Spectroscopic Investigation of the Mechanism of Photocatalysis

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Abstract: Reaction mechanisms of various kinds of photocatalysts have been reviewed based on the recent reports, in which various spectroscopic techniques including luminol chemiluminescence photometry, fluorescence probe method, electron spin resonance (ESR), and nuclear magnetic resonance (NMR) spectroscopy were applied. The reaction mechanisms elucidated for bare and modified TiO2 were described individually. The modified visible light responsive TiO2 photocatalysts, i.e., Fe(III)-deposited metal-doped TiO2 and platinum complex-deposited TiO2, were studied by detecting paramagnetic species with ESR, •O2− (or H2O2) with chemiluminescence photometry, and OH radicals with a fluorescence probe method. For bare TiO2, the difference in the oxidation mechanism for the different crystalline form was investigated by the fluorescence probe method, while the adsorption and decomposition behaviors of several amino acids and peptides were investigated by 1H-NMR spectroscopy.

Keywords: ESR; NMR; OH radical; superoxide radical; H2O2; acetaldehyde; metal doped TiO2; glutathione; amino acids; visible-light responsive photocatalysts

1. Introduction

TiO2 photocatalysts have been widely utilized for the oxidation of organic pollutants [1–4]. For further practical applications, the improvement in the photocatalytic efficiency and the extension of the effective wavelength to visible region are desired. To develop photocatalysts, understanding of the
detailed photocatalytic mechanisms is prerequisite. Recently, the reaction mechanisms of TiO$_2$
photocatalysis have been extensively reviewed [5] and the authors also reviewed the reports published
up to 2011 from the view of the detection of active oxygen species [6]. In this manuscript, recent
development in the reaction mechanism mainly reported by our group was reviewed. Main techniques
used were ESR spectroscopy for the state of photoinduced electron and holes, fluorescence probe
method for the formation of OH radical and NMR spectroscopy for the adsorption and decomposition
of biological molecules in solution.

2. Spectroscopic Methods for Investigating Photocatalysis

2.1. ESR (Electron Spin Resonance) Spectroscopy

ESR spectroscopy is conventionally used to detect unpaired electrons. Photocatalytic reactions
proceed by the two following reactions: reduction of reactants with photoexcited electrons and oxidation
of reactants with holes. Therefore, it is important to examine the generation behavior of these active
species. In TiO$_2$ photocatalytic systems, two kinds of active species (photoexcited electron and hole) are
generated on absorbing photons. Some of the electrons and holes are trapped at Ti and O atoms, to
become Ti$^{3+}$ and O$^-$, respectively. Therefore, by detecting these trapped electrons and holes using ESR
spectroscopy under light irradiation of different wavelengths, the generation behavior of excited
species can be examined. Moreover, the electron transfer between photocatalysts and co-catalysts can
be also examined because if the electron transfer occurs, the amount of the unpaired electron in the
co-catalyst should change before and after light irradiation. Therefore, ESR spectroscopy is very useful
to elucidate photocatalytic reaction mechanism.

2.2. Chemiluminescence Photometry

Reduced oxygen molecules such as superoxide radical (•O$_2^-$) and H$_2$O$_2$ can be detected by
chemiluminescence with luminol (LH$_2$, aminodiazabenzoquinone). The one electron oxidized state of
luminol (•L$^-$) reacts with •O$_2^-$ to form the excited state of 3-aminophthalic acid to emit fluorescence in
alkaline solution [7], where •L$^-$ is formed from LH$_2$ by the oxidation with •O$_2^-$ [8]. Since •O$_2^-$ is rather
stable in alkaline solution, after the irradiation on photocatalyst was stopped, luminol is injected to
measure the amount of •O$_2^-$ by the chemiluminescence intensity. The same chemiluminescence was
obtained from H$_2$O$_2$ by the reaction with L that is two-electron oxidized state of LH$_2$ [9]. To measure
the amount of H$_2$O$_2$ in solution, after mixing luminol, hemoglobin was added to oxidize luminol,
because L is rather unstable [8]. Luminol chemiluminescence method has some problems. It is available
only in alkaline solution, and luminol emits light with SiO$_2$ in the absence of •O$_2^-$ and H$_2$O$_2$. Therefore,
in the case of SiO$_2$ deposited TiO$_2$, instead of luminol, MCLA and lucigenin were employed for the
detection of •O$_2^-$ and H$_2$O$_2$, respectively, by means of chemiluminescence photometry [10].

