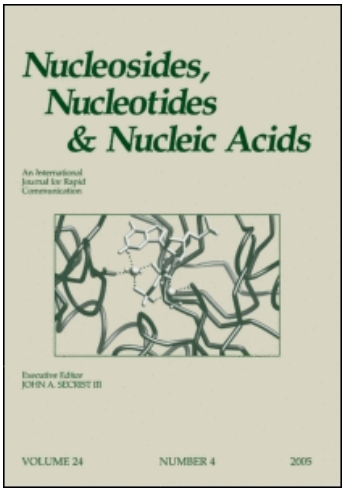

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Effects of Chloride, Bromide, and Iodide Upon Decomposition of Nucleosides Induced by Ultrasound in Neutral Solution

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Online publication date: 19 July 2010

To cite this Article Suzuki, Toshinori , Yamada, Kenji and Inukai, Michiyo(2010) 'Effects of Chloride, Bromide, and Iodide Upon Decomposition of Nucleosides Induced by Ultrasound in Neutral Solution', Nucleosides, Nucleotides and Nucleic Acids, 29: 8, 606 — 615
To link to this Article: DOI: 10.1080/15257770.2010.496279
URL: http://dx.doi.org/10.1080/15257770.2010.496279

Nucleosides, Nucleotides and Nucleic Acids, 29:606–615, 2010

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770.2010.496279

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EFFECTS OF CHLORIDE, BROMIDE, AND IODIDE UPON DECOMPOSITION OF NUCLEOSIDES INDUCED BY ULTRASOUND IN NEUTRAL SOLUTION

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□ *When the ultrasound of 42 kHz was irradiated on a neutral mixture of 2′-deoxycytidine, 2′-deoxyguanosine, 2′-deoxythymidine, and 2′-deoxyadenosine, concentrations of all the nucleosides decreased. Addition of NaCl to the system had no effect. NaBr suppressed the reactions for all the nucleosides, but the efficiency of 2′-deoxyguanosine was low. NaI suppressed the reactions for all the nucleosides more effectively. A comparison with the results of the effects of halides on the reaction of nucleosides by a Fenton system suggested that only half of the nucleoside damage in the ultrasound-irradiated solution was caused by hydroxyl radicals formed from water by the sonication.*

Keywords Ultrasound; nucleoside; halide; hydroxyl radical; hydrogen radical

INTRODUCTION

Ultrasound can penetrate deeply into living tissues and induce various physical and chemical effects on cells.[1,2] Under sonication of isolated or cellular DNA, formation of DNA macroradicals, strand breaks, and various types of base modifications were reported.[3–6] In examination of the cytological effects of therapeutic ultrasound on human fibroblasts in vitro, large increases in mitotic index and chromosomal aberrations with mitotic spindles were observed.[7] In investigating DNA damage by ultrasound in occupationally exposed medical personnel using comet assay, a highly significant increase in levels of DNA damage compared with the control was observed.[8] Ultrasound makes cavitation bubbles in aqueous solutions. The cavitation bubbles undergo violent collapse with the production of localized high temperatures and pressures accompanied by mechanical shear stress and free radical formation.[9] Homolysis of water vapor in the bubble results in formation of hydrogen atoms (H·) and hydroxyl radicals (HO·).[10,11] HO·

Received 31 March 2010; accepted 21 May 2010.
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is a highly reactive oxygen. HO· reacts with DNA at its various sites causing various types of DNA damage such as strand breaks, release of bases, and hydroxylation of bases.[12] Halides, chloride (Cl^-), bromide (Br^-), and iodide (I^-) are essential to humans.[13] Lack or overdose of the halides cause various symptoms. In this study, we examined the effects of the halides on the reaction of deoxynucleosides with ultrasound in a neutral solution, and discussed the reactive species in the sonication system.

MATERIALS AND METHODS

Materials

dCyd, dGuo, dThd, dAdo, Cyt, Thy, and Ade were obtained from Sigma (St. Louis, MO, USA). Gua was obtained from Kohjin (Tokyo, Japan). NaCl (99.99%), NaBr (99.99 + %), and NaI (99.999%) were purchased from Aldrich (Milwaukee, WI, USA). All other chemicals of reagent grade were purchased from Sigma, Aldrich, Cica (Tokyo, Japan), and Nacalai Tesque (Osaka, Japan), and used without further purification. Water was purified with a Millipore Milli-Q deionizer.

