Kinetic study on the protein decomposition by photocatalytic Ti(IV)-doped calcium hydroxyapatite particles

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Kinetic study was done for the decomposition process of lysozyme (LSZ) on the surface of Ti(IV)-doped calcium hydroxyapatite (TiHap) particles. The decomposition of LSZ was examined by changing the weight of TiHap particles dispersed and reaction temperature. The decomposition of LSZ was analyzed by using the first order reaction. The obtained data fairly fit to the first order reaction equation, and the rate constant (k) was obtained. Since a good linear relationship was obtained between k values and particle weigh of TiHap, it was concluded that the decomposition of LSZ molecules occurs on the surface of TiHap. The activation energy of the reaction of LSZ decomposition on the surface of TiHap particles was determined as 30.2 kJ mol⁻¹ by varying the decomposition temperature from 5 to 45°C. The comparison experiments between 0 h UV and 24 h UV methods revealed that the decomposition of LSZ needs appropriate rates of adsorption and decomposition at the TiHap particle surface. It was ascertained that the decomposition temperature at 25–45°C is appropriate to proceed continuous decomposition of LSZ for a long time. The LC-MS measurements revealed that LSZ molecules were decomposed to the small molecules with molecular weight of 1/35–1/50 to LSZ one and they converged to the compounds with low molecular weight of 288–316 after a long time UV irradiation.

Key-words : Ti(IV)-doped calcium hydroxyapatite, Kinetic study, Photocatalytic activity, Decomposition of protein, Lysozyme, LC-MS measurement

1. Introduction

It is well known that a synthetic calcium hydroxyapatite Ca₁₀(PO₄)₆(OH)₂, designated as CaHap, attracts attention as bioceramics, acid-base catalysis in various reactions and adsorbents for separation of biomaterials. One of the authors (M. Wakamura) has developed that the Ti(IV)-doped CaHap (expressed as TiHap), with Ti/(Ca+Ti) atomic ratio (abbreviated as X₅₀) of 0.2 and calcined at 650°C for 1 h, possesses a high photocatalytic activity for decomposition of acetaldehyde and albumin under ultra violet (UV) irradiation. Recently, it was further reported that marine TiHap particles, produced from bones of Atlantic cold fish after treated in appropriate solutions (either Ca₃- or Ti-containing salts) and successively annealed, exhibited photocatalytic activity. The authors investigated the mechanism of photocatalytic activity of TiHap particles by in-situ FTIR measurement applying a self-supporting method and found that strong photocatalytic activity of TiHap particles is attributed to the dissociated OH⁻ ions produced by the heat treatment at 650°C for 1 h in the particles, they can produce OH radicals in the particles without water molecules by the UV irradiation. The authors also disclosed that lysozyme (LSZ) with a rigid molecular structure were readily decomposed by UV irradiation in the presence of TiHap particles, though the decomposition of bovine serum albumin (BSA) molecules with large molecular weight (67200 Da) is hard to take place. The authors also found that a selective photocatalytic decomposition is observed for the LSZ from the BSA (2.5 mg/cm³)-LSZ (1.0 mg/cm³) mixture solution, i.e., only LSZ molecules were decomposed completely from the BSA (2.5 mg/cm³)-LSZ (1.0 mg/cm³) mixture. This selective decomposition of LSZ by TiHap particles was interpreted by higher adsorption affinity of positively charged LSZ to highly negatively charged TiHap together with low molecular weight and rigid structure of LSZ molecules. All the above mentioned studies were done by starting UV irradiation after expended the first 24 h for attaining the protein adsorption equilibrium (called as 24 h UV method). To accelerate protein decomposition, the authors recently examined UV irradiation started at 0 h (called as 0 h UV method), in other words the UV irradiation and LSZ adsorption processes were started at the same time. We reported that the 0 h UV method is efficient for acceleration the LSZ decomposition. However, we still have no fundamental information about decomposition process of LSZ on the surface of TiHap particles. The aim of this study is clarify the decomposition mechanism of LSZ from view point of kinetic procedure. We also applied liquid chromatograph-mass (LC-MS) measurements to elucidate the composition of materials decomposed from LSZ. The results obtained in the present study must serve for giving fundamental data to apply TiHap particles for photocatalysts in aqueous media.

