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Journal of Photochemistry and Photobiology A: Chemistry 192 (2007) 105–113

Journal of Photochemistry and Photobiology A:Chemistry

www.elsevier.com/locate/jphotochem

Adsorption and decomposition of a dipeptide (Ala-Trp) in TiO_2 photocatalytic systems

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Received 11 December 2006; received in revised form 18 March 2007; accepted 10 May 2007

Abstract

The adsorption and photodecomposition of a dipeptide, alanyl-tryptophan (Ala-Trp) on a TiO_2 surface were investigated by zeta potential measurements and ^{1}H NMR spectroscopy in TiO_2 aqueous suspension systems. The photodecomposition rate of Ala-Trp was lower than that of Trp but higher than that of Ala (Ala < Ala-Trp < Trp), reflecting the adsorbability and the photocatalytic reactivity of the component amino acids. The most efficient photocatalysis of Ala-Trp takes place by the adsorption of the heterocyclic ring (3-indolyl group) on the basic terminal hydroxyl sites of TiO_2. The adsorptions of the molecule on the basic terminal OH sites through the carboxyl group or the peptide bond, or on the acidic bridged OH sites through NH_2 group were also suggested. However, the photocatalytic reactivities for such adsorptions are considered to be lower. A plausible adsorption scheme is presented with the decomposition efficiency.

Keywords: Dipeptide; Adsorption; TiO_2; Photocatalysis; Zeta potential; Isoelectric point

1. Introduction

TiO_2 has been widely applied to environmental cleanup, solar cells, antifogging, and self-cleaning of mirrors and glasses due to the intriguing properties such as high oxidizing power, chemical inertness, a photoinduced highly hydrophilic surface, and non-toxicity [1–7]. In addition, TiO_2 has received much attention as one of the most promising nanomaterials capable of a wide variety of applications in medicine and life science, and has been applied to biological fields such as antibacterial effects [8–10], and medical treatments for diseases including cancer [11,12]. Thus, the high reactivity of TiO_2 could provide a new strategy in the photodynamic therapies of some kind of cancers. Besides the photocatalysis, TiO_2 nanoparticles could be utilized for the recognition of biological molecules as one of the efficient biocompatible nanomaterials, now that various nanomaterials have been utilized to facilitate the specific binding and improve the sensitivity in detecting biomolecular recognition [13,14]. However, the mechanism underlying the adsorption and photocatalysis of biological molecules such as proteins/peptides and nucleic acids on TiO_2 surface has not yet been well understood.

Proteins and peptides are composed of various kinds of amino acids that include –COOH and –NH_2 functional groups with various side chains on the α-carbon. The adsorption and decomposition are therefore expected to depend on the molecular structures of the amino acids. The binding of carboxyl groups of the amino acids on the TiO_2 surface is generally acknowledged [15–18]. Hayden et al. suggested that a hydroxyl group adjacent to the ester bridge is eliminated and a bidentate configuration is obtained in which the carboxyl of the amino acid replaces two adjacent bridging oxygens [17]. XPS and IR studies indicated that for Trp and Ala the carboxylic group replaces a basic surface hydroxyl group on a Ti site and binds to this Ti [15,18].

On the other hand, the adsorption of glycine, methionine, serine, and cysteine on partially hydroxylated rutile (1 0 0) and (1 1 0) surfaces was simulated by first principles molecular dynamics [17]. More stable configurations are attained by ester condensation of the carboxyl and terminal OH groups and by the formation of a bond between a deprotonated hydroxyl or thiol group of serine or cysteine, respectively, and a surface Ti.

Our recent studies on the adsorption and decomposition of seven kinds of amino acids (Phe, Ala, Asp, Trp, Asn, His and Ser) in TiO_2 aqueous suspension systems [19,20], indicated that

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1010-6030/$ – see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.jphotochem.2007.05.011

the amino acids that contain –OH (Ser), –NH (Trp, His), or $–NH_2$ (Asn) in their side chain interact with the basic terminal OH sites more preferably by the side chain and vulnerable to photocatalytic oxidation. On the other hand, Ala appeared to interact more favorably with the acidic bridged OH sites on TiO_2, showing the lower decomposition rate.

Proteins and peptides are molecular sequences in which various amino acids combine through a peptide bond (–CONH–). Hence, different from amino acids in a single solution, the adsorption and photocatalysis of a specific constituent amino acid of an amino acid sequence must be affected by many factors, such as the other amino acids in the vicinity, the terminal $–NH_2$ and –COOH groups, the peptide bond, and the secondary and tertiary structure of the whole molecule. In the present study, to obtain more detailed features of adsorption and photocatalysis of proteins/peptides, the adsorption and photocatalysis of a dipeptide (Ala-Trp) in TiO_2 aqueous suspension systems were investigated by zeta potential measurements and ^1H NMR spectroscopy. By comparing the absorptive and the photocatalytic behaviors of Ala-Trp with those of Ala and Trp in single and mixed suspensions, the adsorption related to the photocatalytic reactivity of Ala-Trp will be discussed.

2. Experimental

The photocatalyst powders used were TiO_2 (ST-01) and calcined ST-01 at 973 K. ST-01 was a generous gift from Ishihara Techno and was used as received. The crystal form is 100% anatase with a BET surface area of $320\ m^2\ g^{-1}$ and a particle size of 9 nm [21]. The calcined powders were prepared by calcination in an electric furnace for 3 h at 973 K under air. After calcination, the BET surface area and particle size became $21\ m^2\ g^{-1}$ and 53 nm, respectively. The crystal phase was 100% anatase even after calcination at 973 K, which is consistent with our previous measurements [21,22]. The non-calcined TiO_2 and the calcined TiO_2 are designated as nc-TiO_2 and c-TiO_2, respectively.

