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Hypobromous acid, a powerful endogenous electrophile: Experimental and theoretical studies

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ABSTRACT

Hypobromous acid (HOBr) is an inorganic acid produced by the oxidation of the bromide anion (Br^-). The blood plasma level of Br^- is more than 1,000-fold lower than that of chloride anion (Cl^-). Consequently, the endogenous production of HOBr is also lower compared to hypochlorous acid (HOCl). Nevertheless, there is much evidence of the deleterious effects of HOBr. From these data, we hypothesized that the reactivity of HOBr could be better associated with its electrophilic strength. Our hypothesis was confirmed, since HOBr was significantly more reactive than HOCl when the oxidability of the studied compounds was not relevant. For instance: anisole (HOBr, $k_2 = 2.3 \times 10^2\ M^{-1}\ s^{-1}$, HOCl non-reactive); dansylglycine (HOBr, $k_2 = 7.3 \times 10^6\ M^{-1}\ s^{-1}$, HOCl, $5.2 \times 10^2\ M^{-1}\ s^{-1}$); salicylic acid (HOBr, $k_2 = 4.0 \times 10^4\ M^{-1}\ s^{-1}$, non-reactive); 3-hydroxybenzoic acid (HOBr, $k_2 = 5.9 \times 10^4\ M^{-1}\ s^{-1}$, HOCl, $k_2 = 1.1 \times 10^1\ M^{-1}\ s^{-1}$); uridine (HOBr, $k_2 = 1.3 \times 10^3\ M^{-1}\ s^{-1}$, HOCl non-reactive). The compounds 4-bromoanisole and 5-bromouridine were identified as the products of the reactions between HOBr and anisole or uridine, respectively, *i.e.* typical products of electrophilic substitutions. Together, these results show that, rather than an oxidant, HOBr is a powerful electrophilic reactant. This chemical property was theoretically confirmed by measuring the positive Mulliken and ChelpG charges upon bromine and chlorine. In conclusion, the high electrophilicity of HOBr could be behind its well-established deleterious effects. We propose that HOBr is the most powerful endogenous electrophile.

1. Introduction

There is much evidence that harmful endogenous chemicals are involved in the aetiology and/or progression of chronic inflammatory and degenerative diseases [1–3]. One of the most important primary sources of these chemicals is the one-electron reduction of molecular oxygen to a superoxide anion radical ($O_2^{-\bullet}$) [4]. Other reactive chemicals include hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^\bullet$), peroxyl radical ($ROO^\bullet$), peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl) and hypobromous acid (HOBr) [5–9]. Altogether, these substances are named reactive oxygen or reactive nitrogen species (ROS/RNS) and their harmful chemicals are associated with the ubiquitous presence of easily oxidizable moieties in proteins, lipids and nucleic acids.

Myeloperoxidase (MPO) is an enzyme present in the azurophilic granules of polymorphonuclear leukocytes (PMNs) and plays a fundamental role in the inflammatory and anti-inflammatory processes, including the killing of pathogens, recruitment of PMNs, apoptosis induction in PMNs and production of regulatory molecules inside phagosomes of macrophages [10]. Similarly, eosinophil peroxidase (EPO) is stored in the specific granules of eosinophils, which are specialized human phagocytes that play a critical beneficial role to eliminate tissue-invasive parasites [11]. MPO is able to catalyze the oxidation of both chloride (Cl^-) and bromide (Br^-), whereas EPO, at least at pH 7.0, is much more effective to Br^- [12]. This particularity of MPO is related to the standard reduction potential (E'°) of the redox couple compound I/ferric MPO (1.16 V), which is higher compared with EPO (compound I/ferric EPO, 1.10 V) [13].

Although the production HOCl and HOBr is an essential part of the innate immune defence, there is increasing experimental and clinical studies showing the correlation between hypohalous acid-mediated alteration in biomolecules and the progression of inflammatory-based diseases. Some important findings include the determination of the levels of 3-chlorotyrosine and 3-bromotyrosine in proteins and the identification of higher concentrations in the bronchoalveolar lavage of cystic fibrosis patients [14]; the concentration of 3-chlorotyrosine in circulating HDL has been shown to be an indicator of the risk of cardiovascular disease [15]; the increased level of 3-chlorotyrosine and 3-nitrotyrosine in HDL of rheumatoid arthritis patients [16]; the urinary

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level of 3-bromotyrosine can be used as a biomarker for asthma control and for prediction of the risk of future asthma exacerbation in children [17].

Bromine is a trace element in the human body. Indeed, the Br^- plasma level (2–100 μM) is more than 1,000-fold lower than that of Cl^- (100–140 mM) [18]. However, there is plenty of evidence of the endogenous generation of HOBr and its deleterious effects. For instance, the detection of 3-bromotyrosine can be as effective as 3-chlorotyrosine as a biomarker of MPO/EPO involvement in inflammatory disorders [14,17]. Moreover, the reactivity of HOBr with amino acids can be 30- to 100-fold faster than that of HOCl. Particularly for tyrosine, the ring halogenation by HOBr was 5,000-fold faster than by HOCl [19].

