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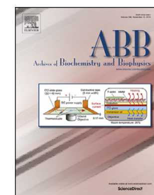

Specific role of taurine in the 8-brominated-2′-deoxyguanosine formation

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ABSTRACT

At the sites of inflammation, hypohalous acids, such as hypochlorous acid and hypobromous acid (HOBr), are produced by myeloperoxidase. These hypohalous acids rapidly react with the primary amino groups to produce haloamines, which are relatively stable and can diffuse long distances and cross the plasma membrane. In this study, we examined the effects of taurine, the most abundant free amino acid in the leukocyte cytosol, on the hypohalous acid-dependent formation of 8-chloro-2′-deoxyguanosine (8-CldG) and 8-bromo-2′-deoxyguanosine (8-BrdG). The reaction of taurine with HOBr yielded taurine bromamine, which is the most stable among other bromamines of amino acids. Taurine also enhanced the bromination of only dG among the four 2′-deoxynucleosides, whereas it inhibited the 8-CldG formation. The specificity of taurine for the enhanced formation of halogenated dG is completely different from that of nicotine, an enhancer of chlorination. The amount of dibrominated taurine (taurine dibromamine) closely correlated with the formation of 8-BrdG, suggesting that taurine dibromamine might be a plausible mediator for the dG bromination *in vivo*.

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1. Introduction

Hypobromous acid (HOBr), a brominating molecule, is generated by eosinophil peroxidase (EPO) through the EPO–H_2O_2–Br^- system. In contrast, hypochlorous acid (HOCl) is produced by myeloperoxidase (MPO) in the presence of H_2O_2 and Cl^-. It is also reported that MPO can produce HOBr and bromine chloride (BrCl) at the concentration of Br^- and Cl^- under physiological conditions [1]. Although these molecules play a major role in the host defense against microorganisms, the excessive amount of both hypohalous acids (HOCl and HOBr) induces oxidative modification of DNA, proteins and lipids. In addition, halogenated nucleosides, such as N_4,5-dichloro-deoxycytidine (N_4,5-diCldC), 8-halogenated 2′-deoxyguanosines (dGs) (8-chloro-2′-deoxyguanosine (8-CldG) and 8-bromo-2′-deoxyguanosine (8-BrdG)) and 5-chlorouracil (5-ClUra), as well as a halogenated amino acid, 3-chlorotyrosine (3-ClTyr), were detected in the liver of lipopolysaccharide (LPS)-treated mice [2,3], the urine of diabetic patients [3] and human atherosclerotic lesions [4,5]. The halogenated nucleosides may increase the mutagenic potential at the site of inflammation [6], i.e., the mis-cording property of 8-BrdG has been demonstrated [7]. Thus, the high concentrations of the halogenating species observed under inflammatory conditions are postulated to contribute to the progression of inflammatory-related diseases including cancer, kidney disease, and atherosclerosis.

Hypohalous acids are not only highly reactive oxidants but also strong electrophiles. Hypohalous acids readily react with amines to form haloamines (chloramine and bromamine) and with the unsaturated bonds of fatty acids to form halohydrins [8]. The

Abbreviations: HOBr, hypobromous acid; EPO, eosinophil peroxidase; MPO, myeloperoxidase; HOCl, hypochlorous acid; BrCl, brmine chloride; dG, 2′-deoxyguanosine; N_4,5-diCldC, N_4,5-dichloro-deoxycytidine; 8-CldG, 8-chloro-2′-deoxyguanosine; 8-BrdG, 8-bromo-2′-deoxyguanosine; 5-ClUra, 5-chlorouracil; 5-BrdC, 5-bromo-2′-deoxycytidine; 8-BrdA, 8-bromo-2′-deoxyadenosine; LPS, lipopolysaccharide; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; TNB, 5-thiol-2-nitrobenzoic acid; LC-MS/MS, HPLC tandem mass spectrometry; NaOCl, sodium hypochlorite; 3-ClTyr, 3-chlorotyrosine.

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predominant species of haloamines are monohaloamines and dihaloamines. Especially, taurine, a semi-essential sulfur-containing β-amino acid, is considered to be the major candidate of haloamines. Taurine is the most abundant free amino acid in most cells of all animals [9], although the concentration of this amino acid is still lower than that for protein-bound amine groups. The concentration of taurine in the plasma and extracellular fluids ranges from 10 to 100 μM [10]. In addition, some types of cells, such as neutrophils, accumulate taurine, the intracellular concentration of which is up to 50 mM [9]. Because the biosynthetic capacity of humans to produce taurine declines with aging and some pathological stages, the diet or supplementation is likely to increase the taurine level in human body [10]. The high concentration of taurine in neutrophils suggests its important role in immune systems, but it is still controversial. For example, the anti-inflammatory properties of taurine is partly explained by its reaction with HOCl resulting in the generation of taurine chloramine (TauCl), a more stable and less toxic anti-inflammatory mediator [10]. In contrast, TauCl oxidizes cellular glutathione and cysteine residues in cellular proteins, suggesting that TauCl is unstable under *in vivo* conditions [11]. Besides, stable long-lived haloamines have been reported to modify nucleosides into halogenated adducts [12]. Tertiary amines, such as nicotine, were also reported to catalytically enhance the chlorination, possibly through chloramine formation [13]. However, to the best of our knowledge, the role of bromamines in the 8-BrdG formation remains to be clarified.

