Modification by Fluoride, Bromide, Iodide, Thiocyanate and Nitrite Anions of Reaction of a Myeloperoxidase–H_2O_2–Cl^- System with Nucleosides

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The influence of fluoride (F^-), bromide (Br^-), iodide (I^-), thiocyanate (SCN^-) and nitrite (NO_2^-) on the reaction of a myeloperoxidase–H_2O_2–Cl^- system with a nucleoside mixture was studied. The reaction was carried out under mildly acidic conditions and terminated by *N*-acetylcysteine. Without the additional anions, quantity of nucleosides consumed fell in the following order: 2′-deoxyguanosine>2′-deoxycytidine≫2′-deoxythymidine>2′-deoxyadenosine≅0. F^- did not affect the reaction. Br^- increased the consumption of 2′-deoxycytidine and 2′-deoxythymidine, but decreased that of 2′-deoxyguanosine. I^-, SCN^- and NO_2^- suppressed the reaction. These results suggest that Br^- has a unique effect in relation to nucleoside damage caused by myeloperoxidase.

Key words myeloperoxidase; nucleoside; bromide; iodide; thiocyanate; nitrite

Hypochlorous acid (HOCl) is generated as an endogenous product of the respiratory burst in mammalian neutrophils by myeloperoxidase from hydrogen peroxide (H_2O_2) and chloride (Cl^-).[1] HOCl generated by myeloperoxidase is of central importance in immune surveillance and host defense mechanisms. However, it also has potential to harm normal tissue and contribute to inflammatory injury. Indeed, reagent HOCl and/or the myeloperoxidase–H_2O_2–Cl^- system have been reported to react with nucleic acid bases to form various compounds.[2—8] We have examined the reaction of 2′-deoxyguanosine (dG) with reagent HOCl and/or the myeloperoxidase–H_2O_2–Cl^- system and found that a diimino-imidazole nucleoside, an amino-imidazolone nucleoside, a spiroiminodihydantoin nucleoside and 8-chloro-2′-deoxyguanosine were generated.[9—12]

In addition to Cl^-, myeloperoxidase can oxidize other halides, (bromide (Br^-) and iodide (I^-)) and a pseudohalide, thiocyanate (SCN^-),[13] as well as nitrite (NO_2^-) in the presence of H_2O_2.[14] However, Cl^- has been assumed to be the physiological substrate for myeloperoxidase, since plasma concentrations of Cl^- are high (100—140 mM),[15] in contrast to 39—84 μM Br^-,[16] 0.46—0.67 μM I^-,[16] 21—134 μM SCN^-[17] and 0.25—0.65 μM NO_2^-.[18] Recently, Byun *et al.*[19] reported that 8-nitro-2′-deoxyguanosine was generated by the reaction of dG with the myeloperoxidase–H_2O_2–Cl^- system in the presence of NO_2^- at plasma concentrations of Cl^- and NO_2^-. Similarly, Henderson *et al.*[20] reported the formation of 5-bromo-2′-deoxycytidine in the reaction of 2′-deoxycytidine (dC) with the myeloperoxidase–H_2O_2–Cl^- system in the presence of Br^-, at plasma concentrations of Cl^- and Br^-. These papers showed that NO_2^- and Br^- were significant regents with respect to nucleoside damage caused by the myeloperoxidase–H_2O_2–Cl^- system. However, in these studies and others of damage caused by myeloperoxidase, specific products and their yields were determined, but data for the whole reaction, *i.e.*, for consumption of the target nucleosides, were extremely limited.

In the present study, we investigated the reaction of a nucleoside mixture with the myeloperoxidase–H_2O_2–Cl^- system in the presence of various anions including F^-, Br^-, I^-, SCN^- and NO_2^-, and the consumption of each nucleoside was determined by HPLC.