2.3. Fluorescence Probe Method

Hydroxyl radical (•OH) has been recognized as a key active species in the oxidation mechanism in
photocatalysis [9,11]. For the detection of •OH we employed coumarin. It reacts with •OH to produce
7-OH coumarin (umbelliferone) which emits strong fluorescence [12]. After the irradiation of a coumarin
aqueous solution containing photocatalysts powder for a given time, the fluorescence intensity of the fluorescent products (umbelliferone) in the solution was measured. The •OH concentration could be calculated from the concentration of umbelliferone with the aid of data of radiation chemistry [12]. Since carboxyl group is known to adsorb on TiO₂, the similar experiments were performed for 3-carboxylic acid derivative of coumarin (CCA, Figure 1), and ensured the reaction with •OH to form OH-CCA as illustrated in Figure 1 [13].

Figure 1. Probing reaction of OH radical with CCA (coumarin 3-carboxy acid) to form fluorescent molecule OH-CCA (7-hydroxy coumarin 3-carboxy acid).

2.4. NMR (Nuclear Magnetic Resonance) Spectroscopy

1H-NMR spectroscopy has been recognized as an effective technique to investigate the behaviors of the reactant molecules in the photocatalytic systems. The adsorption and the decomposition of biomolecules such as amino acids and peptides in the aqueous suspension of photocatalysts can be investigated with 1H-NMR spectroscopy with relatively feasible experimental procedures [14] as follows. Firstly, 1H-NMR of organic molecules dissolved in the solvent are measured. Then certain amount of the photocatalysts is added to the solution. From the initial decrease in the intensity of 1H-NMR peaks of the corresponding reactant molecules the amount of the adsorption can be estimated. Then, by measuring the decrease in the intensities of reactant molecules for various photoirradiation times, one could estimate the photodecomposition rates of the reactants [15].

3. Mechanism of Photocatalysis

3.1. Bare-TiO₂ and Visible-Light Responsive TiO₂ Photocatalysts

General scheme of photocatalysis applied for the oxidation of pollutant is shown in Figure 2. Light absorption in semiconductor corresponds to the formation of an electron (e⁻) in the conduction band (CB) and a hole (h⁺) in the valence band (VB). Usually e⁻ reduces O₂ in air to form •O₂⁻ and H₂O₂.

The photocatalytic oxidation of organic compounds is accelerated with oxygen [16]. The consumption of O₂ at the oxidation site of the photocatalyst has been suggested from the experiment of electrochemical probe reactions at the surface of illuminated TiO₂ photoelectrode [17]. Therefore, the generalized oxidation mechanism of organic molecules (RH) can be illustrated as shown in Figure 2. Organic reactants RH will degrade by losing one carbon atom by releasing CO₂ through the intermediates like aldehyde R’CHO or carboxylate R’COO⁻. Although •OH has been often regarded to play an important role in the actual oxidation mechanism of photocatalytic reactions, •OH is not
involved in the main oxidation process for organic compounds. In place of it, the surface trapped holes play the role of oxidation, which may be acknowledged as the surface adsorbed \( \bullet \text{OH} \) in the de-protonated form as stated below.

**Figure 2.** General reaction processes for the photocatalytic oxidation of organic molecules.

For the extension of the practical applications of photocatalysts, the utilization of visible light has been intensively promoted. Figure 3 shows the energy levels of several representative visible-light responsive photocatalysts. Since the one-electron reduction potential of \( \text{O}_2 \) is very close to that of the CB bottom of \( \text{TiO}_2 \) and the energy level of VB has sufficient oxidation ability, the shift of the VB by doping N (or, C and S) anions has been attempted to absorb visible light (b). In this case, photogenerated holes at the donor level should have the oxidation ability similarly to that of bare \( \text{TiO}_2 \) [9].

**Figure 3.** Classification of visible-responsive photocatalysts by the primary reaction mechanism. (a) Unmodified \( \text{TiO}_2 \); (b) Nitrogen doped \( \text{TiO}_2 \); (c) \( \text{Fe(III)} \) grafted \( \text{TiO}_2 \); (d) \( \text{Fe(III)} \) grafted metal doped \( \text{TiO}_2 \); (e) Platinum complex deposited \( \text{TiO}_2 \). Adopted with permission from [18]. © 2013 American Chemical Society.

Recently, interfacial charge transfer (IFCT) type absorption originating from the excitation of VB electrons to deposited (or grafted) metal ions has been proposed (c). In this case, if the deposited compound has a catalytic ability of \( \text{O}_2 \) reduction, the efficiency is expected to be increased [19,20]. Since the absorbance of IFCT is very small, to increase the absorption, the transferring of the excited electron to the graft level by doping of metal ions was proposed (d). Photocatalysts of
photosensitization type were also proposed, in which the stable metal complex such as PtCl$_6^{2-}$ is deposited as a sensitizer (e). The deposited compound absorbs the visible light and transfers the excited electron to produce a cation radical D$^+$, which can oxidize organic pollutant molecules. In this case, the enough oxidation power with good stability is required for the oxidized sensitizer D$^+$ [21,22]. To confirm the suggested reaction mechanism, several spectroscopic methods have been applied to the detection of the paramagnetic species produced on the catalysts along with the primary products (•O$_2^-$, H$_2$O$_2$, •OH).

