Gelation and Adhesion Behavior of Mussel Adhesive Protein Mimetic Polymer

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ABSTRACT: An acrylamide-type copolymer containing catechol, amino, and hydroxyl groups was synthesized as a mimetic of the natural mussel adhesive protein (MAP). The obtained copolymer in a phosphate buffer solution (pH = 8.0) formed a hydrogel within 2 h under air, whereas gelation did not proceed under argon atmosphere. We confirmed that the crosslinking reaction of the synthesized MAP mimetic copolymer was triggered by aerobic oxidation of catechol moieties to form an adhesive hydrogel. Two aluminum plates were adhered by the gelation of the MAP mimetic copolymer solution under humid air at room temperature. The interfacial region between the two aluminum plates failed at a lap shear strength of 0.46 MPa due to cohesive failure of the hydrogel. The adhesion strength was dominated by mechanical strength of the hydrogel as well as the interface interaction of catechol groups with substrate surface. © 2012 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2013**, *51*, 1058–1065

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INTRODUCTION Mussels in nature are able to firmly anchor to various material surfaces even under wet conditions; however, it is still difficult to artificially develop a comparable adhesion technology. Understanding the materials and mechanisms of the fascinating underwater adhesion system is useful for developing biological glue that can be applied for the adhesion of cells or organisms.

Mussels are able to anchor to underwater surfaces by secretion of byssus adhesive plaque composed of fp-1 to fp-6 proteins.¹⁻⁴ Each of these proteins has a high content of lysine and a "non-standard" amino acid, dihydroxyphenylalanine (DOPA). In particular, the catechol moiety in DOPA is essential for adhesion.^{5,6} The catechol unit plays two roles in adhesion. One is the tethering to surfaces⁷ through stable bidentate hydrogen bonding with metal oxides.⁸⁻¹⁰ The other is the formation of organs by cross-linking. Oxidation of catechol units easily undergo by the oxygen in water to give a catechol quinone. The quinone induces a cross-linking reaction through radical coupling or nucleophilic attack by amine.^{11,12} Therefore, the DOPA is used as a surface modification reagent. When the substrate was immersed into the dopamine aqueous solution, dopamine is readily adsorbed on the substrate surface to form a unique polydopamine thin film by the successive oxidation and cross-link reaction. Further modification of the polydopamine films was also

investigated.¹³ For example, polydopamine film was transformed to a surface initiator layer for the surface-initiated polymerization to form a grafting polymer.¹⁴

During last two decades, various types of catechol-containing polymers have been synthesized to investigate the nature of mussel adhesive protein (MAP) or the functions of the catechol group. Yamamoto chemically synthesized a polypeptide that had a natural fp-1 sequence.¹⁵ The adhesion strength of two metal pieces using an aqueous solution of the polymer was investigated. Deming and coworker synthesized not only a natural-sequence polypeptide but also mimetic polypeptides as well.¹⁶ They synthesized a copolymer composed of lysine and DOPA and measured the adhesion strength of metal adherends. Wilker and coworkers synthesized catechol-containing polystyrene, which improved the adhesion strength of aluminum adherends by the anchoring and cross-linking of catechol units.^{17,18} Adhesion in aqueous media has also been investigated using the strong interaction between catechol groups and metal oxides. Messersmith and coworkers reported that photocured polyethyleneoxide gel containing catechol groups successfully adhered the titanium surfaces in water.¹⁹ Geckos pad-inspired microfabricated pillars coated with catechol-containing polyacrylamide derivatives also achieved adhesion under wet condition.²⁰

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SCHEME 1 Synthesis of MAP mimetic polymer.