Results and Discussion

pH-Dependence of the Reaction of the Nucleoside Mixture with the Myeloperoxidase–H_2O_2–Cl^- System A nucleoside mixture (dG, dC, 2′-deoxythymidine (dT) and 2′-deoxyadenosine (dA); 100 μM each) was incubated with 50 nM myeloperoxidase in 100 mM sodium phosphate buffers of different pH with 200 μM H_2O_2 and 100 mM NaCl at 37 °C for 30 min. The reaction was terminated by addition of *N*-acetylcysteine. The reaction mixture was analyzed by reversed-phase HPLC to determine the remaining concentration of each nucleoside. Figure 1 shows a plot of the concentrations of nucleosides *versus* pH. Consumption of nucleosides was observed under mildly acidic conditions. The myeloperoxidase–H_2O_2–Cl^- system consumed nucleosides in the following order: dG>dC≫dT>dA≅0. The optimal pH for consumption of nucleosides was 4.7. At pH 4.7, total consumption was 67.3 μM corresponding to 33.7% relative to H_2O_2 added.

It has been reported that dC reacts with a myeloperoxi-

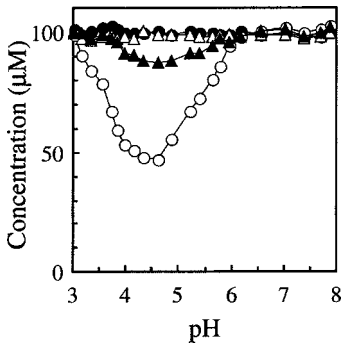

Fig. 1. pH Dependence of the Reaction of the Myeloperoxidase–H_2O_2–Cl^- System with Nucleosides

Concentrations of dG (open circles), dC (closed triangles), dT (open triangles) and dA (closed circles) are plotted against pH. A nucleoside mixture (dG, dC, dT and dA; 100 μM each) was incubated with 50 nM myeloperoxidase in 100 mM sodium phosphate buffer of various pH's with 200 μM H_2O_2, 100 μM diethylenetriaminepentaacetic acid (DTPA) and 100 mM NaCl at 37 °C for 30 min. The reaction was terminated by addition of 400 μM *N*-acetylcysteine. The concentration of each nucleoside was quantified by reversed-phase HPLC analysis.

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dase–H_2O_2–Cl^- system to form 5-chloro-2′-deoxycytidine.[7] At neutral pH, the yield of the chlorinated derivative was extremely low. The yield was greater under mildly acidic conditions, and the optimal pH was 4.5. We have found that dG reacts with a myeloperoxidase–H_2O_2–Cl^- system to generate several products including a diimino-imidazole nucleoside, an amino-imidazolone nucleoside and 8-chloro-2′-deoxyguanosine.[9,12] The yields of these products were extremely low at neutral pH but higher under mildly acidic conditions. The optimal pH was 3.7—4.7 depending on the product. In the present study, no consumption of dC and dG was detected at neutral pH. The optimal pH was 4.7 for consumption of both dC and dG. A ground-state myeloperoxidase uses H_2O_2 as a substrate, oxidized by two electron equivalents, to form a redox intermediate termed compound I.[1] Oxidation of Cl^- by compound I occurs through a single two-electron-transfer reaction to generate HOCl. A previous kinetic study of oxidation of Cl^- by compound I revealed that at pH 7 the second-order rate constant was two orders of magnitude lower than at pH 5.[13] The optimal pH for formation of HOCl by the myeloperoxidase–H_2O_2–Cl^- system was in the mildly acidic region and was dependent on the ratio of H_2O_2 to Cl^-.[21] These previous and present results suggest that the consumption of nucleosides by a myeloperoxidase–H_2O_2–Cl^- system involves the formation of HOCl. Under acidic conditions, HOCl reacts with Cl^- and H^+ to generate molecular chlorine Cl_2.[22] It has been proposed that Cl_2 but not HOCl, is the oxidizing intermediate for the reaction of dC by the myeloperoxidase–H_2O_2–Cl^- system, whereas both HOCl and Cl_2 are the oxidizing intermediate for the reaction of dG with the myeloperoxidase–H_2O_2–Cl^- system.[9,12]

Effect of F^- on the Reaction of Nucleosides with Myeloperoxidase–H_2O_2–Cl^- To evaluate the effect of F^- on the reaction of nucleosides with the myeloperoxidase–H_2O_2–Cl^- system, we examined the reaction of a nucleoside mixture with the myeloperoxidase–H_2O_2–Cl^- system at pH 4.7 or at pH 7.4 in the presence of various concentrations of F^- (0—1000 μM). Figure 2A shows the concentrations of unreacted nucleoside in the reaction mixture at pH 4.7 with various F^- concentrations. The addition of F^- did not affect the consumption of nucleosides. When the reactions were carried out at pH 7.4, no consumption of nucleosides was observed (Fig. 2B).