The amino acids (Ala and Trp) and a dipeptide (Ala-Trp) examined in the present study were supplied by Wako Pure Chemical. Photocatalytic reactions were carried out in an NMR tube of 5 mm diameter. TiO_2 powder (4.9 mg) was dispersed in a 10 mM ($M = mol\ dm^{-3}$) solution of amino acids in 0.5 mL D_2O (99.9% Isotec, Inc.). The final pH was in the range of 6.9–7.9. The pH was not adjusted to avoid the effect of the counter ions on the photocatalytic reactions. ^1H NMR spectra of amino acids were measured with a JEOL EX-400 spectrometer at 400 MHz. The amount of amino acid adsorbed on the TiO_2 surface was estimated from the difference in the NMR signal intensities measured before and 12 h after the addition of TiO_2. The sample was photoirradiated with three 4-W black light bulbs surrounding the NMR sample tube under aerobic conditions. The sample tube was rotated during the irradiation to maintain the powder suspension. The incident light with the wavelength range of 320–380 nm was about $1\ mW\ cm^{-2}$ for each light bulb. To measure the concentration change of the reactant, the NMR spectra were recorded at intervals over the total irradiation time of 3 h. The concentration of the amino acid in the solution was estimated by taking the peak area relative to that of the external standard of DSS (2,2-dimethyl-2-silapentane-5-sulfonate sodium salt) in a glass capillary.

Zeta potential was measured with a zeta potential analyzer (Zeecom, ZC-1500), as described previously [19]. The water was purified with a Milli-Q water ion-exchange system (Millipore Co.) to give a resistivity of $1.8 \times 10^7\ \Omega\ cm^{-1}$.

3. Results and discussion

3.1. Adsorption and decomposition on non-calcined TiO_2

The changes in the concentrations of Ala and Trp in the mixture of Ala and Trp (designated M state hereafter) and dipeptide (Ala-Trp; designated D state hereafter) suspensions were plotted against irradiation time together with those of Ala and Trp in a single suspension (designated S state hereafter) in Fig. 1. The initial concentration of an amino acid and a peptide was 10 mM. The photoirradiation was started 12 h after the addition of TiO_2 to the solution. The decrease in amino acid concentration from 10 mM at irradiation time 0 min corresponds to the amount of adsorbed molecules as described above. The photocatalytic decomposition rates were calculated from the initial slope in Fig. 1 and are summarized in Fig. 2, together with the amounts of adsorption. Though NMR spectra consist of some proton signals of individual amino acids, almost all signals decayed simultaneously, indicating that the adsorption of the decomposed intermediates on the surface after the first decomposition is the rate determining process of the total decomposition. Among the reaction intermediates of Ala acetic acid is exceptionally stable as reported previously [20].

A significant difference in the amount of adsorption was not observed between Trp and Ala, although the amount of adsorption for Ala is slightly higher. For both Ala and Trp, the amount of adsorption in M and D states was almost the same as that in S states. For S state, the initial concentration of Ala and Trp was 10 mM, and for the mixed (M state) and the dipeptide (D state) suspensions, 10 mM of Ala and Trp coexist in the suspension containing 4.9 mg of TiO_2. Therefore, the results suggest that in the mixed (M state) systems, both amino acids would be adsorbed at each site on the solid surface. For D state, upon the adsorption of Ala-Trp on the TiO_2 surface, both constituent amino acids would access simultaneously to the solid surface, showing the same amount of adsorption.

The decomposition profiles of Trp for the three states appeared similar as shown in Fig. 1, but in D state, the decomposition rate was slightly lower as compared to those in S and M states as shown in Fig. 2. On the other hand, the decomposition profile of Ala in M state was distinctively different from those in S and D states although difference in the amount of adsorption among the three states was not significantly large. In S state, Ala was actually photodecomposed with the rate lower than that of Trp. However, in M state, Ala was barely decomposed. Instead, the concentration of Ala in the suspension increased slightly with the irradiation time (Fig. 1), indicating that a small amount of Ala was desorbed from TiO_2 surface along with the decomposition of Trp. This fact suggests that in M state, Trp occupies predominantly the photocatalytic active sites where Ala is decomposed

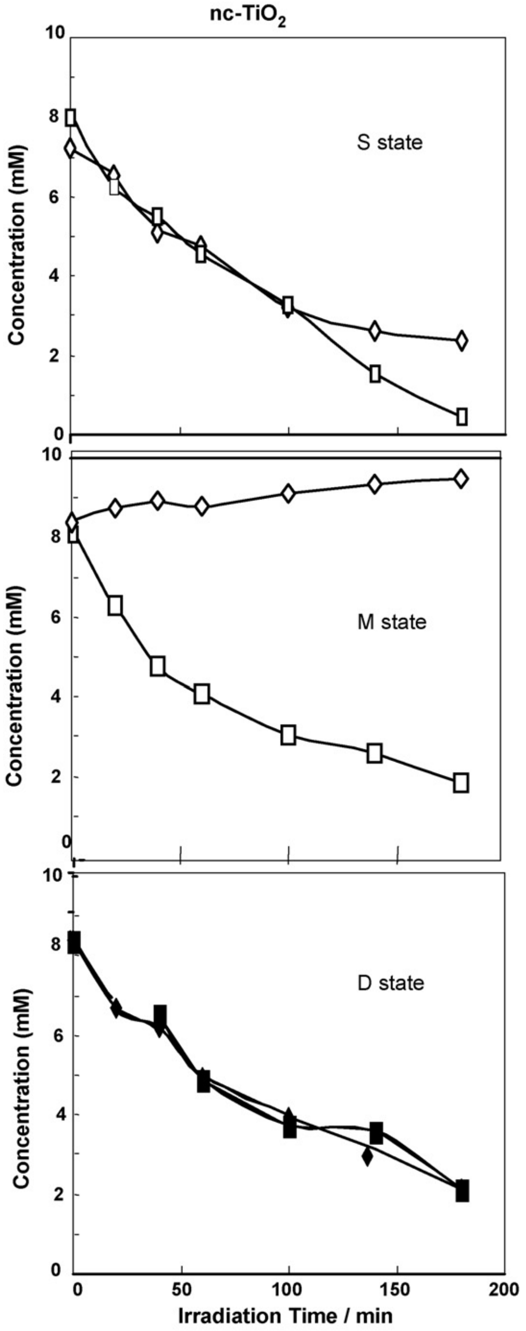

Fig. 1. Change in the concentration of amino acids in a TiO_2 photocatalyst suspension, showing the decrease from 10 mM by the adsorption, and the decay with irradiation time by photocatalytic decomposition for Ala (◊) and Trp (□) in three different states. S state: single solution of Ala and Trp, M state: mixed solution of Ala and Trp, D state: Ala-Trp dipeptide.