2. Experimental

2.1 Materials and Methods

Colloidal TiHap particles doped with Ti(IV) were the same samples as we utilized in our previous papers. These particles were prepared by the coprecipitation method as described briefly below. Ca(NO₃)₂ and Ti(SO₄)₂ were dissolved in 1 dm³ pure water free from CO₂ at an atomic ratio of Ti/(Ca+Ti) abbreviated as X₅₀) was 0.2. The total amount of Ca and Ti in the

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solutions was hold at 0.1 mol; 0.060 mol of H$_2$PO$_4$ was added to the solution and the solution pH was adjusted to 9.0 by adding a 15 M NaOH solution. The resulting suspension was aged in a capped Teflon vessel of 100°C for 6 h. The particles generated were filtered off, thoroughly washed with 5 dm$^3$ deionized pure water, and finally dried at 70°C in an air oven for 24 h. To endow a high photocatalytic activity, TiHap particles with $X_{Ti} = 0.2$ were further heat-treated with a temperature increment of 5°C/min until reaching 650°C and left during 1 h. Since these heat treated particles were stable and maintained high photocatalytic activity, these particles were also preserved in a desiccator. Wakamura et al. revealed by using power neutron scattering measurements that Ca ions in TiHap are exchanged with both columnar and screw-axis sites of Ca ions in CaHap crystal and there is no TiO$_2$ crystal forms in the TiHap particles. The colloidal TiHap particles with $X_{Ti} = 0.2$ thus produced had irregular shape with 30–50 nm in diameter. The specific surface area measured by the $N_2$ adsorption measurement and (Ca+Ti)/P values assayed were 40 m$^2$/g and 1.62, respectively. Since the (Ca+Ti)/P value of TiHap is less than the stoichiometric one of TiHap (1.67), these particles are slightly cation deficient. Our previous paper gave a detailed experimental procedures about characterization of the TiHap particles. TiHap particles exhibit characteristic peaks of CaHap (ICDD 9-432), indicating that Ti(IV) ions are completely incorporated into the crystal lattice of TiHap. It was also reported in our previous paper that TiHap with $X_{Ti} = 0.2$ showed the UV absorption below ca. 370 nm. The band gap energies of TiHap employed in this study estimated from the absorption edges of these UV spectra were 3.13 eV, coincident with the literature value of 3.0–3.2 eV of octahedrally coordinated Ti(IV) such as anatase and rutile TiO$_2$.

Pure waters from Elix (Millipore) were used for the preparation of all solutions. Fresh purified water had conductivities around 0.06 µS/cm. Lysozyme (molecular weight: 14600 Da, isoelectric point: 11.1) were purchased from Sigma Co. (LSZ: L-6876).

### 2.2 Protein decomposition test

The photocatalytic activities were estimated from the decomposition of LSZ solution under 1 mW/cm$^2$ UV irradiation as follows. The TiHap particles with $X_{Ti} = 0.2$ were ultrasonically dispersed in a 10 mL quartz tube with each 8 mL $10^{-4}$ M KCl solution dissolving 1.0 mg/cm$^3$ LSZ. The weight of TiHap in a quartz tube was varied 50, 100 and 150 mg. Unless otherwise specified, all experiments were carried out with 100 mg TiHap particles. The quartz tubes in each system were capped by silicon stops and rotated end-over-end in a thermostat for 24 h. The decomposition experiments of LSZ were taken place at 5, 15, 20, 25, 35 and 45°C with 100 mg TiHap particles. The UV-irradiation with 365 nm in wavelength was started immediately after dispersed the TiHap particles in protein solution in a quartz tube (0 h UV method). To compare the adsorption process on the rate constant of LSZ decomposition, comparison experiments were also done by the 24 h UV method at 5 and 15°C. The concentrations of LSZ in the supernatant after centrifugation during the rotation were determined by a HPLC method (Hitachi L-6200 Intelligent Pump, L-4250 UV–VIS Detector, L-5020 Column Oven and D-2500 Chromato-Integrator) using a YMC-Pack C$_8$-AP column with UV monitoring at 220 nm. LSZ was separated efficiently on the column in acetonitrile (AN)/water/trifluoroacetic acid (TFA) eluents by increase in the AN content in the eluents with a linear gradient from 70/30/0.1 to 10/90/0.1 in AN/water/TFA composition. The LSZ concentrations were related to a calibration curve using the peak area as a measure. Preliminary experiments revealed a high reproducibility of the method using quartz tubes, and no LSZ adsorption onto the quartz tube could be detected. It was also reported in our previous paper that no Ti(IV) ion is dissolved in a 10$^{-4}$ M KCl solution from TiHap particles regardless of with and without UV irradiation [10], the effect of Ti(IV) ions in solution on the LSZ decomposition can be neglected.

