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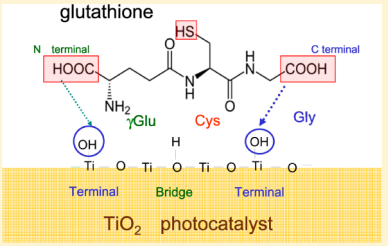
The Behaviors of Glutathione and Related Amino Acids in the TiO_2 Photocatalytic System

Atsuko Y. Nosaka,* Goro Tanaka, and Yoshio Nosaka*

Department of Chemistry, Nagaoka University of Technology, Nagaoka, 940-2188 Japan

ABSTRACT: Glutathione is a tripeptide capable of diminishing active oxygen species in living cells. The photocatalytic decomposition of glutathione and related amino acids in TiO_2 suspension was investigated with ^1H NMR spectroscopy. The results suggest that both glutathione in reduced and oxidative forms is adsorbed on the TiO_2 surface by carboxyl or amino groups but not by the thiol group of the side chain which plays a crucial role in the glutathione cycle, to be degraded. This means that the function of glutathione cycle should be deteriorated in living cells by the adsorption. However, the decomposition rates are considerably low as compared with those of the constituent amino acids (Glu, Cys, and Gly), possibly reflecting the self-defensive property against active oxygen species.



INTRODUCTION

TiO_2 photocatalysts have been utilized extensively for environmental cleanup. Along with the development of visible light responsive photocatalysts,[1] the extensive applications to the medical fields are expected. Owing to the antivirus and antibacterial properties, they are actually applied to the cleanup of operation rooms and therapies. It is believed that the active oxygen species which generate on the photocatalysts such as superoxide, H_2O_2, OH radical, and singlet oxygen are involved in the attack to extinguish the virus and bacteria.[2−6] However, the detailed mechanism has not been completely elucidated. On the other hand, living cells possess strong functions to protect themselves from the attack of active oxygen species. For instance, superoxide dismutase (SOD) diminishes superoxides by catalyzing dismutation; that is, one molecule of superoxide is oxidized and another is reduced ($O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$).[7] Glutathione peroxidase reduces H_2O_2 to water along with the oxidation of glutathione (GSH) ($2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$). Oxidized glutathione (GSSG) is reduced to GSH by the NADPH-dependent enzyme glutathione reductase. This procedure is called a glutathione cycle. Furthermore, GSH has the ability to reduce active oxygen species nonenzymatically.[7] Thus, when the photocatalysts are applied to living materials, the protective functions may compete with the photocatalytic oxidation by the added photocatalysts.

In this report to obtain insights into the influence of TiO_2 on application to living materials, we investigated the behavior of glutathione in the photocatalytic system by means of ^1H NMR spectroscopy.

EXPERIMENTAL SECTION

The commercially available TiO_2 powder ST-01(Ishihara Sangyo Ltd.) was used as received, which is a generous gift from the manufacturer. For the photo-decomposition measurements, 5 mg of TiO_2 powder was suspended in 0.4 mL of D_2O (99.9%). The initial concentration of the samples in the dispersion was fixed at 10 mM. The amino acids and glutathione samples were A.R. grade. The sample in a 5 mm O.D. glass NMR sample tube was UV irradiated outside the NMR probe with two cylindrical black-light lamps (20 W) from two sides. The sample tube was rotated during the photoirradiation. The excitation light intensity at the sample position was 2.2 mW/cm^2. ^1H NMR spectra were acquired at 400 MHz at 298 K on a JMN AL400 NMR spectrometer with 20 s recycle delay with a pulse duration of 9.6 μs. Chemical shifts were measured relative to DSS (sodium 2, 2-dimethyl-2-silapentane-5-sulfonate) solution as an external reference.

RESULTS AND DISCUSSION

As shown in Figure 1, the surface of titanium oxide is amphiphilic, which consists of hydrophobic and hydrophilic parts. The hydrophilic parts involve two kinds of hydroxyl groups, that is, acidic bridged hydroxyl group (pK 2.9) and basic hydroxyl group (pK 12.7).[8] Each part can be adsorbed and/or active sites, depending on the kinds of titanium oxides which are characterized by different particle sizes, surface areas, and crystal forms such as anatase, rutile and brookite.[9−12]

GSH is a tripeptide consisting of L-glutamic acid (Glu), L-cysteine (Cys), and glycine (Gly) residues. The Glu residue is attached to the Cys residue by its γ carboxyl group, rather than its α carboxyl group. Usually, a peptide bond is formed between the amino group and the carboxyl group at the α position but glutathione forms the peptide bond with the carboxyl group at the γ position of Glu and the amino group of Cys residue. The plausible adsorbed sites of glutathione would be the carboxyl group at the C terminal, the amide group at the N terminal, and the thiol group (SH) and the carboxyl group of the side chain (Figure 1).

