \frac{(k_2 + k_7[HPO_4^{2-}])k_8}{k_{-2}[OH^-] + k_{-7}[PO_4^{3-}] + k_8[red]}\right)[OBr^-][SCN^-] \quad (12)$$

$$\frac{-d[OBr^-]}{dt} = \left(k_6 + \frac{k_9k_7[HPO_4^{2-}](k_{10} + k_{11}[HPO_4^{2-}])}{k_{-7}k_{-9}[PO_4^{3-}] + (k_{-7}[PO_4^{3-}] + k_9[red])(k_{10} + k_{11}[HPO_4^{2-}])}\right)[OBr^-][red] \quad (13)$$

the uncertainties of the observed data (vide supra), reliable fits cannot be obtained for any combination of eqs 12 and/or 13; however, the following inferences can be made. First, the rate of reaction 3 is very close to the diffusion-controlled limit, so it is unlikely that the observed 2-fold increase in the observed rate constant (while increasing the buffer concentration only from 0.01 to 0.2 M) is only due to the acceleration of this reaction step (eqs 9−11). Because of experimental limitations, we were unsuccessful in demonstrating a possible retarding effect by employing high concentrations of phosphate buffer. Second, the reaction steps that correspond to $k_{-2}$ and $k_{-7}$ are also likely to be diffusion-controlled (vide supra) (*39*), and there

is only an order of magnitude difference between $K_2$ and $K_7$, so eqs 6−8 alone are also not adequate to explain the observed catalytic effect.

**Product of the Reaction of HOBr and $SCN^-$.** The hypohalous acids generally react as electrophilic halogenating agents. Consequently, they are particularly effective at oxidizing good nucleophiles. In a physiologic setting (in water at neutral pH), most of the intermediates that are produced by the initial electrophilic attack undergo facile hydrolysis to yield products that are chemically equivalent to oxygen atom transfer (eqs 14−15):

$$Nu^- + HOX \rightarrow NuX + OH^- \quad (14)$$

$$NuX + OH^- \rightarrow NuOH + X^- \quad (15)$$

For thiols such as Cys, the corresponding sulfenyl chloride (RSCl) has been reported as a transitory species at high pH (*44*, *45*). For some amines, chloramines and bromamines (RHNX; X = Cl, Br) are observed as transitory species at neutral pH (*46*−*48*). While the initial product that is obtained upon reaction of HOX (X = Cl, Br) with $SCN^-$ is still under investigation, we will note that the same initial product appears to be formed at pH > 10 and the spectroscopic and chemical properties of the species appear to be consistent with a formulation of $OSCN^-$. Thus, the same initial UV spectrum is obtained at high pH regardless of whether HOCl or HOBr is used (*49*). In a double-mixing experiment in which the initial product is produced at high pH, acidification in the presence of excess $SCN^-$ yields a quantitative amount of thiocyanogen ($(SCN)_2$) for both HOCl (*34*) and HOBr (*49*). The rate law that we observe for the latter reaction, which we interpret as comproportionation of HOSCN and $SCN^-$, is first-order in $[SCN^-]$ and $[H^+]$, and the observed rate constants are the same regardless of whether HOCl or HOBr is employed to generate the intermediate at high pH (*49*). The stoichiometry of the reaction of the intermediate and RSH is 1:1, as is expected for the formation of a sulfenyl thiocyanate (*49*). Thus, it appears likely to us that the initial observable product for the reaction of HOX and $SCN^-$ at high pH is $OSCN^-$; however, the situation near physiologic pH is not so clear. We find no evidence for the formation of $OSCN^-$ at pH 7.4 when HOX (X = Cl, Br) is reacted with $SCN^-$ (*49*). When HOCl is reacted with $SCN^-$ in a first-mixing cycle at pH 7.4, followed immediately by a pH-jump to pH 0.3 in a second-mixing cycle, no $(SCN)_2$ is observed (*49*). In contrast, the same experiment with HOBr does not initially produce $(SCN)_2$, but we do subsequently observe the formation of a new intermediate that apparently yields $(SCN)_2$ as one of its decomposition products at low pH (*49*). We are tentatively assigning this reaction to the disproportionation of cyanosulfite ($O_2SCN^-$), a reaction that is expected to yield $OSCN^-$ and cyanosulfate ($O_3SCN^-$). The former species would yield $(SCN)_2$ (observed), and the latter species should undergo hydrolysis to give cyanate ($OCN^-$) and sulfite ($SO_3^{2-}$). Considering the fact that the reaction velocities are expected to achieve their maximum values near neutral pH, the production of $O_2SCN^-$ rather than $OSCN^-$ at pH 7.4 may be due to overoxidation as a consequence of nonturbulent mixing on the time scale of the chemical reaction. Importantly, $OSCN^-$ apparently exhibits transitory stability at pH 7.4. In a triple-mixing experiment, $OSCN^-$ was generated at pH 13; in a second-mixing cycle, the pH was changed to 7.4 for a period of time; finally, in a third-mixing cycle, the pH was adjusted to 0.3 with the observation of a stoichiometric quantity of $(SCN)_2$ (for short delay times) (*49*). The half-life for $[OSCN^-] = 2.5$ mM ($[SCN^-] = 50$ mM) at pH 7.4, as measured by the aforementioned method, is only about 50 s (*49*). It is noteworthy that this half-life is also consistent with a value that we estimated using data that were reported by Stanbury at pH 0−2 (*50*), but it is inconsistent with a large body of literature that reports longevity for $OSCN^-$ at neutral pH. Also, we point out that preliminary studies suggest that the species we assign to $O_2SCN^-$ also reacts with thiols, albeit not as fast as $OSCN^-$ (*49*). However, it is not clear at this point whether $O_2SCN^-$ itself is reacting with the thiols or if perhaps one of its decomposition products (cf. disproportionation to yield $OSCN^-$) is doing so. In either case, these observations raise questions concerning the actual antimicrobials that are produced in the milieu of reactive sulfur species (RSS) that are produced upon oxidation of $SCN^-$.