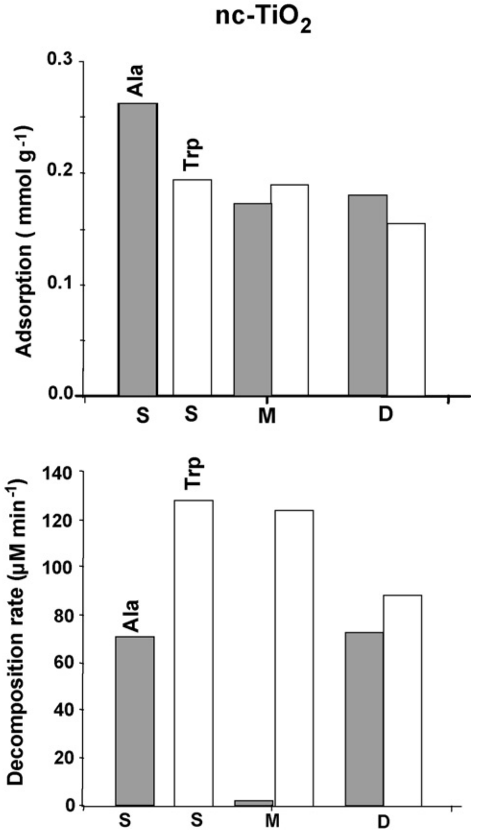

Fig. 2. The amount of adsorption on TiO_2 (nc-TiO_2) estimated from the decrease in solution concentration. (Upper graph) and the photocatalytic degradation rates on TiO_2 calculated from the initial decay of the reactant (lower graph) for Ala (▨) and Trp (□). S, M, and D represent the single suspension of Ala and Trp, the mixed suspension of Ala and Trp, and the suspension of dipeptide (Ala-Trp).

in S state and prevents Ala from photocatalysis, and that the intermediate products generated during the photocatalysis of Trp should possess stronger affinity to the adsorption sites for Ala to expel the Ala from the sites, resulting in the increase of the concentration of Ala in the suspension with irradiation time. Thus, the presence of Trp in M state obviously prevented Ala from photodecomposition. All the adsorption sites for Ala are not necessary the reactive sites. Present results suggest that Trp possesses stronger affinity to the reactive sites for Ala while the decomposition intermediate of Trp possesses the stronger affinity to the other adsorption sites for Ala. In contrast, in D state, as shown in Figs. 1 and 2, Ala showed almost the same decomposition profile and the same rate as those of Trp. In this case, Trp assists Ala to decompose, instead of prevention. For Ala-Trp (D state), Ala binds with Trp through a peptide bond. Therefore, when Trp is adsorbed on the surface, Ala can also access to the vicinity of the TiO_2 surface to be decomposed almost simultaneously with Trp.

3.2. *Zeta potential of TiO_2 on adsorption and the effect of calcinations*

To obtain more detailed features on the adsorption of the peptide, we performed zeta potential measurements for D and M states and compared with those for S state [19]. Recently we demonstrated that the zeta potential measurements in an aqueous suspension of TiO_2 for various amino acids provided useful information on the adsorption of amino acids on TiO_2 [19]. On the surface of TiO_2, there are two kinds of hydroxyl groups: bridged OH (designated B_{OH} hereafter) and terminal OH (designated T_{OH} hereafter). B_{OH} is acidic, while T_{OH} is basic [23–25]. The adsorption of amino acids on T_{OH} enhanced the surface acidity of TiO_2 because the contribution of the remain-

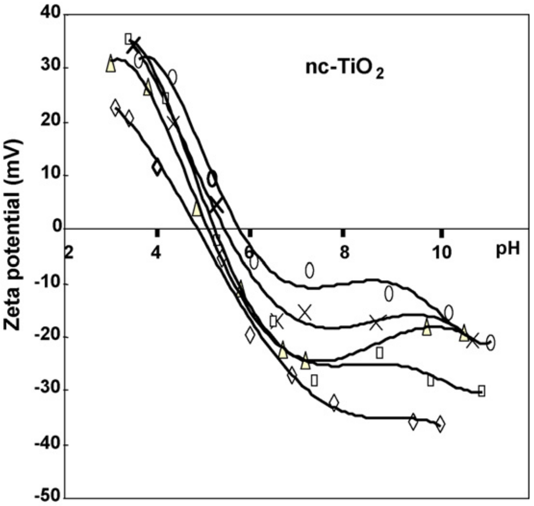

Fig. 3. Zeta potential of TiO$_2$ in an aqueous suspension in water (×) and in solutions containing amino acids and a dipeptide: (◯) Ala, (☐) Trp, (△) Ala and Trp, and (◊) Ala-Trp.

ing acidic bridged sites to the surface charge increased, which lead the shift of the IEP (isoelectric point; defined by the pH having zero potential) to a lower pH with respect to the control TiO$_2$ suspension. On the other hand, the interaction with the acidic B$_{OH}$ made the surface more basic, which lead to the shift of IEP to a higher pH. Thus, the lower shift of IEP observed on the adsorption of Trp was attributed to the more preferable interaction of Trp with T$_{OH}$, while the higher shift observed for Ala was indicative of the favored interaction with B$_{OH}$, which made the surface more basic. The decomposition rates of the seven amino acids (Phe, Ala, Asp, Trp, Asn, His and Ser) were well correlated with the IEP values and became higher with decreasing IEP [19]. Thus the stronger adsorption of the amino acids on the T$_{OH}$ caused a lower shift of IEP, resulting in higher photocatalytic decomposition.