In many countries, tap water is fluoridated to protect against dental caries.[23] The concentration of F^- in human plasma is 0.14—8.7 μM, and shows a good correlation with the F^- concentration in tap water.[24] Although many epidemiological studies have attempted to correlate cancer mortality with concentrations of F^- in the water supply, no association has been proven.[23,25,26] The present results showed no effect on nucleoside damage and suggest that F^- does not affect any potential cancer risk linked to myeloperoxidase in plasma.

Effect of Br^- on the Reaction of Nucleosides with Myeloperoxidase–H_2O_2–Cl^- Br^- had a strong effect on the reaction of nucleosides with the myeloperoxidase–H_2O_2–Cl^- system at pH 4.7. As shown in Fig. 3A, dC consumption was greatly increased by addition of Br^- at above 10 μM, while dG consumption was decreased. In addition, dT consumption was increased by high doses of Br^- (above 100 μM). No consumption of dA was observed over the range of Br^- concentration examined. At 100 μM Br^-, total consumption of nucleosides was 103.8 μM, corresponding to a 51.9% yield relative to the H_2O_2 applied. At pH 7.4, no consumption of nucleosides was observed (Fig. 3B).

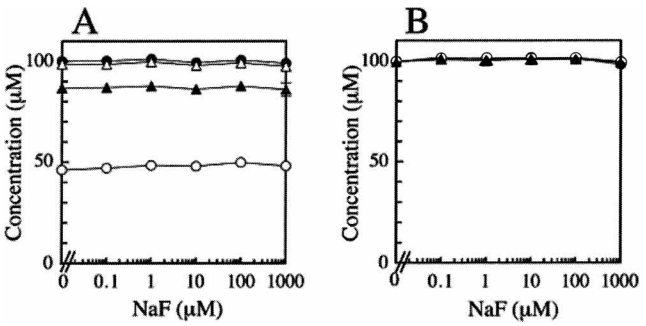

Fig. 2. Effect of F^- on the Reaction of the Myeloperoxidase–H_2O_2–Cl^- System with Nucleosides at pH 4.7 (A) and pH 7.4 (B)

The concentrations of dG (open circles), dC (closed triangles), dT (open triangles) and dA (closed circles) are plotted against the concentration of NaF added. A nucleoside mixture (dG, dC, dT and dA; 100 μM each) was incubated with 50 nM myeloperoxidase in 100 mM sodium phosphate buffer (pH 4.7 or 7.4) with 200 μM H_2O_2, 100 μM DTPA and 100 mM NaCl at 37 °C for 30 min in the presence of 0—1000 μM NaF. The reaction was terminated by addition of 400 μM *N*-acetylcysteine. The concentration of each nucleoside was quantified by reversed phase HPLC analysis. The reactions at pH 4.7 were performed in triplicate and the data points are expressed as means±S.D.

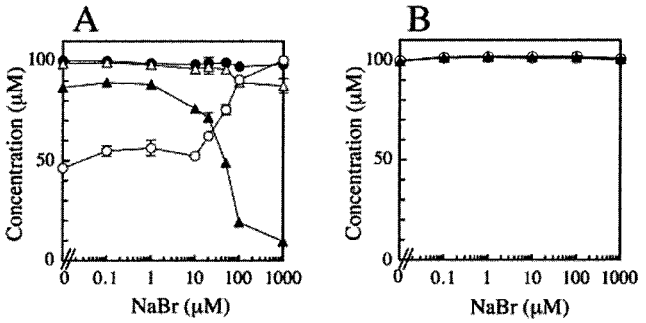

Fig. 3. Effect of Br^- on the Reaction of the Myeloperoxidase–H_2O_2–Cl^- System with Nucleosides at pH 4.7 (A) and pH 7.4 (B)

The concentrations of dG (open circles), dC (closed triangles), dT (open triangles) and dA (closed circles) are plotted against the concentration of NaBr added. The reaction conditions and the analytical methods were as described in Fig. 2, except that the added anion was Br^-.