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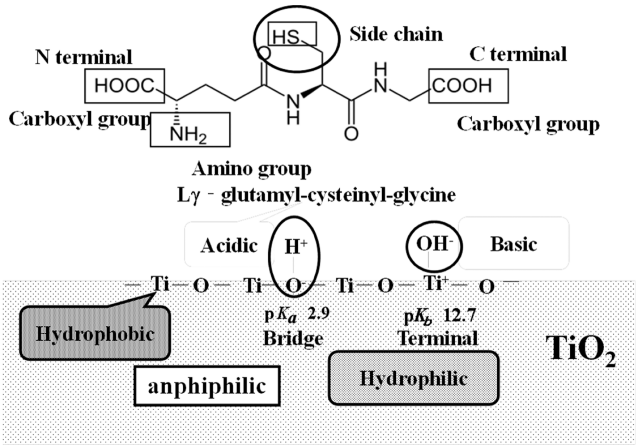


Figure 1. Plausible adsorption sites and active sites on the surface of TiO_2 and possible adsorption sites of glutathione.

The cysteine residue plays an important metabolic role as a constituent of glutathione. As stated above, in cells, GSH reduces active oxygen species with the oxidation of the SH group to convert to the dimer form of GSSG with S−S bond (Scheme 1). In this case, the cysteine residue plays a role in the reduction of active oxygen species. In this regard, first we attempted to investigate the decomposition behavior of cysteine and compare it with that of cystine which is a dimer of cysteine binding with an S−S bond like GSSG. Figure 3 shows the ^{1}H NMR spectra of cysteine and cystine measured in the suspension of ST-01 and those measured after UV irradiation of 100 min. As shown in Figure 2b, the peak intensities of cystine decreased after the UV irradiation because of the decomposition of the molecules. However, no notable peaks could be detected as decomposed products. On the other hand, as shown in Figure 2a, for cysteine, several novel peaks appeared along with the decrease of the inherent peak intensities of cysteine. These novel peaks could be assigned to those of cystine.

In Figure 3, the time profiles of the peak intensity against the UV irradiation time were shown. Figure 3a clearly shows that the concentration of cysteine decreased with an increase of cystine. On the other hand, when cystine was employed as a starting substance, the peak intensities of cystine decreased significantly slowly and no peaks of cysteine as a decomposition product were detected, as shown in Figure 3b. This fact would mean that cysteine is adsorbed on the surface of ST-01 and decomposes under UV light irradiation to produce a dimer (cystine) in which two cysteine residues are bound with a S−S bond. On the other hand, cystine does not possess a SH group to bind with TiO_2. Instead, most probably, it would bind to the TiO_2 surface with the carboxyl and/or amino groups and decompose from the terminal. Taking account of the slow

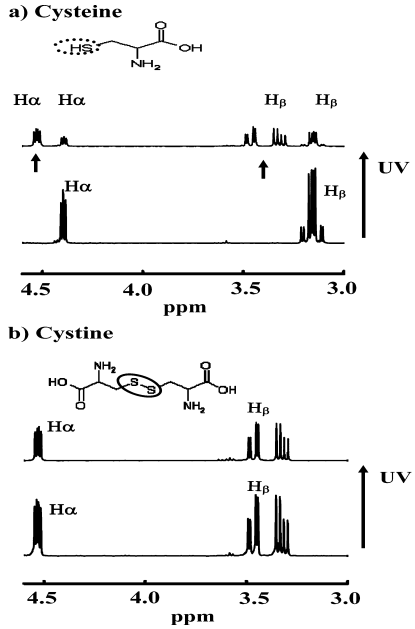


Figure 2. ^{1}H NMR spectra of 10 mM (a) cysteine and (b) cystine in an aqueous suspension of TiO_2 (5 mg/0.4 mL D_2O) before and after 100 min UV irradiation, measured at 297 K.