As shown in Fig. 3, on adsorption of Ala (S state), the zeta potential shifted to the positive direction with respect to the control TiO$_2$ suspension over the entire pH range tested, while those for Trp (S state), a mixed solution of Ala and Trp (M state), and Ala-Trp (D state) shifted to the negative direction. The value of IEP for each state was listed in Table 1. The IEP value of the control TiO$_2$ suspension (without amino acids) was 5.5. For S state, as stated above, Ala is favored to interact with B$_{OH}$ to cause a higher shift of IEP, while Trp caused a lower shift by interacting preferably with T$_{OH}$. The negative shift in M and D states would mean that the adsorption of Trp in M state and Trp part of Ala-Trp (D state) on T$_{OH}$ is more dominated than that of Ala on B$_{OH}$. These results are consistent with those for decomposition rates. In M state, the photocatalysis took place only for Trp but not for Ala (Fig. 1) since Trp predominantly occupies T$_{OH}$ sites. In D state, the dominated interaction of the Trp part of Ala-Trp with T$_{OH}$ sites enables the access of the Ala part in the same molecule to the vicinity of the active sites, resulting in the decomposition of Ala as high as that of Trp (Fig. 2).

The lower shift of IEP observed for D (Ala-Trp) than for S (Trp) and M states may suggest that in addition to the Trp part, a peptide bond (–CONH–) of Ala-Trp should be also involved in the interaction of the dipeptide with T$_{OH}$. For D state, some amount of Ala-Trp may interact with B$_{OH}$ by the Ala part, but the amount would be too small to contribute to the higher shift of the IEP. However, these two factors can be responsible for the lower photodecomposition rate of Trp in D state as compared to those in S and M states (Fig. 2), which can reduce the adsorption of Trp part on the photocatalytic active site, namely T$_{OH}$.

TiO$_2$ is known to show amphiphilic properties. This means that there are both hydrophobic and hydrophilic sites on the solid surface. Hydrophilic sites may include the surface hydroxyl groups. Among various kinds of TiO$_2$, ST-01 shows a characteristic surface change on the thermal treatments [22]. After calcination at 973 K, the absorbed water was eliminated and the re-hydration was significantly slow. The water layer barely recovered even at more than 1 month after the thermal treatment at 973 K, indicating the increase in the hydrophobicity of the surface. ST-01 calcined at 973 K (c-TiO$_2$) could keep the hydrophobic surface for a fairly long time, even in aqueous solution [22]. Consequently, the photocatalytic oxidation of hydrophobic molecules, such as benzoic acid, which consists of a phenyl ring and a carboxyl group, was notably enhanced on

Table 1
IEP values and Adsorption sites of Ala, Trp, and Ala-Trp on non-calcined TiO$_2$ (nc-TiO$_2$)

Solution	Adsorption sites[a]		Decomposition rate	IEP[b]
	Terminal OH (T$_{OH}$)	Bridged OH (B$_{OH}$)		
S				
Ala	–COOH	–NH$_2$	Medium	5.80
Trp	–R$_T$, –COOH	–	High	5.25
M				
Ala		–NH$_2$	Non	5.10
Trp	–R$_T$, –COOH	–	High	
D (Ala-Trp)				
Ala part	–R$_A$	–NH$_2$	Medium	4.90
Peptide bond	–CONH–	–		
Trp part	–R$_T$, –COOH			

[a] R$_T$ and R$_A$ represent the heterocyclic side chain (3-indolyl) of Trp and CH$_3$ of Ala, respectively.

[b] IEP without amino acids is 5.50.

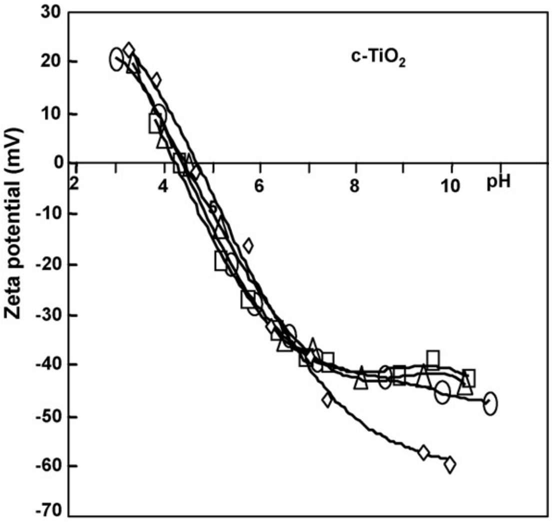

Fig. 4. Zeta potential of TiO_2 calcined at 973 K (c-TiO_2) in an aqueous suspension in water (×) and in solutions containing amino acids and a dipeptide: (○) Ala, (□) Trp, (△) Ala and Trp, and (◊) Ala-Trp.

c-TiO_2 in an aqueous suspension system as compared to that of acetic acid despite the substantial decrease in the surface area by heat treatments [22].

The calcinations at 973 K eliminated almost all T_{OH} on ST-01 with leaving a small amount of thermally more stable B_{OH} on the surface [26]. As shown in Fig. 4, the zeta potential of c-TiO_2 suspension in the absence of amino acid shifted to the negative direction with respect to that of non-calcined TiO_2 (nc-TiO_2), and the IEP was decreased from 5.5 to 4.2, indicating that the TiO_2 surface became more acidic due to the removal of basic T_{OH}. Hence, on c-TiO_2, only hydrophobic sites (designated as H_B, hereafter) and B_{OH} sites would be available for the adsorption of amino acids. The IEP values deduced from the zeta potential measurements for the three states (S, M and D) in c-TiO_2 suspension were listed in Table 2. No change in IEP was observed on addition of Trp (S state) to c-TiO_2 suspension, indicating that Trp does not interact with B_{OH} of TiO_2. On the other hand, for Ala (S state), Ala and Trp mixed solution (M state), and Ala-Trp (D state), it shifted slightly to a higher pH, indicating that Ala in S, M and D states interacts with B_{OH} to induce a higher shift of IEP, although the amount of B_{OH} sites must be reduced as compared to that on nc-TiO_2 after calcination.