3.1.1. Fe(III) Grafted TiO$_2$ Based Photocatalysts

Fe(III) grafted TiO$_2$ (Fe/TiO$_2$) showed the photocatalytic activity under visible light irradiation. The quantum efficiency of Fe/TiO$_2$ prepared under optimized condition was reported to be 22% [20]. We examined the photocatalytic reaction mechanism of the Fe/TiO$_2$ using ESR spectroscopy [23]. As shown in Figure 4A, under visible light irradiation, the ESR signal assigned to Fe$^{3+}$ ($g = 4.3$) was decreased and the ESR signal assigned to trapped holes ($g = 2.01$) at the TiO$_2$ host was observed. In the case of TiO$_2$ without the grafting of Fe$^{3+}$, the trapped hole signal was scarcely observed as compared to the Fe/TiO$_2$ under visible light irradiation. This means that electrons at VB are directly transferred to the grafted Fe$^{3+}$ rather than CB (Figure 4B). Using ESR spectroscopy, we could reveal for the first time that the direct electron transfer from the VB of TiO$_2$ to the Fe$^{3+}$ is the origin of the visible light response.

Figure 4. (A) ESR spectra of (a) Fe$^{3+}$ and (b) holes for Fe/TiO$_2$ and (c) holes for TiO$_2$ before and after light irradiation with different wavelengths; (B) Photocatalytic reaction mechanism for Fe/TiO$_2$ under visible light irradiation. Reprinted with permission from [23]. © 2012 American Chemical Society.

Photocatalytic reactions cannot proceed when the photogenerated electrons do not react with molecular oxygen, which is the only molecule to be reduced in ambient atmosphere even though the
redox potential of the photogenerated hole is positive enough to decompose organic compounds. Therefore, it is important to confirm the reduction of O$_2$ into •O$_2^-$ by one-electron or H$_2$O$_2$ by two-electron reductions. Under visible light irradiation, for Fe/TiO$_2$, the production of H$_2$O$_2$ was dominant as compared to •O$_2^-$. This means that the excited Fe$^{2+}$ can reduce O$_2$ to H$_2$O$_2$ through two-electron process. Since the electrons having a potential energy of +0.695 V (vs. SHE at pH = 0) can reduce O$_2$ to H$_2$O$_2$ by two-electron process [24], the redox potential of the grafted Fe$^{3+}$ to Fe$^{2+}$ was equal to or less than +0.695 V (vs. SHE at pH = 0). Since photogenerated electrons were consumed by the reduction of O$_2$ to H$_2$O$_2$, holes remained at valence band could decompose organic substances efficiently, resulting in the high performance.

Moreover, after the grafting of Fe$^{3+}$ on the TiO$_2$ doped with metal (M) ions such as Ru, Ir, Rh or Cr ions (Fe/M:TiO$_2$), the photocatalytic activities were enhanced compared to the Fe/TiO$_2$ as shown in Figure 5A. The visible light response was increased in the order Ir > Cr > Ru > Rh. In the case of the Fe/Ru:TiO$_2$, based on the measurements by ESR spectroscopy, Ru ions were doped as tetravalent and played a role as an acceptor level. Then, the photoexcited Ru ions, by receiving electrons from the VB, immediately transfer electrons to the grafted Fe$^{3+}$ under visible light irradiation. This indirect electron transfer from the VB to the Fe$^{3+}$ via the doped Ru ions occurred in addition to the direct electron transfer from the VB to the Fe$^{3+}$, leading to the enhancement of photocatalytic activity.

**Figure 5.** (A) Photocatalytic activities of the Fe/M:TiO$_2$ against gaseous acetaldehyde under visible light irradiation of (a) $\lambda$ = 470 nm and (b) $\lambda$ = 625 nm; (B) The amount of (a) •O$_2^-$ and (b) H$_2$O$_2$ generated on the M:TiO$_2$ and the Fe/M:TiO$_2$, respectively under visible light irradiation of $\lambda$ = 625 nm; (C) Schematic energy level diagram for the Fe/M:TiO$_2$. Reprinted with permission from [25]. © 2014 Elsevier B. V.

