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Breakage of λ-DNA by Inorganic Tin and Organotin Compounds as Environmental Pollutants

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In proportion to the environmental pollution problems caused by organotin compounds, the genotoxicities of tin compounds in the environments have become of interest so as to estimate their safety in recent years. In this work, isolated λ-DNA (double-strand DNA) was incubated with inorganic tin(II) and tin(IV) and five organotin compounds [n-butyltin trichloride, di(n-butyltin) dichloride, methyltin trichloride, dimethyltin dichloride and trimethyltin chloride] in reaction systems both with and without hydrogen peroxide (H_2O_2) content. The tin compounds tested in this study did not induce DNA breakage in the absence of hydrogen peroxide. Divalent inorganic tin ($SnCl_2$) and tetravalent inorganic tin ($SnCl_4$) caused DNA breakage in the presence of hydrogen peroxide (10 mM), and the DNA damage activity of inorganic tin was much more potent in divalent inorganic tin ($SnCl_2$) than in tetravalent inorganic tin ($SnCl_4$). Divalent inorganic tin ($SnCl_2$) induced DNA breakage in a concentration-dependent fashion at concentrations greater than 0.1 mM of $SnCl_2$ in the presence of hydrogen peroxide (10 mM). DNA breakage was not caused by n-butyltin compounds and methyltin compounds either in the presence or in the absence of hydrogen peroxide.

Keywords: DNA breakage; inorganic tin; tin(II) dichloride; tin(IV) tetrachloride $SnCl_4$; hydrogen peroxide; genotoxicity; environmental pollution; organotin species

INTRODUCTION

Environmental pollution by organotin compounds has arisen with their increasing industrial uses.[1–5] The usage of tri(n-butyl)tin compounds as antifouling agents has been regulated in many countries since 1982[6] because of their high toxicity for aquatic organisms.[4,7,8] As a result of marine pollution, various organotin compounds, such as mono-n-butyltin and methyltin compounds, have been found as environmental metabolites in aquatic and sedimentary samples.[9–11] Mono-n-butyltin compounds have also been detected as degradation products of tri(n-butyl)tins or the di(n-butyl)tins used in industry.[12] Methyltin compounds could be produced by methylation of inorganic tins in the environments.[13,14] Degradation or methylation of tin compounds could be induced by chemical and biological action in the environment.[5,12] Therefore, it is meaningful to study the toxicities of organotin compounds found in the environment.

Their acute toxicities for various organisms or their toxicities towards a target organ have been reviewed,[15–17] including information on the genotoxicity of organotin compounds. A study of the genotoxicity of organotin compounds is very important to estimate their safety. The genotoxicities of some organotin compounds (e.g. di(n-butyl)tin dichloride, bis[tri(n-butyl)tin]oxide, dimethyltin dichloride, trimethyltin chloride) have been studied in recent years.[18–28] We have previously investigated the genotoxicities of 14 organotin compounds, including the four organotin compounds mentioned above, and have shown that several of these compounds damaged DNA in *Bacillus subtilis*,[29] were SOS inducers in *Escherichia coli* PQ37 strain[29] and were mutagens in *Salmonella typhimurium* TA100 and TA98 strains by an induced mutation frequency test.[30] However, the mechanisms of their genotoxicity have not been clarified.

Recently, DNA damage by hydroxyl free radicals produced from hydrogen peroxide (H_2O_2) with some heavy metals have received attention from the viewpoint of significance of biological activity.[31–33] In this study the authors tried to

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investigate the mechanism of the appearance of genotoxicities in tin compounds in reaction system between a tin compound and isolated λ-DNA, both with and without H_2O_2. There is the possibility that H_2O_2 acts as an endogenous promoter or carcinogen. DNA-damaging activities of five organotin compounds [n-butyltin trichloride, di(n-butyl)tin dichloride, methyltin trichloride, dimethyltin dichloride, trimethyltin chloride] and inorganic tins [divalent inorganic tin ($SnCl_2$), tetravalent inorganic tin ($SnCl_4$)], existing in the environment, against isolated λ-DNA (of commercial origin) were studied in the absence or presence of H_2O_2, using the agarose gel electrophoresis method.