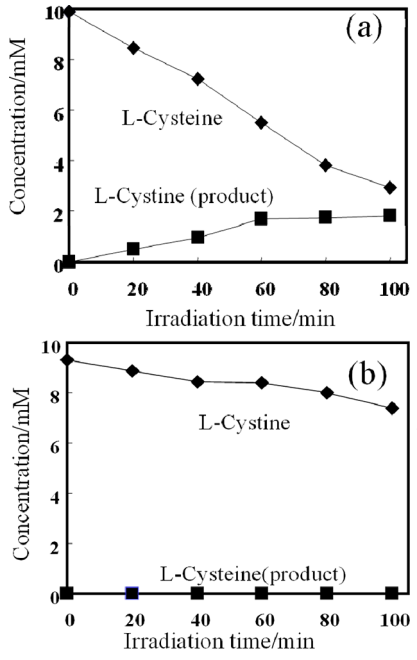
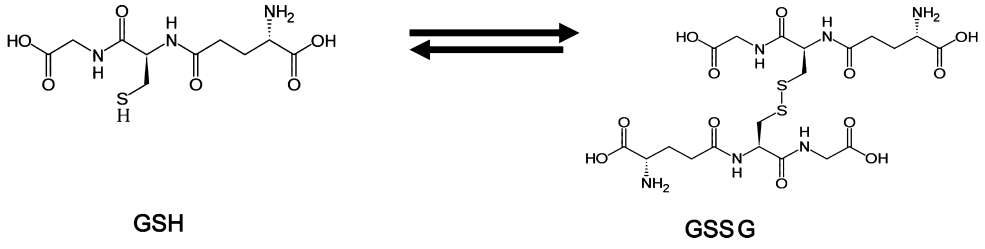


Figure 3. The time profiles of the concentrations of (a) cysteine and (b) cystine in aqueous suspension of TiO_2 (5 mg/0.4 mL D_2O) under the UV irradiation at 297 K.

Scheme 1



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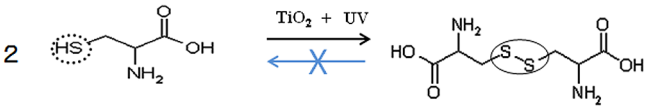
decomposition rate, it might not bind to the active site directly. Thus, as shown in Scheme 2, these facts indicate that, under

Scheme 2



photocatalysis, monomer cysteine decomposes through the production of a dimer (cystine). However, cystine does not decompose to a monomer (cysteine).

These results are consistent with the previous reports on the interaction of L-cysteine and cystine with TiO_2.[13−23] Atman et al. studied the adsorption of L-cysteine on a rutile TiO_2(110) surface by X-ray photoelectron spectroscopy.[17−19] They suggested the dissociative interaction between the molecular thiol groups and the surface. It was attributed to a dissociative bond to the bridging oxygen vacancies. Most likely, the thiol groups are deprotonated and a bond is formed between the thiolates and defects. In addition, they suggested that L-cysteine binds dissociatively to the 5-fold-coordinated Ti atoms of the surface through their deprotonated carboxyl groups.[19] The simulation by first principles molecular dynamics on the adsorption of Cys on partially hydroxylated rutile (100) and (110) suggested that the NH_3^+-group of the zwitterion showed only weak interaction with the surface oxygen atoms.[20]

Next, we investigated the photocatalytic decomposition of glutathione in reduced (GSH) and oxidative (GSSG) forms. Figure 4 shows the ^{1}H NMR spectra of GSH (Figure 4a) and

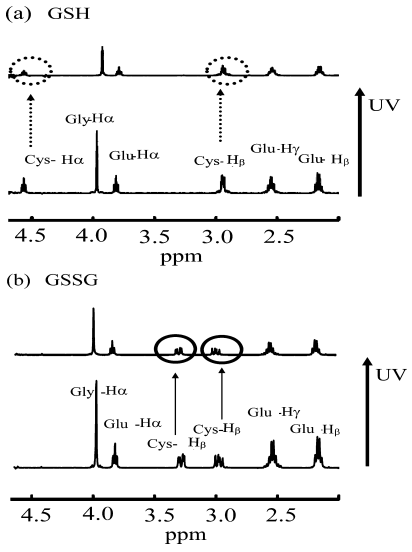


Figure 4. ^{1}H NMR spectra of 10 mM glutathione (a) GSH (reduced form) and (b) GSSG (oxidative form) in aqueous suspension of TiO_2 (5 mg/0.4 mL D_2O) before and after 100 min UV irradiation, measured at 297 K.