**Production of HOBr by Phagocytes, Inflammatory Disease, and Antioxidants.** Given the differences that are observed for the reaction properties of HOCl and HOBr (*16*, *17*, *29*), it is important to consider the origins of these ROS in a physiologic setting. Neutrophils and monocytes both possess MPO, but eosinophils employ EPO instead. Monocytes lose their MPO activity during differentiation into tissue macrophages (*51*), but macrophages may reacquire MPO from their environment by pinocytosis or from ingested neutrophils (*52*). While neutrophils are typically the most abundant leukocytes and are the first to respond to sites of inflammation, macrophages play a larger role in most chronic inflammations. In particular, macrophages are associated with atherosclerotic lesions (*53*). However, it is not always the case that macrophages are involved in the advanced stages of infection, in that neutrophils dominate throughout all stages of periodontal disease (*54*), for example. In contrast to neutrophils, which play a key role in the immunological clearance of pathogenic bacteria, eosinophils appear to be responsible for combating infection by parasites (*55*), a defensive mechanism that generally involves extracellular release of killing agents, an event that should improve the chances of collateral damage to the host. Eosinophils also play a role in the allergic response, are considered the main effector cells in asthma pathogenesis (*56*), and are in particular associated with disease severity. Extracellular release of EPO is observed during eosinophil degranulation in connection with certain pathological conditions such as Hodgkin's disease, chronic myelogenous leukemia, and endomyocardial fibrosis (*57*). Given the cytotoxic nature of many of the aforementioned ROS, it is critical that mechanisms are available to govern their reactivities. The human body contains a plethora of antioxidants, including small molecules (e.g., retinol, ascorbic acid, and α-tocoperol) (*3*) and enzymes. Nonetheless, "oxidative stress" occurs when the defensive mechanisms are overwhelmed. Biomarkers (thermodynamically stable derivatives that are produced as end products in the chemistry of reactive species) have been employed to access oxidative stress. There is naturally significant interest in interpreting the speciation of biomarkers in terms of their origin and kinetics that have played a significant role in such analyses.

**Biomarkers.** There has been extensive modeling of the reactions of HOCl and HOBr with proteinaceous components in the context of identifying biomarkers for oxidative stress (*17*, *58*). Halogenated tyrosine residues are often used as evidence for oxidative stress, with chlorinated tyrosine (Cl-Tyr) and brominated tyrosine (Br-Tyr) being attributed to MPO and EPO, respectively. However, it has been recently suggested (*17*) that the use of X-Tyr as biomarkers for oxidative stress may overestimate the role of EPO, because HOBr reacts ca. 5000

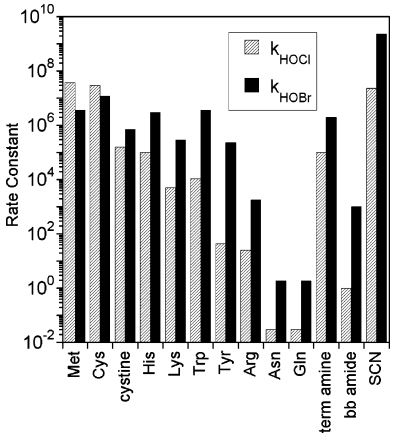


**Figure 6.** Summary of the effective second-order rate constants (log scale and corrected for pH 7.4) for the reactions of HOCl (hashed bars) and HOBr (filled bars) with various proteinaceous components and thiocyanate. Adapted from ref *17*. Note the general trend of ca. 100-fold larger rate constants for HOBr as compared with HOCl, with the notable exceptions of Cys and Met.