Furthermore, we examined the desirable character of doped metal ions for photocatalytic performance in detail. Figure 5B shows the amounts of •O$_2^-$ and H$_2$O$_2$ generated on the M:TiO$_2$ and the Fe/M:TiO$_2$, respectively, under visible light irradiation. The electrons having a potential energy of +0.38 V (vs. SHE at pH = 0) can reduce O$_2$ into •O$_2^-$ under the experimental conditions (pH = 11.5) [24]. Therefore, by measuring the amount of •O$_2^-$, the redox potential of dopants can be relatively estimated. For the
non-doped TiO₂, the generation amount of •O₂⁻ was larger than that of the M:TiO₂. For the non-doped TiO₂, O₂ was reduced to •O₂⁻ by an electron excited at conduction band from defect level under visible light irradiation. This result indicated that all kinds of dopant used played a role as acceptor, because if they play a role as donor, the generation amount of •O₂⁻ should increase due to electron excitations from dopants to CB. Among the M:TiO₂, the generation amount of •O₂⁻ was decreased in the order of Rh > Cr > Ru > Ir. Since the order of the redox level of dopants should be consistent with that of the •O₂⁻ amount, their redox levels would be more negative in the order of Rh > Cr > Ru > Ir. Secondly, when electrons transfer to the grafted Fe³⁺, the excited Fe²⁺ can reduce O₂ to H₂O₂ by a two-electron process as mentioned below. Therefore, we can determine the degree of electron transfer to the Fe³⁺ from the dopants by the measurement of amount of H₂O₂. The generation amount of H₂O₂ was decreased in the order of Ir > Ru > Cr > Rh (Figure 5B). This H₂O₂ generation tendency was opposite to that of •O₂⁻. This means that through the dopant with more positive redox potential, electrons can transfer more easily to the Fe³⁺ as illustrated in Figure 5C. This is due to the small energy loss of electrons when the redox potential of dopant was close to that of the Fe³⁺. Therefore, we concluded that the high photocatalytic activity of the Fe/Ir:TiO₂ under visible light irradiation was attributable to the acceptor level due to Ir⁴⁺ formed close to the redox potential of the grafted Fe³⁺ (Figure 5C).

In the case of TiO₂ codoped with Rh and Sb ions, the efficiency of the indirect electron transfer to the Fe³⁺ was lowered compared to the TiO₂ doped with Rh ions alone (Figure 6A). By codoping with Sb ions, Rh⁴⁺ was reduced to Rh³⁺ and the formed Rh³⁺ played a role as donor [26,27]. This indicated that the efficiency of the indirect electron transfer of Rh³⁺ → CB → Fe³⁺ was lower than that of VB → Rh⁴⁺ → Fe³⁺ (Figure 6B).

**Figure 6.** (A) The co-doped effect on photocatalytic activities for CO₂ formation from gaseous acetaldehyde under visible light irradiation of λ = 470 nm. (a) Before and (b) after grafting of Fe³⁺; (B) Indirect electron transfer paths to the Fe³⁺ via (a) donor level and (b) acceptor level. Reprinted with permission from [25]. © 2014 Elsevier B. V.
The energy gap of the redox potential between conduction band and the grafted Fe$^{3+}$ was larger than that between the doped Rh$^{3+}$ and the grafted Fe$^{3+}$ and therefore electrons photoexcited at CB could not effectively transfer to the Fe$^{3+}$ because of the large energy loss. From these results, forming acceptor level closed to the redox potential of the grafted Fe$^{3+}$ was important for high performance of Fe/TiO$_2$ based photocatalysts under visible light irradiation [25]. Recently, a better energy level matching in Fe/M:TiO$_2$ was achieved by employing Fe$^{3+}$ as a doping metal ion [28].

3.1.2. Pt Chloride Deposited TiO$_2$ Photocatalysts

Pt$^{4+}$ chloride deposited TiO$_2$ (PtCl/TiO$_2$) also showed a photocatalytic activity under visible light irradiation and its quantum efficiency was 9.8% [20]. In the past, Kisch et al., reported a mechanistic hypothesis to explain PtCl/TiO$_2$ activity [21,29]. The proposed hypothesis was that the PtCl undergoes homolytic Pt-Cl cleavage by absorbing of light, generating a Pt$^{3+}$ intermediate and a chlorine atom, the Pt$^{3+}$ injects an electron to the conduction band of TiO$_2$, and then the Cl radical oxidizes organic compounds. However, it is not clear whether the Pt-Cl cleavage in the PtCl/TiO$_2$ system would occur. In addition, there is no sufficient evidence to support the injection of electron from Pt$^{3+}$ to the conduction band of TiO$_2$. Therefore, we clarified the charge transfer between the PtCl and TiO$_2$ under visible-light irradiation using ESR spectroscopy [30].

