Application of Enzymatically Gelled Chitosan Solutions to Water-resistant Adhesives

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Received 9 October 2006; revised 16 November 2006; accepted 18 November 2006
DOI 10.1002/app.25833
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: An investigation was undertaken on the application of dilute chitosan solutions gelled by tyrosinase-catalyzed reaction with 3,4-dihydroxyphenethylamine (dopamine). The tyrosinase-catalyzed reaction with dopamine conferred water-resistant adhesive properties to the semidilute chitosan solutions. The viscosity of the chitosan solutions increased highly by the tyrosinase-catalyzed reaction and the subsequent reactions between *o*-quinone compounds and chitosan. These highly viscous, gel-like modified chitosan materials were allowed to spread onto the surfaces of the glass slides, which were tightly lapped together and held them in water. Tensile shear adhesive strength of over 400 kPa was observed for the modified chitosan samples. The increase in the amino group concentration of the chitosan solutions and the molecular mass of the chitosan used effectively led to the increase in adhesive strength of the glass slides. In addition, in the case where the chitosan solution was gelled by the enzymatic reaction with dopamine in the presence of poly(ethylene glycol), adhesive strength sharply increased at shorter reaction times concomitantly with the increase in the viscosity of the chitosan solutions because the tyrosinase activity effectively was retained by poly(ethylene glycol). © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 104: 1818–1827, 2007

Key words: mushroom tyrosinase; chitosan; dopamine; PEG; 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 marine animals such as mussels to adhere to wet or submerged surfaces. These animals are well known to use a polyphenolic adhesive protein that is rich in lysine, hydroxyproline, and dihydroxyphenylalanine residues.[1] A catechol oxidase enzyme converts these *o*-diphenolic residues to *o*-quinone residues that are 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. Although chemical characterization of the enzymatic reactions has been less well understood, some studies suggest that dihydroxyphenylalanine residues are required for water-resistant adhesion.[2,3] Adhesive proteins can be extracted from mussels. However, biotechnological routes for mussel glue production are very complicated and cost-effective for low value adhesive applications. In addition, synthetic methods for mussel glue formation are 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 a different biological analogy and a different biopolymer.[6] The tyrosinase enzyme oxidizes low molecular mass sclerotizing precursors such as *N*-acetyldopamine. The *o*-quinones generated from this reaction undergo the subsequent nonenzymatic crosslinking reactions with proteins. This ''quinone tanning'' yields a hardened outer integument. These reaction mechanisms were schematically illustrated in detail in our previous article.[6]

By analogy to insect sclerotization, we used tyrosinase to oxidize a low molecular mass compound, 3,4-dihydroxyphenethylamine (dopamine). Further, to facilitate quinone reactions, we used a biopolymer that has amino groups with a moderately low pK value. Here, a polysaccharide, chitosan, was chosen as the biopolymer in place of a protein. Chitosan has primary amino groups with the p*K* value of 6 to 6.5.[7] The tyrosinase-catalyzed and subsequent nonenzymatic reactions lead to substantial increases in the viscosity of the chitosan solutions.[2,3,5,6] 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. Adhesive strength of over 400 kPa was observed for the modified chitosan samples. These results demonstrated that a

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water-resistant adhesive was prepared from a dilute chitosan solution through the tyrosinase-catalyzed and subsequent nonenzymatic reactions.

Many research groups have reported that the activity of oxidoreductases such as tyrosinase and soybean and horseradish peroxidases was retained by adding a small amount of additives. The enzymatic reactions should be initiated at the optimum pH value and temperature to obtain a high enzymatic activity. The activity of tyrosinase and peroxidase are gradually decreased by either various interactions with chemical species enzymatically generated from the substrates.[8–10] Therefore, the enzymatic activity can be considered to be retained by restraining the interactions mentioned above. In fact, it has been empirically demonstrated that the activity of tyrosinase and peroxidase was retained by adding the additives such as poly(ethylene glycol) (PEG).[11–14] The retention of the enzymatic activity by the addition of PEG is qualitatively explained in terms of the formation of water-soluble complex between PEG and chemical species enzymatically generated. Although the mechanism of these interactions should be explored in more detail, we can safely say that some unfavorable influence to enzymes can be undoubtedly limited. On the basis of the above-mentioned foundation, PEG was used to restrain the decrease in the activity of tyrosinase PEG in this study.

In this study, the dependences of the increased viscosity of the chitosan solutions enzymatically modified and adhesive strength on molecular mass of chitosans and their amino group concentration were investigated in detail. In addition, we tried to shorten the enzymatic gelation of the chitosan solutions by adding a small amount of PEG. If a high-viscosity modified chitosan was prepared at shorter reaction time, this technique will be applicable to various fields.

EXPERIMENTAL

Chemicals

Mushroom tyrosinase (EC 1.14.18.1) of the specific activity of 2590 U/mg-solid (activity determined by supplier) and 3,4-dihydroxyphenetylamine (dopamine) were purchased from Sigma Chemical (St Louis, USA). Six kinds of the chitosan samples were obtained from Sigma Chemical and Wako Pure Chemical (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.[15–18] After the pH values of 50 cm^3 sample solutions of chitosan at 0.02–0.025 mg/cm^3 were adjusted to 3 to 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_{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 measuring turbidity at 420 nm. The degrees of deacetylation of the chitosan samples used were calculated from the weight concentration of chitosan, $C_{chitosan}$ mg/cm^3, and the volume of the titrant, V_{KPVS} cm^3, of using eqs. (1)–(3).

