Oxidative Cell Damage in *Kat-sod* Assay of Inorganic Halogen Compounds and Their Formation by Ozonation

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Introduction

Ozonation has recently been utilized in the water purification process for controlling trihalomethanes and removal of undesirable organoleptic properties. When raw water contains bromide (Br^-), ozone (O_3) oxidizes Br^- to form bromate (BrO_3^-),¹⁾ which causes renal cancer in rats.²⁾ This carcinogenesis might be associated with oxidative DNA damage which is caused by active oxygen species formed during the reduction of BrO_3^- with glutathione (GSH) and cysteine.³⁾ However, little study has been conducted on oxidative DNA damage of the other oxyhalides concerning disinfection.

In the present study, we investigated oxidative cell damage of oxyhalides by *Kat-sod* assay. To identify oxyhalides formed by ozonation, the occurrence of ozonation by-products of Br^- and iodide (I^-) was also investigated.

Materials and Methods

Oxyhalides — The following compounds were tested for *Kat-sod* assay and Ames assay: hypochlorite (NaClO), chlorine dioxide (ClO_2), chlorite (NaClO_2), chlorate (NaClO_3), hypobromite (NaBrO), dissolved iodine (NaI_3), iodate (NaIO_3) and metaperiodate (NaIO_4).

Toxicological Assays — *Kat-sod* assay was carried out with 4 strains of *E. coli* DSH56 (*wild*), catalase (CAT)-deficient DSH19 (*kat-EG*), superoxide dismutase (SOD)-deficient DSH56 (*sodAB*) and CAT-SOD-deficient DSH67 (*katEG sodAB*), and judged by the difference between the distance of growth inhibition of the wild strain and the other strains.

Analysis of Ozonation By-products — Neutralized solutions (200 ml) containing various concentrations of Br^- and I^- were ozonated with

10 mg/l O_3 gas (2.5 l/min) for 1 to 30 min, with or without 1.0 mg/l NH_4^+ . The samples were analyzed for oxyhalides (differential titration method) and inorganic nitrogen compounds (spectrophotometry).

Results and Discussion

Without cysteine, ClO_2 , NaClO_2 and NaIO_3 were highly cytotoxic in SOD- and CAT-SOD-deficient strains. NaBrO_3 and NaIO_4 showed O_2^- -relating cytotoxicity in the presence of cysteine. NaBrO_3 also showed oxidative mutagenicity with cysteine or GSH ethyl ester. The ozonation of I^- resulted in the dominant formation of IO_3^- regardless of the addition of NH_4^+ . This suggests that oxidative intermediates formed during the ozonation may not react with NH_4^+ . The ozonation of Br^- led to the formation of BrO^- and BrO_3^- . The amount of BrO^- was relatively low throughout ozonation, whereas the amount of BrO_3^- increased with ozonation. The formation of BrO_3^- was suppressed by the addition of NH_4^+ , and the co-existence of I^- did not affect the decline of BrO_3^- by NH_4^+ . However, when the pH during ozonation was adjusted to 8, like seawater, the formation of BrO_3^- was high even with the addition of NH_4^+ .

These findings indicate that BrO_3^- is toxicologically one of the most remarkable oxyhalides detectable in drinking water, and the ozonation condition to lower BrO_3^- is to keep it neutral in the presence of NH_4^+ .

References

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