MATERIALS AND METHODS

Chemicals

λ-DNA (250 μg/961.5 μl, MW = 3.2×10^6) was purchased from GIBCO BRL. Trimethyltin chloride was purchased from Kanto Chemical Co. Di(n-butyl)tin dichloride and dimethyltin dichloride were from Merck. n-butyltin trichloride and methyltin trichloride were from Aldrich. $SnCl_2.2H_2O$ and $SnCl_4.5H_2O$ were from Wako Pure Chemicals Co. and Hayashi Pure Chemical Co., respectively. All these reagents were analytical grade.

Methyltin trichloride, dimethyltin dichloride, trimethyltin chloride, $SnCl_2$ and $SnCl_4$ were dissolved in deionized sterile water and stored under acidified condition with HCl (heavy metal analysis grade). n-Butyltin trichloride and di(n-butyl)tin dichloride were dissolved in deionized sterile water with an ultrasonicator. These solutions were diluted with deionized sterile water to the desired concentration. All reagents which were used for the extraction of λ-DNA and electrophoresis were of specialized grade for RNA/DNA experiments.

Experimental procedure

The experimental procedures were mainly based on the method of Sugioka *et al.*:[34] 30 μl of λ-DNA (18.1 μg ml^{-1} in tested solution) was incubated

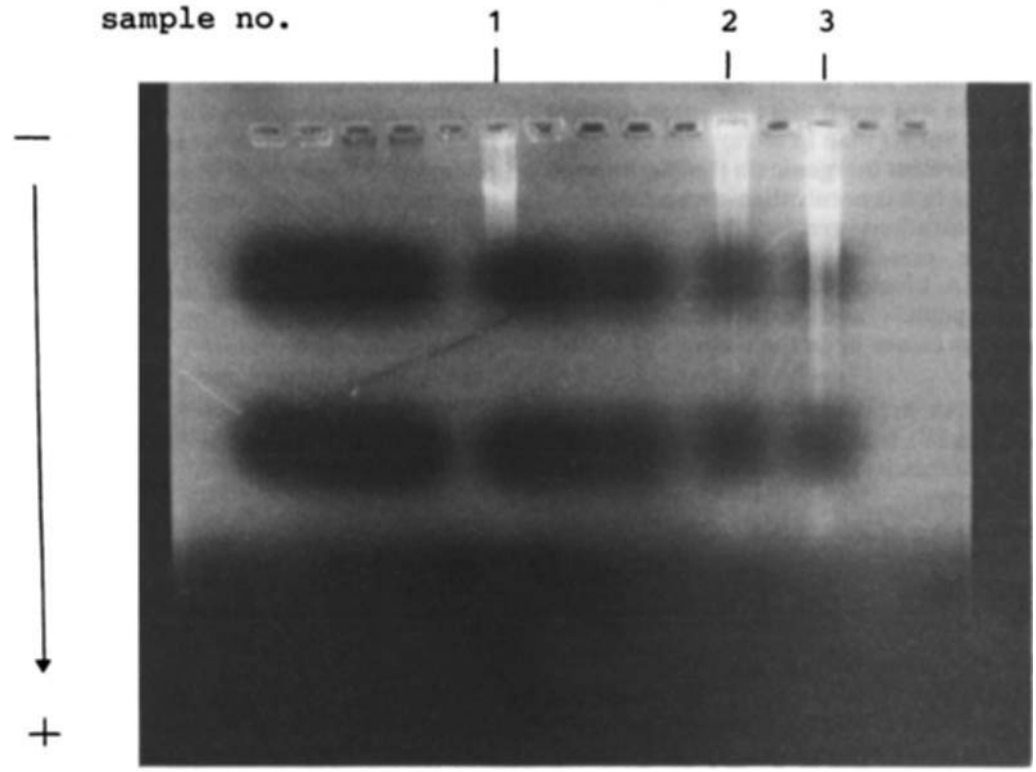

Figure 1 Agarose gel electrophoresis for the reaction of divalent inorganic tin ($SnCl_2$) at 0.1 mM and 0.5 mM in the presence of H_2O_2 (10 mM): lane 1, H_2O_2; 0.1 mM Sn(II) + H_2O_2; 0.5 mM Sn(II) + H_2O_2.

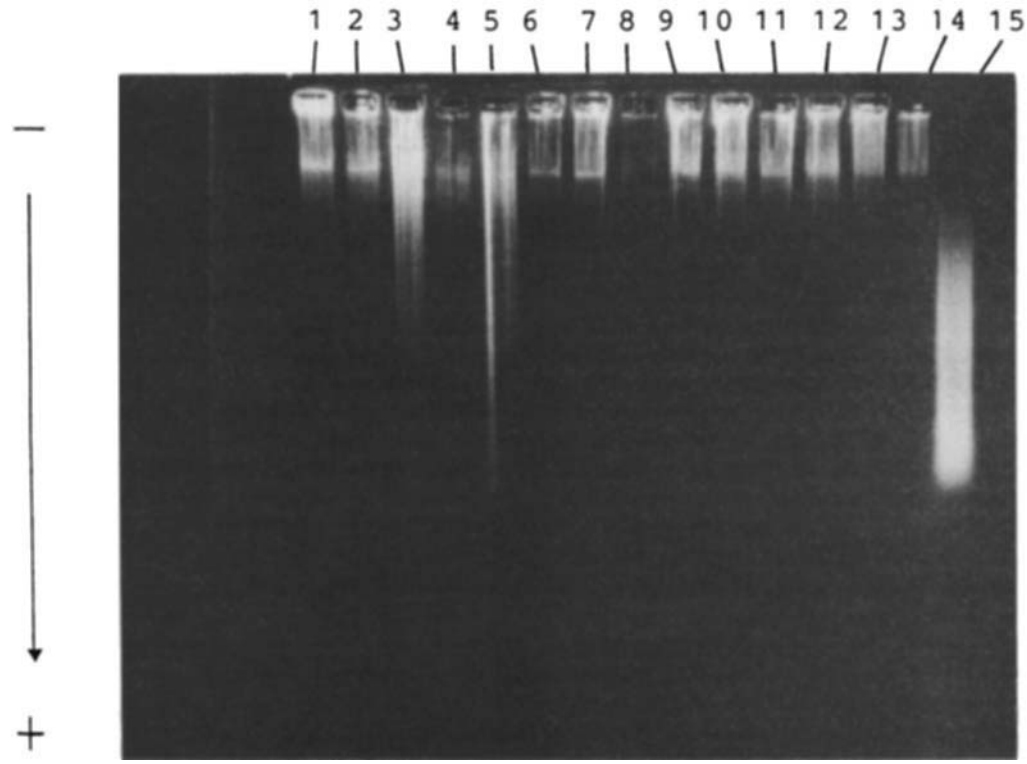

Figure 2 Agarose gel electrophoresis for the reaction of divalent inorganic tin ($SnCl_2$) and tetravalent inorganic tin ($SnCl_4$) with and without 10 mM H_2O_2: lane 1, negative control (sodium phosphate buffer, pH 7.4); 2, H_2O_2; 3, 0.1 mM Sn(II) + H_2O_2; 4, 0.5 mM Sn(II) + H_2O_2; 5, 1 mM Sn(II) + H_2O_2; 6, 0.1 mM Sn(II); 7, 0.5 mM Sn(II); 8, 1 mM Sn(II); 9, 0.1 mM Sn(IV) + H_2O; 10, 0.5 mM Sn(IV) + H_2O_2; 11, 1 mM Sn(IV) + H_2O_2; 12, 0.1 mM Sn(IV); 13, 0.5 mM Sn(IV); 14, 1 mM Sn(IV); 15, positive control (1 mM $FeCl_2$ + 10 mM H_2O_2).