For a bare TiO$_2$ without deposition of PtCl, under visible light irradiation, both ESR signals assigned to trapped electrons and holes were not observed (Figure 7A(a)). For the PtCl/TiO$_2$, a signal assigned to Pt$^{3+}$ was observed. This means that Pt$^{4+}$ chloride complexes were charge-separated into Pt$^{3+}$ and Cl radicals. Then in the TiO$_2$ host, trapped electrons ($g \approx 1.98$) were observed (Figure 7A(b)). These results proved that TiO$_2$ could receive electrons from excited Pt$^{3+}$ as well as the hypothetical mechanism. However, unlike the hypothetical mechanism, trapped hole signal ($g = 2.01$) was also observed. Based on the results, some electrons in the VB of TiO$_2$ would be excited to the orbital of the Cl radicals similarly to the case of direct electron transfer from the VB of TiO$_2$ to the grafted Fe$^{3+}$ for the Fe/TiO$_2$ photocatalysts. Since the redox potential (+3.0 V vs. SHE at pH = 0) of the VB of rutile TiO$_2$ is more positive than that (2.47 V vs. SHE at pH = 0) of Cl/Cl$^-$ [31], the high photocatalytic activity of PtCl/TiO$_2$ would be owing to the generation of holes in the TiO$_2$ host.

Generation behaviors of •O$_2^{-}$ and H$_2$O$_2$ under visible light irradiation were also examined for the PtCl/TiO$_2$ as shown in Figure 6B. •O$_2^{-}$ was predominantly generated compared to H$_2$O$_2$. This was opposite behavior to the Fe/TiO$_2$ for which H$_2$O$_2$ was dominantly generated rather than •O$_2^{-}$. This means that photoexcited electrons have a higher potential energy than +0.38 V (vs. SHE at pH = 0) and therefore the reduction of O$_2$ to •O$_2^{-}$ was produced by the electrons photoexcited at CB of TiO$_2$, which supported the ESR results [30].

A plausible reaction mechanism for the PtCl/TiO$_2$ photocatalyst is illustrated in Figure 7C. Photoexcited Pt$^{3+}$ generated by ligand- metal charge transfer in deposited PtCl complex by adsorption of visible light gives an electron to the TiO$_2$ CB and then the electron is consumed by reduction of O$_2$ into •O$_2^{-}$. Some of the photogenerated Cl radicals can decompose organic substances and the other receive electrons by photo-excitation from the valence band of TiO$_2$, resulting the generation of holes in TiO$_2$. The organic substances can be efficiently decomposed by the generated holes in TiO$_2$ with strong oxidation ability.
3.1.3. Comparison of the Visible-Light Responsive TiO₂ Photocatalysts

The reaction mechanisms of various modified TiO₂ were investigated by detecting •OH quantitatively by means of a coumarin fluorescence probe method [18]. The photocatalysts investigated were nitrogen-doped, Fe(III)-grafted, Fe(III)-grafted Ru-doped TiO₂, and Pt-complex-deposited, whose diffuse reflectance spectra are shown in Figure 8A. On the irradiation with 470 nm light in the presence of coumarin, the concentration of umbelliferone was increased (Figure 8B). From the slope, the formation rate of •OH was calculated. Then, the •OH quantum yield was calculated with the absorbed light intensity which was evaluated from the absorption and irradiance spectra in Figure 8A. The quantum yield ranged from $10^{-5}$ for N-TiO₂ to $4 \times 10^{-4}$ for Fe/TiO₂ [18]. In the presence of 0.14 mM H₂O₂, the •OH yield decreased for N-TiO₂ while it increased for Fe/TiO₂. The increase for Fe/TiO₂ suggests that H₂O₂ is a reaction intermediate for producing •OH.

The photocatalytic activity was evaluated by the rate of CO₂ generation associated with acetaldehyde decomposition and then it was plotted in Figure 9A as a function of the •OH formation rate for each photocatalyst. The CO₂ generation rates of the photocatalysts were positively correlated with those of the •OH formation. However, the formation rates of CO₂ were extremely larger (10³ times) than those of •OH. This finding indicates that the oxidation reaction predominantly takes place at the photocatalyst surface with the trapped holes. The good correlation in the figure suggests that •OH in the bulk solution is equilibrated with trapped holes (Equation (1)), but the equilibrium is significantly shifted to the surface trapped holes.

\[
\text{•OH} + \text{Ti}^{4+}[^\text{TiO}_2] \leftrightarrow \text{•O}^-\text{Ti}^{4+}[\text{TiO}_2] + \text{H}^+ \quad (1)
\]

Figure 7. (A) ESR spectra of electrons and holes for (a) TiO₂ and (b) PtCl/TiO₂; (B) (a) •O₂⁻ and (b) H₂O₂ generated on the PtCl/TiO₂ under visible light irradiation. (C) Photocatalytic reaction mechanism of PtCl/TiO₂. Reprinted with permission from [30]. © 2012 American Chemical Society.
Figure 8. (A) Absorption spectra as the complement of the reflectance (1-R) and the irradiance spectra of LED used in the study; (B) Concentration of umbelliferone generated under 470-nm irradiation in aqueous coumarin solution was plotted as a function of the irradiation time. Reprinted with permission from [18]. © 2013 American Chemical Society.

