# Application of Chitosan Solutions Gelled by *melB* Tyrosinase to Water-Resistant Adhesives

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**ABSTRACT:** An investigation was undertaken on the application of dilute chitosan solutions gelled by *melB* tyrosinase-catalyzed reaction with 3,4-dihydroxyphenethylamine (dopamine). The tyrosinase-catalyzed reaction with dopamine conferred water-resistant adhesive properties to the semi-dilute chitosan solutions. The viscosity of the chitosan solutions highly increased by the tyrosinase-catalyzed quinone conversion and the subsequent nonenzymatic reactions of *o*-quinones with amino groups of the chitosan chains. The viscosity of chitosan solutions highly increased in shorter reaction times by addition of *melB* tyrosinase. Therefore, in this study, the gelation of a chitosan solution was carried out without poly(ethylene glycol) (PEG), which was added for the gelation of chitosan solutions using mushroom tyrosinase. The highly viscous, gel-like modified chitosan materials were allowed to spread onto the surfaces of the glass slides, which were tightly lapped together and were held under water. Tensile shear adhesive strength of over 400 kPa was observed for the modified chitosan samples. An increase in either amino group concentration of the chitosan solutions or molecular mass of the chitosan samples used effectively led to an increase in adhesive strength of the glass slides. Adhesive strength obtained by chitosan materials gelled enzymatically was higher than that obtained by a chitosan gel prepared with glutaraldehyde as a chemical crosslinking agent. In addition, the use of *melB* tyrosinase led to a sharp increase in adhesive strength in shorter reaction times without other additives such as PEG.  J Appl Polym Sci 107: 2723–2731, 2008

**Key words:** *melB* tyrosinase; chitosan; dopamine; adhesive strength; water-resistant adhesive

## INTRODUCTION

There is a considerable interest in mimicking nature to generate high-performing, environment-friendly materials. One of the most studied examples is a water-resistant adhesive protein used by mussels to adhere to wet or submerged surfaces. Mussels are well known to use a polyphenolic adhesive protein that is rich in lysine, hydroxyproline, and dihydroxyphenylalanine (DOPA) residues.[1] A catechol oxidase enzyme converts these *o*-diphenolic residues into the corresponding *o*-quinone residues that are highly reactive and undergo the subsequent nonenzymatic reactions. One of the enzymatic reactions is the crosslinking of secreted proteins and the formation of a highly viscous gel that confers adhesive strength. Some studies suggest that DOPA residues are required for water-resistant adhesion.[2,3] However, chemical characterization of the enzymatic reactions has been less well-understood. Biotechnological routes for mussel glue production are very complicated and cost-effective, although adhesive proteins can be extracted from mussels. In addition, although synthetic methods for mussel glue formation have also been reported, synthesis is also complicated by the need to produce a high molecular mass protein.[4,5]

In our previous study, we attempted to mimic the function of the adhesive protein, using mushroom tyrosinase and chitosan.[6,7] Mushroom tyrosinase oxidizes a low molecular mass compound, 3,4-dihydroxyphenethylamine (dopamine), and the subsequent nonenzymatic crosslinking reaction between *o*-quinones enzymatically generated, and chitosan leads to substantial increases in the viscosity of the chitosan solutions.[2,3,6] These reaction mechanisms were schematically illustrated in detail in our previous article.[6,7] After high-viscosity modified chitosans were spread on the surfaces of the glass slides, the glass slides lapped and clipped together were submerged in water. Then, tensile shear adhesive strength of over 400 kPa was observed for highly viscous or gelled chitosan materials. These results demonstrated that a water-resistant adhesive was prepared from a dilute

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chitosan solution through the tyrosinase-catalyzed and subsequent nonenzymatic reactions. Commercially available tyrosinase used in the above studies is the one derived from mushroom. In addition, when poly (ethylene glycol) (PEG) was added to a chitosan solution containing dopamine and mushroom tyrosinase, the viscosity of the chitosan solutions increased in shorter reaction times. It is empirically demonstrated by many research groups that PEG protects the activity of the oxidoreductases such as tyrosinase and peroxidase from enzymatically generated species.[8–11] A research group of Obata published that a tyrosinase-encoding gene (*melB*) specifically expressed in solid state culture (koji) from *Aspergillus oryzae* was cloned, and a novel tyrosinase was isolated and characterized.[12] However, little have reported on biological or technical application of *melB* tyrosinase. As one of the practical usages of *melB* tyrosinase we focused attention on the preparation of highly viscous or gelled chitosan materials and their application to a water-resistant adhesive.

In this study, first, the dependences of the increased viscosity of the chitosan solutions containing dopamine with *melB* tyrosinase were investigated on molecular mass of chitosans and their amino group concentration and *melB* tyrosinase concentration in much detail. In addition, the application of highly viscous or gelled chitosan materials prepared was also estimated from tensile shear adhesive strength of the glass slides.

## EXPERIMENTAL

### Enzyme

*MelB* tyrosinase (EC 1.14.18.1) was favorably supplied from Gekkeikan Sake (Kyoto, Japan). This enzyme had the specific activity equivalent to 936 or 1170 U/mg-solid (determined by supplier) for a commercially available mushroom tyrosinase. Cloning of a tyrosinase-encoding gene and its overexpression in solid state culture of *Aspergillus oryzae* were previously reported by the research group of Gekkeikan Sake and National Research Institute of Brewing.[12] The gene carries six exons interrupted by five introns and has an open reading frame encoding 616 amino acid residues. This gene was designated as *melB*. The amino acid sequence of *melB* tyrosinase showed 26% homology to other fungal tyrosinases such as *Agaricus bisporus* and *Neurospora crassa*. However, the highly conserved sequences of copper-binding domains in these tyrosinases were illustrated, in which amino acid residues essential for tyrosinase activity were indicated.