with 0.2 ml of the tested chemical and 0.2 ml of 10 mM sodium phosphate buffer (pH 7.4) at 37 °C for 2 h with and without 10 mM H_2O_2. After the reaction, the reaction mixtures were treated with 0.4 ml of phenol, which was saturated with 0.3% NaCl, and shaken to remove excess protein. Then, the reacted λ-DNA was extracted with 0.4 ml of chloroform. Ethanol (0.8 ml) and 10 μl of 5 M NaCl were added to the chloroform solution and left at −80 °C for 2 h. After centrifugation, the precipitated λ-DNA was redissolved in 60% glycerol solution containing 0.03% xylene cyanol and 0.03% Bromophenol Blue. DNA damage was assessed by electrophoresis on 0.8% agarose gel containing 0.1 mg ethidium bromide buffered with TAE buffer (40 mM Tris, 20 mM sodium acetate, 2 mM EDTA; pH 8.5) at 4 °C with 60 mA for 4 h. The plate was photographed under ultraviolet light.

RESULTS AND DISCUSSION

In the presence of hydrogen peroxide (H_2O_2; 10 mM), λ-DNA breakage by divalent tin ($SnCl_2$) was observed. The results of the reaction between λ-DNA and 0.1 mM and 0.5 mM of divalent inorganic tin ($SnCl_2$) in the presence of 10 mM H_2O_2 are shown in Fig. 1. DNA breakage at 0.5 mM divalent inorganic tin ($SnCl_2$) (lane no. 3) was more intensive than at 0.1 mM $SnCl_2$ (lane no. 2) in Fig. 1.

Breakage of λ-DNA by divalent inorganic tin $SnCl_2$ and tetravalent inorganic tin ($SnCl_4$) are shown in Fig. 2. In the presence of 10 mM H_2O_2, λ-DNA breakage by divalent inorganic tin ($SnCl_2$) was induced more extensively than with tetravalent tin ($SnCl_4$). In the absence of hydrogen peroxide, neither of the inorganic tin compounds ($SnCl_2$, $SnCl_4$) broke the DNA. The most

Table 1 Results of agarose gel electrophoresis for the reaction between λ-DNA and tin compounds[a]

Chemical	Tested concentration (mM)	DNA breakage[b]
Inorganic tin(II)–H_2O_2 (10 mM)	0.1	+
	0.5	++
	1.0	+++
Inorganic tin(IV)–H_2O_2 (10 mM)	0.1	−
	0.5	±
	1.0	±

[a] In the absence of H_2O_2 all the chemicals tested in this study did not cause DNA breakage.

[b] −, DNA breakage was not observed; ±, some DNA breakage was observed, but broken DNA was observed in much smaller pieces than for Bromophenol Blue; +, broken DNA was observed at nearly the same positions as for Bromophenol Blue; ++, broken DNA was observed at places between Bromophenol Blue and xylene cyanol by electrophoresis; +++, broken DNA was observed at the same places as for xylene cyanol.

Note: The following compounds tested at 0.1, 0.5 and 1.0 mM concentration did not cause any DNA breakage: $nBuSnCl_3$, nBu_2SnCl_2, $MeSnCl_3$, Me_2SnCl_2, Me_3SnCl, all with H_2O_2 at 10 mM. Similarly inorganic tin(II) and tin(IV) with H_2O_2 at 1 mM did not cause breakage.

intensive λ-DNA breakage was observed for 1.0 mM divalent inorganic tin ($SnCl_2$) with 10 mM H_2O_2 in lane 5 in Fig. 2. At 0.5 mM divalent inorganic tin ($SnCl_2$) with 10 mM, H_2O_2 DNA breakage was not shown clearly in lane no. 4 in Fig. 2 because of weak fluorescence, but a higher intensity of DNA damage at 0.5 mM $SnCl_2$ compared with 0.1 mM $SnCl_2$ was observed by the naked eye. In the presence of 10 mM H_2O_2, divalent inorganic tin ($SnCl_2$) caused DNA breakage in a concentration-dependent fashion at concentrations over 0.1 mM $SnCl_2$.