As stated above, tissue damage associated with ROS/RNS is related to the susceptibility of biomolecules to oxidation. However, the oxidizing capacity does not seem to be the major factor that influences the deleterious properties of HOBr. Indeed, though HOBr reacts faster with several biomolecules [19], HOCl is the stronger two-electron oxidant (E'° HOCl/Cl^- = 1.28 V, E'° HOBr/Br^- = 1.13 V) [20]. On the other hand, taking into account the electrophilicity of these chemicals, an inversion is observed, since HOBr is more reactive than HOCl under non-physiological experimental conditions [21]. Hence, considering the importance of bromination reactions in the deleterious pathways that underline chronic inflammatory diseases and the low concentration of Br^- in the physiological medium, we hypothesized that the electrophilicity of HOBr could be mainly responsible by its effects. Hence, we selected and studied endogenous and non-endogenous compounds for which the relative electrophilic potency of the hypohalous acids could be compared without, or with less, interference of oxidative process. We found that HOBr was able to act as an authentic electrophile and with higher efficacy than HOCl.

2. Materials and methods

2.1. Chemicals

Dansylglycine, anisole, acetophenone, salicylic acid, 3-hydroxybenzoic acid, tryptophan, adenine, cytosine, guanine, thymine, uracil, adenosine, cytidine, guanosine, thymidine, uridine, 5-bromouridine and 4-bromoanisole were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Stock solutions of anisole 4-bromoanisole and acetophenone (10 mM) were prepared in ethyl alcohol. Stock solution of dansylglycine (5 mM) was prepared in 10 mM hydrochloric acid. Stock solution of amino acids, nitrogen bases and nucleosides (5 mM) were prepared in 50 mM phosphate buffer at pH 7.0. The concentration of the commercial HOCl solution was determined spectrophotometrically after dilution in 0.01 M NaOH at pH 12 (ε_{292nm} = 350 $M^{-1}\,cm^{-1}$) [22]. HOCl was prepared immediately before the assays to give an aqueous stock solution of 100 mM. HOBr was synthesized by combining 100 mM HOCl and 200 mM NaBr in water immediately before the assays [23].

2.2. Determination of rate constants

The fast-kinetic experiments were performed using a single-mixing stopped-flow system equipped with high intensity LED source and cut-off filters (SX20/LED Stopped-Flow System, Applied Photophysics, UK). The photophysical properties of the studied compounds and their intrinsic reactivity determined the experimental conditions used to monitor each reaction.

2.2.1. Uridine

The measurements were performed by its absorbance using a 280 nm LED. The experiments were performed using pseudo-first-order conditions where the hypohalous acids were used in excess (500–1250 μM) compared with nucleoside (50 μM) in 50 mM phosphate buffer, pH 7.0 at 25 °C. The observed pseudo-first-order constant (k_{obs}) was obtained by fitting the absorbance decay of the nucleoside in a single exponential decay equation, as follows:

$$F = F_0 * e^{-k_{obs} * t}$$

From the k_{obs} values obtained at various nucleoside concentrations, the bimolecular rate constants (k_2) were calculated from the slope of the linear regression as follows:

$$\text{Reaction rate} = k_2{}^*[A]^*[B]$$

where [A] is the hypohalous acid and [B] is the studied compound.

If [A] >> [B] → reaction rate = $k_{obs}{}^*$[B].

Then, $k_{obs} = k_2{}^*$[A].

The k_2 is the slope of the linear fit of k_{obs} versus [A].

2.2.2. Dansylglycine

As above with the following modifications: dansylglycine 50 μM and HOCl 520 to 3200 μM, and dansylglycine 5 μM and HOBr 50 to 125 μM. The reactions were monitored by the intrinsic fluorescence of dansylglycine (excitation using a 360 nm LED and emission using a 475 nm cut-off filter).

2.2.3. Salicylic acid

As above with the following modifications: salicylic acid 50 μM and HOBr 500 to 1250 μM. The reactions were monitored by the intrinsic fluorescence of salicylic acid (excitation using a 280 nm LED and emission using a 375 nm cut-off filter).

2.2.4. 3-Hydroxybenzoic acid

As above with the following modifications: 3-hydroxybenzoic acid 50 μM and HOBr 125 to 500 μM, and 3-hydroxybenzoic acid 50 μM and HOCl 250 to 2000 μM. The reactions were monitored by its intrinsic fluorescence (excitation using a 280 nm LED and emission using a 375 nm cut-off filter).

2.2.5. Tryptophan

As above with the following modifications: tryptophan 50 μM and HOCl 125 to 375 μM, and tryptophan 5 μM and HOBr 10 μM. The reactions were monitored by the intrinsic fluorescence of tryptophan (excitation using a 280 nm LED and emission using a 325 nm cut-off filter).

2.2.6. Anisole

As above with the following modifications: anisole 50 μM and HOBr 125 to 375 μM, and anisole 50 μM and HOCl 500 μM. The measurements were performed by its absorbance using a 280 nm LED.

2.3. HPLC studies

The nitrogenous bases, nucleosides and anisole (1 mM) were submitted to the reactions with the hypohalous acids (1 mM) in phosphate buffer 0.05 mM, pH 7.0 and 25 °C, and the consumption after 30 min was monitored by HPLC analysis. The consumption of nitrogenous bases and nucleosides were chromatographically evaluated by HPLC (Jasco, Easton, MD, USA) in line with a UV-visible (UV–vis) detector set at 280 nm. The analyses were carried out isocratically on a Synergi Polar reversed-phase column (150 mm × 4.6 mm, 4 μm), with 0.1% formic acid in water and 0.1% formic acid in acetonitrile (95:5, v:v) as mobile phase, and flow rate of 1.0 mL/min. The analyses of consumption of anisole and formation of 4-bromoanisole were carried out isocratically on a Shim-pack C18 CLC-ODS reversed-phase column (150 mm × 6.0 mm, 4 μm), with 0.1% formic acid in water and 0.1% formic acid in acetonitrile (70:30, v:v) as mobile phase, and flow rate of 1.0 mL/min.