3.3. Adsorption and decomposition on calcined TiO_2

To investigate the effect of the interactions of Ala-Trp with H_B and B_{OH} on the photocatalysis, the adsorption and the photodecomposition of the constituent amino acids for Ala-Trp (D state) in the c-TiO_2 suspension were measured by observing the changes of the concentration of the constituent amino acids in an aqueous suspension of c-TiO_2 against the photoirradiation time. The decomposition profiles were shown in Fig. 5, together with those for S and M states. The initial concentration of the peptide and the amino acids was 10 mM. The decrease in amino acid concentration from 10 mM at irradiation time 0 min corresponds to the amount of adsorbed molecules as described above. Although for c-TiO_2, the amount of adsorption and the decomposition rates were substantially decreased, Trp actually decomposed slowly in S (Trp), M and D states similarly (Fig. 5). This suggests that Trp should decompose on H_B, since Trp does not interact with B_{OH}, as deduced from the no change of IEP on addition of Trp to c-TiO_2. Since the active site is different from that in the case of nc-TiO_2, the prevention for Ala in M state by Trp was not observed in the case of c-TiO_2.

Contrary to Trp, the photocatalytic oxidation of hydrophobic molecules, such as benzoic acid, was notably enhanced on c-TiO_2 in aqueous suspension system because the direct adsorption of the aromatic ring of benzoic acid on the H_B is more promoted on the highly hydrophobic surface of c-TiO_2 to lead the more effective photodecomposition [22]. For nc-TiO_2, for which T_{OH} is available as adsorption sites, the typical hydrophobic amino acid Phe showed a very low decomposition rate which was even lower than that of Ala [19]. Therefore, it was considered that the photo-oxidization through the direct adsorption of an aromatic ring on H_B sites on nc-TiO_2 would barely take place. Instead, Phe would interact with T_{OH} by its carboxyl group or amino group to be photo-oxidized since Phe showed a relatively large lower shift of IEP, indicating a strong interaction with T_{OH}. Different from benzoic acid, aromatic amino acids possess an

Table 2
IEP values and adsorption sites of Ala, Trp, and Ala-Trp on TiO_2 calcined at 973 K (c-TiO_2)

Solution	Adsorption sites[a]		Decomposition rate	IEP[b]
	Hydrophobic site	Bridged OH		
S				
Ala	$-R_A$	$-NH_2$	Lower	4.45
Trp	$-R_T$	–	Low	4.25
M				
Ala	$-R_A$	$-NH_2$	Lowest	4.40
Trp	$-R_T$	–	Low	
D (Ala-Trp)				
Ala part	$-R_A$	$-NH_2$	Low	4.50
Trp part	$-R_T$	–		

[a] R_T and R_A represent the heterocyclic side chain (3-indolyl) of Trp and CH_3 of Ala, respectively.

[b] IEP without amino acids is 4.20.

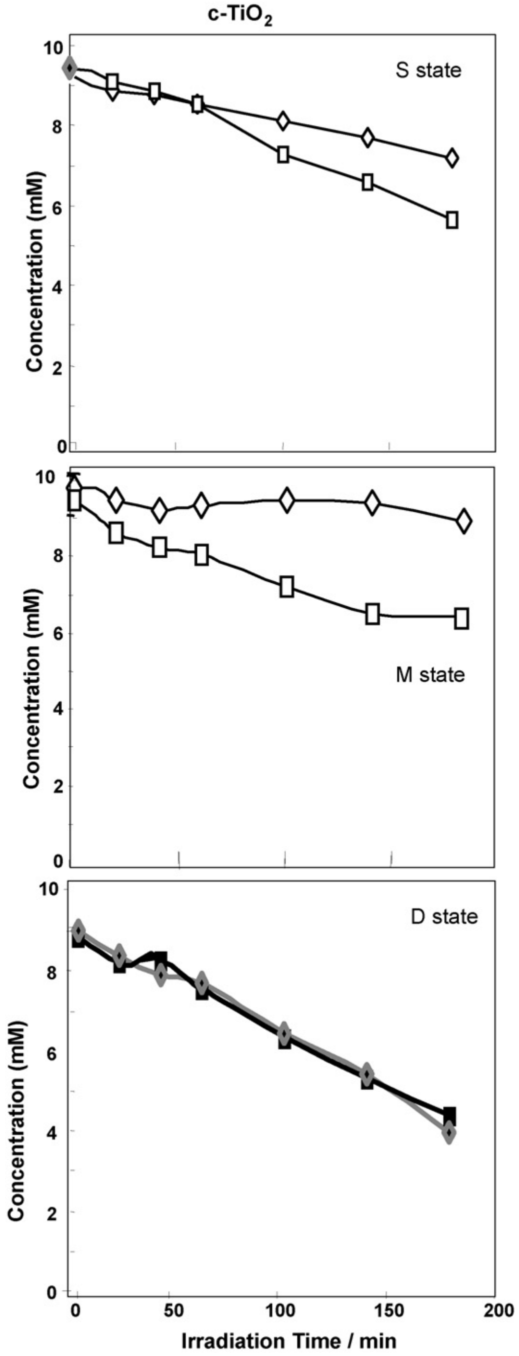

Fig. 5. Change in the amino acids concentration in TiO_2 calcined at 973 K (c-TiO_2) suspension, showing the decrease from 10 mM by the adsorption, and the decay with irradiation time by photocatalytic decomposition for Ala (◇) and Trp (□): S state: single solution of Ala and Trp, M state: mixed solution of Ala and Trp, D state: Ala-Trp.

amino group in addition to a carboxyl group and an aromatic side chain. In the pH range for the NMR measurements, it exists in a zwitter-ionic form of $–COO^-$ and $–NH_3^+$, which might be highly stabilized by the interaction with the surrounding water molecules to prevent the amino acids from directly accessing the hydrophobic sites of TiO_2. However, in the presence of the surface hydroxyl groups, the functional groups of the amino acids could directly interact with nc-TiO_2 surface to be photodecomposed, indicating that the photo-oxidization on nc-TiO_2 takes place mainly on surface hydroxyl groups but not through the direct adsorption of aromatic ring on the hydrophobic sites of nc-TiO_2 [19]. The present results indicated that for c-TiO_2, because of the lack of terminal OH and the increased hydrophobicity of c-TiO_2 surface, the heterocyclic ring of Trp became accessible to the hydrophobic sites to be photodecomposed. However, by taking into account of the much lower decomposition as compared to that of benzoic acid, the interaction of Trp with the hydrophobic sites of c-TiO_2 would be much weaker than that for benzoic acid.

