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Titanium dioxide photocatalyzes DNA damage via the secondary generation of hydrogen peroxide in the presence of sugars

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ABSTRACT

Photocatalytic DNA damage by titanium dioxide was enhanced by the addition of glucose and galactose. Furthermore, the oxidized products of sugars by titanium dioxide photocatalysis induced DNA damage in the presence of copper(II) ion. This DNA damage was inhibited by catalase, indicating the contribution of hydrogen peroxide. The hydrogen peroxide generation from the oxidized sugars was confirmed by fluorometry. These findings suggest that the generation of secondary reactive oxygen species contributes to biomolecular damage by a titanium dioxide photocatalyst.

KEYWORDS: titanium dioxide, photocatalyst, DNA damage, hydrogen peroxide, sugar

1. INTRODUCTION

Titanium dioxide (TiO_2) is a well-known photocatalyst [1-3]. Many efforts have been devoted to clarifying the reactive species generated at the irradiated TiO_2 surface, which is essential for understanding the mechanism of photocatalysis. These reactive species include holes, either free or trapped hydroxyl radicals ($OH^{\bullet}$), superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and singlet oxygen, among others. TiO_2 photocatalysts have been found to kill cancer cells, bacteria, viruses, and algae under ultraviolet illumination [1, 2, 4-7]. One of the potential applications of the TiO_2 photocatalyst is photodynamic therapy, which is a promising treatment for cancer and some non-malignant conditions [8-10]. In general, the mechanism of cytotoxicity by the photocatalysis of TiO_2 is based on cell membrane damage via the above reactive species [1, 2, 4-7]. Furthermore, DNA damage in human cells by the TiO_2 photocatalyst has also been reported [11]. The direct DNA damage by TiO_2 photocatalyst *in vitro* has been also studied [12, 13]. However, the DNA-damaging mechanism *in vivo* is not well-understood because the incorporation of the TiO_2 particle in the nucleus is difficult [4]. Since the TiO_2 photocatalytic reaction occurs in a complex biological environment, an interaction between TiO_2 particles and biomaterials may contribute in the generation of reactive species to induce DNA damage. In this study, the effect of sugars, which are ubiquitous biomaterials, on DNA damage photocatalyzed by TiO_2 was examined.

2. MATERIALS AND METHODS

TiO_2 particles (anatase, average diameter: 50-100 nm) were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Diethylenetriamine-*N,N,N',N'',N''*-pentaacetic acid (DTPA) and bathocuproinedisulfonic acid were from Dojindo Molecular Technologies, Inc. (Kumamoto, Japan). Calf thymus DNA, catalase (45,000 units/mg from bovine liver), and

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superoxide dismutase (SOD) (3,000 units/mg from bovine erythrocyte) were from Sigma-Aldrich Co. LLC. (St. Louis, MO, USA). Methional (3-methylthiopropionaldehyde) was from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Copper(II) chloride dihydrate ($CuCl_2$), ethanol, glucose, galactose, D-mannitol, piperidine, and sodium formate were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The ^{32}P-5'-end-labeled DNAs, namely, 211-base pair (bp) [14] and 261-bp [15] fragments, were prepared as shown in previous reports.

DNA damage was examined using ^{32}P-5'-end-labeled DNA fragments through electrophoresis, as previously reported [12]. The sample solution to examine the photocatalytic DNA damage contained the ^{32}P-labeled DNA fragment, 20 μM-bp calf thymus DNA, 20 μM $CuCl_2$, 5 μM DTPA, and 8 μg mL^{-1} TiO_2 with or without sugars (glucose or galactose) in 100 μL of a 10 mM sodium phosphate buffer (pH 7.8). The solutions were irradiated with a 10 W UV lamp (λ_{max} = 365 nm, 1 mW cm^{-2}, UVP, Inc., CA, USA). Subsequently, the DNA was treated with 1 M piperidine for 20 min at 90°C. Piperidine treatment can cleave DNA at the damaged base. Non-damaged DNA is not cleaved by this treatment. The DNA fragments were subjected to electrophoresis on an 8 M urea/8% polyacrylamide gel. The autoradiogram was obtained by exposing an X-ray film to the gel. The sample solutions to examine DNA damage by the secondary reactive oxygen species contained a 100 μg mL^{-1} TiO_2 dispersion with or without 10 mM sugars (glucose or galactose) in 200 μL of a 10 mM sodium phosphate buffer (pH 7.8). The solutions were exposed to UVA light using a 10 W UV lamp (λ_{max} = 365 nm, 1 mW cm^{-2}). The TiO_2 particles were removed by centrifugation after this photocatalytic reaction. The reaction mixture incubated in a 1.5-mL microtube contained a ^{32}P-labeled DNA fragment, 20 μM-bp calf thymus DNA, 20 μM DTPA, and 1 mM of treated sugars in 200 μL of a 10 mM sodium phosphate buffer (pH 7.8) (60 min, 37ºC). DNA fragments were treated and analyzed as mentioned above to obtain the autoradiogram. The concentration of H_2O_2 generated from the oxidized sugars was measured by fluorometry using folic acid according to the literature [16].