MAPs in nature have not only catechol group but also hydroxyl and amino groups in the amino acids. Each functional group must have a unique role in achieving stable adhesion in an aqueous environment. As mentioned above, catechol groups worked as anchoring sites on metal oxide surface. Hydroxyl groups improve the hydrophilicity of the proteins. Amino groups are expected to promote the crosslinking reaction through the aerobic oxidation. In this study, we designed MAP mimetic acrylamide copolymers containing these three functional groups to investigate the effect of each groups on the adhesion properties (Scheme 1). Acrylamides are the useful water soluble monomers for synthesis of the functional copolymers with desired compositions by a conventional radical polymerization. In addition, water soluble "vinyl polymers" containing catechol moiety has not been reported so much until now. We synthesized water soluble acrylamide copolymers containing hydroxyl, amino, and catechol units with various compositions. The relationship between adhesion properties and the compositions of the each functional group in the copolymers were investigated. The effect of oxidant reagent on the gelation process and adhesion ability of the obtained copolymer under wet condition were also investigated.

EXPERIMENTAL

Materials

Dry tetrahydrofuran (THF, Kishida Chem., Osaka, Japan) purified using an organic solvent pure unit (GlassContour Solvent Dispensing System, Nikko Hansen, Osaka, Japan) was used. Dimethylformamide (DMF, Wako Pure Chemical, Osaka, Japan) was dried on a molecular sieve 4A and distilled from calcium hydride before use. Triethylamine (Wako) was dried over potassium hydroxide. Methanol (MeOH, Wako) was distilled before use. 2,2'-Azobis(isobutyronitrile) (AIBN, Wako) was recrystallized from methanol before use. N-(2-Hydroxyethyl)acrylamide (Tokyo Chemical, Tokyo, Japan) was purified by flash column chromatography using aluminum oxide (Merck, Tokyo, Japan) before use. 3-Hydroxytyramine hydrochloride, acryloyl chloride, chlorotriethylsilane, N-(tert-butoxycarbonyl)-1,6-diaminohexane, and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were purchased from Tokyo Chemical and used as received. Aluminum plates ($20 \times 50 \text{ mm}^2$) for the adhesion tests were washed with hexane, ethanol, and water and dried under nitrogen gas. Glass plates ($10 \times 40 \text{ mm}^2$) were washed with a mixture of sulfuric acid and hydrogen peroxide (7/3, v/v)at 100 °C for 1 h, rinsed with water, and dried under nitrogen gas. Polytetrafluoroethylene (PTFE) plates (10 imes 40 mm²) were washed with ethanol and dried under nitrogen.

Synthesis of *N*-(2-[3,4-Dihydroxyphenyl]ethyl) acrylamide (<u>1</u>)

3-Hydroxytyramine hydrochloride (10.0 g, 52.8 mmol) and triethylamine (7.31 mL, 52.7 mmol) were dissolved in MeOH (100 mL), and cooled on an ice bath. THF solution (5 mL) of acryloyl chloride (5.11 mL, 63.2 mmol) and MeOH solution (11 mL) of triethylamine (11.0 mL, 79.1 mmol) were alternately added dropwise to the hydroxyramine solution maintaining the pH at 9. After adding the reagent, the reaction mixture was stirred at room temperature for 1 h. Solvent was removed under vacuo. The residue was dissolved in ethyl acetate and washed with 1 M hydrochloric





FIGURE 1 ¹H NMR spectra of protected polymer (in CD₃OD) and target polymer (in D₂O).

acid (HCl) and brine. The organic layer was dried over sodium sulfate, then filtrated, and concentrated by evaporation. The product was dissolved in ethyl acetate to repeat the recrystallization, giving the compound (1) (7.06 g, 34.1 mmol) in a 65% yield as a white solid.

¹H NMR (400 MHz, CD₃OD, δ ppm); 6.67 (1H, d, $J_1 = 8.0$), 6.64 (1H, d, $J_1 = 2.0$), 6.52 (1H, dd, $J_1 = 8.0$, $J_2 = 2.1$), 6.19 (2H, m), 5.62 (1H, dd, $J_1 = 6.8$, $J_2 = 5.3$), 3.40 (2H, t, $J_1 = 7.4$), 2.66 (2H, t, $J_1 = 7.4$).