HPLC Conditions

The high-performance liquid chromatography (HPLC) system consisted of Shimadzu LC-10ADvp pumps and an SCL-10Avp system controller. Online ultraviolet (UV) spectra were obtained with a Shimadzu SPD-M10Avp UV-vis photodiode-array detector. For the RP-HPLC, an Inertsil ODS-3 octadecylsilane column of 4.6 × 250 mm and particle size 5 μm (GL Science, Tokyo, Japan) was used. For analyses, 20 mM triethylammonium acetate buffer (pH = 7.0) containing methanol was used as the eluent. The methanol concentration was increased from 0 to 50% for 30 minutes in linear gradient mode. The column temperature was 40°C and the flow rate was 1.0 mL/min.

Ultrasound Irradiation

The ultrasound bath (Bransonic 3510J-DTH, 100 W, 42 kHz; Branson, CT, USA) filled with tap water up to the designated operating level was used. The NMR sample tube (ST500-7, 5 mm O.D. × 178 mm; Norell, NJ, USA) containing the nucleosides mixture (1 mL) was dangled by suspension of the cap of the nuclear magnetic resonance (NMR) sample tube through a hole of a paper tape, which stretched horizontally on a clamp. The sample tube was located a distance of 10 cm from the surface of the tap water of the ultrasound bath to the bottom of the sample tube. When the NMR sample tube was fixed by the clamp directly, the reaction of the nucleosides by the ultrasound irradiation suppressed greatly.

Quantitative Procedures

The concentrations of all the compounds in the reaction mixture were evaluated from integrated peak areas on HPLC chromatograms compared with those of authentic standard solutions. The detection wavelength was 260 nm. All the experiments reported here were carried out independently tree times. The results are expressed as means ± S.D.

RESULTS AND DISCUSSION

A nucleosides mixture of 2′-deoxycytidine (dCyd), 2′-deoxyguanosine (dGuo), 2′-deoxythymidine (dThd), and 2′-deoxyadenosine (dAdo) (100 μM each) with 100 mM potassium phosphate buffer (pH = 7.4) in an NMR sample tube was irradiated with ultrasound in a ultrasound bath (100 W, 42 kHz) at 37°C for 60 minutes. Figure 1 shows the reversed phase high performance liquid chromatography (RP-HPLC) chromatogram of the reaction mixture (the solid line) and the control mixture without irradiation (the dotted line). In addition to peaks of starting nucleosides, several product peaks including nucleobases, cytosine (Cyt), guanine (Gua), thymine