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

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

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

where $n_{chitosan}$ and n_{chitin} are the amounts of substance of the chitosan and chitin segments of the chitosan samples used, respectively. The quantities of 197.62 and 203.19 are the molecular mass of the chitosan chloride salt and chitin segments, respectively. The molecular mass of the chitosan samples was calculated from the intrinsic viscosities determined using an Ubbelohde viscometer at 25°C. The constants, K and a, in Mark–Houwink–Sakurada equation were 1.81×10^{-3} cm^3/g and 0.93, respectively.[19,20] The flow times of the 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 viscosities 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.

The PEG samples of molecular mass of 1.0×10^3, 4.6×10^3, 1.0×10^4, 2.0×10^4, 1.0×10^5, 2.0×10^5, and 3.0×10^5 were obtained from Sigma Chemical and Wako Pure Chemical and named 1 K-PEG, 4.6 K-PEG, 10 K-PEG, 20 K-PEG, 100 K-PEG, 200 K-PEG, and 300 K-PEG, respectively.

Reaction of enzymatically generated quinone with chitosan

Homogeneous reaction

One cubic centimeter of an aqueous tyrosinase solution (2.34 mg/cm^3) was added to an aqueous dopamine solution of 100 cm^3 at 10 m*M* in the absence and presence of PEG at 25°C (concentration in the reaction solution = 60 U/cm^3), and then the UV-visible spectra of the reaction mixture solutions were measured at prescribed time intervals on a

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)	$\overline{M}\eta$ (g/mol)
C48	71.3	48.0	438	6.15×10^5
C51	78.2	51.0	444	6.25×10^5
C560	81.3	561	672	9.75×10^5
C890	87.3	888	839	1.24×10^6
C1100	87.3	1119	1020	1.53×10^6
C2500	89.8	2500	1072	1.62×10^6

Shimadzu UV-visible recording spectrophotometer UV 260.[21,22]

Preparation of chitosan films and heterogeneous reaction

A chitosan solution (1 w/v %) was prepared by adding 1.0 g of chitosan flakes to 100 cm^3 of distilled water and adding 2*M* HCl to keep the pH values of the chitosan solutions of 3–4. After most of the added chitosan flakes were dissolved, the solutions were vacuum-filtered to remove the insoluble parts using a G3 glass filter. The chitosan films were prepared by pipetting a chitosan solution of 3.0 cm^3 into a Petri dish of diameter 3.5 cm. The solutions were allowed to dry in an oven at 60°C. The chitosan films (average thickness: 20.2 μm) removed from the Petri dishes 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 the dopamine + tyrosinase solutions in the presence or absence of PEG at 25°C. The chitosan films incubated in the dopamine + tyrosinase solutions were washed with pure water, and then dried under reduced pressure. Adsorption spectra of the chitosan films were recorded in the range of 250–600 nm on the UV-visible spectrophotometer. The modified chitosan films were placed perpendicular to the light path in the spectrophotometer such that the light directly passed through the chitosan films.[21,22]

Gelation of chitosan and adhesive strength measurements

Chitosan solutions were diluted to the amino group concentrations of 10–30 m*M* with water on the basis of the degrees of deacetylation of the chitosan samples. The pH values of the chitosan solutions were adjusted to 5.8–6.0 by adding a small amount of 1–2*M* NaOH solutions prior to the enzymatic reaction.

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 10–30 m*M*. The tyrosinase concentration of the reaction solutions prepared was 60 U/cm^3. The steady shear viscosities of the chitosan mixtures were measured using a Brookfield DV II+ viscometer with S18, S25, and S34 spindles at a rotation speed of 1 rpm.[6] We selected a glass microscope slide (26 × 76 mm, thickness: 1.3 mm) as the adherend. The glass slides 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. 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 overlapping surface area. After the overlapping surfaces were pressed together, the glass slides were clipped together with two binder clips and immersed in water.

Adheive strength was measured at room temperature with an Orientec universal testing machine STA 1225. 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 in until failure. The values of adhesive strength were calculated by dividing the force to separate the bonded glass slides by the overlapping surface area.[6,23–25]

RESULTS AND DISCUSSION

Effect of addition of PEG on quinone oxidation

The degrees of deacetylation determined by colloid titration and the average molecular mass calculated from the intrinsic viscosities obtained using a Ubbelohde viscometer are summarized in Table I for six kinds of chitosan samples used in this study. The amino groups appended to the chitosan chains were protonated at pH 3–4 and the degrees of protonation of the amino groups for the chitosan samples decreased with an increase in the pH value from 100% at pH 4.0 to 60% at pH 6.0.

When tyrosinase was added to an aqueous dopamine solution (10 m*M*), a progressive increase in the absorbances at 390 and 470 nm is observed over the reaction time. The peak at 390 nm is assigned to dopaminoquinone. The peak at 470 nm is characteristic of dopaminochrome as an intramolecular cyclized product of intermediate quinone.[6,7,26–28] It has been reported that when quinone enzymatically generated is complexed with PEG, the enzymatic reaction is enhanced.[29,30] Therefore, the effect of the addition of PEG on the retention of the tyrosinase activity was investigated by varying the concentra-

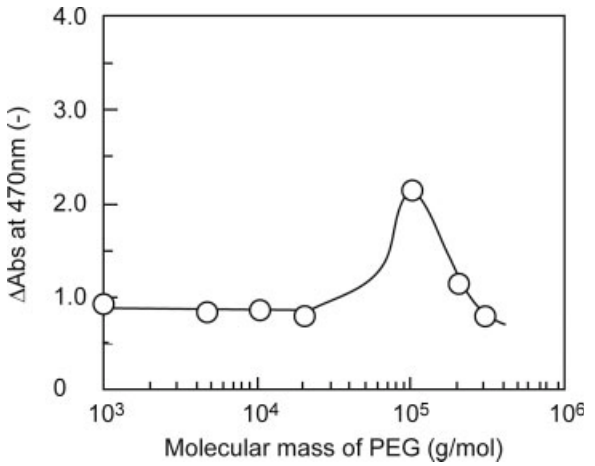

Figure 1 The effect of molecular mass of PEG (1.0 mg/cm^3) added to a dopamine (10 m*M*) + tyrosinase (60 U/cm^3) solution on the increment in the absorbance at 470 nm at 60 min. Reaction temperature: 25°C.