The highest photocatalytic activity in the suspension system was obtained for Fe(III)-deposited Ru-doped TiO$_2$ (Fe(III)/Ru:TiO$_2$) whose reaction mechanism is shown in Figure 9B. On the basis of the ESR and chemiluminescence experiments mentioned above [23], the CB electrons are formed by two step excitation with visible light irradiation and O$_2$ is reduced to H$_2$O$_2$. The grafted Fe$^{3+}$ is reduced by Ru$^{3+}$ or by IFCT and then the formed Fe$^{2+}$ produces •OH from H$_2$O$_2$. The •OH produced in solution is adsorbed on the TiO$_2$ surface to form trapped holes which could oxidize organic compounds, such as acetaldehyde, leading to CO$_2$.

Figure 9. (A) Relationship between the formation rates of CO$_2$ and •OH under the irradiation of 470 nm LED. The CO$_2$ formation rate is a measure of the photocatalytic reaction rate in the acetaldehyde decomposition in aqueous suspension system; (B) Schematic illustration of reaction mechanism of Fe(III)-grafted Ru-doped TiO$_2$ photocatalyst based on the detection of •OH and CO$_2$. Key: IFCT, interfacial charge transfer; h$^+$tr, surface trapped hole. Reprinted with permission from [18]. © 2013 American Chemical Society.
3.2. Photocatalysis with Bare TiO$_2$

3.2.1. Reactivities of Rutile and Anatase Surfaces

The photogeneration of molecular oxygen at rutile TiO$_2$ electrode is a famous historical reaction [32]. To investigate the oxidation mechanism, •OH formation was measured by employing three electrodes of rutile TiO$_2$ (100), (110), and (001) [33]. Figure 10A shows the amount of the produced umbelliferone, which is normalized to the number of charges used in the reaction. For all electrodes, the photocurrent efficiency of •OH was less than 1%, while that of O$_2$ was about 100%. This observation implies that the conventionally proposed mechanism to produce O$_2$ via •OH formation is not a major mechanism in water oxidation at TiO$_2$ surface. Figure 10B shows the plausible reaction steps in the formation of O$_2$ and •OH through surface peroxo (Ti-O-O-Ti). By cleaving Ti-O bond in the peroxo, O$_2$ is formed as shown in Figure 10B(a) [34]. When O-O bond is cleaved instead of Ti-O bond, •OH is formed as a byproduct. The •OH formation in Figure 10A increases in the order of (001) < (110) < (100), which can be explained by the strength of Ti-O bond deduced from the surface structure [33].

Though rutile TiO$_2$ shows high activity for O$_2$ evolution, anatase TiO$_2$ is known to have a higher activity in the photocatalytic oxidation of organic molecules [35]. The difference in the generation process of •OH between rutile and anatase was investigated by using CCA and coumarin [36]. Figure 11A shows the quantum yields of •OH generation measured with coumarin and CCA together with the adsorbed fraction of CCA. As shown in Figure 11A, anatase and anatase-contained TiO$_2$ (ST-01, P25, and F1) generated •OH in the substantial yields. The quantum yield for OH-CCA was much larger than that for umbelliferone, indicating that •OH is formed at the TiO$_2$ surface and diffused into bulk solution. Furthermore, this observation indicates that the active site is different from the adsorption site of $-\text{COO}^-$ group. Since H$_2$O$_2$ is produced in photocatalysis, the effect of H$_2$O$_2$ on the
•OH generation was investigated. Figure 11B shows the effect of the addition of H₂O₂ on the formation rate for (a) OH-CCA and (b) umbelliferone. On the addition of H₂O₂, the •OH generation for pure anatase TiO₂ decreased but increased for rutile and rutile-contained TiO₂. This phenomenon has been reported previously for other several TiO₂ powders [37]. The amount of •O²⁻ was significantly increased with the addition of H₂O₂ [37]. Although the formation of •OH from H₂O₂ by CB electrons is commonly suggested in the •OH generation mechanism, the fact that the •O²⁻ was significantly increased with H₂O₂ denied the one-electron reduction of H₂O₂.