### Chemicals

Four kinds of the chitosan samples were purchased from Sigma Chemicals (St. Louis, MO) and Wako Pure Chemicals (Tokyo, Japan). The 1.0 wt % chitosan solutions were prepared in an aqueous acetic acid solution of 1.0 w/v% and their viscosities were measured by a Brookfield DV II+ viscometer with S18, S25, or S34 spindles at a rotation speed of 1 rpm. The degrees of deacetylation of the chitosan samples used were determined by the colloid titration method.[8,13–16] After the pH values of 50-cm$^3$ sample solutions of chitosan at $C_{\text{chitosan}}$ = 0.020–0.025 mg/cm$^3$ were adjusted to 3–4 with 0.1–2*M* HCl, the chitosan solutions were titrated with an aqueous potassium poly(vinyl alcohol) sulfate (KPVS) solution at a sulfate group concentration, $C_{\text{KPVS}}$, of 0.00251 mmol/cm$^3$ using an ART-3 type HIRAMA automatic recording titrator. The end point of the titration was determined by the turbidity at 420 nm. The degrees of deacetylation of the chitosan samples used were calculated from the weight concentration of chitosan, $C_{\text{chitosan}}$, and volume of the titrant, $V_{\text{KPVS}}$ cm$^3$, using eqs. (1)–(3).

$$n_{\text{chitosan}} = C_{\text{KPVS}} V_{\text{KPVS}} = 0.00251 V_{\text{KPVS}} \quad (1)$$

$$n_{\text{chitin}} = \frac{50 C_{\text{chitosan}} - n_{\text{chitosan}} \cdot 197.62}{203.19} \quad (2)$$

$$\text{Degree of deacetylation} = \frac{n_{\text{chitosan}}}{n_{\text{chitosan}} + n_{\text{chitin}}} \quad (3)$$

where $n_{\text{chitosan}}$ and $n_{\text{chitin}}$ are the amounts of substance of the chitosan and chitin segments in the chitosan solutions, respectively. The quantities of 197.62 and 203.19 are molecular mass of the chitosan chloride salt and chitin segments, respectively. Molecular mass of the chitosan samples was calculated from the values of intrinsic viscosity determined using an Ubbelohde viscometer at 25°C. The constants $K$ and $a$ in Mark–Houwink–Sakurada equation were $1.81 \times 10^{-3}$ cm$^3$/g and 0.93, respectively.[8,17,18] The flow times of chitosan solutions were measured at different concentrations in an aqueous acetic solution of 0.1*M* containing NaCl of 0.2*M*, and then the intrinsic viscosity values were graphically determined. The degrees of deacetylation and viscosity-average molecular mass of the chitosan samples determined by the above-mentioned methods are summarized in Table I. Dopamine was obtained from Sigma Chemicals. Other chemicals were used as received without any purification.

### Preparation of chitosan solutions

Chitosan solutions of 1.6–2.0 w/v% were prepared by adding chitosan flakes to 100 cm$^3$ of water and intermittently adding HCl to maintain the pH values at 3–4. After the mixtures were stirred for 24 h, insoluble parts were removed from the chitosan solutions using a G3 glass filter by vacuum filtration. For

**TABLE I**
**The Degrees of Deacetylation and Viscosity-Average Molecular Mass of the Chitosan Samples Used in This Study**

| Sample | Degree of deacetylation (%) | Viscosity (mPa s) | [η] ($cm^3/g$) | $\overline{M_\eta}$ ($\times 10^6$ g/mol) |
|---|---|---|---|---|
| C560 | 81.3 | 561 | 672 | 9.75 |
| C890 | 87.3 | 888 | 839 | 1.24 |
| C1100 | 87.3 | 1119 | 1020 | 1.53 |
| C2500 | 89.8 | 2500 | 1072 | 1.62 |

individual experiments, the chitosan solutions were diluted with water so that the amino group concentration was adjusted to 10–30 m*M* on the basis of the weight concentration and degrees of deacetylation of each chitosan sample. Before conducting the enzymatic reaction, the pH values of the chitosan solutions were adjusted to 5.8–6.0 using small amounts of 2*M* NaOH solution.

### Reaction of enzymatically generated quinone with chitosan

#### Homogeneous reaction

One cubic centimeter of an aqueous *melB* tyrosinase solution (the concentration in the reaction solution = 20 U/$cm^3$) was added to an aqueous dopamine solution of 100 $cm^3$ (10 m*M*) at 25°C, and then the UV–visible spectra of the reaction solutions were measured at prescribed time intervals on a Shimadzu UV–visible recording spectrophotometer UV 260.[19,20]

#### Preparation of chitosan films and heterogeneous reaction

The chitosan films were prepared by pipetting an 1 w/v% chitosan solution of 3.0 $cm^3$ into a Petri dish of diameter 3.5 cm. The chitosan solutions were allowed to dry in an oven at 60°C. The chitosan films (average thickness: 20.2 μm) were thoroughly washed with 1*M* NaOH solution and pure water to neutralize the amino groups, and then dried under reduced pressure.