Synthesis of *N*-(2-[3,4-Ditriethylsilyloxyphenyl]ethyl)acrylamide (2)

Triethylchlorosilane (1.75 g, 11.6 mmol) was added dropwise to a mixture of compound <u>1</u> (1.00 g, 4.83 mmol) and triethylamine (2.41 mL, 17.4 mmol) dissolved in DMF (10 mL) on an ice bath. The reaction mixture was stirred for 1 h at 0 °C and 2 h at room temperature. Chloroform was added to the reaction mixture, which was washed with 1 N HClaq, sat. NaHCO₃aq, and brine. The organic layer was dried over sodium sulfate, filtrated, and dried *in vacuo*. The crude product was purified by silica gel column chromatography using hexane/ethyl acetate (3/1, v/v) as an eluent. The compound (<u>2</u>) was obtained as a viscous colorless liquid (2.07 g, 4.75 mmol) in 98% yield.

¹H NMR (400 MHz, CDCl₃, δ ppm); 6.75 (1H, d, $J_1 = 8.0$), 6.61–6.65 (2H, m), 6.24 (1H, dd, $J_1 = 17.0$, $J_2 = 1.4$), 6.00 (1H, dd, $J_1 = 16.9$, $J_2 = 10.3$), 5.62 (1H, dd, $J_1 = 10.3$, $J_2 =$ 1.4), 5.51 (1H, s), 3.54 (2H, m), 2.72 (2H, t, $J_1 = 6.8$), 0.98 (18H, m,), 0.74 (12H, m).

Synthesis of *N*-[*N*'-(Tert-butoxycarbonyl)-6-aminohexyl] acrylamide (3)

To a solution of *N*-(*tert*-butoxycarbonyl)-1,6-diaminohexane (3.01 g, 13.9 mmol) and triethylamine (2.88 mL, 20.8 mmol) diluted with THF (30 mL), THF solution (2 mL) of acryloyl chloride (1.34 mL, 16.6 mmol) was added dropwise at 0 $^{\circ}$ C, and the mixture was stirred at room temperature for 1 h. After the reaction mixture was evaporated, the residue was

dissolved in ethyl acetate, washed with 10% citric acid solution, sat. NaHCO₃aq, and brine. The organic layer was dried on sodium sulfate, filtrated, and dried *in vacuo*. The resulting solid was recrystallized from ethyl acetate to give the compound (3) (3.23 g, 12.0 mmol) in 72% yield as a white solid.

¹H NMR(400 MHz, CDCl₃, δ ppm); 6.28 (1H, dd, $J_1 = 17.0$, $J_2 = 1.6$), 6.10 (1H, dd, $J_1 = 17.0$, $J_2 = 10.3$), 5.80(1H, s), 5.63 (1H, dd, $J_1 = 10.3$, $J_2 = 1.6$), 4.54 (1H, s), 3.27 (2H, m), 3.12 (2H, m), 1.55 (2H, m), 1.48 (2H, m), 1.44 (9H, s), 1.35 (4H, m).

MAP Mimetic Copolymer Synthesis

A methanol solution (5 mL) of monomer 2 (218 mg, 0.500 mmol), 3 (135 mg, 0.499 mmol), hydroxyethylacrylamide (575 mg, 0.499 mmol), and AIBN (19.4 mg, 0.116 mmol) was degassed by the freeze-thaw method three times. The reaction vessel was sealed *in vacuo*, and stirred for 14 h at 60 °C. The reaction mixture was diluted with methanol and poured into ether to precipitate the resulting polymer, which was collected by centrifugation (3000 rpm, 10 min), washed with ether three times, and dried *in vacuo*. The precopolymer with $M_n = 91,600$ and $M_w/M_n = 3.6$ was obtained in a 90% yield.