Figure 11. (A) Quantum yields of •OH using the different probe molecules, coumarin (blue) and CCA (brown), and the fraction of adsorbed CCA (green) for four kinds of TiO₂ powders; (B) Effect of the addition of 0.14 mM H₂O₂ on the formation rates of (a) OH-CCA and (b) umbelliferone. Reprinted with permission from [36]. © 2014 American Chemical Society.

Since the increase is remarkable for anatase than rutile TiO₂, with the addition of H₂O₂, the generation of •OH at anatase surface was replaced by the oxidation of H₂O₂ to form •O²⁻, as illustrated in Figure 12A.

For rutile TiO₂ adsorbed H₂O₂ is equivalent to the surface peroxy, Ti-O-O-Ti and promotes the formation of •OH as stated above. The detailed generation mechanism of •OH on anatase and rutile TiO₂ surfaces can be proposed as shown in Figure 12B. On the anatase surface, photogenerated valence band holes, h⁺, are trapped at the surface oxygen to form trapped holes (Ti−O•) that can be regarded as the adsorbed •OH in the deprotonated form (•O⁻) [18] then an •OH is released to the solution as represented by Equation (1). On the other hand, for rutile TiO₂, since the crystalline structure is packed more tightly than that for anatase, the stability of the surface trapped holes may be different. By trapping of h⁺ predominantly near the trapped hole, Ti-peroxo is formed. As described above in Figure 10B(b), •OH radical is produced by h⁺ from H₂O with Ti-peroxo, which plays the role of a catalyst. Thus, the increase of the •OH generation with H₂O₂ for rutile TiO₂ can be explained.
3.2.2. Adsorption and Decomposition of Glycine Related Peptides

The application of photocatalysts to biological fields for their antibacterial effect and in medical treatments for diseases, including cancer, has been proceeding extensively [38,39]. It is believed that the active oxygen species generated on the photocatalysts such as H₂O₂, •OH, and singlet oxygen are involved in the attack to kill various kinds of virus and bacteria [40]. However, the mechanism underlying the photobiological activity is not yet well understood. Since the photocatalytic process is expected to occur at the interface between the photocatalysts and the liquid medium, the interface between protein molecules and inorganic materials has recently received much attention.

Proteins and peptides are composed of various kinds of amino acids. For a proper understanding of the adsorptive and photocatalytic interactions between the surface of the photocatalysts and proteins/peptides, fundamental knowledge on the adsorption and photocatalytic reactivity of individual constituent amino acids would be necessary.

TiO₂ is widely used for practical applications as a photocatalyst. The surface of TiO₂ is amphiphilic, which consists of hydrophobic and hydrophilic parts [41]. The hydrophilic parts involve two kinds of hydroxyl group, that is, the acidic bridged hydroxyl group and the basic terminal hydroxyl group. Both groups can be adsorptive and/or photocatalytic active sites, depending on the kinds of titanium dioxide which are characterized by different particle size, surface area, and crystal forms such as anatase, rutile and brookite. The photocatalyst with different characteristic surface shows different adsorbability and photocatalytic activity [40].

It was demonstrated that both hydrophilic and hydrophobic sites are adsorptive sites but that only hydrophobic sites are photocatalytically active for ST-01 TiO₂ (100% anatase crystal form with a BET surface area of 320 m²·g⁻¹ and a particle size of 9 nm; Ishihara Sangyo Ltd., Osaka, Japan) [15].
After the calcinations at 973 K hydrophilic parts of the surface of ST-01 can be eliminated and a highly hydrophobic surface (designated as HT-TiO2) is created without changing the crystal form [15]. By employing these characteristics, the adsorption and decomposition sites of the simplest amino acid glycine, whose adsorbability on the TiO2 surface is still controversial [42], and its homopeptides (Gly-Gly and Gly-Gly-Gly) were investigated by 1H-NMR spectroscopy [43]. For Gly-Gly and Gly-Gly-Gly the carboxylic group and the peptide bond were assigned as the adsorptive sites of the peptides on the surface of ST-01. The adsorption feature of Gly-Gly-Gly on TiO2 (ST-01) are illustrated in Figure 13; the peptide would adsorb by the C-terminal carboxyl group most probably with the terminal hydroxyl group at 5-coordinated Ti of TiO2 as is generally believed [42]. The photo decomposition took place by the weak adsorption of the peptide bonds on the surface of TiO2 (ST-01).

**Figure 13.** Schematic presentation of the adsorption of the peptide (Gly-Gly-Gly) on the hydrophilic and hydrophobic surface of TiO2 (ST-01). Reprinted with permission from [43] © 2014 American Chemical Society.