The chitosan films were immersed in 10 m*M* dopamine solutions containing *melB* tyrosinase at 25°C. The chitosan films incubated in the dopamine + tyrosinase solutions were washed with water, and then dried under reduced pressure. Adsorption spectra of the chitosan films incubated were recorded on the UV–visible spectrophotometer. The incubated chitosan films were placed perpendicular to the light path in the spectrophotometer such that the light directly passed through the chitosan films.[8,19,20]

### Gelation of chitosan and adhesive strength measurements

The glass slides (26 × 76 $mm^2$, thickness: 1.3 mm) used as the adherend were first cleaned by soaking for 24 h in a mixture of water, $H_2SO_4$, and $K_2Cr_2O_7$ (10 : 5 : 1). After rinsing with pure water, the glass slides were oven-dried at 60°C. The gelation was initiated by adding 0.192 g of dopamine (10 m*M*) and 1.0 $cm^3$ of an aqueous tyrosinase solution to the chitosan solutions of 100 $cm^3$ at the amino concentrations of 10–40 m*M*. The melB tyrosinase concentration of the reaction solutions prepared was adjusted to 60 U/$cm^3$. The steady shear viscosities of the reaction solutions were measured using a Brookfield DV II+ viscometer with S18, S25, and S34 spindles at a rotation speed of 1 rpm.[5,6] Approximately 50 mg of the reaction mixture was spread onto each face of two glass slides, and the faces were placed in contact with a 26 × 26 $mm^2$ overlapping surface area. After the overlapping surfaces were tightly pressed together, the glass slides were clipped together with two binder clips and immersed in water.

An Orientec universal testing machine STA 1225 was used for tensile shear adhesive strength measurements. One end of each glass slide sample was attached to the load cell and the other end to the actuator of the testing machine. The glass slide samples were loaded with a strain rate of 3 mm/s until failure. The values of tensile shear adhesive strength were calculated by dividing the force to separate the bonded glass slides by the overlapping surface area.[6,21–23]

## RESULTS AND DISCUSSION

### Quinone formation by *melB* tyrosinase

The degrees of deacetylation determined by colloid titration and viscosity-average molecular mass calculated from the intrinsic viscosity values obtained using a Ubbelohde viscometer are summarized in Table I for four kinds of chitosan samples used in this study. The amino groups appended to the chitosan chains were protonated at pH 3–4 and the degree of protonation of the amino groups decreased with an increase in the pH value from 100% at pH 4.0 to 55–60% at pH 6.0.[8] When *melB* tyrosinase (20 U/$cm^3$) was added to a chitosan (amino group concentration: 10 m*M*) solution containing dopamine (0.5 m*M*), the solution was observed to change from colorless to reddish-brown and then to blackish-brown for about 1 h, and the absorbance peaks at 360 and 470 nm gradually increased.

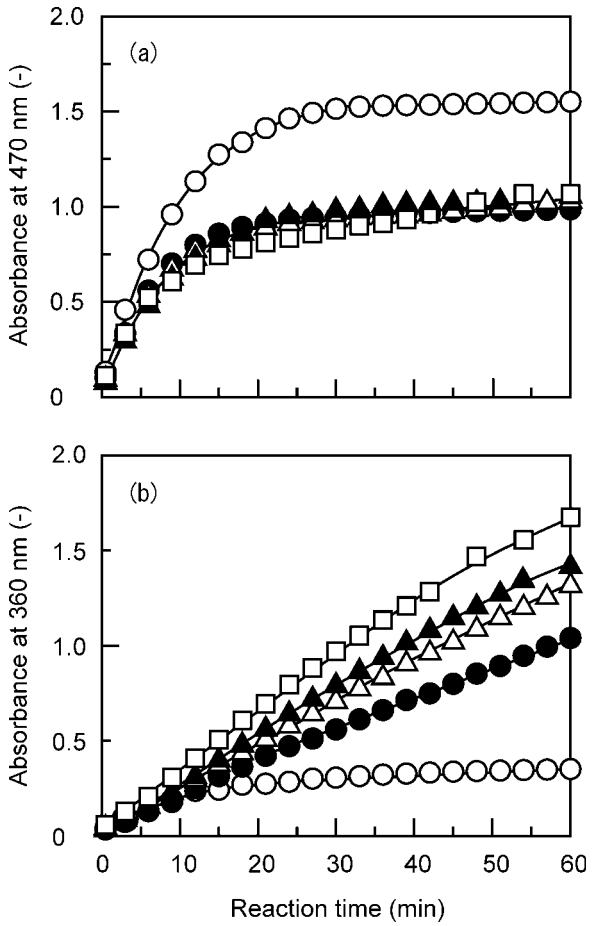
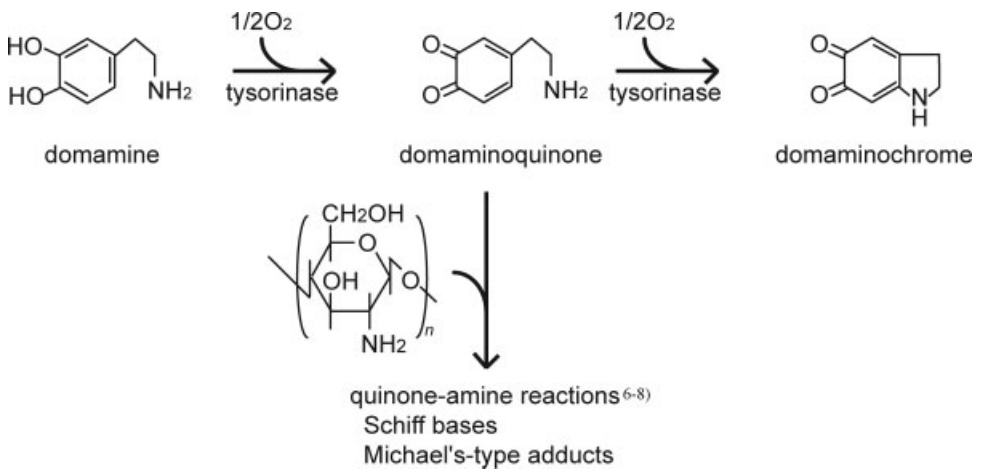