tion and molecular mass of PEG added to the dopamine + tyrosinase solutions. PEG samples with different molecular mass were added to a dopamine + tyrosinase solution at a PEG concentration of 1.0 mg/cm^3. Figure 1 shows the effect of the molecular mass of PEG (1.0 mg/cm^3) added on the increment of absorbance at 470 nm. The increase in the absorbance against the reaction time had the maximum for 100 K-PEG. There was no difference in the increment of absorbance between 1, 4.6, 10, or 20 K-PEGs and a dopamine + tyrosinase solution without PEG. This result means that the addition of PEG samples with low molecular mass is ineffective in retaining or enhancing the tyrosinase activity. However, when the molecular mass of PEG further increased from 1.0×10^5, the increment in the absorbance sharply decreased. The optimum molecular mass of PEG added to retain the enzymatic activity would vary with the kind and concentration of the enzymes and substrates used. Therefore, the optimum molecular mass of PEG is required to be determined in each case. Here, the optimum molecular mass of PEG was determined to be 1.0×10^5 from Figure 1.

A few articles have been reported on the retention of the enzymatic activity by addition of PEG and the decrease in the enzyme dosage.[20,31] It is well known that the decrease in the enzymatic activity or deactivation is restrained by adding PEG for the radical formation from phenol compounds by peroxidase. A qualitative interpretation was given for the retention of enzymatic activity by the addition of PEG. It is considered that the addition of PEG suppresses the deactivation of tyrosinase by enzymatically generated chemical compounds and adsorption of tyrosinase molecules on chemical compounds present in the solution which inhibit the contact of a substrate molecule on the active site of the enzyme molecule. Since it was found from Figure 1 that the activity of tyrosinase in the solution was retained by the addition of PEG, subsequently the effect of the PEG concentration on the activity of tyrosinase was investigated.

Figure 2(a) shows the increases in the absorbance at 470 nm of the dopamine + tyrosinase solutions in the presence of 100 K-PEG at different concentrations with the reaction time. The increment of the absorbance in the early reaction stage increased with an increase in the 100 K-PEG concentration. In addition, the absorbances at 470 nm of the dopamine + tyrosinase solutions containing 10, 200, and 300 K-PEGs at different concentrations were measured against the reaction time. As shown in Figure 2(b), the absorbance increased against the reaction time except for 20 K-PEG. In the case where 100 K-PEG was added, the absorbance considerably increased at lower concentrations and black precipitates were

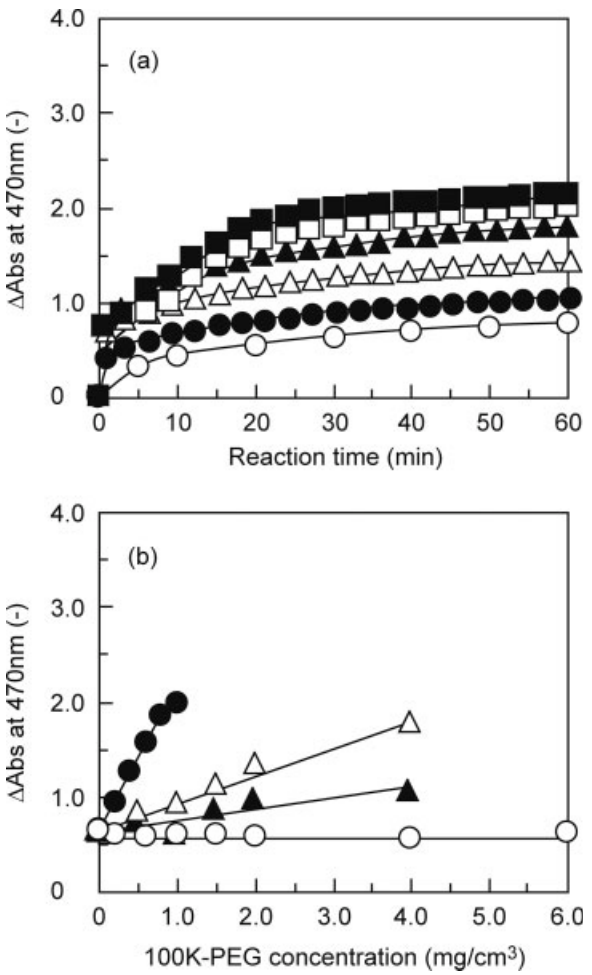

Figure 2 The effects of (a) the 100 K-PEG concentration and (b) molecular mass of PEG on the increment in the absorbance at 470 nm of the dopamine (10 m*M*) + tyrosinase (60 U/cm^3) solutions at 25°C. (a) 100 K-PEG concentration (mg/cm^3)—○: 0, ●: 0.2, △: 0.4, ▲: 0.6, □: 0.8, ■: 1.0. (b) PEG sample—○: 20 K-PEG, ●: 100 K-PEG, △: 200 K-PEG, ▲: 300 K-PEG. Reaction time: 60 min.