3. RESULTS AND DISCUSSION

Photo-irradiated TiO_2 caused DNA cleavage in the presence of Cu(II) ion, an endogenous metal ion, after piperidine treatment (Figure 1). A previous study also demonstrated the photocatalytic DNA damage by TiO_2 under a similar condition [12]. This DNA damage can be mainly explained by the oxidation of guanine and thymine residues through the photocatalytic formation of H_2O_2. In the case of anatase, a high concentration of TiO_2 can damage DNA at every nucleobase by $OH^{\bullet}$ generation in the absence of Cu(II). Typical free $OH^{\bullet}$ scavengers inhibited this Cu(II)-independent DNA damage. These results indicate that free $OH^{\bullet}$ partly contributes to DNA damage photocatalyzed by TiO_2. On the other hand, scavengers of $OH^{\bullet}$, such as a sugar (mannitol), ethanol, and formate, enhanced the Cu(II)-dependent DNA damage [12]. These scavengers themselves did not induce DNA damage. Since $OH^{\bullet}$ can oxidize most biomaterials, the oxidized products of biomaterials

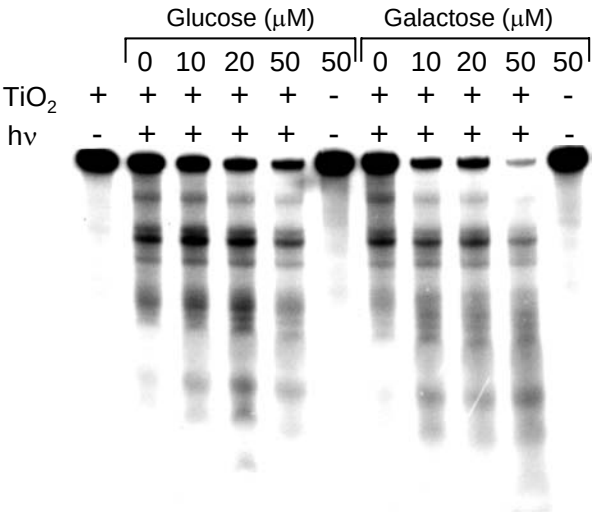

Figure 1. Autoradiograms of a ^{32}P-5'-end-labeled DNA fragment photocatalyzed by TiO_2 in the absence or presence of sugars. The reaction mixtures containing the ^{32}P-5'-end-labeled 211-bp DNA fragment, 20 μM-bp calf thymus DNA, 20 μM $CuCl_2$, 5 μM DTPA, 8 μg mL^{-1} TiO_2, and the indicated concentration of glucose or galactose in a 10 mM sodium phosphate buffer (pH 7.8) were irradiated (λ_{max} = 365 nm, 10 J cm^{-2}). The DNA fragments were analyzed by electrophoresis after piperidine treatment as described in Materials and Methods.