Although bromine is one of the most abundant trace elements, essential roles in cells have been difficult to demonstrate.[27] Br^- can replace Cl^- as a substrate in many biological processes including enzyme activation and inhibition. The first identified physiological function of Br^- in humans appears to be a role in defense mechanisms against parasites mediated by the preferential oxidation of Br^- by eosinophil peroxidase.[28] An eosinophil peroxidase–H_2O_2–Br^- system in the presence of a plasma concentration of Cl^- can react with nucleosides to form brominated nucleosides.[29—31] More recently, it has been reported that the myeloperoxidase–H_2O_2–Cl^- system in the presence of a plasma concentration of Br^- can generate 5-bromo-2′-deoxycytidine from dC.[20] In the present study, we found that Br^- strongly affects nucleoside consumption during the reaction with myeloperoxidase. The concentration of Br^- in plasma is fairly high and ranges over levels of 39—84 μM.[17] In the presence of Br^- at concentrations in this range, more dC was consumed than dG, and at >100 μM Br^-, more dT was consumed than dG by the myeloperoxidase–H_2O_2–Cl^- system. The total consumption

of nucleosides was increased by addition of Br^-. These results imply that Br^- can have important effects on nucleoside damage caused by myeloperoxidase. It has been proposed that bromine chloride (BrCl), molecular bromine (Br_2) or hypobromous acid (HOBr) is the oxidizing intermediate for the reaction of nucleosides by the myeloperoxidase–H_2O_2–Cl^-–Br^- system as well as by the eosinophil peroxidase–H_2O_2–Cl^-–Br^- system.[20,29—31] In the present study, the main reaction target of the myeloperoxidase–H_2O_2–Cl^- system was altered from dG to dC by the addition of Br^-. The oxidizing intermediate (BrCl, Br_2 or HOBr) formed by the myeloperoxidase–H_2O_2–Cl^-–Br^- system may react with dC more preferentially than dG.

Effect of I^- on the Reaction of Nucleosides with Myeloperoxidase–H_2O_2–Cl^- Figure 4 shows concentrations of unreacted nucleosides in the reaction mixture at various concentrations of I^-. The addition of I^- suppressed the reaction at pH 4.7 (Fig. 4A), and at 10 μM I^-, the consumption of dG was almost totally suppressed. However, this suppressive effect was weak for dC. At pH 7.4, no consumption of nucleosides was observed at the I^- concentration range examined (Fig. 4B).

Iodine is an essential micronutrient for human. Lack of iodine causes delay of physical and mental development in humans, a condition known as iodine deficiency disorder.[32] To prevent this, iodized table salt is used in many countries. On the other hand, it has been reported that intake of high doses of I^- can promote cancer in animals.[33] In the present study, I^- efficiently inhibited the reaction of nucleosides, especially dG, with the myeloperoxidase–H_2O_2–Cl^- system. However, since the plasma concentration of I^- is low (0.46—0.67 μM),[16] it is unlikely that I^- affects nucleoside damage caused by myeloperoxidase in plasma. It has been proposed that hypoiodous acid (HOI) or molecular iodine (I_2) is generated as the oxidizing intermediate by the reaction of I^- with a myeloperoxidase–H_2O_2 system or with HOCl.[13,34,35] The reactivity of the oxidizing intermediate formed in the myeloperoxidase–H_2O_2–Cl^-–I^- system (possibly HOI or I_2) would be lower than that of HOCl.

Effect of SCN^- on the Reaction of Nucleosides with Myeloperoxidase–H_2O_2–Cl^- Figure 5 shows concentrations of unreacted nucleosides in the reaction mixture at various concentrations of SCN^-. The addition of SCN^- inhibited the reaction at pH 4.7 (Fig. 5A). By 50 μM SCN^-, the reactions with both dG and dC were suppressed almost totally. At pH 7.4, no consumption of nucleosides was observed in the SCN^- concentration range examined (Fig. 5B).