The concentrations of decomposed compounds of LSZ after UV irradiation for 60, 72 h and 10 days at 25°C with 100 mg TiHap particles were determined by a liquid chromatography–mass spectrometer (LC-MS, Agilent Technologies Co., USA, 1260 infinity-Brucker microTOF-QIII) equipment with a Agilent Tech–Poroshell 120 EC-C18 column (3 × 50 mm, particle size 2.7 µm). To avoid an effect of electrolyte’s impurity, these measurements were carried out without KCl. The decomposed compounds of LSZ were separated efficiently on the column in acetonitrile (AN)/water eluents. The decomposed compounds were eluted by increase in the AN content in the eluents with a linear gradient from 5/95 to 95/5 in AN/water composition at a flow rate of 0.20 mL/min. The analysis time was 55 min. The LC-MS was equipped with ionization by electronic spray ions (ESI) and it was operated in the secondary MS ion scanning mode. The maximum MS data accumulation time was 300 ms. The capillary voltage was 4500 V. The nebulizer pressure was 1600 hPa. The flow rate of the drying gas (N$_2$, 200°C) was 8.0 L/min. The collision voltage was 8.0 eV.

### 3. Results and discussion

#### 3.1 Effects of TiHap weight on the rate of LSZ decomposition

The changes of concentration of LSZ ([LSZ]) as a function of the UV irradiation time at 25°C are shown in Fig. 1(a). It is known that the first order reaction can be expressed as Eq. (1),

$$ln \frac{C_0}{C_t} = kt$$

where $C_0$ and $C_t$ are [LSZ] at the reaction time of 0 and $t$ (h), respectively, and $k$ is the rate constant. As following the Eq. (1), $ln \frac{C_0}{C_t}$ values can be plotted as a function of time in Fig. 1(b). It can be recognized that $ln \frac{C_0}{C_t}$ vs. $t$ represents a good linear relationship through the origin. Hence we could obtain $k$ values for three different particle weight systems from the slope of each line. The fitting lines in Fig. 1(a) were drawn by using the obtained $k$ values in Fig. 1(b). The fitting lines highly coincide to the obtained results. Here it should be noticed that $k$ value of the system with 50 mg TiHap was obtained at early stage of the reaction (0–6h). Because the fitting curves in Fig. 1(a) for these systems by using $k$ values obtained from whole reaction time did not fit well. This result can be explained by the less surface area of TiHap particles to proceed continuous decomposition of LSZ for a long time. The relationship of $k$ and particle weight was illustrated in Fig. 1(c). A good linear relationship can be seen between $k$ values and particle weight of TiHap. Since the particle weight is directly proportional to the surface area of TiHap particles dispersed, this result strongly suggests that the decomposition of LSZ molecules takes place on the surface of TiHap.

#### 3.2 Effect of temperature on the LSZ decomposition

The effect of temperature on the decomposition of LSZ was examined at 5, 15, 20, 25, 35 and 45°C with 100 mg TiHap
particles. The [LSZ] profiles as a function of the UV irradiation time for the 0 h UV method were shown in Fig. 2. All the [LSZ] profiles were decreased with increase in the UV irradiation time. To make clear the difference among six temperatures, we also analyzed these profiles by fitting to the Eq. (1) as well as Fig. 1(b). The ln $C_0/C_t$ vs. $t$ plots for six temperatures were shown in Fig. 3 and the rate constant $k$ values were obtained by the slope of each line. Here the $k$ values for the systems carried out at low temperature of 5–20°C was obtained at early stage of the reaction (0–12 h) as the same reason as for the system on 50 mg in Fig. 1. The lines as drawn in Fig. 2 are fitted lines using the obtained $k$ values of each temperature in Fig. 3. It is easy to recognize that the lines (shown by dotted line) obtained for low temperatures of 5–20°C in Fig. 3 do not fit well and they do not pass through the origin even though the $k$ values for these systems were obtained at early stage of the reaction. Indeed the fitting curves at these low temperatures are not compatible with the plots as were shown by dotted lines in Fig. 2 (upper figures). On the other hand, the lines obtained for high temperature of 25–45°C fit well and their $ln C_0/C_t$ vs. $t$ plots pass through the origin in Fig. 3 (shown by solid line). The fitting lines, as shown by solid lines in Fig. 2 (bottom figures), for the systems carried out at high temperature are exactly consisted with the plots. To discuss the effects of UV irradiation temperature on the rate constant $k$, $ln k$ values were plotted as a function of $1/T$ in Fig. 4 by using the Arrhenius Eq. (2),