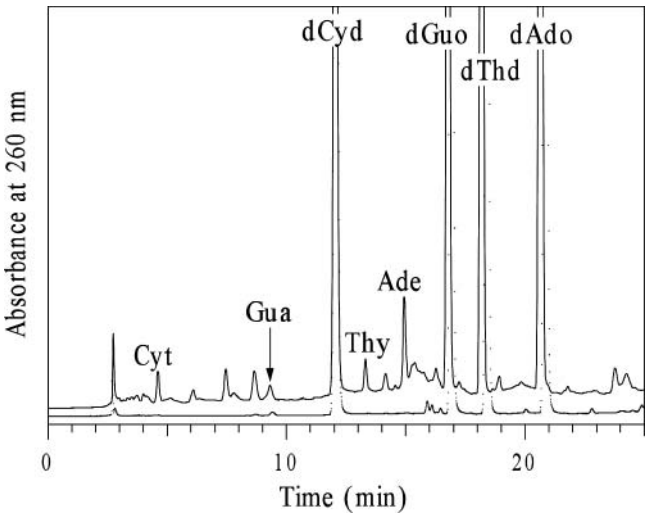

FIGURE 1 Reversed phase high performance liquid chromatography (RP-HPLC) chromatogram for an ultrasound irradiated nucleosides mixture detected at 260 nm. A solution of dCyd, dGuo, dThd, and dAdo (100 μM each) with 100 mM potassium phosphate buffer (pH = 7.4) in an NMR sample tube was irradiated with ultrasound in a ultrasound bath (100 W, 42 kHz) at 37°C for 60 minutes. The dotted line presnts the control nucleosides mixture before the ultrasound irradiation with the same scale. The HPLC system consisted of Shimadzu LC-10ADvp pumps and an SCL-10Avp system controller. Online UV spectra were obtained with a Shimadzu SPD-M10Avp UV-vis photodiode-array detector. For the RP-HPLC, an Inertsil ODS-3 octadecylsilane column of 4.6 × 250 mm and particle size 5 μm (GL Science, Tokyo, Japan) was used. For analyses, 20 mM triethylammonium acetate buffer (pH = 7.0) containing methanol was used as the eluent. The methanol concentration was increased from 0 to 50% for 30 minutes in linear gradient mode. The column temperature was 40°C and the flow rate was 1.0 mL/min.

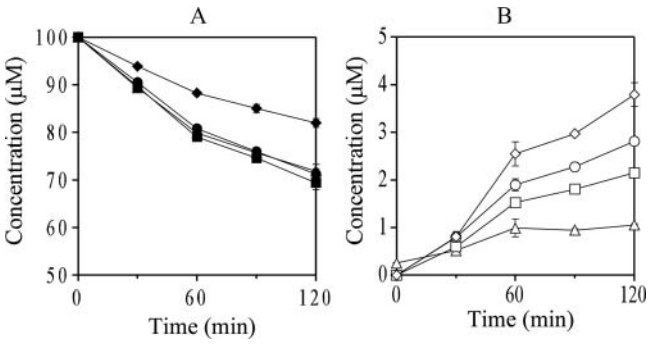

FIGURE 2 The time course of the concentration changes in (A) dCyd (closed circle), dGuo (closed triangle), dThd (closed square), and dAdo (closed rhombus) and in (B) Cyt (open circle), Gua (open triangle), Thy (open square), and Ade (open rhombus), when a solution mixture of dCyd, dGuo, dThd, and dAdo (100 μM each) in 100 mM potassium phosphate buffer (pH = 7.4) was irradiated by the ultrasound at 37°C. The nucleoside and nucleobase concentrations were determined by RP-HPLC analysis detected at 260 nm. Means ± SD (n = 3) are shown.

(Thy), and adenine (Ade) appeared by the ultrasound irradiation. These nucleobase peaks were identified by coincidence of the retention time and the online UV spectrum of the peak with those of authentic samples. Figure 2 shows the time course of the reaction. All the nucleosides decreased with increasing irradiation time, although the reaction efficiency for dAdo was low (Figure 2A). The nucleobases increased with increasing irradiation time (Figure 2B). The concentration of Ade was the highest. Ade has been reported to have the lowest reactivity among the four nucleobases by ultrasound irradiation.[14] We investigated the effects of halides (Cl^-, Br^-, and I^-) on the reaction of nucleosides by ultrasound irradiation. Figure 3 shows

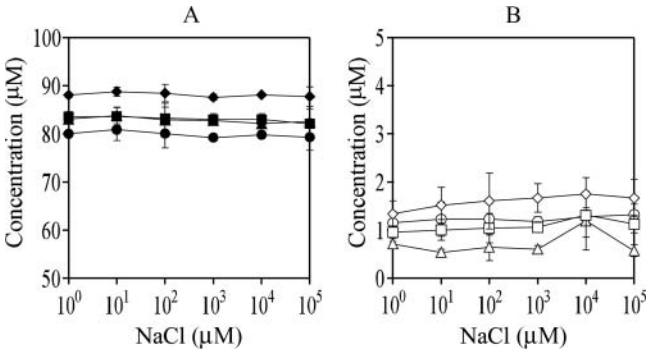

FIGURE 3 The NaCl dose dependence of the concentration changes in (A) dCyd (closed circle), dGuo (closed triangle), dThd (closed square), and dAdo (closed rhombus) and in (B) Cyt (open circle), Gua (open triangle), Thy (open square), and Ade (open rhombus), when a solution mixture of dCyd, dGuo, dThd, and dAdo (100 μM each) in 100 mM potassium phosphate buffer (pH = 7.4) containing from 1 μM to 100 mM NaCl was irradiated by ultrasound at 37°C for 60 minutes. The nucleoside and nucleobase concentrations were determined by RP-HPLC analysis detected at 260 nm. Means ± SD (n = 3) are shown.