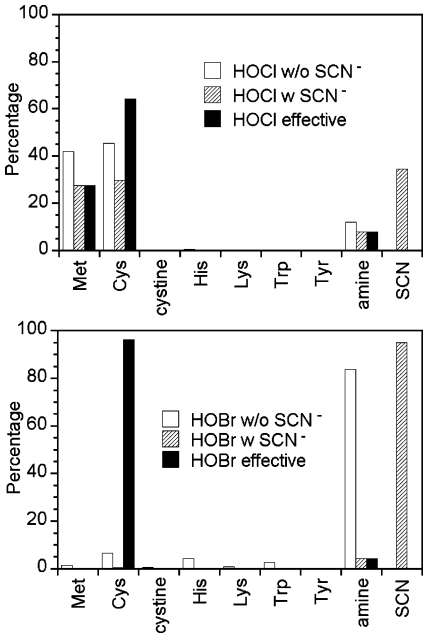


**Figure 7.** Histogram illustrating the expected partitioning (initial rates) of HOCl (top) and HOBr (bottom) between free amino acids and thiocyanate in plasma using three models: (1) the original model as proposed by Pattison and Davies in ref *17* (open bars), (2) the same model with the inclusion of $SCN^-$ with a normal reference value of 50 $\mu$M (hashed bars), and (3) the latter model after taking into account that the $OSCN^-$ that is produced by the reaction of HOX and $SCN^-$ is expected to subsequently react with Cys to yield, after hydrolysis, effective oxidation (filled bars).

times faster than HOCl for a given concentration of HOX (Figure 6). Importantly, the most effective proteinaceous scavengers (e.g., the Cys and Met residues of HSA) react *more slowly* with HOBr than with HOCl (Figure 6), thereby reinforcing the incongruence between HOX concentration in vivo and the expected occurrence of X-Tyr (*17*, *29*, *34*, *49*, *59*). Figure 7 illustrates a model for the partitioning of HOX between the free amino acids in plasma as previously proposed by Pattison and Davies (open bars) (*17*). This model predicts HOCl will react principally with sulfur-containing components, whereas HOBr will react largely with nitrogen-containing species. We have augmented this model by including the effects of $SCN^-$ using a normal reference value of 50 $\mu$M (hashed bars). The effect of $SCN^-$ on the partitioning of HOCl is minimal, but a marked influence on the partition of HOBr is predicted because $SCN^-$ is expected to compete effectively for most of the HOBr, presumably to produce $OSCN^-$. Given the apparent selectivity of $OSCN^-$ for thiols (*20*−*23*) in a reaction that effectively yields the same oxidation products that would have resulted if HOBr had reacted directly with Cys (*23*), the anticipated net effect of the intervening $SCN^-$ is to cause the oxidation of Cys (solid bars), not nitrogen-derivatives as predicted in the original model. Importantly, the altered reaction pattern that emerges when $SCN^-$ is included in the model has a marked effect on the relative amounts of X-Tyr. For equal molar amounts of HOCl and HOBr in the absence of $SCN^-$, about 1800 times more Tyr-Br is expected than Tyr-Cl. If 50 $\mu$M $SCN^-$ is included in the model, the ratio drops to 140:1. Importantly, we are not proposing that the inclusion of $SCN^-$ in the model of Pattison and Davies (*17*) yields an accurate model. On the contrary, this example illustrates the challenges that are faced in developing such kinetic models of physiological chemistry, wherein the addition of a single new parameter (in this case the reaction of HOBr with $SCN^-$) has the effect of significantly altering the outcome.

## Conclusion

The kinetics and mechanism of the reaction of $SCN^-$ with $HOBr/OBr^-$ have been determined. The proposed mechanism includes parallel pathways with $Br^+$ transfer to $SCN^-$ by general acid catalysis and by direct reaction with HOBr. HOBr reacts with $SCN^-$ with a second-order rate constant ($2.3 \times 10^9$ $M^{-1}$ $s^{-1}$) that is 5 orders of magnitude larger than that for $OBr^-$ ($3.8 \times 10^4$ $M^{-1}$ $s^{-1}$). Since the p$K_a$ of HOBr is 8.59, the HOBr pathway is expected to dominate at neutral pH. On a molar basis, thiocyanate is apparently the most efficient scavenger of HOBr in a physiological setting that has been reported to date. The next best scavengers, Cys ($1.2 \times 10^7$ $M^{-1}$ $s^{-1}$) and Met ($3.6 \times 10^6$ $M^{-1}$ $s^{-1}$), are 200 and 650 times less effective on a per mole basis, respectively. Furthermore, if we assume the rate constant for free Cys represents the upper limit for the reaction of HSA and HOBr (see the Introduction), we arrive at the remarkable conclusion that $SCN^-$ is more than 30 times more effective at sequestering HOBr in human plasma. The facile reaction of $SCN^-$ with HOBr leads us to suggest $SCN^-$ may serve as a scavenger that restricts the capacity of HOBr to inflict host tissue damage during inflammatory response, particularly during conditions of eosinophilia (*60*). In addition, our results raise questions as to whether extracellular HOBr plays a significant role in the clearance of parasites by eosinophil cells in a plasma setting where the HOBr that is produced by degranulated EPO is expected to react rapidly with $SCN^-$ to produce $OSCN^-$. Thus, $OSCN^-$ may play a larger role in the killing of parasites than that for which it has previously been given credit.

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