Although the DNA-damaging activity of divalent inorganic tin ($SnCl_2$) was much more potent than tetravalent inorganic tin ($SnCl_4$), very weak λ-DNA breakage was observed for tetravalent tin ($SnCl_4$) with H_2O_2 (10 mM) (Fig. 2). However, a significant difference of DNA breakage with an increasing concentration of tetravalent inorganic tin ($SnCl_4$) was not observed. This may be due to the presence of some tin(II) (Sn^{2+}) ions, produced from an equilibrium between Sn^{4+} and Sn^{2+} ions in the reaction mixture. DNA breakage by 0.1 mM–1.0 mM solutions of both divalent and tetravalent inorganic tin compounds was not observed at 1 mM H_2O_2.

It is well known that DNA damage can be induced by generation of hydroxyl radicals through the reaction between H_2O_2 and several heavy metals, e.g. Co, Mn, Ni, Fe, Cu. Breakage of λ-DNA in the reaction between Sn^{2+} ions and H_2O_2 may be caused by hydroxyl radicals (·OH) through a similar pathway to the Haber–Weiss reaction.

In our experiments, DNA breakage was not observed with five tested organotin compounds.

The positive experimental results in this study are summarized in Table 1.

We have already reported that the five organotin compounds which were tested in this study were genotoxicants in the rec-assay, SOS-Chromotest or the induced mutagenicity frequency test.[29,30] However, it became apparent that these organotin compounds did not directly break λ-DNA by themselves and that they did not modify the activity of hydrogen peroxide.

Interactions between di- and tri-organotin compounds and native DNA have been studied. Westendorf *et al.*[23] found that di(n-octyl)tin dichloride (DOTC) interacted with DNA cultured V79 Chinese hamster cells, although Sagelsdorff *et al.*[24] reported that DOTC ([^{14}C]DOTC) did not bind covalently to calf thymus DNA. In recent years, the interactions between calf thymus DNA and some di- or tri-organotin compounds have been investigated by Barbieri and co-workers using ^{119}Sn Mössbauer spectrometry.[25–27] Formation of complex tin species [$R_2Sn(O_2PXY)_2$ or $R_3Sn(O_2PXY)$] produced by interaction between the di- or tri-organotin compound and

phosphodiester groups of the nucleic acid in 0.1 mmol dm^{-3} ethanol solutions was observed and a higher ability for coordination to nucleic acid was observed in the less lipophilic organotin compounds (e.g. methyl- or ethyl-tin compounds).[25-27] Lipid peroxidation by diorganotin compounds and a possible lipid peroxidation mechanism by formation of a triorganostannylperoxy free radical in the membranes were proposed.[35,36] The appearance of genotoxicity in organotin compounds in previous studies[29,30] may be the result of coordination between the organotin compound and DNA in the tested bacteria or of generation of peroxy radicals (ROO•) through lipid peroxidation in the membrane by the organotin compound.

In previous experiments, Hamasaki *et al.*[29,30] reported that DNA damage in rec-assay, and SOS-Chromotest and mutagenicity was not induced by inorganic tin on the bacteria tested. This may be based on the disappearance of active oxygen in these bacteria, caused by the action of enzymes which eliminated active oxygen. Although the effects of inorganic tin on DNA damage in cells are not clear, it was confirmed that DNA breakage was induced through a modification of DNA damaging activity related to hydrogen peroxide, particularly by divalent and tetravalent inorganic tin in our model experimental systems.

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