Modification by Fluoride, Bromide, Iodide, Thiocyanate and Nitrite Anions of Reaction of a Myeloperoxidase–H$_2$O$_2$–Cl$^-$ System with Nucleosides

Toshinori SUZUKI and Hiroshi OHSHIMA*

International Agency for Research on Cancer; 150 Cours Albert Thomas, F 69372 Lyon Cedex 08, France.

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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$_2$O$_2$–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$^{9,9}$. 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$_2$O$_2$) 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$_2$O$_2$–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$_2$O$_2$–Cl$^-$ system and found that a diimino-imidazole nucleoside, an amino-imidazolone nucleoside, a spiroimino-dihydrantoin 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$_2$O$_2$.$^{14}$ 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 $\mu$M Br$^-$, 0.46—0.67 $\mu$M I$^-$, 21—134 $\mu$M SCN$^-$,$^{16}$ and 0.25—0.65 $\mu$M NO$_2^-$. Recently, Byun et al.$^{19}$ reported that 8-nitro-2'-deoxyguanosine was generated by the reaction of dG with the myeloperoxidase–H$_2$O$_2$–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'-deoxyctydine in the reaction of 2'-deoxyctydine (dC) with the myeloperoxidase–H$_2$O$_2$–Cl$^-$ system in the presence of Br$^-$. These papers showed that NO$_2^-$ and Br$^-$. were significant regents with respect to nucleoside damage caused by the myeloperoxidase–H$_2$O$_2$–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$_2$O$_2$–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$_2$O$_2$–Cl$^-$ System A nucleoside mixture (dG, dC, 2'-deoxythymidine (dT) and 2'-deoxyadenosine (dA); 100 $\mu$M each) was incubated with 50 nM myeloperoxidase in 100 mM sodium phosphate buffers of different pH with 200 $\mu$M H$_2$O$_2$ 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 reverse-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$_2$O$_2$–Cl$^-$ system consumed nucleosides in the following order: dG > dC > dT > dA.$^@$ The optimal pH for consumption of nucleosides was 4.7. At pH 4.7, total consumption was 67.3 $\mu$M corresponding to 33.7% relative to H$_2$O$_2$ added.

It has been reported that dC reacts with a myeloperoxi-

\[\text{Fig. 1. pH Dependence of the Reaction of the Myeloperoxidase–H}_2\text{O}_2\text{–Cl}^-\text{ System with Nucleosides}\]

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 $\mu$M each) was incubated with 50 nM myeloperoxidase in 100 mM sodium phosphate buffer of various pH's with 200 $\mu$M H$_2$O$_2$. The reaction was terminated by addition of 400 $\mu$M N-acetylcysteine. The concentration of each nucleoside was quantified by reversed-phase HPLC analysis.
has been proven. The present results showed no effect on nucleoside damage and suggest that F- enhances the reaction of nucleosides with the myeloperoxidase–H2O2–Cl2 system at pH 4.7. As shown in Fig. 2A, the concentration of dC (open triangles), dT (open triangles), dA (closed circles), and dG (closed triangles) are plotted against the concentration of NaF added. A nucleoside mixture (dG, dC, dT, and dA, 100 μM each) was incubated with 50 μM myeloperoxidase in 100 mM sodium phosphate buffer (pH 4.7 or 7.4) with 200 μM H2O2, 100 μM DTPA and 100 μM 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 reaction of HOCl by the myeloperoxidase–H2O2–Cl2 system is shown in Fig. 3. The concentrations of dG (open circles), dC (closed triangles), dT (open triangles), and dA (closed circles) are plotted against the concentration of NaBr added. A nucleoside mixture (dG, dC, dT, and dA, 100 μM each) was incubated with 50 μM myeloperoxidase in 100 mM sodium phosphate buffer (pH 4.7 or 7.4) with 200 μM H2O2, 100 μM DTPA and 100 μM NaCl at 37 °C for 30 min in the presence of 0—1000 μM NaBr. The reaction was terminated by addition of 400 μM N-acetylcysteine. The concentration of each nucleoside was quantified by reversed phase HPLC analysis. The reaction of HOCl by the myeloperoxidase–H2O2–Cl2 system is shown in Fig. 3. The concentrations of dG (open circles), dC (closed triangles), dT (open triangles), and dA (closed circles) are plotted against the concentration of NaBr added. A nucleoside mixture (dG, dC, dT, and dA, 100 μM each) was incubated with 50 μM myeloperoxidase in 100 mM sodium phosphate buffer (pH 4.7 or 7.4) with 200 μM H2O2, 100 μM DTPA and 100 μM NaCl at 37 °C for 30 min in the presence of 0—1000 μM NaBr. The reaction was terminated by addition of 400 μM N-acetylcysteine. The concentration of each nucleoside was quantified by reversed phase HPLC analysis. The reaction of HOCl by the myeloperoxidase–H2O2–Cl2 system is shown in Fig. 3.
of nucleosides was increased by addition of \( \text{Br}^- \). These results imply that \( \text{Br}^- \) can have important effects on nucleoside damage caused by myeloperoxidase. It has been proposed that bromine chloride (\( \text{BrCl} \)), molecular bromine (\( \text{Br}_2 \)) or hypobromous acid (\( \text{HOBr} \)) is the oxidizing intermediate for the reaction of nucleosides by the myeloperoxidase–\( \text{H}_2\text{O}_2–\text{Cl}^-–\text{Br}^- \) system as well as by the eosinophil peroxidase–\( \text{H}_2\text{O}_2–\text{Cl}^-–\text{Br}^- \) system.\(^{20,29–31}\) In the present study, the main reaction target of the myeloperoxidase–\( \text{H}_2\text{O}_2–\text{Cl}^- \) system was altered from \( \text{dG} \) to \( \text{dC} \) by the addition of \( \text{Br}^- \). The oxidizing intermediate (\( \text{BrCl} \), \( \text{Br}_2 \) or \( \text{HOBr} \)) formed by the myeloperoxidase–\( \text{H}_2\text{O}_2–\text{Cl}^-–\text{Br}^- \) system may react with \( \text{dC} \) more preferentially than \( \text{dG} \).