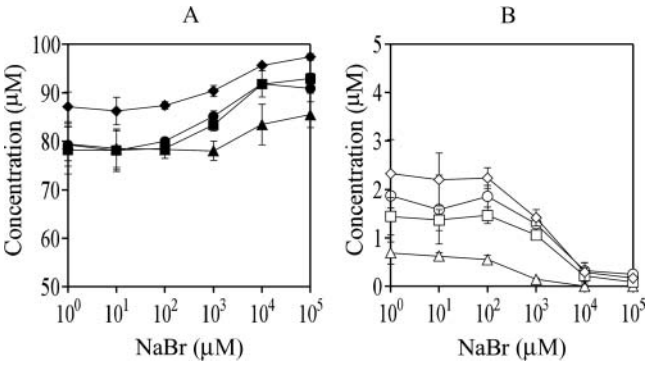

FIGURE 4 The NaBr dose dependence of the concentration changes in (A) dCyd (closed circle), dGuo (closed triangle), dThd (closed square), and dAdo (closed rhombus) and in (B) Cyt (open circle), Gua (open triangle), Thy (open square), and Ade (open rhombus), when a solution mixture of dCyd, dGuo, dThd, and dAdo (100 μM each) in 100 mM potassium phosphate buffer (pH = 7.4) containing from 1 μM to 100 mM NaBr was irradiated by ultrasound at 37°C for 60 minutes. The nucleoside and nucleobase concentrations were determined by RP-HPLC analysis detected at 260 nm. Means ± SD (n = 3) are shown.

the NaCl dose dependence of the reaction when the nucleoside mixture containing from 1 μM to 100 mM NaCl was irradiated by ultrasound at 37°C for 60 minutes. NaCl in this dose range did not affect consumption of nucleosides (Figure 3A) or formation of nucleobases (Figure 3B). Figure 4 shows the NaBr dose dependence of the reaction. The addition of NaBr up to 100 μM showed no effect. Above 1 mM, NaBr suppressed consumption of all the nucleosides, although the suppression efficiency for dGuo was low (Figure 4A). However, at a high dose (100 mM) of NaBr, significant amounts of nucleosides were still consumed. Formation of nucleobases was also suppressed by addition of NaBr above 1 mM (Figure 4B). At 100 mM of NaBr, the amounts of nucleobases formed were low. Figure 5 shows the NaI dose dependence of the reaction. NaI suppressed the consumption of all the nucleosides more efficiently than NaBr (Figure 5A). However, at a high dose (100 mM) of NaI, almost half of the nucleosides were still consumed. NaI also efficiently suppressed the formation of all the nucleobases (Figure 5B). At 100 mM NaI, concentrations of all the nucleobases were nearly zero. To compare the effects of halides upon the sonication with those on a HO· reaction, a Fenton reaction system was conducted for the nucleosides in the absence and presence of the halides. Each nucleoside mixture (100 μM) was incubated with 5 mM $FeSO_4$ and 10 mM H_2O_2 in 100 mM potassium phosphate buffer (pH = 7.4) at 37°C for 60 minutes without or with 100 mM halides. Table 1 shows the nucleoside concentrations in the reaction mixture by the Fenton system. Without halides, consumption of all the nucleosides by the Fenton system was greater than those by the sonication for 60 minutes. Addition of NaCl showed no effect on the

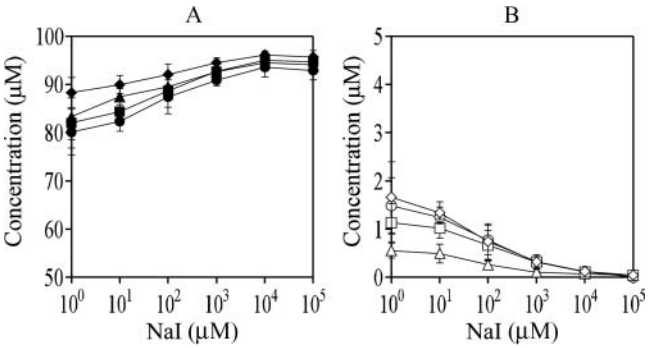

FIGURE 5 The NaI dose dependence of the concentration changes in (A) dCyd (closed circle), dGuo (closed triangle), dThd (closed square), and dAdo (closed rhombus) and in (B) Cyt (open circle), Gua (open triangle), Thy (open square), and Ade (open rhombus), when a solution mixture of dCyd, dGuo, dThd, and dAdo (100 μM each) in 100 mM potassium phosphate buffer (pH = 7.4) containing from 1 μM to 100 mM NaI was irradiated by ultrasound at 37°C for 60 minutes. The nucleoside and nucleobase concentrations were determined by RP-HPLC analysis detected at 260 nm. Means ± SD (n = 3) are shown.