**Figure 1** The effect of the amino group concentration of C1100 chitosan on the increment in the absorbances at (a) 470 and (b) 360 nm at 25°C. Dopamine (0.5 m*M*) and *melB* tyrosinase (20 U/cm$^3$) were added to a C1100 chitosan solution. Amino group concentration of C1100 chitosan (m*M*): ○, 0; ●, 2.5; △, 5.0; ▲, 7.5; □, 10.0.

Figure 1 shows the effect of the chitosan's amino group concentration on the increment in absorbances at 360 and 470 nm, in the case where *melB* tyrosinase was added to C1100 chitosan solutions at different amino group concentrations containing dopamine (0.5 m*M*). When *melB* tyrosinase was added to an aqueous dopamine solution (0.5 m*M*), a progressive increase in the absorbances at 300 and 470 nm is observed. This difference of the UV–visible spectra suggests that the reaction sequence for enzymatic formation of dopaminochrome from dopamine is altered in the presence of chitosan. The enzymatic reaction in the absence and presence of chitosan is illustrated in Scheme 1 on the basis of systematic studies, which were carried out by Payne et al. on the quinone conversion of dopamine and chlorogenic acid by mushroom tyrosinase and subsequent reaction of quinones enzymatically generated with chitosan.[6,7,24,25] The emergence of the peak at 470 nm shows that dopaminochrome was enzymatically generated as an intramolecular cyclized product of the intermediate quinone. Although the absorbance at 470 nm sharply increased in the initial reaction stage for C1100 chitosan solutions containing dopamine irrespective of the chitosan's amino group concentrations, the absorbances were lower than that for an aqueous dopamine solution without chitosan. The increase in the chitosan's amino group concentration constricted an increase in the absorbance at 470 nm and the absorbance at 60 min was limited to 1.0. The peak little emerged at 360 nm even by adding *melB* tyrosinase to a dopamine solution. On the other hand, the absorbance at 360 nm increased over the reaction time for a chitosan solution containing dopamine. The emergence of this peak suggests that quinones enzymatically generated underwent the reaction with chitosan's amino groups. In addition, as the chitosan's amino group concentration increased, the absorbance at 360 nm more sharply increased. This indicates that the increase in the chitosan's amino group concentration accelerated nonenzymatic reaction of quinones enzymatically generated with chitosan's amino groups.




**Scheme 1** Schematic depiction of *melB* tyrosinase-catalyzed reaction to create a water-resistant adhesive from chitosan.

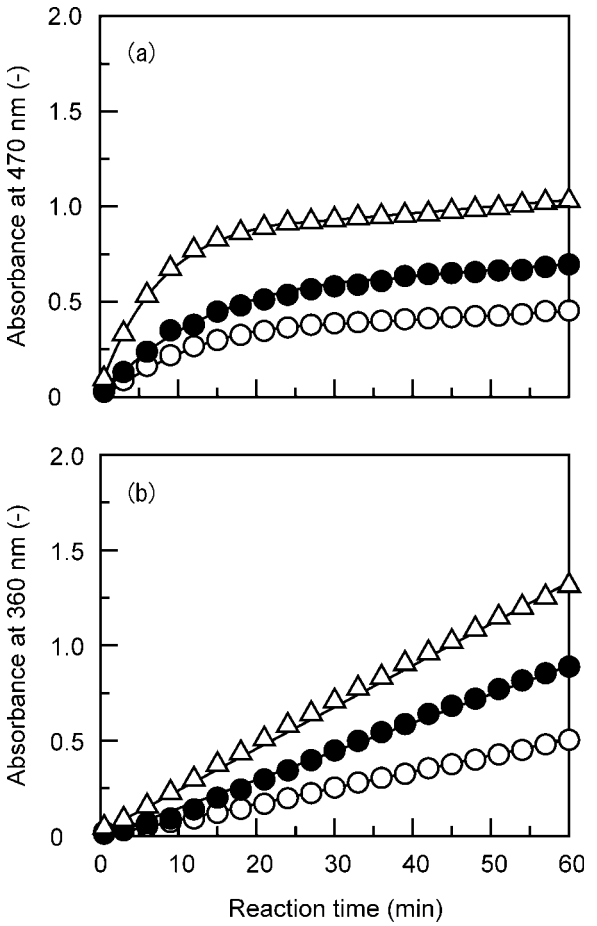



**Figure 2** The effects of *melB* tyrosinase concentration on the increment in the absorbances at (a) 470 and (b) 360 nm at 25°C for the C1100 chitosan solutions at an amino group concentration of 30 m*M* containing dopamine (0.5 m*M*) and *melB* tyrosinase. *MelB* tyrosinase concentration (U/cm$^3$): ○, 5; ●, 10; △, 20.