Aqueous hydrochloric acid solution (2 M, 7 mL) was added to the methanol solution (7 mL) of precopolymer (730 mg) at 0 °C and was stirred at room temperature for 14 h to proceed the acidic hydrolysis of silyloxy groups on catechol units and the removal of Boc groups from amino groups. The reaction mixture was poured into ether to precipitate the deprotected polymer, which was collected by centrifugation, washed with ether three times, and dried *in vacuo*. The residue was dissolved in water and dialyzed to remove the hydrochloric acid and then lyophilized. The MAP mimetic polymer (608 mg) was obtained as a white powder. The polymer was stored at -20 °C in a freezer and was stable under these this conditions.

Characterization

 ^1H NMR spectra were recorded in chloroform-d (CDCl_3), CD_3OD, or D_2O on a Bruker AV400N (^1H 400.13 MHz, ^{13}C

TABLE 1 Code Name of the MAP Mimetic Copolymers and

 Contents of Catechol and Amine Units

Polymor Codo	<i>x/y/z</i> (Molar Ratio) ^b	Contents (mol %)	
Name ^a		Catechol	Ammonium
P(10H-1C-1A)	10/1/1	0.08	0.08
P(5H-1C-1A)	5/1/1	0.14	0.14
P(6H-1C)	6/1/0	0.14	0.00
P(11H-1C)	11/1/0	0.08	0.00
P(20H-1C-1A)	20/1/1	0.045	0.045
P(21H-1C)	21/1/0	0.045	0.00
P(10H)	10/0/0	0.00	0.00
P(10H-1A)	10/0/1	0.00	0.08

 $^{\rm a}$ Remarks: (H) = N-(2-hydroxyethyl) acrylamide, (C) = N-(2-[3,4-dihydroxyphenyl]ethyl) acrylamide, (A) = 6-(acrylamidehexyl)ammonium chloride.

 $^{\rm b}$ Molar ratio of x/y/z corresponds to H/C/A unit ratio in the copolymers, respectively.

100.61 MHz) spectrometer, with tetramethylsilane or solvent as an internal standard. Number- and weight-average molecular weights (M_w and M_n) were estimated by size-exclusion column chromatography on a Shimazu HPLC system (Shimadzu Corporation, Kyoto, Japan), equipped with two consecutive polymethylmethacrylate gel columns (TSKgel SuperAW4000 and TSKgel SuperAW5000, Tosoh Corporation, Tokyo, Japan) and refractive index and ultraviolet (254 nm) detectors, using DMF solution of 0.01 M LiBr at an eluent flow rate of 1.0 mL/min, calibrated with polystyrene standards. Water content of adhesive layers was determined from weight loss during heating, recorded by a Pyris TGA (PerkinElmer Japan, Kanagawa, Japan).

A gelation process was evaluated by a rheometer (Anton Paar Physica MCR 101) with parallel geometry (diameter 50 mm). MAP mimetic polymer solution (5 wt %) in phosphate buffer (0.1 M, pH = 8.0) was prepared and quickly put on a sample stage (thinkness: 0.2 mm). Then storage modulus (G') and loss modulus (G') were measured at 5 min intervals (strain 1%, frequency 1 Hz, 25 °C).

The adhesion strength was determined by measuring the lap shear adhesion force with a tensile tester (Shimadzu EZ-Graph) at 298 K in an ambient atmosphere. An amount of 10 mg of 10 wt % solution of MAP mimetic polymer in phosphate buffer solution (0.1 M, pH = 8.0) was mounted

on the aluminum plate and was bound with the other plate. The adhesion area was 100 mm². The substrates were allowed to stand under humid air (>90% relative humidity) at room temperature for 24 h. The sample was held at both ends with two mechanical chucks connected to a load cell and a tensile tester base anchor. The crosshead speed was set to 1 mm/min in tensile mode. The lap shear strength was defined as the force corresponding to the failure force divided by an adhesion area of 100 mm².