nucleosides consumption. NaBr suppressed the reaction of dCyd, dThd, and dAdo greatly, but not that of dGuo. NaI suppressed all the reactions of nucleosides almost completely.

Collapse of cavitation bubbles results in the homolysis of water vapor in the bubble to create hydrogen atoms and hydroxyl radicals.[10,11]

$$H_2O + \text{ultrasound} \rightarrow H\cdot + HO\cdot \quad (1)$$

HO· is a highly reactive oxygen species with a standard oxidation potential (E°) of +2.72 V versus normal hydrogen electrode (NHE).[15] E° values of the nucleosides are reported to be +2.12 V for dCyd, +1.47 V for dGuo, +2.09 V for dThd, and +1.94 V for dAdo.[16] Thus, HO· can react with all the four nucleosides, resulting in consumption of all the nucleosides. E° values of the halides are −1.36 V for Cl^-, −1.07 V for Br^-, and −0.54 V for I^-.[13]

TABLE 1 Effects of halides on Fenton reaction of nucleosides[a]

Halides (100 mM)	dCyd (μM)	dGuo (μM)	dThd (μM)	dAdo (μM)
None	55.1 ± 1.6	72.4 ± 1.2	56.2 ± 2.0	74.0 ± 1.4
NaCl	52.1 ± 0.4	69.3 ± 0.2	52.6 ± 0.1	71.6 ± 0.5
NaBr	98.6 ± 0.1	69.7 ± 0.2	99.3 ± 0.2	99.4 ± 0.1
NaI	99.5 ± 0.1	100.3 ± 0.2	100.0 ± 0.1	100.2 ± 0.1

[a]A solution of dCyd, dGuo, dThd, and dAdo (100 μM each) with 100 mM potassium phosphate buffer (pH = 7.4) in a micro tube was incubated with 5 mM $FeSO_4$ and 10 mM H_2O_2 in the presence of 100 mM halides at 37°C for 60 minutes. The nucleoside concentrations were determined by RP-HPLC analysis. Means ± SD (n = 3) are shown.

Thus, HO· can react with Cl^-, Br^-, and I^-, forming chlorine radicals (Cl·), bromine radicals (Br·), and iodine radicals (I·), respectively.[17–19]

$$HO\cdot + X^- \rightarrow HO^- + X\cdot \ (X: \text{Cl, Br, or I}) \quad (2)$$

These halogen radicals can react with halides generating dihalogen radical anions ($X_2\cdot^-$).[17–19]