by the TiO_2 photocatalyst may damage DNA via the generation of secondary reactive oxygen species. The addition of sugars, glucose and galactose, which are ubiquitous biomolecules, enhanced the DNA damage photocatalyzed by TiO_2 (Figure 1). Enhancement of DNA damage by sugars has seldom been reported, and these sugars themselves could not induce DNA damage. Therefore, the products of the photocatalytic reaction of these sugars by TiO_2 should induce Cu(II)-dependent damage to DNA. Indeed, the glucose and galactose oxidized by the TiO_2 photocatalytic reaction caused DNA damage in the presence of Cu(II) ion (Figure 2). Figure 3 shows the inhibitory effect of various scavengers for DNA damage by the photo-oxidized products of glucose by TiO_2. Catalase inhibited DNA damage by the photocatalyzed glucose, indicating the involvement of H_2O_2. Bathocuproine, which is a chelator of Cu(I) ion, also inhibited DNA damage, suggesting the involvement of Cu(I) ion. The free $OH^{\bullet}$ scavengers had no or little inhibitory effect on DNA damage. The inhibitory effect of superoxide dismutase (SOD) was weak, suggesting that $O_2^{\bullet-}$ itself is not the main reactive species for DNA damage. Similar results were observed in the case of galactose. Fluorometry using folic acid [16] demonstrated the formation of H_2O_2 from the photocatalyzed sugars (Figure 4). The amount of H_2O_2 generation was comparable with that of other H_2O_2-mediated DNA-damaging drugs [17]. H_2O_2 generation was not observed in the absence of Cu(II). These results showed that the oxidized products of sugars generate H_2O_2 during the reaction with Cu(II), resulting in secondary DNA damage.

These sugars act as an electron donor for the photocatalytic reaction [18, 19]. Partially oxidized sugars, such as aldehyde compounds, are possibly produced through this photocatalytic oxidation. The mechanism of DNA damage by the photocatalyzed product of sugars is proposed in Figure 5. Aldehydes can generate H_2O_2 via its further oxidation [20], though these sugars themselves are stable compounds. Many studies have reported the DNA damage by H_2O_2 and Cu(II) [21]. Various chemical compounds, including aldehydes, easily produce $O_2^{\bullet-}$ through their autooxidation process. The autooxidation is

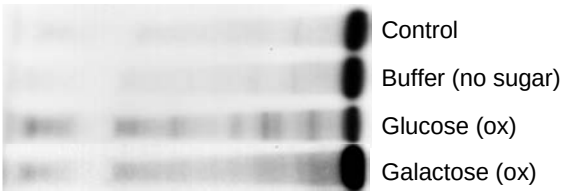

Figure 2. DNA damage induced by the photocatalyzed sugars in the presence of Cu(II) ion. The reaction mixtures containing the ^{32}P-5'-end-labeled 211-bp DNA fragment, 20 μM-bp calf thymus DNA, 20 μM $CuCl_2$, 5 μM DTPA, and 1 mM of treated sugars (Glucose (ox) and Galactose (ox)) in a 10 mM sodium phosphate buffer (pH 7.8) were incubated (60 min, 37ºC). The DNA fragments were analyzed by electrophoresis after piperidine treatment as described in Materials and Methods. The buffer solution with or without 10 mM sugars was previously irradiated (λ_{max} = 365 nm, 6 J cm^{-2}) with 100 μg mL^{-1} TiO_2. The TiO_2 particles were removed by centrifugation, and the solution containing the oxidized sugars was used.

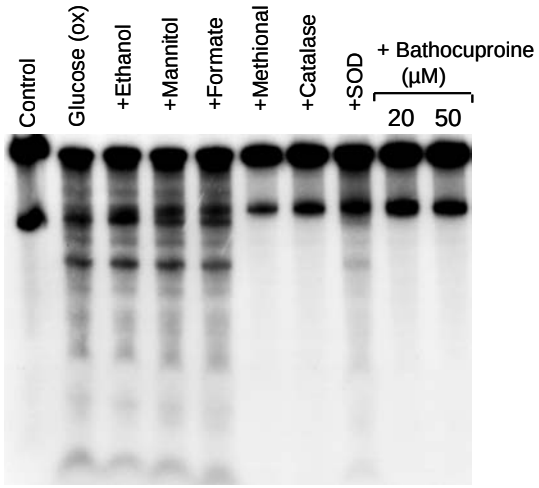

Figure 3. Effects of scavengers on DNA damage induced by the oxidized glucose in the presence of Cu(II) ion. One mL of reaction mixtures containing the ^{32}P-5'-end-labeled 261-bp DNA fragment, 20 μM-bp calf thymus DNA, 20 μM $CuCl_2$, 5 μM DTPA, and 1 mM of treated glucose was incubated (60 min, 37°C) in the absence or presence of the scavengers. The concentrations of scavengers were as follows: 5 v% ethanol, 0.1 M mannitol, 0.1 M sodium formate, 0.1 M methional, 30 units of SOD, and 50 units of catalase. The glucose solution was previously treated as described in the caption of Figure 2. The DNA fragments were analyzed by electrophoresis after piperidine treatment as described in Materials and Methods.