**Effect of \( \text{I}^- \) on the Reaction of Nucleosides with Myeloperoxidase–\( \text{H}_2\text{O}_2–\text{Cl}^- \)** Figure 4 shows concentrations of unreacted nucleosides in the reaction mixture at various concentrations of \( \text{I}^- \). The addition of \( \text{I}^- \) suppressed the reaction at pH 4.7 (Fig. 4A), and at 10 \( \mu \text{M} \) \( \text{I}^- \), the consumption of \( \text{dG} \) was almost totally suppressed. However, this suppressive effect was weak for \( \text{dC} \). At pH 7.4, no consumption of nucleosides was observed at the \( \text{I}^- \) 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 \( \text{I}^- \) can promote cancer in animals.\(^{33,34,35}\) In the present study, \( \text{I}^- \) efficiently inhibited the reaction of nucleosides, especially \( \text{dG} \), with the myeloperoxidase–\( \text{H}_2\text{O}_2–\text{Cl}^- \) system. However, since the plasma concentration of \( \text{I}^- \) is low (0.46–0.67 \( \mu \text{M} \)),\(^{10}\) it is unlikely that \( \text{I}^- \) affects nucleoside damage caused by myeloperoxidase in plasma. It has been proposed that hypiodous acid (\( \text{HOI} \)) or molecular iodine (\( \text{I}_2 \)) is generated as the oxidizing intermediate by the reaction of \( \text{I}^- \) with a myeloperoxidase–\( \text{H}_2\text{O}_2 \) system or with \( \text{HOCl} \).\(^{13,34,35}\) The reactivity of the oxidizing intermediate formed in the myeloperoxidase–\( \text{H}_2\text{O}_2–\text{Cl}^-–\text{I}^- \) system (possibly \( \text{HOI} \) or \( \text{I}_2 \)) would be lower than that of \( \text{HOCl} \).

**Effect of \( \text{SCN}^- \) on the Reaction of Nucleosides with Myeloperoxidase–\( \text{H}_2\text{O}_2–\text{Cl}^- \)** Figure 5 shows concentrations of unreacted nucleosides in the reaction mixture at various concentrations of \( \text{SCN}^- \). The addition of \( \text{SCN}^- \) inhibited the reaction at pH 4.7 (Fig. 5A). By 50 \( \mu \text{M} \) \( \text{SCN}^- \), the reactions with both \( \text{dG} \) and \( \text{dC} \) were suppressed almost totally. At pH 7.4, no consumption of nucleosides was observed in the \( \text{SCN}^- \) concentration range examined (Fig. 5B).

\( \text{SCN}^- \) is a well known catalyst for nitration caused by nitrous acid.\(^{36}\) Since \( \text{SCN}^- \) concentration in saliva of smokers are three times higher than those of non-smokers, \( \text{SCN}^- \) is recognised as a cancer risk factor.\(^{37}\) However, the present results suggest that \( \text{SCN}^- \) might act protectively against nucleoside damage caused by myeloperoxidase. Hypothiocyanous acid (\( \text{HOSCN} \)) has been proposed as the oxidizing intermediate generated in the myeloperoxidase–\( \text{H}_2\text{O}_2–\text{Cl}^-–\text{SCN}^- \) system.\(^{38}\) The reactivity of \( \text{HOSCN} \) to nucleosides may be lower than that of \( \text{HOCl} \).

**Effect of \( \text{NO}_2^- \) on the Reaction of Nucleosides with Myeloperoxidase–\( \text{H}_2\text{O}_2–\text{Cl}^- \)** Figure 6 shows concentrations of unreacted nucleosides in the reaction mixture at various \( \text{NO}_2^- \) concentrations. At pH 4.7, \( \text{NO}_2^- \) inhibited the reaction, but with low efficiency. 1000 \( \mu \text{M} \) of \( \text{NO}_2^- \) was required to suppress the reaction almost entirely (Fig. 6A). At pH 7.4, no consumption of nucleosides was observed in the \( \text{NO}_2^- \) concentration range examined (Fig. 6B).

\( \text{NO}_2^- \) is a well known cancer risk factor. However, although many efforts have been made to determine whether high intake of \( \text{NO}_2^- \) is a cause of cancer, this remains unclear.\(^{39}\) In the present study, \( \text{NO}_2^- \) inhibited the reaction of nucleosides with low efficiency. As the plasma concentration of \( \text{NO}_2^- \) is
less than 1 μM, NO3 in plasma seems unlikely to affect nucleoside damage caused by myeloperoxidase, although the formation of a nitro derivative of dG has been reported. It has been proposed that nitrogen dioxide (NO2) or nitryl chloride (NO2Cl) is generated in the myeloperoxidase–H2O2–Cl– NO3 system. The reactivity of NO2 and NO2Cl 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–H2O2–Cl– system with a nucleoside mixture. F− showed no effect, while I−, SCN− and NO3 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%), Naf (99.999%), NaSCN (99.99% +) and NaNO3 (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 octade-cylsilane (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 μM myeloperoxidase in 100 mM sodium phosphate buffer containing 100 mM NaCl, 200 μM H2O2, and 100 μM diethylenetriaminepentaacetic acid (DTPA) at 37 °C for 30 min in the absence or presence of anions (F−, Br−, I−, SCN− or NO3; 0—1000 μM). The reaction was terminated by addition of 400 μM N-acetylcyesteine.

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

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