Figure 2 shows the effect of the concentration of *melB* tyrosinase added to the chitosan solutions at an amino group concentration of 30 m*M* containing dopamine at 0.5 m*M* on the increment in the absorbances at 360 and 470 nm. Both absorbances more sharply increased with an increase in the *melB* tyrosinase concentration. The results obtained from Figures 1 and 2 indicate that most of dopamine was converted to quinones 20 min after *melB* tyrosinase was added to a C1100 chitosan solutions containing dopamine. It has been reported that the decrease in the activity of oxidoreductases such as mushroom tyrosinase and horseradish or soybean peroxidases was restrained by adding PEG.[8,26–29] However, since dopamine was, here, effectively converted to quinones in shorter reaction times without PEG as mentioned above, we investigated the heterogeneous reaction of quinones enzymatically generated from dopamine by *melB* tyrosinase with a chitosan film in the absence of PEG in the next section.

### Quinone tanning on chitosan film

Here, the effects of the *melB* tyrosinase concentration and molecular mass of chitosan on the reaction of enzymatically generated quinone with chitosan were investigated using chitosan films prepared from four kinds of chitosan samples shown in Table I. When a chitosan film was immersed in a dopamine (0.5 m*M*) + *melB* tyrosinase (20 U/cm$^3$) solution, quinones nonenzymatically reacted with chitosan's amino groups through Schiff base or Michael-type reaction and the peak at 330 nm emerged.[4,30,31] The increase in the absorbance at 330 nm against the incubation time was shown in Figure 3 for C1100 chitosan films at different *melB* tyrosinase concentrations. The C1100 chitosan films were colored blackish brown due to quinone-tanning.[6–8] The absorbance at 330 nm increased over the incubation time and the increase in *melB* tyrosinase concentration led to a considerable increase in the absorbance. The results shown in Figures 1 and 3 indicate that the increase in concentration of quinones at higher *melB* tyrosinase concentrations accelerated the reaction of quinones with amino groups at the chitosan surfaces. The chitosan films prepared by the other three kinds of chitosan samples shown in Table I were also incubated in a dopamine + *melB* tyrosinase solution. Figure 4 shows the change in the absorbance at 330 nm with the incubation time for C360, C890, and C2500 chitosan films as well as C1100 chitosan film. The absorbance at 330 nm increased over the incubation time irrespective of molecular mass of chitosan used. This indicates that the quinone-tanning reaction at the surfaces of the chitosan films is independent of molecular mass of chitosan used. Here, since the qui-

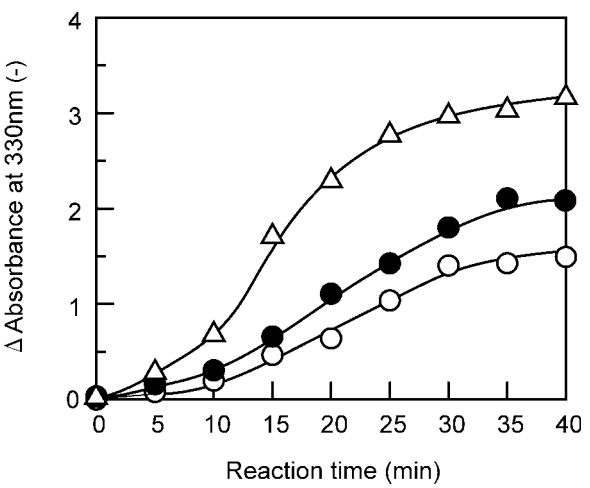



**Figure 3** The increment in the absorbance at 330 nm for the C1100 chitosan films incubated in the dopamine (0.5 m*M*) + *melB* tyrosinase mixtures against the reaction time at 25°C. *MelB* tyrosinase concentration (U/cm$^3$): ○, 5; ●, 10; △, 20.

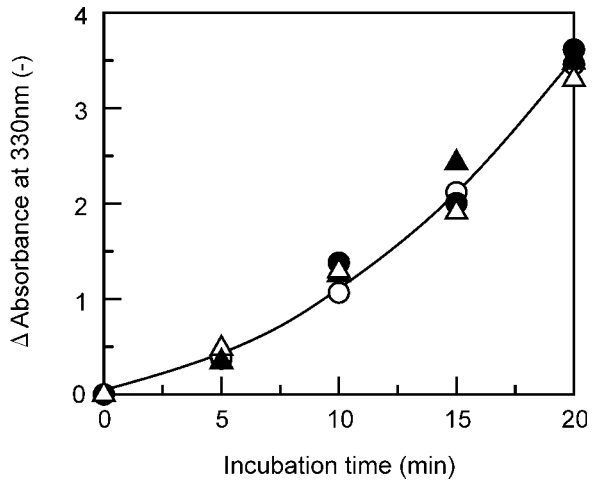



**Figure 4** The increment in the absorbance at 330 nm for the C560 (○), C890 (●), C1100 (△), and C2500 (▲) chitosan films incubated in a dopamine (0.5 m*M*) + *melB* tyrosinase (20 U/cm$^3$) mixture against the reaction time at 25°C.

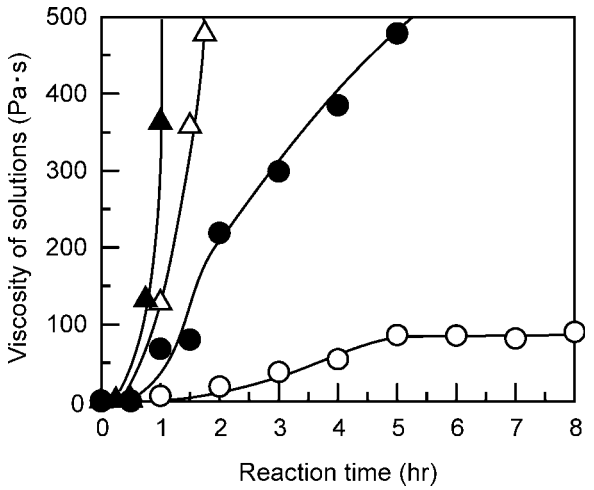



**Figure 6** The effect of the amino group concentration of C1100 chitosan on the increase in the viscosity with the enzymatic conversion of dopamine and subsequent nonenzymatic reaction with C1100 chitosan at 25°C. Amino group concentration of C1100 chitosan (m*M*): ○, 10; ●, 20; △, 30; ▲, 40.

none-tanning reaction was effectively progressed in relatively shorter incubation times, the incubation of the chitosan films in a dopamine + *melB* tyrosinase solution was carried out without adding any PEG.