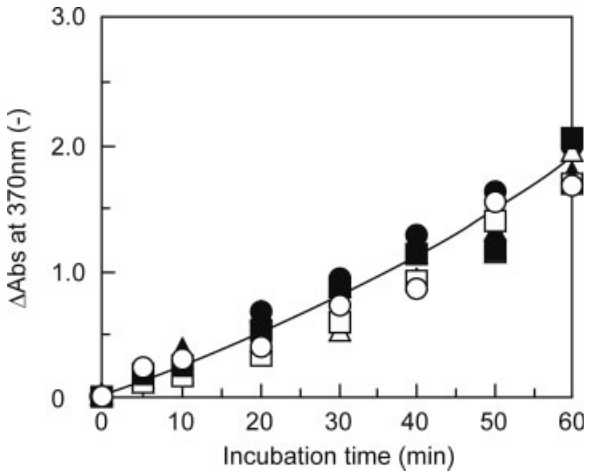

Figure 3 The increment in the absorbance at 370 nm for the C48 (○), C51 (●), C560 (△), C890 (▲), C1100 (□), and C2500 (■) chitosan films incubated in a dopamine (10 m*M*) + tyrosinase (60 U/cm^3) solution against the reaction time at 25°C.

generated at the 100 K-PEG concentration of 1.2 mg/cm^3. This is probably due to oligomerization of dopaminoquinone enzymatically generated from dopamine through 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid by autooxidation.[29,32,33] On the other hand, even if the concentration of 20 K-PEG added was increased to 6.0 mg/cm^3, the increase in the absorbance against the reaction time was almost the same as that of a dopamine + tyrosinase solution without PEG. It was found from Figures 1 and 2 that the most effective quinine oxidation of dopamine by tyrosinase was obtained at a 100 K-PEG concentration of 1.0 mg/cm^3.

Reaction of enzymatically generated quinone with chitosan

First, the dependence of the reaction of enzymatically generated quinone with chitosan on molecular mass of chitosan was investigated using chitosan films prepared by six kinds of chitosan samples shown in Table I. A peak at 370 nm emerged through two different types of reactions with chitosan's amino groups, Schiff base and Michael-type reactions in the UV-visible spectra for the chitosan films incubated in a dopamine + tyrosinase solution, and the chitosan films were colored blackish brown due to quinone-tanning.[6,7,26,27] The increase in the absorbance at 370 nm of the chitosan films against the incubation time in the absence of PEG was shown in Figure 3. The absorbance increased over the incubation time irrespective of molecular mass of chitosan used. These results indicate that quinone reaction on the chitosan films is independent of the molecular mass of chitosan used. Subsequently, the chitosan films were incubated in the dopamine + tyrosinase solution containing PEGs of different molecular mass. And the chitosan films were colored blackish brown due to quinone-tanning.[6,7,26,27]

Figure 4(a) shows the effects of the molecular mass of PEG added to the dopamine + tyrosinase solution on the increment in the absorbance at 370 nm at 60 min. The absorbance increased with an increase in the molecular mass of PEG added and the increment of absorbance had the maximum for 100 K-PEG. This dependence on the molecular mass of PEG was in good agreement with that obtained in Figure 2. This indicates that the quinone-tanning of the chitosan films is caused by the increase in the concentration of quinone generated by the addition of PEG. The increment in the absorbance of the chitosan films incubated in the dopamine + tyrosinase solutions containing 100 K-PEG at different concentrations linearly increased against the PEG concentration as shown in Figure 4(b). It was found that

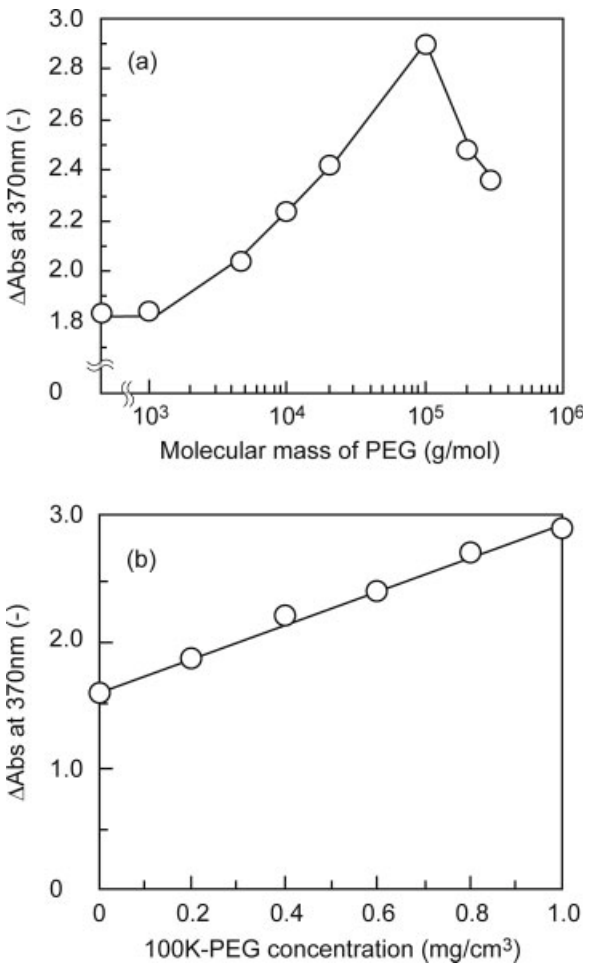

Figure 4 The effects of (a) molecular mass of PEG added and (b) 100 K-PEG concentration on the increment in the absorbance at 370 nm for the C1100 chitosan films incubated in the dopamine (10 m*M*) + tyrosinase (60 U/cm^3) solutions containing PEG at 25°C.