In this study, we examined the effect of taurine on the halogenated DNA formation focusing on the 8-halogenated dGs, which are the promising biomarkers during the early inflammation stage [3]. Our results show that taurine selectively enhanced the bromination, but not the chlorination, of dG. Only the bromination of dG was promoted by taurine in contrast to the other 2′-deoxynucleosides. Furthermore, the amount of dibrominated taurine (taurine dibromamine) is closely correlated with the production of 8-BrdG, suggesting that taurine dibromamine might be a possible mediator for dG bromination.

2. Materials and methods

2.1. Materials

2′-Deoxyadenosine, 2′-deoxycytidine, 2′-deoxyguanosine, 2′-deoxythymidine, L-lysine, Nα-t-Boc-L-lysine, L-taurine, L-alanine, β-alanine, L-methionine, L-serine, L-phenylalanine, L-glutamic acid, L-asparagine, 5, 5′-dithiobis-2-nitrobenzoic acid (DTNB), nicotine and sodium hypochlorite (NaOCl) were obtained from Wako Pure Chemicals Industries. HOBr was prepared from an equimolar solution of NaOCl and KBr as described [14].

2.2. HPLC analysis of deoxynucleosides modification by hypohalous acids

As typical conditions, 1 mM deoxynucleosides and 1 mM hypohalous acids (HOCl and HOBr) with or without taurine or nicotine were reacted in 50 mM phosphate buffer (pH 7.4) at 37 °C for 60 min. The reaction was terminated by adding methionine (10 mM). The formations of halogenated deoxynucleosides were analyzed by reverse-phase HPLC (Develosil C30-UG, 4.6 × 150 mm) as described below. The chromatographic separation was performed by a gradient elution using two solvents, 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), as follows: 0–5 min, 3% B; 5–20 min, linear gradient to 15% B; 20.1–30 min, linear gradient to 100% B; 30–30.1 min, linear gradient to solvent A; 30.1–40 min, 100% A at a flow rate of 1.0 ml/min with monitoring at 280 nm. The amounts of products were estimated by comparison with authentic standards, which were purchased or synthesized as described below.

2.3. Bromamine synthesis and evaluation

The *N*-bromamines of amino acids were prepared by drop wise addition of 2 mM HOBr to 2 mM amino acids in 50 mM phosphate buffer (pH 7.4) at 4 °C. After incubation of reaction mixture at 37 °C for 15 s, samples were kept on ice. Then, half of the reaction mixture was incubated at 37 °C for 30 min. Bromamine concentrations were determined with 5-thiol-2-nitrobenzoic acid (TNB) as described previously [15].

2.4. The reaction of deoxynucleosides and hypohalous acids with amino acids

2 mM deoxynucleosides and 2 mM hypohalous acids (HOCl and HOBr) were reacted with 1 mM amino acids in 50 mM phosphate buffer (pH 7.4) at 37 °C for 60 min. The reaction was terminated by adding L-methionine (10 mM).

2.5. Synthesis of 8-CldG and 8-BrdG

8-CldG and 8-BrdG were prepared as described previously [3]. In brief, (A) 8-CldG: dG (2 mM) was supplemented with nicotine (20 μM) in 50 mM phosphate buffer (pH 8.0). The reaction was initiated by adding NaOCl (1 mM) to the reaction mixture at 37 °C for 1 h, and terminated by adding L-methionine (10 mM). (B) 8-BrdG: dG (2 mM) was supplemented with taurine (1 mM) in 50 mM phosphate buffer (pH 7.4). The reaction was initiated by adding HOBr (1 mM) to the reaction mixture at 37 °C for 1 h, and terminated by adding L-methionine (10 mM). The structures and the purity of 8-CldG and 8-BrdG were elucidated according to the previous report [3].

2.6. Conditions of detection of 8-modified dGs by LC-tandem mass spectrometry (LC-MS/MS)

LC-MS/MS analyses were performed on an API 2000 triple quadrupole mass spectrometer (Applied Biosystems, Framingham, MA, USA) through a TurboIonSpray source as described [3]. Chromatography was carried out on a Develosil ODS-HG-3 column (2.0 × 50 mm) using an Agilent 1100 HPLC system. 8-halo-dGs: The chromatographic separation was performed by a gradient elution using two solvents, 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Gradient program is as follows: 0–5 min, solvent A (hold); 5–18 min, linear gradient to 26% solvent B; 18–18.1 min, linear gradient to 100% solvent B; 18.1–24 min, 100% solvent B (hold); 24–24.1 min, linear gradient to solvent A; 24.1–34 min, solvent A (hold); flow rate = 0.2 ml/min. The instrument response was optimized by infusion experiments of the standard compounds using a syringe pump at a flow rate of 5 μl/min 8-halo-dGs were detected using electrospray ionization tandem mass spectrometry in the multiple reactions monitoring mode. Specific transitions used to detect products in the positive ionization mode were those between the molecular cation of the products and the characteristic fragment ion formed from the loss of the 2′-deoxyribose moiety.

2.7. Characterization of monohaloamine and dihaloamine

Taurine and 2 mM hypohalous acids (HOCl and HOBr) were reacted in 50 mM phosphate buffer (pH 7.4) at 37 °C for 15 s. After incubation, samples were kept on ice. Haloamine concentrations were determined by UV absorption spectra to assure the

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