![Fig. 1.](image1)

![Fig. 2.](image2)

![Fig. 3.](image3)

![Fig. 4.](image4)
the adsorption of LSZ onto TiHap is endothermic process,\(^16\) i.e., particles. As the authors disclosed in our previous literature that explained by their low adsorption ability of LSZ onto the TiHap after analyzed for whole reaction time. This result can be activation energy \(E_a\) of the reaction of LSZ decomposition was 21 kJ/mol.

\[ \ln k = \ln A - \frac{E_a}{T}, \]  

(2)

where \(A\), \(E_a\) and \(T\) are frequency factor, activation energy and absolute temperature, respectively. The obtained \(\ln k\) values are plotted in Fig. 4 as a function of \(1/T\) by open circles. The \(\ln k\) vs. \(1/T\) plot obtained is found to be a linear (solid line). The activation energy \(E_a\) of the reaction of LSZ decomposition was estimated from the slope of the Arrhenius plot in Fig. 4 (solid line). The \(E_a\) value thus obtained was 30.2 kJ/mol. It has been reported that the \(E_a\) values for decomposition of \(\text{H}_2\text{O}_2\) are 46 and 21 kJ/mol in the presence of Pt colloidal particles and catalase as catalysts, respectively.\(^10\) Also, the \(E_a\) value for the decomposition of sucrose in the presence of hydrogen ions and saccharase has been reported as 111 and 48 kJ/mol, respectively.\(^17\) Hence, it can be considered that the obtained 30.2 kJ/mol in the present study is reasonable.

Now we should consider the decomposition of LSZ at low temperatures (5–20°C). These systems gave less curve fitting after analyzed for whole reaction time. This result can be explained by their low adsorption ability of LSZ onto the TiHap particles. As the authors disclosed in our previous literature that the adsorption of LSZ onto TiHap is endothermic process,\(^16\) i.e., the adsorption of LSZ is driven by entropy change of LSZ with changing their conformation. Hence, the adsorption of LSZ is inhibited at low temperature condition. To avoid this problem, we utilized the 24 h UV method at the systems employed at 5 and 15°C. In this 24 h UV method, after LSZ molecules are completely adsorbed on the surface of TiHap particles, the decomposition of adsorbed LSZ molecules was started. The result gained for the 24 h UV method was shown in Figs. 5(a) and 5(b). In these figures, the [LSZ] was plotted as a function of UV irradiation time. The [LSZ] was decreased ca. 0.5 mg/cm\(^2\) within 24 h in both systems, indicating that the adsorption of LSZ reached equilibrium. The decomposition of LSZ was started at 24 h. The [LSZ] profile obtained at 5 and 15°C after 24 h was shown in Figs. 5(a)′ and 5(b)′, respectively. These figures, [LSZ] at 24 h was regarded as unity for each system. The insets of Figs. 5(a)′ and 5(b)′ represent \(\ln \frac{C_0}{C}\) vs. \(t\) plot of each reaction after started the UV irradiation. Hence 24 h was expressed as 0 h in these insets. The curves drawn in Figs. 5(a)′ and 5(b)′ are fitting curves using the \(k\) values obtained from the insets. Both curves much more fit well to the plots rather than those observed at 5 and 15°C in Fig. 2 (upper figures). The obtained \(\ln k\) values are plotted in Fig. 4 as filled circles. Clearly, the \(\ln k\) values obtained were decreased, especially the \(\ln k\) value obtained at 5°C exhibit extremely low value than the solid line. The \(E_a\) value of the reaction of LSZ decomposition for the 24 h UV system from the slope of dotted line in Fig. 4 was estimated to be 61.6 kJ/mol. This value is about twice as was obtained from 0 h UV method (30.2 kJ/mol), indicating that the adsorbed LSZ molecules depressed the UV absorption efficiency of TiHap particles. This results strongly indicate that the decomposition of LSZ needs appropriate rates of adsorption and decomposition at the TiHap particle surface. In other words, the temperature above 25°C proceeds both steps and gave a good curve fitting in Fig. 2.