On the other hand, when a hydrophobic side chain Leu is incorporated, in addition to the peptide bonds and the carboxylic group, the adsorption of hydrophobic leucyl residue on the hydrophobic parts of TiO2 surface would take place. As shown in Figure 14, for HT-TiO2, the adsorption of the Leu- containing peptides increased with the increase of the number of the peptide bond that is, Leu < Leu-Gly, Gly-Leu < Leu-Gly-Gly (Figure 14C).

However, the decomposition rates are almost the same (Figure 14D). These facts suggest that both the peptide bond and leucyl side chain could adsorb on the hydrophobic surface of TiO2 but photocatalytic decomposition should take place through the adsorption of the leucyl side chain which would adsorb preferably on the photocatalytic active part of the hydrophobic TiO2 surface. Thus leucyl residue would adsorb preferably on the active site of the hydrophobic part of TiO2 instead of the peptide bonds and photocatalysis proceeds. The adsorption feature of Leu-Gly-Gly, on TiO2 (ST-01) are illustrated in Figure 15.
Figure 14. Equilibrium adsorption and rate of decomposition under the UV irradiation measured at 297 K for Leu, Leu-Gly, Gly-Leu, and Leu-Gly-Gly in the aqueous suspensions of TiO$_2$; (A, B) for untreated TiO$_2$ and (C, D) for HT-TiO$_2$ (TiO$_2$ calcined at 973 K). Reprinted with permission from [43] © 2014 American Chemical Society.

However, as shown in Figure 14A,B it was found that Leu-Gly showed remarkably low adsorbability and decomposition rate as compared to Gly-Leu due to the specific conformation, in which the positively charged amino group and negatively charged carboxyl group interact strongly by electrostatic force [43]. Thus, when a peptide or proteins take a specific conformation, photocatalysis does not work effectively. For the effective use of TiO$_2$ it would be necessary to acquire information
on the surface conformation of the corresponding proteins/peptides to access the surface of the photocatalysts. By combining the information about the surface conformation of proteins/peptides and the active sites of TiO₂ (hydrophobic or hydrophilic), we could design the TiO₂ effective to diminish the specific virus, bacteria or environmental hazardous materials.

3.2.3. Glutathione and Related Amino Acids

With increased applications of TiO₂ nanoparticles, the concerns about their potential human toxicity and their environmental impact have also increased. Although details of human biological responses to TiO₂ exposure are still unavailable, numerous *in vitro* examinations concerning cellular responses induced by TiO₂ have been reported [44–46].

Glutathione is a tri-peptide capable of diminishing active oxygen species in living cells. In spite of the importance of glutathione in defense against oxidative stress, its actual affects and the mechanism for the TiO₂-induced cytotoxicity and genotoxicity have not been completely elucidated yet.

The photocatalytic decomposition of glutathione and related amino acids in TiO₂ suspension was investigated with ¹H NMR spectroscopy [47]. The results suggest, that as shown in Figure 16A, both glutathione in reduced (GSH) and oxidative forms (GSSG) are adsorbed on the TiO₂ surface by carboxyl or amino groups but not by the thiol group (SH) of the side chain which plays a crucial role in the glutathione cycle (Scheme 1), to be degraded. This suggests that the function of glutathione cycle should be deteriorated in living cells by the adsorption. However, the decomposition rates are considerably slow as compared with those of the constituent amino acids (Glu, Cys and Gly) as shown in Figure 16B, possibly reflecting the self-defensive property against active oxygen species.

**Figure 16.** (A) Schematic presentation of the plausible adsorption of glutathione on the surface of TiO₂ (ST-01); (B) (a) Adsorption and (b) decomposition rates of glutathione (GSH and GSSG) and the constituent amino acids (Glu, Cys, and Gly) in aqueous suspension of TiO₂ (5 mg/0.4 mL D₂O) under UV irradiation at 297 K. Reprinted with permission from [47] © 2012 American Chemical Society.
4. Conclusions

TiO₂ photocatalysts have been utilized for the oxidation of organic pollutants. For the development of further practical applications, the improvement of the activity with the aid of an understanding of the detailed mechanism(s) of action is a prerequisite. The primary process of photocatalysis reported in the literatures still have some confusion. To clarify the reaction mechanism, the proper and reliable detection of primary active species, such as trapped electrons, trapped holes, •O₂⁻ and •OH, in photocatalytic systems is required. By employing various spectroscopic techniques we have succeeded in elucidating some of the mechanisms of important photocatalytic reactions. Further investigations are proceeding in our laboratory.

Author Contributions

Main contributions of each author follow; Yoshio Nosaka; Sections 2.2, 2.3, 3.1, 3.1.3, 3.2.1, Masami Nishikawa; Sections 2.1, 3.1.1, 3.1.2, and Atsuko Y. Nosaka; Sections 2.4, 3.2.2, 3.2.3. All of the authors participated in the revision and corrections of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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