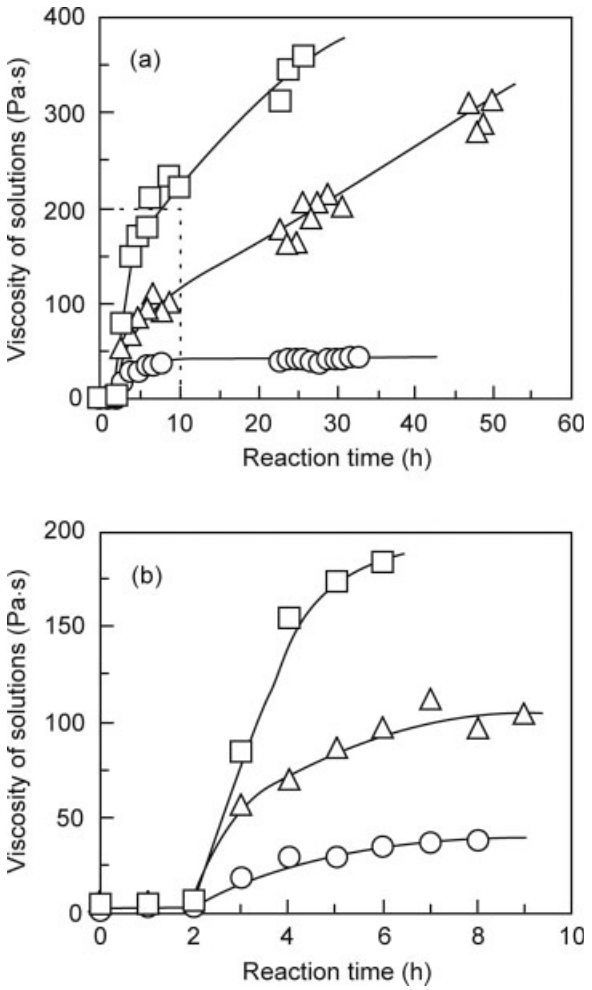

Figure 5 The effect of chitosan's amino group concentration on the increase in the viscosity with the enzymatic conversion of dopamine and subsequent nonenzymatic reaction with C1100 chitosan at 25°C. (b) is an expanded view in the range of below 10 h. Amino group concentration of C1100 chitosan (m*M*)—○: 10, △: 20, □: 30.

the increase in the quinone generation by the addition of 100 K-PEG accelerated the heterogeneous reaction of quinone with the chitosan films.

Gelation of chitosan solutions

The effects of both amino group concentration and molecular mass of chitosan were investigated on the increase in the viscosity with the enzymatic conversion of dopamine and subsequent nonenzymatic reaction. Figure 5(a) shows the effect of chitosan's amino group concentration on the increase in the viscosity with the enzymatic conversion of dopamine to quinone and subsequent nonenzymatic reaction with C1100 chitosan at 25°C. When dopamine (10 m*M*) and tyrosinase (60 U/cm^3) were added to the C1100 chitosan solutions of the amino group concentrations of 10–30 m*M*, the solutions first became yellow, then orange–red, and finally dark brown. Then, the viscosity of the solution sharply increased 2 h after tyrosinase was added to the chitosan solution, and higher viscosities were obtained at higher amino group concentrations. The increase in viscosity is caused by crosslinking of 1100 chitosan with quinone enzymatically generated through Schiff base or Michael-type reaction.[27] The viscosities of the solutions at an amino group concentration of 30 m*M* prepared from chitosan samples of different molecular mass increased over the reaction time except for C48 and C51 chitosan as shown in Figure 6. The results from Figures 5 and 6 show the viscosities of the chitosan solutions more sharply increased with an increase in the amino group concentration irrespective of the molecular mass of chitosan used, and the increase in the molecular mass of chitosan

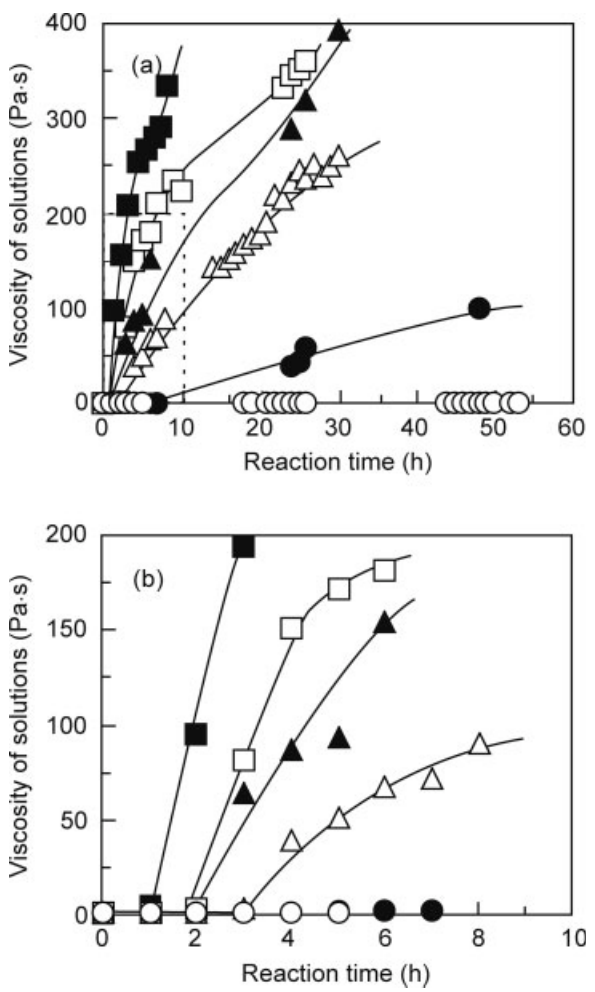

Figure 6 The effect of molecular mass of chitosan on the increase in the viscosity with the enzymatic conversion of dopamine and subsequent nonenzymatic reaction with chitosan at 25°C. (b) is an expanded view in the range of below 10 h. Chitosan sample—○: C48, ●: C51, △: C560, ▲: C-890, □: C1100, ■: C2500. Chitosan's amino group concentration: 30 m*M*.

led to the increase in the viscosity of the solution. Subsequently, a reduction of the time required for gelation was investigated by the addition of PEG to a C-1100 chitosan solution.