SCN^- is a well known catalyst for nitrosation caused by nitrous acid.[36] Since SCN^- concentration in saliva of smokers are three times higher than those of non-smokers, SCN^- is recognised as a cancer risk factor.[37] However, the present results suggest that SCN^- might act protectively against nucleoside damage caused by myeloperoxidase. Hypothiocyanous acid (HOSCN) has been proposed as the oxidizing intermediate generated in the myeloperoxidase–H_2O_2–Cl^-–SCN^- system.[38] The reactivity of HOSCN to nucleosides may be lower than that of HOCl.

Effect of NO_2^- on the Reaction of Nucleosides with Myeloperoxidase–H_2O_2–Cl^- Figure 6 shows concentrations of unreacted nucleosides in the reaction mixture at various NO_2^- concentrations. At pH 4.7, NO_2^- inhibited the reaction, but with low efficiency. 1000 μM of NO_2^- was required to suppress the reaction almost entirely (Fig. 6A). At pH 7.4, no consumption of nucleosides was observed in the NO_2^- concentration range examined (Fig. 6B).

NO_2^- is a well known cancer risk factor. However, although many efforts have been made to determine whether high intake of NO_2^- is a cause of cancer, this remains unclear.[39] In the present study, NO_2^- inhibited the reaction of nucleosides with low efficiency. As the plasma concentration of NO_2^- is

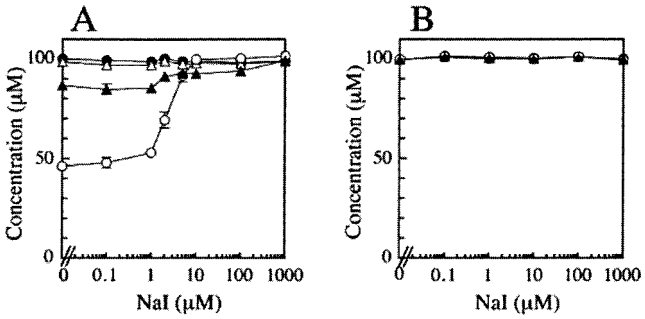

Fig. 4. Effect of I^- on the Reaction of the Myeloperoxidase–H_2O_2–Cl^- System with Nucleosides at pH 4.7 (A) and pH 7.4 (B)

The concentrations of dG (open circles), dC (closed triangles), dT (open triangles) and dA (closed circles) are plotted against the concentration of NaI added. The reaction conditions and the analytical methods were as described in Fig. 2, except that the added anion was I^-.

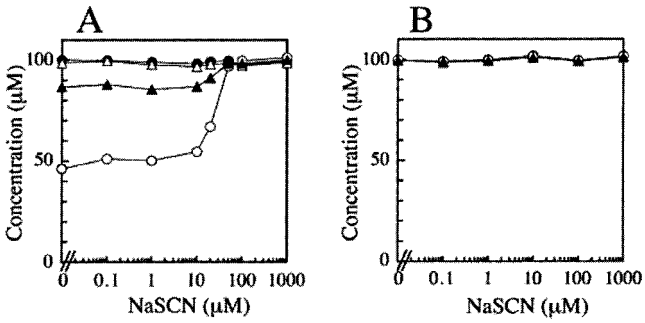

Fig. 5. Effect of SCN^- on the Reaction of the Myeloperoxidase–H_2O_2–Cl^- System with Nucleosides at pH 4.7 (A) and pH 7.4 (B)

The concentrations of dG (open circles), dC (closed triangles), dT (open triangles) and dA (closed circles) are plotted against the concentration of NaSCN added. The reaction conditions and the analytical methods were as described in Fig. 2, except that the added anion was SCN^-.