### Gelation of chitosan solutions

The effects of the *melB* tyrosinase dose and the amino group concentration and molecular mass of chitosan were investigated on the increase in the viscosity through the enzymatic conversion of dopamine into quinone and subsequent nonenzymatic reaction with chitosan. Figure 5 shows the effect of *melB* tyrosinase concentration on the increase in the viscosity at an amino group concentration of 30 m*M* for C1100 chitosan at 25°C. When *melB* tyrosinase was added to the C1100 chitosan solution containing dopamine (10 m*M*), the solutions first became yellow, then orange-red, and finally blackish-brown about 1 h after the enzymatic reaction was started. The viscosity slightly increased 6 h after the enzymatic reaction started at a *melB* tyrosinase concentration of 2 U/cm$^3$. On the other hand, the viscosity sharply increased for only 1–2 h at 5 U/cm$^3$. When the *melB* tyrosinase concentration was further increased, the viscosity was more sharply increased.

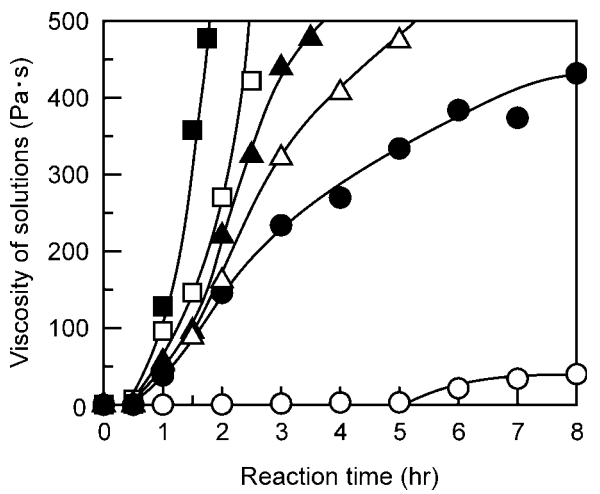



**Figure 5** The effect of *melB* tyrosinase concentration on the increase in the viscosity with the enzymatic conversion of dopamine and subsequent nonenzymatic reaction with C1100 chitosan at 25°C. *MelB* tyrosinase concentration (U/cm$^3$): ○, 2; ●, 5; △, 10; ▲, 20; □, 40; ■, 60.

Subsequently, Figure 6 shows the effects of chitosan's amino group concentration on the increase in the viscosity for C1100 chitosan. The viscosity gradually increased against the reaction time at an amino group concentration of 10 m*M*. At further increased amino group concentrations, the viscosity sharply increased at around 1 h and higher viscosities were obtained. We can safely say that the increase in viscosity shown in Figures 5 and 6 is caused by crosslinking of C1100 chitosan with quinones enzymatically generated through Schiff base or Michael-type reaction,[6,8,25] since no increase in solution viscosity was observed for control chitosan solution containing either *melB* tyrosinase or dopamine.

In addition, the viscosity of the chitosan solutions at 30 m*M* prepared from chitosan samples of different molecular mass increased against the reaction time as shown in Figure 7. For gelation of the chitosan solutions by dopamine and mushroom tyrosinase, the viscosity of solutions sharply increased 2 h after mushroom tyrosinase was added.[6,8] In addition,

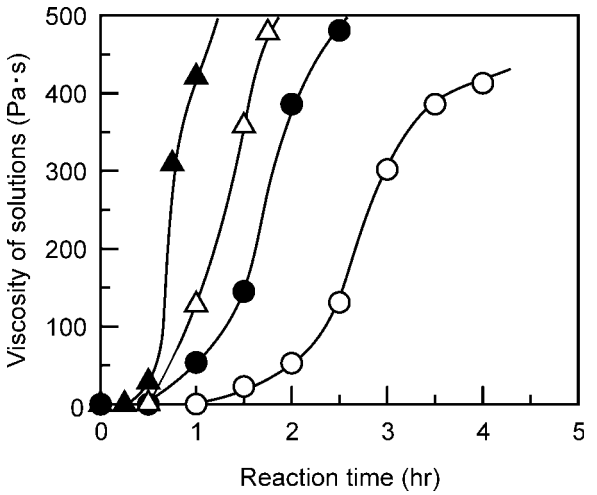



**Figure 7** The effect of molecular mass of chitosan on the increase in the viscosity of the chitosan solutions at an amino group concentration of 30 m*M* containing dopamine (10 m*M*) and *melB* tyrosinase (60 U/cm$^3$). Chitosan sample: ○, C560; ●, C890; △, C1100; ▲, C2500.

when the enzymatic reaction was initiated in the presence of PEG of molecular mass of $1.0 \times 10^5$ at 1.0 mg/cm$^3$, the viscosity increased in shorter reaction times. It is said that PEG suppresses deactivation of mushroom tyrosinase by enzymatically generated chemical species. Here, the viscosity of C1100 chitosan solutions containing *melB* tyrosinase increased in shorter reaction times than that containing mushroom tyrosinase in the presence of PEG reported in our previous article.[8] Consequently, the gelation of the chitosan solutions by *melB* tyrosinase was carried out without PEG. The use of chitosan materials gelled to a water-resistant adhesive was examined in the next section.