Since the gelation was observed by the reaction of quinone enzymatically generated from dopamine with chitosan, the effect of the addition of PEG on the gelation of the chitosan solutions was investigated. The enzymatic reaction was initiated by adding tyrosinase to the chitosan solutions at an amino group concentration of 30 m*M* containing dopamine in the presence of PEGs of different molecular mass. The effect of molecular mass of PEG added on the increase in the viscosity of the C-1100 chitosan solution containing dopamine and tyrosinase is shown in Figure 7(a). Compared with a chitosan solution without PEG, the viscosity of the 100 K-PEG-added C1100 chitosan solutions increased at shorter reaction times. In the case when 100 K-PEG was added to the C1100 chitosan solution, the viscosity most sharply increased. This finding was in agreement with the result shown in Figure 2. Based on the results obtained in Figure 7(a), the effect of the molecular mass of PEG on the gelation of the chitosan solution was investigated using 100 K-PEG. As shown in Figure 7(b), the viscosity sharply increased 0.5 h after 100 K-PEG was added to the chitosan solution irrespective of the PEG concentration. The viscosity more sharply increased with an increase in the 100 K-PEG concentration. It was found that the addition of PEG to the chitosan solutions containing dopamine and tyrosinase is an effective procedure in increasing the viscosity of the chitosan solution at shorter reaction times. At an 100 K-PEG concentration of 1.0 mg/cm^3 a highly viscous solution was obtained only 1–2 h after the enzymatic reaction started. The inhibition of the tyrosinase activity was considered to be depressed by the interaction between PEG chains and enzymatically generated quinones. As shown in the above section, the in-depth interpretation has been made by Nappi et al. in terms of the configuration of tyrosinase and interaction between tyrosinase and enzymatically generated compounds.[29]

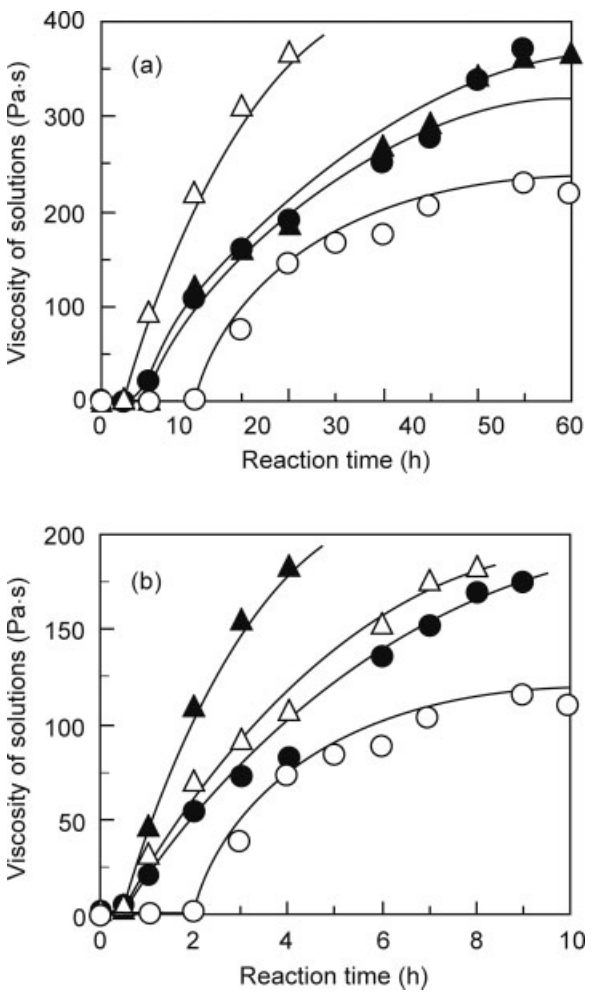

Figure 7 The effects of (a) molecular mass of PEG and (b) 100 K-PEG concentration on the increase in the viscosity with the enzymatic conversion of dopamine and subsequent nonenzymatic reaction with C1100 chitosan in the presence of PEG at 25°C. (a) PEG sample—○: without PEG, ●: 4.6 K-PEG, △: 100 K-PEG, ▲: 200 K-PEG. (b) 100 K-PEG concentration (mg/cm^3)—○: 0, ●: 0.2, △: 0.6, ▲: 1.0. C1100 chitosan's amino group concentration: 30 m*M*.