The higher shift of IEP for M and D states indicates the interaction of Ala with B_{OH} sites. Trp does not interact with B_{OH}, while Ala would be able to adsorb both on H_B and B_{OH} sites on c-TiO_2. In M state Ala decomposed much less than that in S state. Since the heterocyclic ring (3-indolyl group) of Trp would possess stronger affinity to H_B than CH_3 of Ala, Trp would dominantly adsorb on H_B to be photodecomposed for M state, resulting in the reduction of decomposition of Ala as compared to that for S state. Therefore, this fact suggests that the photocatalysis of Ala takes place mainly on H_B but not on B_{OH} sites. For D state, the Ala part of Ala-Trp can access the vicinity of H_B when the Trp part of the same molecule interacts with H_B, and be attacked by the active species photogenerated on TiO_2 surface to be decomposed almost simultaneously with the Trp part as shown in Fig. 5 (D state). As stated above, on nc-TiO_2, the hydrophobic sites are not involved in the photocatalysis of amino acids, and for M state, Trp occupies predominantly the photocatalytic active site to prevent the photocatalysis of Ala. However, by taking into account that Trp does not interact with B_{OH}, Ala in M state must interact with B_{OH} on nc-TiO_2. However, Ala did not show photodecomposition. This fact means that B_{OH} is not involved in the effective photocatalysis of Ala. Ala in M state did not decompose on nc-TiO_2 (M states in Figs. 1 and 2) but appeared to decompose slightly on c-TiO_2 (M states in Figs. 5 and 6) although it is much less than those in S and D states. Therefore, even in M state despite the presence of Trp a small amount of Ala may be able to be adsorbed on the highly hydrophobic surface of c-TiO_2 to be photodecomposed.

3.4. Plausible adsorption and decomposition scheme

As shown in Figs. 2 and 6, with c-TiO_2, the significant reduction in the amount of the adsorption on the surface was observed for all the three states (S, M, and D) both for Ala and Trp as compared to those for nc-TiO_2. This may be attributed to the elimination of T_{OH} and/or the decrease of the surface area. It is noted that the amounts of the adsorption of Ala and Trp in M state are smaller than those in D state (Fig. 6) although for nc-TiO_2 they were almost the same as those in D state (Fig. 2). On c-TiO_2, in S state some amounts of Ala adsorb on B_{OH} and H_B. In M state, due to the stronger hydrophobic interaction of Trp with H_B on c-TiO_2 the amount of Ala adsorbed on H_B sites would be reduced. On the other hand, since in D state the same amount of Ala as that of Trp becomes accessible to H_B, when the Trp part of Ala-Trp adsorbed on H_B.

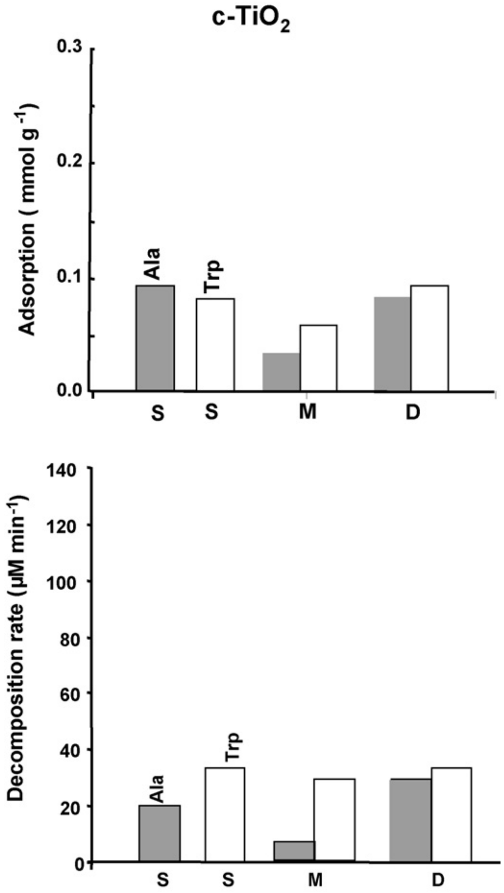

Fig. 6. The amount of adsorption on TiO_2 calcined at 973 K (c-TiO_2) estimated from the decrease in solution concentration (upper graph) and the photocatalytic degradation rates on TiO_2 (c-TiO_2) calculated from the initial decay of the reactant (lower graph) for Ala (■) and Trp (□). S, M, and D represent the single suspension of Ala and Trp, the mixed suspension of Ala and Trp, and the suspension of dipeptide (Ala-Trp).

In Tables 1 and 2, the decomposition efficiencies and IEP values obtained in the present study are summarized. The experimental results obtained for nc-TiO_2 and c-TiO_2 revealed several aspects for the adsorption and photodecomposition for dipeptide (1) Ala interacts with B_{OH} but Trp does not. (2) The photocatalysis mainly takes place on T_{OH} and B_{OH} is not involved in the efficient photocatalysis. (3) The photocatalysis on H_B is negligibly small for nc-TiO_2, while for c-TiO_2, owing to the enhanced hydrophobic surface by heat treatments, both Ala and Trp become accessible to the H_B sites to be photodecomposed.

For nc-TiO_2, Ala in S state was actually photodecomposed as shown in Fig. 1. However, the hydrophobic interaction is considered to be negligibly small and the B_{OH} site is supposed not to be involved in the efficient photocatalysis. Therefore, some amount of Ala must adsorb on the T_{OH} to be photodecomposed. The higher shift of IEP would mean that the amount of the adsorption on B_{OH} sites is more dominated than that on T_{OH}. This means that Ala interacts more preferably with B_{OH} but a small amount of Ala interacts with T_{OH} sites to be photodecomposed. Thus Ala can be adsorbed on T_{OH} sites either by its NH_2 or COOH group in addition to B_{OH} sites on nc-TiO_2. On the other hand, Trp cannot interact with B_{OH} but only with T_{OH} for nc-TiO_2 and induced a relatively large lower shift of IEP. In the previous study, among seven amino acids (Phe, Ala, Asp, Trp, Asn, His, Ser), only Ala showed a higher shift of IEP [19]. The geometry around B_{OH} on the TiO_2 surface might allow only small amino acids like Ala to access B_{OH} sites. This may be supported by the fact that IEP of Gly showed also a higher shift both for nc- and c-TiO_2 (data are not shown).