### Adhesive strength in water

The effects of the *melB* tyrosinase dose and the amino group concentration and molecular mass of chitosan on the increase in tensile shear adhesive strength of the glass slides were investigated using gelled chitosan materials under water.

Figure 8 shows the increase in the water-resistant adhesive properties against the immersion time under water for a C1100 chitosan solution of 30 m*M* gelled for 2.5 h by *melB* tyrosinase at 60 U/cm$^3$. The increase in the immersion time led to the increase in adhesive strength for the lapped glass plates under water after spread of a C1100 chitosan solution, which was allowed to react with dopamine and tyrosinase for 2.5 h. This indicated that the gelation of chitosan solutions will be advanced between the lapped glass plates in water after the spread. However, it is difficult to measure the viscosity after the spread of chitosan materials. In a similar manner, the increase in adhesive strength against the immersion time was also observed for chitosan materials gelled by mushroom tyrosinase.[6]

In our previous article,[6] adhesive strength obtained by chitosan materials enzymatically gelled was compared with that obtained by a chitosan gel prepared with a chemical crosslinking agent, glutaraldehyde (1 m*M*). Adhesive strength for the chitosan gel chemically crosslinked with glutaraldehyde was 280 kPa. As shown in Figure 8, adhesive strength for C1100 chitosan was higher than that obtained for the chemically crosslinked chitosan gel. These results will open up the possibility of chitosan materials gelled through the enzymatic reaction toward the practical application as a water-resistant adhesive.

Next, a C1100 chitosan solution of 30 m*M* was gelled by *melB* tyrosinase at 10–60 U/cm$^3$. Figure 9 shows the increase in adhesive strength against the reaction time for a C1100 chitosan solution of 30 m*M*. Here, on the basis of the results obtained from Figure 8, the gelled chitosan materials were spread on two clean and dry glass slides, and then the glass slides were lapped together, clipped, and immediately submerged in water for 48 h. Adhesive strength gradually increased against the reaction time at 10 U/cm$^3$. At further increased *melB* tyrosinase concentrations, adhesive strength sharply increased in shorter reaction times. The increase in adhesive strength is caused by crosslinking of chitosan with quinone enzymatically generated as shown in Figure 3. In addition, the maximum adhesive strength was obtained at 2.5–3 h. It is considered that the formation of nonenzymatic quinone-crosslinking is faster than decomposition of generated

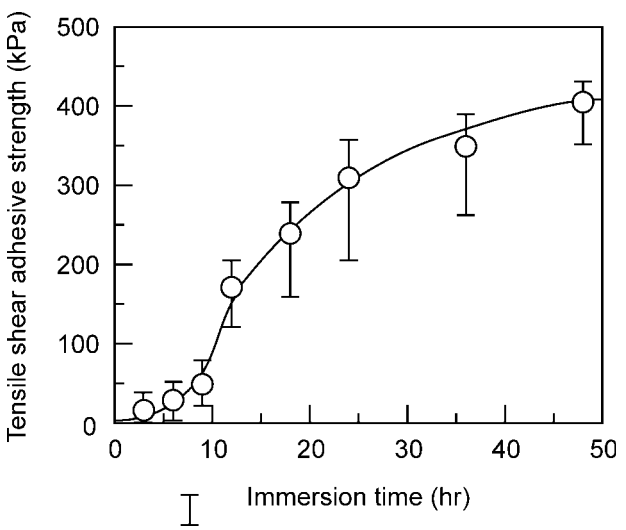



**Figure 8** An increase in tensile shear adhesive strength of the glass plates coated with C1100 chitosan gels with the immersion time. Reaction time: 2.5 h. Dopamine (10 m*M*) and *melB* tyrosinase (60 U/cm$^3$) were added to a C1100 chitosan solution at an amino group concentration of 30 m*M*.

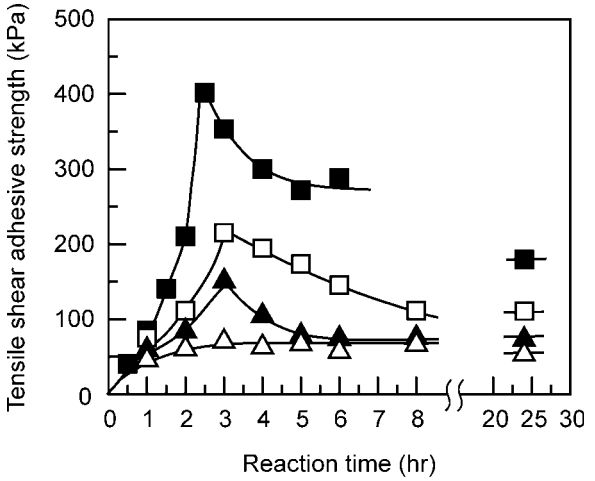



**Figure 9** The effect of *melB* tyrosinase concentration on the increase in tensile shear adhesive strength of the glass slides coated with C1100 chitosan gels prepared at different tyrosinase concentrations. *MelB* tyrosinase concentration (U/cm$^3$): △, 10; ▲, 20; □, 40; ■, 60. Dopamine (10 m*M*) and *melB* tyrosinase (60 U/cm$^3$) were added to a C1100 chitosan solution at an amino group concentration of 30 m*M*. The lapped slides were immersed in water for 48 h.

quinone crosslinking in the range that adhesive strength increased. The reaction of quinones enzymatically generated with chitosan's amino groups would be completed between the glass plates during immersion in water for 48 h. However, the decrease in adhesive strength is considered to be caused by decomposition of quinone crosslinking. Although the viscosity of a chitosan solution of 30 m*M* gelled with *melB* tyrosinase at 60 U/cm$^3$ exceeded 500 Pa s as the measuring limit of the viscometer used at the reaction time of 1.5 h, the viscosity dropped down to 120 Pa s at 120 h and then gradually decreased against the reaction time. This result supports our consideration that decomposition of quinone crosslinking occurred between the glass slides after spread of gelled chitosan materials.