Adhesive strength under water

Here, the effects of both amino group concentration and molecular mass of chitosan were investigated on the increase in adhesive strength of the glass slides under water. Figure 8(a) shows the increase in the water-resistant adhesive properties against the reaction time for the chitosan solutions of an amino group concentration of 30 m*M* in the absence of PEG. After reaction for different times, the material was spread onto two clean and dry glass slides. The glass slides were then lapped together, clipped, and immediately submerged in water for 48 h. Adhesive strength was little developed irrespective of the reaction time and amino group concentration for C48 and C51 chitosan. A main reason given is that a considerable increase in the viscosity was little observed as shown in Figure 7 for C48 and C51 chitosan. To the contrary, adhesive strength increased over the reaction time for other four kinds of chitosan samples, and increased with an increase in the molecular mass of the chitosan samples used. Subsequently, the effect of amino group concentration on the increase in adhesive strength using C1100 chitosan is shown in Figure 8(b). As the amino group concentration of C1100 chitosan increased, adhesive strength increased. This is because the viscosity of the solutions more sharply increased with an increase in the amino group concentration of C1100 chitosan as shown in Figure 5. Figure 8 shows that when the reaction mixture contained chitosan, dopamine, and

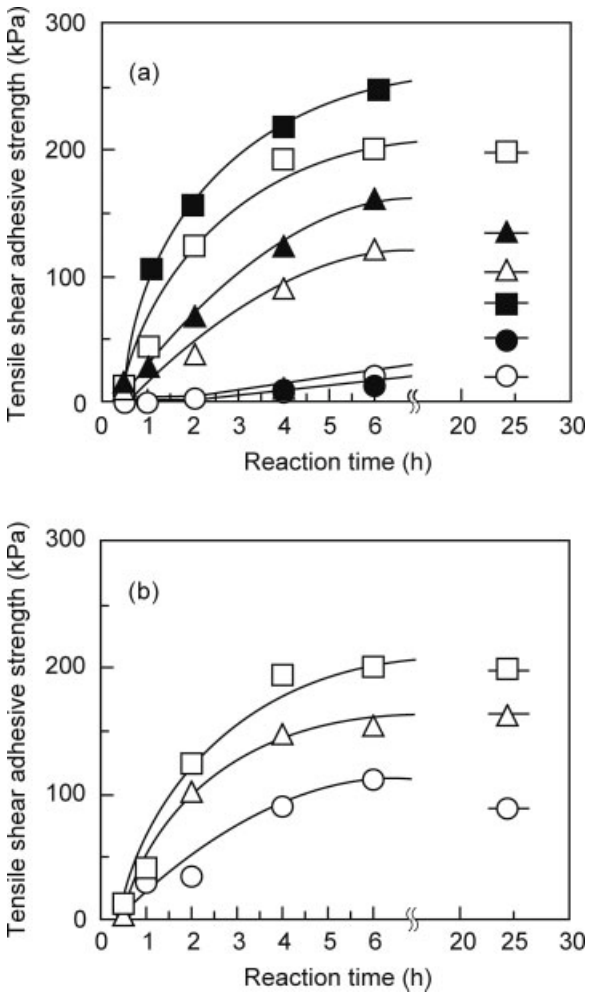

Figure 8 The effects of (a) molecular mass of chitosan and (b) C1100 chitosan's amino group concentration on the increase in adhesive strength of the glass slides. (a) Chitosan sample—○: C48, ●: C51, △: C560, ▲: C890, □: C1100, ■: C2500. Chitosan's amino group concentration: 30 m*M*. (b) C1100 chitosan's amino group concentration (m*M*)—○: 10, △: 20, □: 30. The reaction solutions contained chitosan, dopamine (10 m*M*), and tyrosinase (60 U/cm^3). The lapped glass slides were immersed in water for 48 h.

tyrosinase, considerable adhesive strength was required to separate the glass slides. If the reaction mixture contained only two components such as chitosan and tyrosinase, chitosan and dopamine, and tyrosinase and dopamine, the glass slides separated immediately after unclapping. In addition, the results in Figure 8 indicate that tyrosinase and dopamine can confer water-resistant adhesive properties to chitosan. It was found that as molecular mass of chitosan and chitosan's amino group increased, adhesive strength more sharply increased.

Effect of PEG on adhesive strength

Adhesive strength was also estimated of chitosan materials gelled in the presence of PEG, since gelation proceeded at shorter reaction times by the addition of PEG as shown in Figure 7(a,b). Gelation was initiated by adding PEGs of different molecular mass to a C1100 chitosan solution of 30 m*M*, and then chitosan materials were spread on the glass plates. Figure 9(a) shows the effect of molecular mass of PEG added on the increase in adhesive strength for the C1100 chitosan solutions of 30 m*M*. Here, the tightly lapped glass slides were immersed in water for 48 h after the highly viscous, gel-like modified chitosans were spread on the glass slides.

Even though 4.6 or 200 K-PEG was added to a chitosan mixture, a tendency to increase adhesive strength against the reaction time was left almost unchanged compared with a C1100 chitosan solution gelled in the absence of PEG. When C1100 chitosan materials pre-

