Singlet Oxygen Generation Photocatalyzed by TiO_2 Particles and Its Contribution to Biomolecule Damage

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The study of near-infrared emission has shown that singlet oxygen ($^1O_2$) is generated via superoxide by the photocatalytic reaction of titanium dioxide (TiO_2) dispersed in solvents. The $^1O_2$ was deactivated on the TiO_2 surface and could not oxidize nicotinamide adenine dinucleotide, which has no affinity to the TiO_2 surface. The generation of photocatalytic $^1O_2$ was enhanced in the phospholipid membrane, and a water soluble protein acted as an effective scavenger of the generated $^1O_2$. These findings suggest that $^1O_2$ generated by TiO_2 contributes to the phototoxic effect through the oxidation of the membrane protein.

Titanium dioxide (TiO_2) is a well-known semiconducting photocatalyst.[1] When exposed to ultraviolet A light (320–400 nm), the reduction–oxidation activity of TiO_2 has a significant biological impact, as is exemplified by its bactericidal activity. Moreover, the phototoxic effect of TiO_2 has been studied for medical application,[1,2] such as photodynamic therapy (PDT).[3] Reactive oxygen species, including hydroxyl radicals, superoxide (O_2^-), and hydrogen peroxide (H_2O_2), play an important role in the phototoxic effect.[1,4] The contribution of singlet oxygen ($^1O_2$) to the TiO_2 photocatalytic reaction has also been reported.[5,6] Recently, the $^1O_2$ generation by TiO_2 photocatalysis has been demonstrated by the emission measurement of $^1O_2$, which is assigned to the transition from $^1O_2(^1\Delta_g)$ to $^3O_2(^3\Sigma_g)$.[6] Because $^1O_2$ is considered to be an important reactive species in the PDT process,[7] the clarification of the contribution of $^1O_2$ generated by TiO_2 photocatalysis is closely related to a design of photocatalysts for medical applications. In the present study, the generation of $^1O_2$ in the TiO_2 photocatalysis and the importance on the biomolecular damage were examined.

TiO_2 particles (anatase and rutile; particle size: 100–300 nm, Kanto Kagaku Co.) were dispersed in solvents (D_2O, ethanol, and dichloromethane). TiO_2 particles dispersed with liposome were prepared using a commercially available liposome kit (Sigma Chemical Co.) using an ultrasonication method.[8] The near-infrared emission spectrum was measured during irradiation (355 nm) according to a previous report.[9]

The typical emission of $^1O_2$ at ca. 1270 nm was observed during irradiation of TiO_2 (Figure 1). A relatively strong emission of $^1O_2$ was observed in nonpolar organic solvents. The quantum yield (Φ_Δ) of the $^1O_2$ generation by TiO_2 photocatalysis was roughly estimated from the comparison of the $^1O_2$ emission intensities by TiO_2 and Methylene Blue ($\Phi_\Delta = 0.52$)[10] and the apparent absorbance of the TiO_2 dispersions.[11] It has been reported that the lifetime of $^1O_2$ generated by TiO_2 photocatalysis (5 μs)[6] is shorter than that by the photosensitized reaction of Methylene Blue (12 μs).[12] Because the emission intensity is proportional to the lifetime, the Φ_Δ was corrected by its lifetime. The estimated value of Φ_Δ by both types of TiO_2, anatase and rutile, was about 0.02 in ethanol. This value of Φ_Δ is sufficiently large to induce oxidative damage to biomolecules. The $^1O_2$ emission in D_2O was completely quenched by the addition of superoxide dismutase, which is the enzyme to dismutate O_2^- into H_2O_2. These findings suggest that $^1O_2$ is produced via oxidation of O_2^-, which is generated from the photocatalytic reduction of molecular oxygen by TiO_2. The $^1O_2$ emission by rutile was markedly larger than that of anatase in D_2O (data not shown). This difference can be reasonably explained by the fact that, in

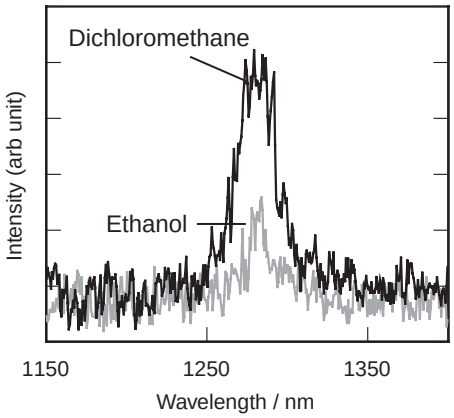

Figure 1. Emission spectra of $^1O_2$ generated by the TiO_2 photocatalytic reaction. Dispersed TiO_2 particles (140 μg mL^{-1}, anatase) in organic solvents were irradiated (ex: 355 nm).

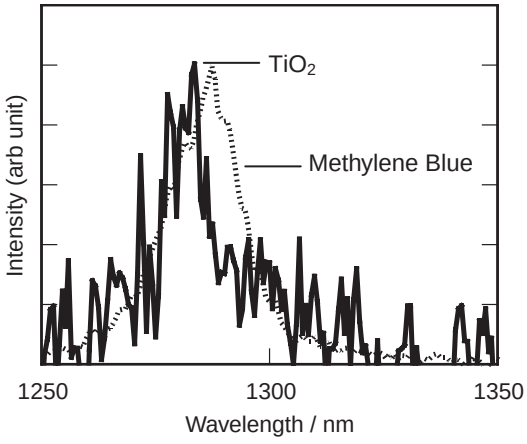

Figure 2. Emission spectra of $^1O_2$ generated by the TiO_2 photocatalytic reaction and the photosensitized reaction of Methylene Blue. A dispersed TiO_2 particle (140 μg mL^{-1}, anatase) or Methylene Blue (absorbance: 0.05 at 355 nm) in ethanol was irradiated (ex: 355 nm).