### 3.3 Decomposition products of LSZ molecules by UV irradiation

The authors already reported that LSZ molecules can be decomposed by UV irradiation as well as the present study. However, the decomposed products of LSZ molecules should prove to be of value in applying TiHap as a catalyst in aqueous media. To clear this point, we employed the LC-MS measurements for the solutions after UV irradiation for 60, 72 h and 10 days. The LC charts were illustrated in Fig. 6. It should be emphasized that four main peaks exhibited for both the solutions UV irradiated for 60 h (peaks 1, 2 and 3) and 72 h (peaks 1, 2, 3 and 5). In the case for the solution on UV irradiated for 10 days appears Peaks 1, 2, 3′ together with Peaks 4 and 5. The \(m/z\) values of each peak assayed by MS were shown in the table in Fig. 6. It is noteworthy that molecular weight is not much change even though the peaks are slightly shifted. The decomposed materials possess about \(m/z\) values of 288–425. This result indicate that LSZ molecules (MW: 14600 Da) were decomposed by UV irradiation as well as the present study. The [LSZ] at 24 h was regarded as unity for each system. The insets of Figs. 5(a) and 5(b) are plotting in Fig. 4 as a function of UV irradiation time carried out at various temperatures by the 0 h UV method as shown in Fig. 3. Full circles represent the results obtained by the 24 h UV method.

![Fig. 3. Plots of \(\ln \frac{C_0}{C}\) as a function of UV irradiation time carried out under various temperatures as shown in Fig. 2. Temperatures: \(\bullet\) 5, \(\square\) 15, \(\triangle\) 20, \(\odot\) 25, \(\triangle\) 35 and \(\square\) 45°C.](image1)

![Fig. 4. Arrhenius plots for the decomposition reaction of LSZ carried out at various temperatures by the 0 h UV method as shown in Fig. 3. Full circles represent the results obtained by the 24 h UV method.](image2)
4. Conclusion

From the information presented in this publication, we can draw the following conclusions. The decomposition of LSZ fairly fits to the first order reaction equation, and the rate constant \((k)\) was obtained. Since a good linear relationship was obtained between \(k\) values and particle weigh of TiHap, it was proved that the decomposition of LSZ molecules occurs on the surface of TiHap particles. The activation energy of the reaction of LSZ decomposition on the surface of TiHap particles was determined as 30.2 kJ/mol. The comparison experiments between 0 h UV and 24 h UV method revealed that the decomposition of LSZ needs appropriate rates of adsorption and decomposition at the TiHap particle surface. It was ascertained that the decomposition temperature at 25–45°C is appropriate to proceed continuous decomposition of LSZ for a long time. The LC-MS measurement revealed that LSZ molecules were decomposed to the small molecules with molecular weight of 1/35–1/50 to LSZ one and they

Fig. 5. Changes of the concentration of LSZ as a function of UV irradiation time under (a), (a') 5°C and (b), (b') 15°C with 100 mg TiHap particles. The experiments were done under the 24 h UV method with LSZ 1.0 mg/cm³. The lines in the bottom figure are the fitting curves drawn by using rate constants obtained from insets in each figure. The insets in (a) and (b') represent \(\ln C_0/C_t\) vs. \(t\) plot at each reaction temperature.

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<tr>
<th>Peak</th>
<th>Meas. mg²</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>3'</th>
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<th>5</th>
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<td></td>
<td></td>
<td>288.287</td>
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<td>301.136</td>
<td>301.139</td>
<td>425.285</td>
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Fig. 6. LC charts of the decomposed LSZ solutions after UV irradiation for 60, 72 h and 10 days at 25°C with 100 mg TiHap particles. Data of MS for each peak are shown in the table.
converge to the substances with low molecular weight of 288–316 after a long time UV irradiation.

References