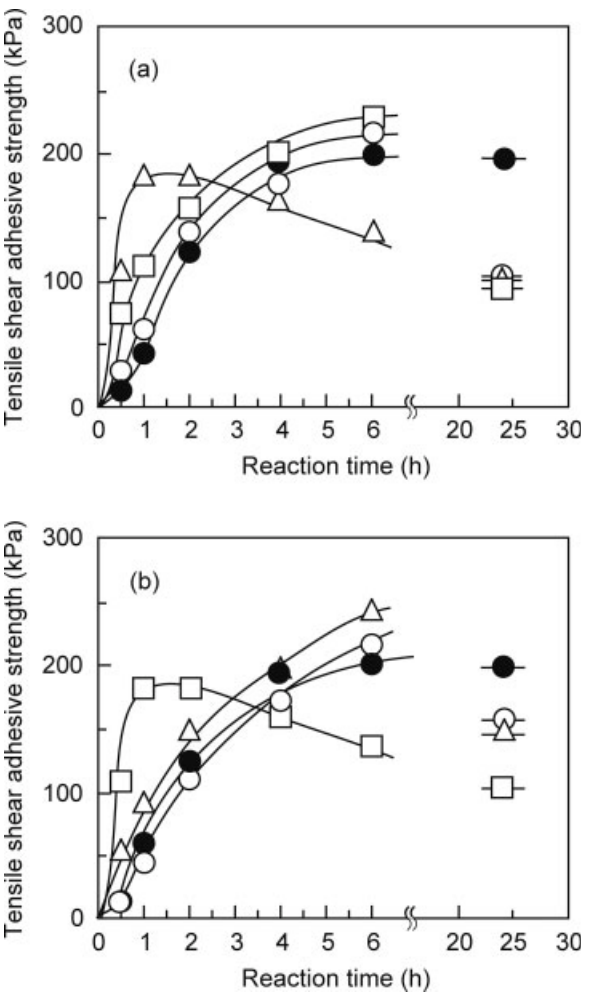

Figure 9 The effects of (a) molecular mass of PEG and (b) 100 K-PEG concentration on the increase in adhesive strength of the glass slides. (a) PEG sample—●: without PEG, ○: 4.6 K-PEG, △: 100 K-PEG, □: 200 K-PEG. (b) 100 K-PEG concentration (mg/cm^3)—●: 0, ○: 0.2, △: 0.6, □: 1.0. The reaction solutions contained C1100 chitosan (amino group concentration: 30 m*M*), dopamine (10 m*M*), tyrosinase (60 U/cm^3), and PEG. The lapped glass slides were immersed in water for 48 h.

pared in the presence of 100 K-PEG were spread on the glass slides, adhesive strength sharply increased at shorter reaction times. However, adhesive strength gradually decreased at further reaction times. It is because the viscosity of the chitosan mixture was too high and crosslinking reaction heterogeneously occurred. In addition, the changes in adhesive strength with the reaction time for C1100 chitosan solutions of 30 m*M* in the presence of 100 K-PEG at different concentrations are shown in Figure 9(b). Adhesive strength increased over the reaction time at the 100 K-PEG concentrations of 0.2 and 0.6 mg/cm^3. When the 100 K-PEG concentration further increased up to 1.0 mg/cm^3, adhesive strength sharply increased at shorter reaction times. Subsequently, the effect of the immersion time of the tightly lapped glass slides under water on adhesive strength for chitosan materials gelled in the presence of PEG. A chitosan solution reacted for 4 h was spread on the surfaces of the glass slides, and then the glass slides were lapped and submerged them in water.

Figure 10 shows the changes in adhesive strength with the immersion time for the C1100 chitosan solutions gelled for 4 h in the presence of PEGs of different molecular mass. 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 24 h in the absence of PEG because gelation of chitosan solutions will proceed between the lapped glass plates in water after the spread. By the addition of PEG (reaction time = 4 h), adhesive strength increased up to value comparable with that obtained for a C1100 chitosan mixture gelled for 24 h in the absence of PEG irrespective of molecular mass of PEG used. It was found that the addition of PEG led to the increase in adhesive strength at shorter reaction times. In addition, adhesive strength linearly increased with an increase in the immersion time of the tightly lapped glass slides either in the presence or absence of PEG. Gelation of chitosan materials spread would be more advanced between the glass slides, although it is impossible to measure the viscosity after the spread of chitosan materials. In our previous article,[6] adhesive strength obtained by chitosan materials gelled enzymatically 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 crosslinked with glutaraldehyde was 280 kPa. As shown in Figure 8, adhesive strength obtained for C2500 chitosan came up to that obtained for the chitosan gel crosslinked with glutaraldehyde. In addition, it was found from Figure 10 that adhesive strength went over the value obtained for the chitosan gel crosslinked with glutaraldehyde by increasing the immersion time. The results mentioned above will open up the possibility of chitosan materials gelled through the enzymatic reaction toward the practical application as a water-resistant adhesive.

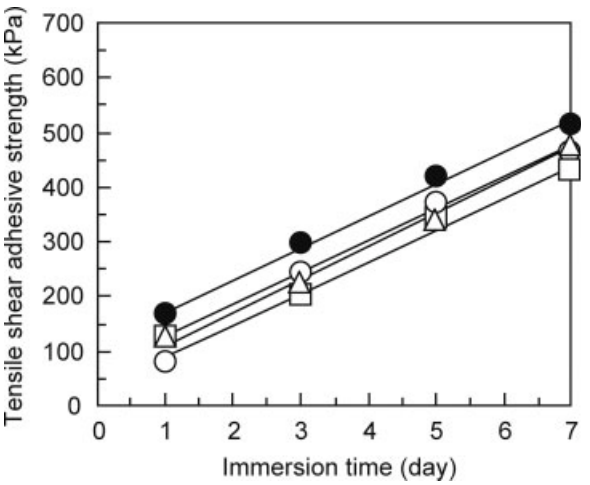

Figure 10 The effect of immersion time on the increase in adhesive strength of the glass slides. PEG sample—●: without PEG, ○: 4.6 K-PEG, △: 100 K-PEG, □: 200 K-PEG. Reaction time (h)—open: 4, shaded: 24. The reaction solutions contained C1100 chitosan (amino group concentration: 30 m*M*), dopamine (10 m*M*), and tyrosinase (60 U/cm^3) in the presence of PEG (1.0 mg/cm^3).

CONCLUSIONS

The experimental results obtained in this study indicate that tyrosinase-catalyzed reactions of dopamine can be used to confer water resistant adhesive properties to dilute chitosan solutions. The observed 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. In addition, the addition of PEG led to the increase in the viscosity of the chitosan solutions and adhesive strength at shorter reaction times. Since chitosan is typically obtained from wastes generated from crustacean processing, these materials are renewable and environmentally friendly.

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