$$X\cdot + X^- \rightarrow X_2\cdot^- \ (X: \text{Cl, Br, or I}) \quad (3)$$

Cl^- showed no effect to the nucleoside sonication system (Figure 3), although Cl^- can be oxidized by HO· resulting in formation of Cl·. In the presence of a high concentration of Cl^-, Cl· further reacts with Cl^- forming $Cl_2^-\cdot$. $Cl_2^-\cdot$ is a highly reactive species with E° of +2.09 V,[15] which is greater than those for dGuo and dAdo and comparative to those for dCyd and dThd. Reportedly, the reaction of $Cl_2^-\cdot$ to nucleosides can be understood in the same way as the reaction of HO·.[20] Similarly, Br^- can be oxidized by HO· resulting in formation of Br·. In the presence of a high concentration of Br^-, Br· further reacts with Br^- forming $Br_2^-\cdot$. E° of $Br_2^-\cdot$ was calculated to be +1.63 V.[15] Recently, we showed that ultraviolet light irradiation on a neutral solution of deoxynucleosides and hydrogen peroxide caused great decreases in concentration of all the deoxynucleosides.[21] Addition of hydroxyl radical scavengers suppressed the reaction. Addition of bromide in the system suppressed the reactions of dCyd, dThd, and dAdo, but not that of dGuo. The result of dose dependency of bromide suggests that $Br_2^-\cdot$ is the reaction species to react only with dGuo. In the present sonication system, the formed $Br_2^-\cdot$ can irreversibly oxidize only dGuo among the nucleosides resulting in decrease of dGuo concentration. This would be the reason why dGuo consumption was suppressed by Br^- with a lower efficiency than other nucleosides in this study (Figure 4). I^- can be oxidized by HO· resulting in formation of I·. In the presence of a high concentration of I^-, I· further reacts with I^- forming $I_2^-\cdot$. E° of $I_2^-\cdot$ is low and calculated to be +1.03 V.[19] $I_2^-\cdot$ could not react with any nucleosides used in this study. In the Fenton reaction, 100 mM of Br^- and I^- almost completely suppressed the reactions of nucleosides except for dGuo in the presence of Br^-. However, in the sonication system, 100 mM of Br^- and I^- suppressed only a half of the nucleosides consumptions, while the nucleobase formations were almost completely suppressed (Figures 4 and 5). This implies that only a half of the reaction was attributable to HO·. In the sonication system, H· should be formed in the same amount as HO· as shown in reaction (1). H· is also a reactive species. However the efficiency of H· on the reaction with DNA is very low. The absolute efficiency of H· on the reaction with DNA is estimated to be 50-fold less than that of HO·.[22] In the presence of O_2, H· reacts with

O_2 to generate $HO_2\cdot$, which dissociates to H^+ and superoxide anion radical ($O_2^-\cdot$), the other reactive oxygen species, at neutral pH.[23]

$$H\cdot + O_2 \rightarrow HO_2\cdot \quad (4)$$

$$HO_2\cdot \rightarrow H^+ + O_2^-\cdot \quad (5)$$

However, it has been reported that $O_2^-\cdot$ does not react with DNA bases at all.[24] In addition, Br^- and I^- can react with H· with first order rate constants $k = 2.8 \times 10^7$ and $3.4 \times 10^7\ M^{-1}\ S^{-1}$, respectively, although k for Cl^- is smaller than $1 \times 10^5\ M^{-1}\ S^{-1}$.[25] Considerable amounts of H· would be trapped by Br^- and I^- at the high concentrations (100 mM). Thus, the possibility would be low that H· and its derivatives in the aqueous solution contribute to the consumption of nucleosides as reactive species. The action of ultrasound is complex.[26,27] Other reactive species may generate from the components of the solution, water, dissolved oxygen, phosphate, and halides. In addition to the formation of the reactive species, mechanical forces caused by ultrasound may act on the nucleosides directly causing this decomposition.

Plasma concentrations of Cl^- are high (100–140 mM).[28] Since 100 mM Cl^- did not affect the nucleosides decomposition by ultrasound in the present study (Figure 3), the effects of Cl^- for ultrasound would be negligible in humans. Plasma concentrations of Br^- are low (39–84 μM).[29] In this range, the effects of Br^- for nucleosides decomposition were low (Figure 4). However, Br^- is used in the treatment of patients with refractory seizures, particularly in pediatrics. The therapeutic serum concentration of Br^- ranges over levels of 10–35 mM.[30] In addition, excess cola consumption causes elevation of serum Br^- concentration up to 40 mM.[31] Br^- is generated from brominated vegetable oil through the biotransformation in humans. Brominated vegetable oil is widely used as an emulsifier in citrus-flavored soft drinks in USA. At such dose levels, Br^- would suppress the nucleoside decomposition caused by ultrasound. Plasma concentrations of I^- are very low (0.46–0.67 μM).[29] Since 1 μM I^- did not affect the nucleosides decomposition by ultrasound in the present study (Figure 5), the effects of I^- for ultrasound would be low in the normal plasma concentration levels. Marine algae (seaweeds) contains high amount of iodine. The coastal populations of Japan and China frequently eat excessive quantity of marine algae and intake iodine up to 200 mg a day per person.[32,33] The I^- concentration of these populations may high enough to suppress the nucleoside decomposition by ultrasound.

In conclusion, we found that ultrasound caused decomposition of nucleosides in neutral solutions, and that addition of large amounts of Br^- and I^- suppressed only a half of the nucleoside consumption. It is suggested that a half of the reaction is attributable to HO· but the cause of the remaining half is unclear.

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