RESULTS AND DISCUSSION

Synthesis of MAP Mimetic Polymer

MAPs in nature are mainly composed of certain amino acids containing hydroxyl, amino, and catechol groups. Each functional group plays a unique role in achieving stable adhesion in an aqueous environment. Catechol groups form bidentate type hydrogen bonds with metal oxide surfaces to anchor the proteins. Catechol and amino groups are used for crosslinking reaction of proteins followed by aerobic oxidation. Hydroxyl groups are useful for improving water solubility of the proteins. Based on this knowledge, the target material was designed as an acrylamide terpolymer containing catechol, amino, and hydroxyl groups. To obtain this target polymer, triethylsilyl-protected catechol monomer 2 and Boc-protected amino monomer 3 were copolymerized with hydroxyl monomer (H) by AIBN-initiated radical polymerization. ¹H NMR spectrum of the polymer [Fig. 1(a)] showed characteristic peaks attributed to each monomer unit, such as triethylsilyl groups (1.5 and 1.0 ppm) and aromatic proton (6.6 ppm) in catechol monomer 2, tert-butyl group (1.4 ppm) in amino monomer 3, and oxymethylene group (3.6 ppm) in hydroxyl monomer H. All protection groups were successively removed by acid treatment simultaneously. Removal of each protection group was confirmed by ¹H NMR spectrum [Fig. 1(b)]. Signals from the tert-butyl group and triethylsilyl group disappeared completely. Monomer ratio in the obtained polymer was estimated from this spectrum. The peaks at 3.5 ppm ($-CH_2$ -OH) for hydroxyl group, at 2.5 ppm ($-CH_2$ -Ar) for catechol group, and at 2.8 ppm $(-CH_2-NH_2)$ for amino group were used for the estimation. The ratio of each peak area was determined as 10/0.97/ 0.97, which agreed well with feed ratio (10/1/1). These results clearly indicate that the target polymer was quantitatively obtained. The monomer units of 2 and 3 in the copolymer were successfully converted to catechol unit (C) and ammonium chloride unit (A), respectively.



FIGURE 2 Result of the gelation test of MAP polymer solution in phosphate buffer (pH = 8.0). (a) Gel formed in air with 2 h. (b) Gel formed tyrosynase containing solution with 20 min. (c) Polymer solution under argon atmosphere after 24 h.





FIGURE 3 Time course of storage modulus (*G*') and loss modulus (*G*') (strain 1%, frequency 1 Hz, 25 °C) during gelation of MAP mimetic P(10H-1C-1A) phosphate buffer solution (pH = 8.0).

We prepared several types of copolymers having various comonomer ratio of x/y/z corresponding to the unit ratio of H/C/A, as listed in Table 1. The copolymer samples were named after the comonomer ratio, such as P(10H-1C-1A), which is the copolymer consisting of H, C, and A monomer units at the molar ratio of 10/1/1.

Main component of mussel adhesion protein (fp-1) is composed from decapeptide repeating units and contain one Dopa and two lysine residues in the repeating unit.¹ We set the catechol group composite almost same with fp-1, and same ratio of amino group was adopted. In this report, gelation and adhesion properties of P(10H-1C-1A) were mainly discussed.

Gelation of MAP Mimetic Polymer Solution by Aerobic Oxidation

To achieve adhesion, fluid adhesives have to be solidified for formation of adhesive layer and anchoring adhesives to the surfaces.²¹ Therefore, gelation of polymer solution is essential in this system. Gelation of polymer solution was investigated first.

A solution of P(10H-1C-1A) formed a gel under ambient air conditions. This polymer had catechol moieties, cross-linking reaction triggered by aerobic oxidation of catechol groups in the polymer. The synthesized P(10H-1C-1A) was dissolved in basic phosphate buffer solution. The pH of the solution was set at 8.0. It is known that the oxidation reaction of catechol is accelerated under basic conditions,²² such as sea water of which pH is almost 8. The solution gradually became deep

TABLE 2 Lap Shear Adhesive Strength of MAP MimeticPolymers and Reference Polymers

Polymers	Lap Shear Adhesive Strength
P(10H-1C-1A)	0.46 \pm 0.11 MPa
Synthesized MAP ^a	0.25 MPa
P(10H)	No adhesion
P(10H-1A) + adipic acid + EDC	0.024 \pm 0.01 MPa
^a Ref. 14.	