Thus, five potential interaction sites of Ala-Trp, i.e. the terminal amino and carboxyl groups, a peptide bond, and the two side chains would compete for the interaction with T_{OH} sites which are considered to be the most efficient photocatalytic sites on nc-TiO_2.

On the basis of the XPS and IR studies it was reported that for Trp and Ala the carboxylic group replaces a basic surface hydroxyl group on a Ti site and binds to this Ti [15,18]. Therefore, we can reasonably assume that for S state, some amounts of Ala interact with T_{OH} through the carboxyl group to be photodecomposed, and the others with B_{OH} through the NH_2 group. On the other hand, most of the Trp interact with T_{OH} through the N atom of the heterocyclic ring and the less amounts interact through the carboxyl group, to be photodecomposed. For Ala-Trp a peptide bond interacts also with T_{OH}.

Based on the discussion stated above, potential adsorption sites of Ala and Trp in state S, M and D on nc-TiO_2 and c-TiO_2 are summarized with the decomposition efficiency in Tables 1 and 2, respectively. And a plausible adsorption and photodecomposition scheme for each state is illustrated in Fig. 7. For nc-TiO_2, the affinity of the heterocyclic ring to the T_{OH} is considered to be the largest as described above. The interaction of the amino acids would become stronger in the order of B_{OH}–NH_3^+ (Ala) > T_{OH}–R_T > T_{OH}–(–CONH–) > T_{OH}–COO^-(Ala, Trp) (≫H_B–R_T > H_B–R_A) (R_T and R_A represent the heterocyclic side chain of Trp and CH_3 of Ala, respectively, and (–CONH–) represents a peptide bond). On the other hand, for c-TiO_2, from which T_{OH} is eliminated, only H_B and B_{OH} are available for the adsorption. The removal of T_{OH} and the enhancement of the hydrophobicity of the surface enabled the access of R_T and R_A to H_B, resulting in the slow photodecomposition. In M state Trp prevents the adsorption of Ala on H_B, to lead the decrease of the photodecomposition of Ala. However, in D state the Ala part becomes accessible to H_B along with the adsorption of the Trp part in the same molecule and shows almost the same photodecomposition as that for the Trp part. This means that the molecule in the vicinity of the surface can be photodecomposed as effective as the adsorbed molecules because it is quite unlike that after the photodecomposition of Trp part of Ala-Trp, the released Ala part adsorbs on the photocatalytic site of the surface to be photodecomposed by taking into account of the same decomposition profile of Ala and Trp part of Ala-Trp. Thus the photodecomposition efficiency on c-TiO_2 increases in the order of H_B–R_T > H_B–R_A.

Thus, Ala-Trp showed the lower photodecomposition rate than Trp but higher than Ala (Ala < Ala-Trp < Trp) on nc-TiO_2, reflecting the adsorbability and photocatalytic reactivity of the constituent amino acids. More detailed studies are progressing in our laboratory with various peptides and different kinds of

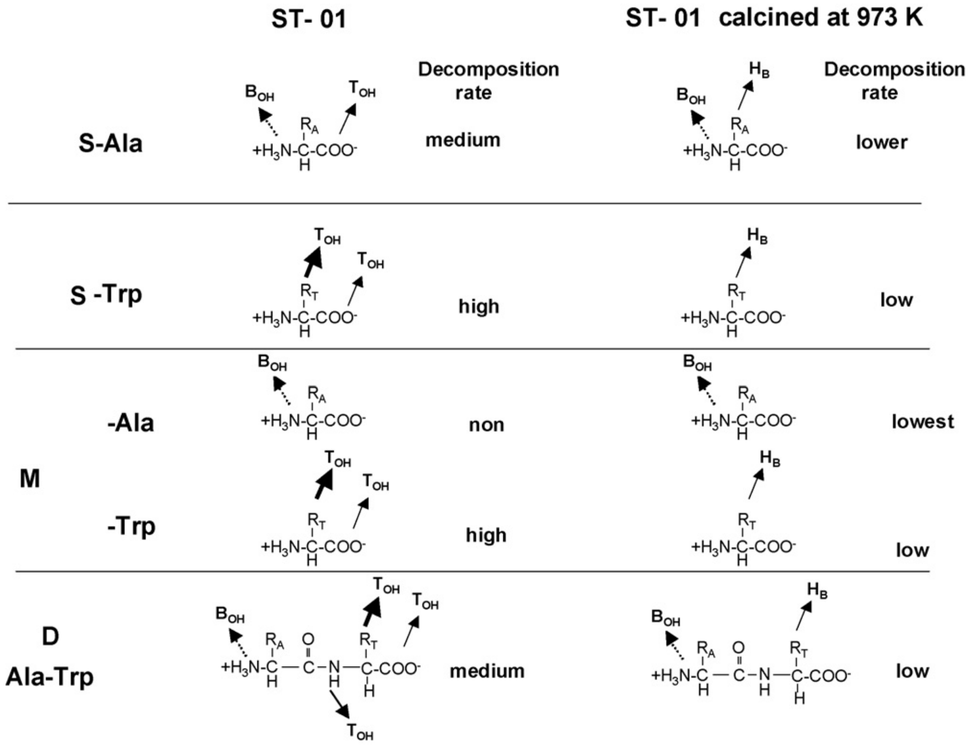

Fig. 7. Plausible scheme representing the interaction of Ala and Trp with non-calcined (nc) and calcined (c)-TiO_2 surfaces. T_{OH}, B_{OH} and H_B represent the terminal and bridged OH groups, and hydrophobic site on TiO_2, respectively. (–CONH–) is a peptide bond. R_T and R_A represent the heterocyclic side chain (3-indolyl group) of Trp and CH_3 of Ala, respectively.