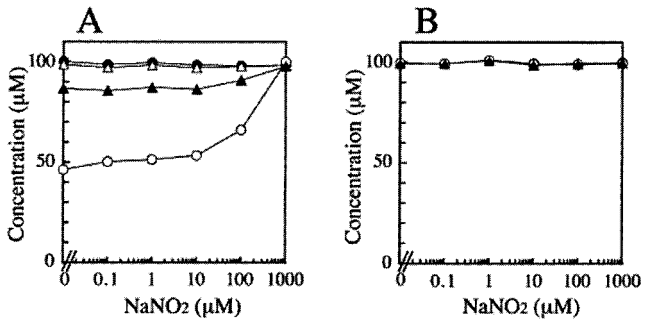

Fig. 6. Effect of NO_2^- on the Reaction of the Myeloperoxidase–H_2O_2–Cl^- System with Nucleosides at pH 4.7 (A) and pH 7.4 (B)

The concentrations of dG (open circles), dC (closed triangles), dT (open triangles) and dA (closed circles) are plotted against the concentration of $NaNO_2$ added. The reaction conditions and the analytical methods were as described in Fig. 2, except that the added anion was NO_2^-.

less than 1 μM, NO_2^- in plasma seems unlikely to affect nucleoside damage caused by myeloperoxidase, although the formation of a nitro derivative of dG has been reported.[19] It has been proposed that nitrogen dioxide (NO_2) or nitryl chloride (NO_2Cl) is generated in the myeloperoxidase–H_2O_2–Cl^-–NO_2^- system.[19] The reactivity of NO_2 and NO_2Cl to nucleosides may be lower than that of HOCl.

In conclusion, we have studied the influence of several anions on the reaction of a myeloperoxidase–H_2O_2–Cl^- system with a nucleoside mixture. F^- showed no effect, while I^-, SCN^- and NO_2^- acted as inhibitors. The most notable effect on nucleoside damage caused by myeloperoxidase was due to Br^-, since at concentrations of Br^- found in plasma, the predominant target nucleoside was altered and total nucleoside consumption was increased. Further studies are required to clarify the role of Br^- on inflammatory tissue injury caused by neutrophils.

Experimental

Materials NaF (99.99%), NaCl (99.999%), NaBr (99.999%), NaI (99.999%), NaSCN (99.99+%) and $NaNO_2$ (99.99+%) were obtained from Aldrich (Milwaukee, WI, U.S.A.). dC, dT and dA were purchased from Sigma (St. Louis, MO, U.S.A.), and dG from Fluka (Buchs, Switzerland). All other chemicals of reagent grade were purchased from Sigma, Aldrich or Fluka, and used without further purification. Myeloperoxidase (EC 1.11.1.7, from human leukocytes) was purchased from Alexis Biochemicals (Lausen, Switzerland).

HPLC Conditions The HPLC system consisted of an HP1050 series pumping system (Hewlett Packard, CA, U.S.A.). On-line UV spectra were obtained with a Spectra Focus UV–visible photodiode-array detector (Spectra Physics, CA, U.S.A.). For reversed phase HPLC, an Ultrasphere octadecylsilane (ODS) column (4.6×250 mm, particle size 5 μm; Beckman, CA, U.S.A.) was used. The eluent used was 20 mM sodium phosphate buffer (pH 7.0) containing acetonitrile. The acetonitrile concentration was increased from 0 to 12.5% over 15 min in a linear gradient mode. The column temperature was 30 °C and the flow rate 1 ml/min.

Reaction of Nucleoside Mixture with Myeloperoxidase A nucleoside mixture (dG, dC, dT and dA; 100 μM each) was incubated with 50 nM myeloperoxidase in 100 mM sodium phosphate buffer containing 100 mM NaCl, 200 μM H_2O_2 and 100 μM diethylenetriaminepentaacetic acid (DTPA) at 37 °C for 30 min in the absence or presence of anions (F^-, Br^-, I^-, SCN^- or NO_2^-; 0—1000 μM). The reaction was terminated by addition of 400 μM *N*-acetylcysteine.

Quantitative Procedures The reaction mixture was injected onto HPLC immediately after termination by *N*-acetylcysteine. The concentration of each nucleoside was evaluated from the integrated peak areas on HPLC chromatograms detected at 260 nm.

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