FIGURE 4 Lap shear adhesive strength of aluminum plates using MAP mimetic copolymers with various molar ratio of hydroxyl, catechol, and ammonium functional groups. Adhesive was aqueous solution of copolymer containing tyrosynase and buffers (pH = 8). Lap shear adhesive strength was measured under air atmosphere at 25 °C.

orange and viscous to lose fluidity. It took 2 h to form a hydrogel eventually [Fig. 2(a)]. Conversely, when tyrosinase was added to the P(10H-1C-1A), the viscosity quickly increased. A gel was formed within 20 min [Fig. 2(b)]. This result clearly indicates that the tyrosinase accelerated oxidation rate to induce rapid gel formation.

However, gelation did not occur under oxygen-free condition even if the tyrosinase was contained. Figure 2(c) represents gelation test under argon atmosphere. After oxygen gas in the buffer solution was removed by argon bubbling, MAP mimetic P(10H-1C-1A) was dissolved in the deoxygenized buffer solution under argon atmosphere. In this case, the polymer solution kept fluidity even after 24 h, and no gelation occurred. These results clearly indicate that gelation was triggered by aerobic oxidation of catechol units. Synthesized mimetic polymer showed aerobic gelation properties like a native adhesive protein.

Viscoelasticity of the copolymer solution during the gelation process was measured by rotational rheometer. MAP mimetic P(10H-1C-1A) dissolved in phosphate buffer solution (pH = 8.0) was placed between the stage and the parallel plate under air, then time course of G' and G" was measured (Fig. 3). The G' and G" of the solution increased with time until the 1000 min, indicating that the cross-linking reaction of P(10H-1C-1A) proceeded to form a gel by aerobic oxidation.

Adhesion of Aluminum Plates by Aqueous Gel Formation Triggered by Aerobic Oxidation

A portion of the aqueous solution of P(10H-1C-1A) containing tyrosinase and buffers was put on an aluminum plate and sandwiched with two aluminum plates. The sample was fixed by clamps and stored under humid atmosphere at room temperature for 24 h. Two substrates were adhered through gelation of the polymer solution. Lap shear adhesion strength is summarized in Table 2. The aluminum plates adhered by synthesized MAP mimetic P(10H-1C-1A) broke at **TABLE 3** Lap Shear Adhesive Strength of Various Substrates

 Using P(10H-1C-1A)

Substrates	Lap Shear Adhesive Strength (MPa)
Stainless steel	0.45 ± 0.02
Glass	0.34 ± 0.08
PTFE	0.011 ± 0.001

a lap shear strength of 0.46 MPa. This value was similar to the adhesion strength of synthesized MAP proteins reported previously by Yamamoto.¹⁵ Broken hydrogel remained on the failed face of each aluminum plate, indicating that cohesive failure took place at the polymer gel. The polymer interacted so strongly with the aluminum oxide surface that it broke inside the hydrogel layer.

As a reference experiment, P(10H) and P(10H-1A) bearing no catechol unit were used for adhesives instead of MAP mimetic polymer. A solution of the homopolymer P(10H) was sandwiched between two aluminum plates and stored under humid air for 24 h. Even after 24 h, the P(10H) remained as a viscous solution between the aluminum plates with no adhesion. A mixture of P(10H-1A), adipic acid, and EDC formed adhesive gel layer of the aluminum plates; however, the lap shear adhesion strength was lower level as 0.024 MPa. Therefore, adhesion by MAP mimetic polymer requires two factors; gel formation and strong interaction of polymers with substrate surface owing to catechol groups.