TiO_2. The systematic analysis of the data must provide a clue to understand the photodecomposition of larger proteins and assist the design of the novel bio-interfaces.

4. Conclusions

The adsorption and photodecomposition of Ala-Trp on TiO_2 surface were investigated by zeta potential measurements and ^1H NMR spectroscopy in TiO_2 aqueous suspension systems. It was revealed that among the potential photocatalytic sites on TiO_2, the terminal hydroxyl group (T_{OH}) is the most efficient photocatalytic sites, while hydrophobic and bridged OH (B_{OH}) sites are not involved in the efficient photocatalysis.

Ala adsorbs on TiO_2 by its carboxyl group on T_{OH} or by its NH_2 group on B_{OH} sites, showing low decomposition rate. On the other hand, Trp adsorbs on TiO_2 through the heterocyclic ring or the carboxyl group on T_{OH} sites, showing high decomposition rate. Trp does not interact with the B_{OH}.

The most efficient photocatalysis of Ala-Trp takes place by the adsorption of heterocyclic 3-indolyl group on the basic T_{OH} sites of TiO_2. The adsorption of the molecule on the basic T_{OH} through the carboxyl group or the peptide bond, or on the acidic B_{OH} sites through NH_2 group was also suggested. However, the photocatalytic efficiencies for such adsorptions are considered to be lower. The photodecomposition rate of Ala-Trp is lower than that of Trp but higher than that of Ala (Ala < Ala-Trp < Trp). The binding of peptide bond on T_{OH} and NH_2 on B_{OH} reduced the amount of the adsorption of side chain on T_{OH}, leading to the decrease of the photodecomposition rate.

The present results indicate that the photodecomposition of the peptides obviously reflect the adsorbability and photocatalytic sensitivity of the constituent amino acids. In addition it was suggested that a peptide bond can reduce the photocatalytic efficiency for some cases.

Acknowledgments

This work was supported in part by a Grant-in-Aid on Priority Areas (417) and a 21st COE Program (for THT) from the Ministry of Education, Culture, Science and Technology (MEXT) and also by Core Research for Evolution Science and Technology (CREST), under the auspices of the Japan Science and Technology Agency (JST).

References

[1] O. Ollis, H. El-Akabi (Eds.), Photocatalytic Purification and Treatment of Water and Air, Elsevier, New York, 1993.
[2] A. Fujishima, K. Hashimoto, T. Watanabe, TiO_2 Photocatalysis: Fundamentals and Application, BKC Publishing, Tokyo, 1999.
[3] M. Kaneko, I. Ohkura (Eds.), Photocatalysis—Science and Technology, Kodansha Springer, Tokyo, 2002.
[4] M.R. Hoffmann, S.T. Martin, W. Choi, D.W. Bahnemann, Chem. Rev. 95 (1995) 69.
[5] A. Mills, S.L. Hunte, J. Photochem. Photobiol. A 108 (1997) 1.
[6] A. Fujishima, T.N. Rao, D.A. Tryk, J. Photochem. Photobiol. C: Photochem. Rev. 1 (2000) 1.
[7] M.W. Peterson, J.A. Turner, A.J. Nozik, J. Phys. Chem. 95 (1991) 221.
[8] K. Sunada, T. Watanabe, K. Hashimoto, J. Photochem. Photobiol. A 156 (2003) 227.

[9] P. Amezaga-Madrid, R. Silveyra-Morales, L. Cordoba-Fierro, G.V. Nevarez-Moorillon, M. Miki-Yoshida, E. Orrantia-Borunda, F.J. Solis, J. Photochem. Photobiol. B 70 (2003) 45.

[10] Z.-X. Lu, L. Zhou, Z.-L. Zhang, W.-L. Shi, Z.-X. Xie, H.-Y. Xie, D.-H. Pang, P. Shen, Langmuir 19 (2003) 8765.

[11] Y. Kubota, T. Shuin, C. Kawasaki, M. Hosaka, H. Kitamura, R. Cai, H. Sakai, K. Hashimoto, A. Fujishima, Br. J. Cancer 70 (1994) 1107.

[12] T. Saito, T. Iwase, J. Horie, T. Morioka, J. Photochem. Photobiol. B 14 (1992) 369.

[13] T. Hayashi, K. Sano, K. Shiba, Y. Kumashiro, K. Iwahori, I. Yamashita, M. Hara, Nano Lett. 6 (2006) 515.

[14] K. Sano, K. Shiba, J. Am. Chem. Soc. 125 (2003) 14234.

[15] G. Martra, S. Horikoshi, M. Anpo, S. Coluccia, H. Hidaka, Res. Chem. Intermed. 28 (2002) 359.

[16] W. Langel, L. Menken, Surf. Sci. 538 (2003) 1.

[17] B.E. Hayden, A. King, M.A. Newton, J. Phys. Chem. B 103 (1999) 203.

[18] M. Schmidt, S.G. Steinemann, Fresen. J. Anal. Chem. 341 (1991) 412.

[19] T.H. Tran, A.Y. Nosaka, Y. Nosaka, J. Phys. Chem. B 110 (2006) 25525.

[20] M. Matsushita, T.H. Tran, A.Y. Nosaka, Y. Nosaka, Catal. Today 120 (2007) 240.

[21] Y. Nosaka, M. Nakamura, T. Hirakawa, Phys. Chem. Chem. Phys. 4 (2002) 1088.

[22] A.Y. Nosaka, J. Nishino, T. Fujiwara, H. Yagi, H. Akutsu, Y. Nosaka, J. Phys. Chem. B 110 (2006) 8380.

[23] G.D. Parfitt, Prog. Surf. Memb. Sci. 11 (1976) 181.

[24] H.P. Boehm, Discuss. Faraday Soc. 52 (1971) 264.

[25] A.Y. Nosaka, Y. Nosaka, Bull. Chem. Soc. Jpn. 178 (2005) 1595.

[26] K.I. Hadjiivanov, D.G. Klissurski, Chem. Soc. Rev. 25 (1996) 61, references therein.