Subsequently, the effects of the amino concentration and molecular mass of chitosan on adhesive strength were investigated. The chitosan solutions of 10–30 m*M* were gelled by *melB* tyrosinase at 60 U/cm$^3$. The increase in adhesive strength was shown in Figure 10 for the C1100 chitosan solutions of 10–30 m*M* and chitosan solutions of 30 m*M* with different molecular mass. When the amino concentration of C1100 chitosan increased from 10 to 30 m*M* or molecular mass of chitosan increased from C560 to C1100, adhesive strength more sharply increased in shorter reaction times and the maximum adhesive strength values went up. On the other hand, the maximum adhesive strength value decreased for the C1100 chitosan of 40 m*M* and C2500 of 30 m*M*. A decrease in adhesive strength against the reaction time was also observed for gelation of a C1100 chitosan solution by mushroom tyrosinase in the presence of 100K PEG.[7] It is probably because the viscosity of the chitosan mixture was too high and crosslinking reaction heterogeneously occurred for chitosan samples at higher amino group concentrations or the chitosan samples were of higher molecular mass. However, the experimental results mentioned above will open up the possibility of chitosan materials gelled through the enzymatic reaction toward the practical application as water-resistant adhesive.

## CONCLUSIONS

The experimental results obtained in this study show that tyrosinase-catalyzed reactions of dopamine can be used to confer water-resistant adhesive properties to dilute chitosan solutions. The observed

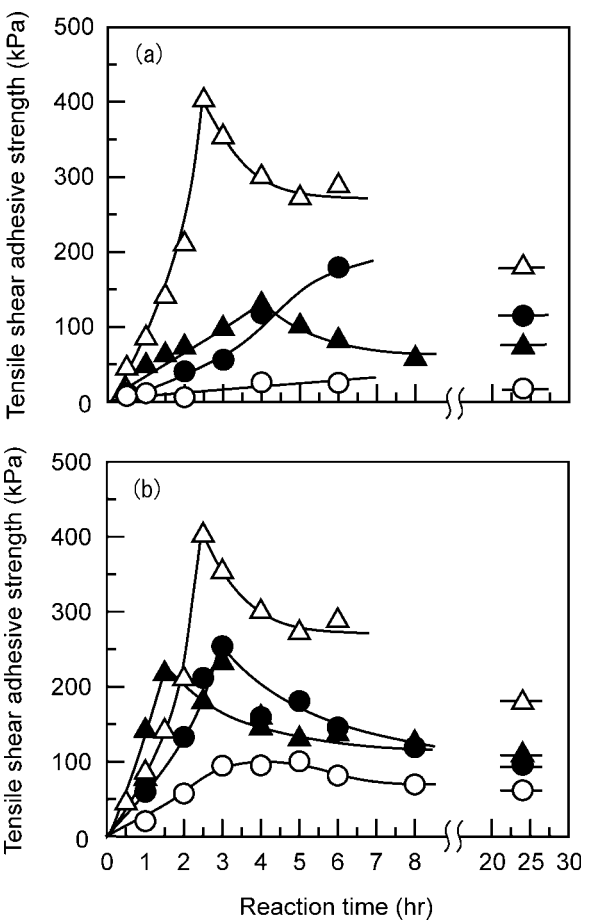



**Figure 10** The effects of (a) C1100 chitosan's amino group concentration and (b) molecular mass of chitosan (amino group concentration: 30 m*M*) on tensile shear adhesive strength of the glass slides. (a) Amino group concentration of C1100 chitosan (m*M*): ○, 10; ●, 20; △, 30; ▲, 40. (b) Chitosan sample: ○, C560; ●, C890; △, C1100; ▲, C2500. Dopamine (10 m*M*) and *melB* tyrosinase (60 U/cm$^3$) were added to chitosan solutions. The lapped slides were immersed in water for 48 h.

adhesive properties appear to be related to the increased viscosity of the modified chitosan materials. Practically, this work demonstrates the potential for developing modified chitosan materials as water-resistant adhesives. Adhesive strength increased by increasing the molecular mass of the used chitosan samples and their amino group concentration. A chitosan solution was gelled in shorter reaction times and adhesive strength sharply increased by *melB* tyrosinase in lieu of mushroom tyrosinase. In addition, the viscosity of chitosan solutions was sharply increased in shorter reaction times by *melB* tyrosinase without PEG as an additive to retain the activity of tyrosinase. Adhesive strength increased up to 400 kPa for highly gelled materials obtained from a chitosan solutions at an amino group concentration of 30 m*M*. Unfortunately, the enzymatically gelled chitosan materials were colored blackish-brown. However, this technique is environmentally friendly in that a water-resistant adhesive is developed without use of organic solvent and chitosan typically obtained from wastes generated from crustacean processing is used as a main low material.

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