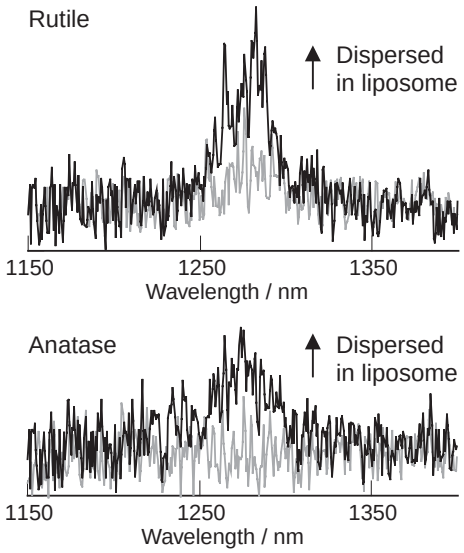

Figure 3. Emission spectra of $^1O_2$ generated by the TiO_2 photocatalytic reaction with or without liposome. Dispersed TiO_2 particles (140 μg mL^{-1}) in D_2O were irradiated (ex: 355 nm).

aqueous solution, H_2O_2 generation, rather than O_2^- generation, proceeds in the photocatalysis of anatase, whereas O_2^- is the main product from oxygen photoreduction mediated by rutile.[13] These results support the mechanism of $^1O_2$ generation via O_2^- by TiO_2 photocatalysis.

The emission spectrum of $^1O_2$ by TiO_2 (in the cases of anatase and rutile) shifted to blue (about 4 nm) slightly more than in the case of Methylene Blue (Figure 2), suggesting that the surroundings of the $^1O_2$ by TiO_2 are different from those of Methylene Blue. The $^1O_2$ generated by the photosensitized reaction of Methylene Blue becomes deactivated in the homogeneous media of solvent molecules. Consequently, a possible explanation of the blue shift of the $^1O_2$ emission by TiO_2 is that most of the $^1O_2$ becomes deactivated on the TiO_2 surface.

The $^1O_2$ emission was significantly increased in liposome in the cases of anatase and rutile (Figure 3). A possible reason for the enhancement of the $^1O_2$ emission is the elongation of the lifetime of $^1O_2$ or the promotion of the photocatalytic reaction. This result has shown that the phospholipid membrane is an important environment for the phototoxic reaction mediated by $^1O_2$ generation in the TiO_2 photocatalysis. It has been reported that TiO_2 particles show affinity with a cell membrane.[2] Therefore, a cell membrane should be an important environment of the TiO_2 photocatalytic reaction. Since the amino acid residues in proteins can be oxidized by $^1O_2$,[10] a membrane protein should be the target biomolecule in a cell membrane. Indeed, $^1O_2$ emission was quenched by the addition of bovine serum albumin, a water-soluble protein (data not shown), suggesting the scavenging of $^1O_2$ through the oxidation of a protein.

In vivo, nicotinamide adenine dinucleotide (NADH) is one of the most important target biomolecules oxidized by $^1O_2$.[14] NADH is easily oxidized into the oxidized form, and the typical absorption peak at ca. 340 nm is diminished. In the present study, dispersed anatase and rutile particles did not oxidize NADH in H_2O and D_2O during irradiation (data not shown). Because NADH is rarely adsorbed on a TiO_2 surface, $^1O_2$ could not effectively oxidize NADH in solution. In a previous study, we reported that DNA is damaged mainly through H_2O_2 and hydroxyl radicals in the photocatalytic reaction of TiO_2 and that the contribution of $^1O_2$ is negligible.[4] These findings and those of previous reports have shown that the $^1O_2$ generated by the TiO_2 photocatalytic reaction does not play an important role in the mechanism of damage to these biomolecules, which do not interact with the TiO_2 surface.

In summary, $^1O_2$ can be generated via the oxidation of O_2^-, which is produced through the photocatalytic reduction of molecular oxygen, on the surface of a TiO_2 photocatalyst during photoirradiation. The $^1O_2$ on the TiO_2 surface scarcely oxidized NADH, because most of the $^1O_2$ deactivated on the TiO_2 surface. On the other hand, the $^1O_2$ generation by TiO_2 photocatalysis could be enhanced in the microenvironment of the phospholipid membrane. These findings suggest that $^1O_2$ contributes to the phototoxicity of TiO_2 through oxidation of the membrane protein.

This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (417) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of the Japanese Government.

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11 The precise estimation of the Φ_Δ is difficult because scattering by the suspended TiO_2 powder makes the calculation of absorbed light intensity difficult. In the present study, the Φ_Δ was estimated using the apparent absorbance of TiO_2. The calculated value indicates the lowest limit of the Φ_Δ by the TiO_2 photocatalysis in ethanol.
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Published on the web (Advance View) June 27, 2006; doi:10.1246/cl.2006.832