GSSG (Figure 4b) measured in the suspension of ST-01 and those measured after the UV irradiation of 100 min. As shown in Figure 4a and b, the peak intensities of both GSH and GSSG decreased after the UV irradiation because of the decomposition of the molecules but no notable novel peaks could be detected as decomposed products. This fact means that in the photocatalytic system neither the transition from GSH to GSSG nor from GSSG to GSH occurs different from the case of cysteine and cystine. Therefore, for GSH, the decomposition by the adsorption on the TiO_2 surface with the SH of the cysteine residue would not take place. Presumably, the adsorption sites of GSH would be the carboxyl group or amino group. In Figure 5, the time profiles of the peak intensity

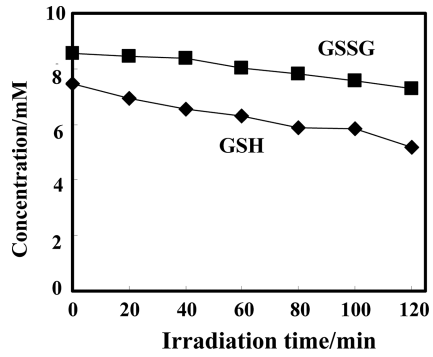


Figure 5. The time profiles of the concentrations of GSH ◆ and GSSG ■ in aqueous suspension of TiO_2 (5 mg/0.4 mL D_2O) under the UV irradiation at 297 K.

for GSH and GSSG against the UV irradiation time were shown. As is expected, the time profiles of both molecules are not different significantly. However, the adsorption and the decomposition are slightly larger for GSH than GSSG, probably because of the steric hindrance of GSSG whose molecular size is larger than that of GSH.

In Figure 6, the adsorption and decomposition rates of GSH and GSSG were compared with those of the constituent amino

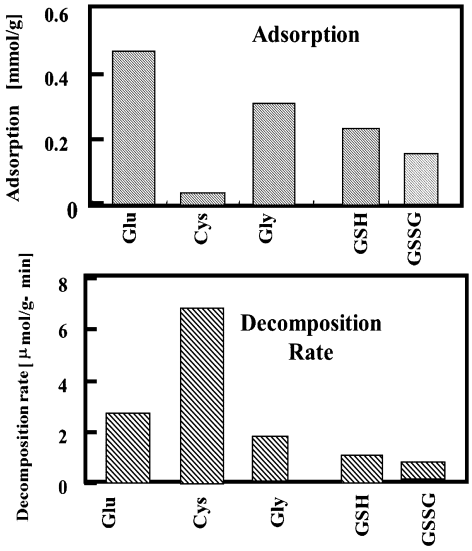


Figure 6. (a) Adsorption and (b) decomposition rates of glutathione (GSH and GSSG) and the constituent amino acids (Glu, Cys, and Gly) in aqueous suspension of TiO_2 (5 mg/0.4 mL D_2O) under UV irradiation at 297 K.

acids, that is, Glu, Cys, and Gly. The amount of the adsorption was estimated from the difference in the intensities of the sample measured before and after the addition of TiO_2 powder. It is noted that for Cys which can be adsorbed with SH the amount of the adsorption on the surface of TiO_2 is very low. Nevertheless, the decomposition rate is significantly high. This fact may indicate that the SH group is adsorbed preferentially

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on the active site of the TiO_2 to decompose efficiently. On the other hand, Glu and Gly are adsorbed on TiO_2 by the carboxyl group or amino group. In spite of the high amount of the adsorption, the decomposition rates are considerably low as compared to that of Cys. Therefore, these two amino acids would not be adsorbed on the active sites of the surface efficiently. The simulation by first principles molecular dynamics on the adsorption of Gly and Cys on partially hydroxylated rutile (100) and (110) suggested that the NH_3^+-group of the zwitterion showed only weak interaction with the surface oxygen atoms. More stable configurations are attained by ester condensation of the carboxyl group and a basic surface hydroxyl group and by the formation of a bond between a SH group and a surface Ti.[20] Stable adsorption configurations of Glu and Cys on anatase (101) and (001) and rutile (100) as well as (110) were reported by Car−Parrinello simulations of aqueous solutions. The negatively charged side chain carboxyl group of Glu had a great affinity to positively charged protonated surface bridging oxygen, with the O−H distance being around 1.4 Å. The SH group of Cys preferentially adsorbs in oxygen vacancies. A backbone COO^- oxygen forms a coordinative bond to a 5-fold coordinated surface Ti, without interference by adsorbed water, and independently of pH value and surface or from molecular adsorbed H_2O. Only small interaction of amino groups with the stoichiometric surface was indicated.[21]

As shown in Figure 6, the adsorption and decomposition rates for GSH and GSSG are lower than those of the constituent amino acids except Cys. This is consistent with the fact that these peptides are adsorbed on the TiO_2 surface with the terminal carboxyl group to be exerted by photocatalysts. The fact that the decomposition rates of GSH and GSSG are less than every composing amino acid may mean that glutathione should be resistant against the attack by the active oxygen species generated on the TiO_2 surface. Although a usual peptide bond for most of the peptides is formed between the amino group and the carboxyl group at the α position, as stated above, glutathione forms the peptide bond with the carboxyl group at the γ position of Glu and the amino group of the Cys residue. This characteristic configuration may be related to the stability of the molecule against the attack of active oxygen species.