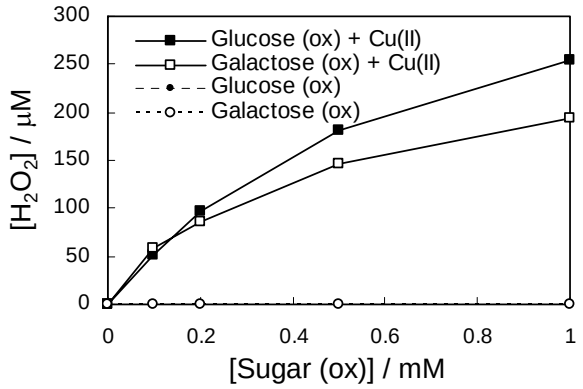

Figure 4. Hydrogen peroxide formation from TiO_2-photooxidized glucose and galactose. The sugar solutions were previously treated as described in the caption of Figure 2. One mL of solution containing the treated sugars (Sugar (ox)) and 10 μM of folic acid was incubated (60 min, 37°C) in the absence or presence of 20 μM $CuCl_2$, and the fluorescence intensity was measured (excitation: 360 nm, detection: 450 nm). The concentration of the generated H_2O_2 was determined by the calibration curve method.

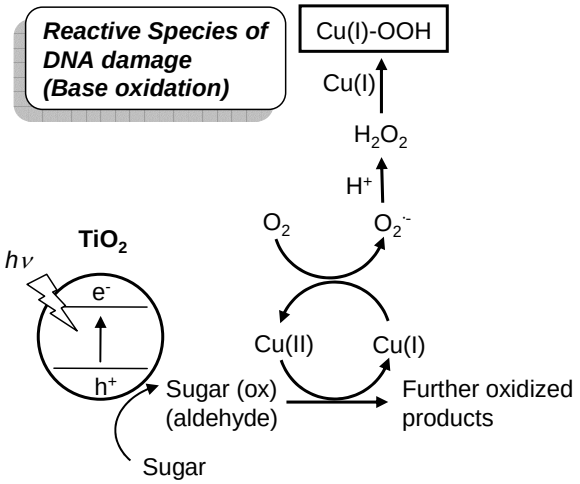

Figure 5. Proposed mechanism of DNA damage induced by the photocatalyzed sugars.

markedly enhanced by Cu(II) ion, which is an essential component of chromatin [22]. The formed $O_2^{\bullet-}$ is rapidly dismutated into H_2O_2. Although the generated H_2O_2 itself cannot damage DNA, H_2O_2 reduces Cu(II) into Cu(I), leading to the activation of H_2O_2 through the formation of reactive species [23], such as Cu(I)-OOH [12, 17, 21]. Indeed, methional, a scavenger of Cu(I)-OOH, inhibited the DNA damage (Figure 3). This reactive species cannot be scavenged by the free $OH^{\bullet}$ scavengers; however, it can effectively oxidize the nucleobases [12, 17, 21].

Although TiO_2 is not likely to be incorporated in a cell nucleus [4], H_2O_2 generated via a photocatalytic reaction can be easily diffused and incorporated in a cell nucleus. This DNA-damaging mechanism via H_2O_2 generation may participate in the phototoxicity of TiO_2. *In vivo*, the cell membrane is an important reaction field for the TiO_2 photocatalyst because TiO_2 particles show affinity with a cell membrane [4]. Further, a part of the TiO_2 particles can become incorporated into the cell. Sugars on the cell membrane and cytoplasm may be oxidized by the TiO_2 photocatalytic reaction. The generated hole and $OH^{\bullet}$ can oxidize these sugars, leading to the formation of secondary H_2O_2 from their photooxidized products.

CONCLUSION

In summary, sugars enhance the DNA damage photocatalyzed by TiO_2 particles. This enhancement of DNA damage is due to the secondary generation of a reactive oxygen species, H_2O_2, which can diffuse in the cell and damage cellular DNA. These findings suggest that the secondary H_2O_2 generation contributes to the phototoxicity of TiO_2 more than the direct formation of reactive oxygen species does.

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