The low decomposition rates for GSH and GSSG are considered to suggest their self-defensive properties. However, no concrete measure of the decomposition rates has been provided yet to ensure the self-defensive properties so far. Because the present photocatalytic reaction is considered to be so-called light-intensity limited, i.e., photoabsorption is the rate-determining step, under very weak UV light irradiation, the decomposition of GSH and GSSG can be negligibly small.

GSH is present in all animal cells in high concentrations (1−10 mM). It reduces H_2O_2 to become an oxidative dimer form of GSSG, which reverts to GSH enzymatically. This procedure is called a glutathione cycle. In living cells, the molecular ratio of GSH to GSSG is retained as about 10. When GSSG is excessively produced, it is excluded from the cell to maintain the ratio. Although details of human biological responses to TiO_2 exposure are still unavailable, numerous in vitro examinations concerning cellular responses induced by TiO_2 have been reported.[24−31] Park et al. showed the cytotoxicity of titanium dioxide nanoparticles with the induction of active oxygen species and the decreased level of intracellular GSH in cultured BEAS-3B cells. With the induction of active oxygen species, the expressions of oxidative stress-related genes including heme oxygenase-1 or inflammation-related genes including IL-8 were increased.[29] Active oxygen species can cause DNA damage and impair protein function. The mechanism of the genotoxicity of TiO_2 nanoparticles is believed to involve alterations in redox homeostasis, which includes both increased production of active oxygen species and depletion of antioxidant defenses. Numerous studies have demonstrated that TiO_2 can induce active oxygen species and promote oxidative DNA damage.[25] Antioxidant enzymes, such as SOD, catalase, and nitric oxide synthase, convert active oxygen species into less damaging compounds, while enzymes such as glutathione peroxidase, glutathione S-transferase, aldo-keto reductase, and aldehyde dehydrogenase detoxify the reactive intermediates. GSH plays a central role in these intracellular antioxidant defense processes as a free-radical scavenger.[25] In spite of the importance of GSH in defense against oxidative stress, its actual effects and the mechanism for the TiO_2-induced cytotoxicity and genotoxicity have not been completely elucidated yet.

The present results suggest that in living cells TiO_2 should deteriorate the function of the glutathione cycle by the adsorption even without UV illumination. The consequence would be the facilitated accumulation of active oxygen species, leading to the deactivation or damaging of the several molecules such as DNA. When TiO_2 coating is applied to environmental cleanup, the active oxygen species generated on the TiO_2 surface extinguish bacteria and virus. On the other hand, when TiO_2 is accumulated in living cells for some reason, the adsorption of GSH and GSSG on the surface of TiO_2 would result in the deterioration of the function to diminish active oxygen species.

The amount of TiO_2 (5 mg/0.4 mL) employed in the present study is much higher than those employed in the studies for living systems (several μg/mL) such as virus, bacteria, and mammalian cells. Although the feature of adsorption of glutathione on the surface of the TiO_2 is considered to be similar in the living cells, the interaction mechanism might be different in the actual living cells which contain a number of competing adsorbents.

To obtain more detailed insights into the genotoxicity and cytotoxicity caused by TiO_2 in living cells, more detailed molecular level investigations on the interaction of TiO_2 with the antioxidant molecules such as SOD are in progress in our laboratory.

CONCLUSION

With increased applications of TiO_2 nanoparticles, the concerns about their potential human toxicity and their environmental impact have also increased. In the presence of TiO_2 nanoparticles, the generations of active oxygen species and GSH depletion in living cells have been recognized to be closely related. The present results demonstrated that, in the photocatalytic system where active oxygen species are generated by UV illumination, both GSH and GSSG are adsorbed on the TiO_2 surface to be degraded, which must lead to the dysfunction of the glutathione cycle in living cells. This dysfunction would take place in actual cells where active oxygen species are persistently generated even without UV illumination, if TiO_2 nanoparticles are accumulated. The consequence would be the facilitated accumulation of active oxygen species, leading to the deactivation or damaging of cell constituents.

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However, the lower decomposition rates of GSH and GSSG compared to those for the composing amino acids obtained in the present study suggested their resistance against the attack by the active oxygen species in the photocatalytic systems.

AUTHOR INFORMATION

Corresponding Authors

*E-mail: nosaka@nagaokaut.ac.jp (Y.N.); aynosaka@mst.nagaokaut.jp (A.Y.N.). Fax: +81-258-47-9315.

Notes

The authors declare no competing financial interest.

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