MAP contains lysine unit as well as the Dopa. The amino group in lysine work as pH-controller keeping the adhesion environment to be basic condition to promote the oxidation of Dopa. However, the further detail role is still unclear. In this study, adhesion strength of the copolymer with or without amino group was investigated. As shown in Figure 4, copolymer solution containing amino group showed higher lap shear adhesive strength. The pH of the polymer solution was adjusted to be 8, ammonium salt in polymer was converted to amino group. P(5H-1C-1A) and P(10H-1C-1A) revealed slightly higher adhesion strength compared with copolymer without amino group, such as P(6H-1C) and P(11H-1C). We supposed that amino groups promoted the oxidation of catechol and cross-linking reaction improving the mechanical strength of the resulting hydrogel.

Adhesion Strength of Various Substrates by MAP Mimetic Polymer

It is known that catechol units strongly interact with metal oxides surface through stable bidentate hydrogen bonding, whereas interactions of catechol with polymers are weak.²³ We adapted stainless steel, glass, and PTFE plates for adhesion test using test pieces. MAP mimetic P(10H-1C-1A) solution containing tyrosinase and buffers was put between two substrates, clumped, and stored under humid air for 24 h. As shown in Table 3, lap shear adhesion strength for stainless steel and glass was 0.45 and 0.34 MPa, respectively. Adhesion layer was broken with cohesive failure manner, which is the same behavior observed with aluminum plate adhesion. These results indicate that the interaction between the polymer gel and these material surfaces is much stronger than the shear strength of gel. Conversely, the lap shear strength of PTFE adhesion was 0.011 MPa. When the substrate was separated by lap shear test, the gel layer peeled off smoothly from one substrate. The failure took place at the interface between gel and PTFE surface.

Mechanical strength of MAP mimetic polymer gel formed between the substrates would be independent on the substrate materials. Therefore, low adhesion strength on PTFE substrate must be caused by the weak interaction of catechol groups in the gel and PTFE surface. Conversely, catechol units strongly interacted with metal oxide. The lap shear adhesion strength was dominated by mechanical strength of the hydrogel because the gel ruptured before the interface interaction was broken. Consequently, the improvement of physical strength of the hydrogel would be expected to give large lap shear strength.

Effect of Water on Adhesion by Aerobic Oxidation

The functions and structure of this polymer were learned from the natural system of MAPs. Mussels adhere to surfaces even in the aqueous environment using these adhesive proteins containing catechol groups. MAP mimetic polymer inspired by MAPs is also expected to work as an adhesive in aqueous condition. Thus, the effect of water on adhesion of the MAP mimetic polymer gel was examined by following two

Oxidation	Adhesion Process ^b	Lap Shear Adhesive Strength (MPa)	Water Content (%)
Aerobic oxidation	In humid air for 24 h	0.46 ± 0.11	10.5
Aerobic oxidation	In water for 24 h ^c	0.036 ± 0.16	83.1
Aerobic oxidation	In humid air for 24 h and in water for 24 h ^d	0.22 ± 0.01	13.8
$H_2O_2+Fe^{3+}$	In humid air for 24 h	0.48 ± 0.14	14.0
$H_2O_2 + Fe^{3+}$	In water for 24 h ^c	0.15 ± 0.05	16.8

TABLE 4 Lap Shear Adhesive Strength of P(10H-1C-1A)^a

^a Under air at 25 °C.

^b At 25 °C.

^c Adhesion Procedure 1.

^d Adhesion Procedure 2.





FIGURE 5 (a) Gradient gel formed by aerobic oxidation of aqueous solution of P(10H-1C-1A) in the presence of tyrosynase and buffers (pH = 8) under air for 24 h at 25 °C, and (b) swollen state of the resulting gradient gel in citric acid solution at 25 °C.

procedures. First one is adhesion in water. (Procedure 1) Immediately after the 10 wt % P(10H-1C-1A) solution containing tyrosinase was placed between the two aluminum plates, the plates were immersed in water. Twenty-four hours later, lap shear strength of the resulting plates was measured by tensile tester under air without any drying. The other method is adhesion in humid air and successive immersion in water. (Procedure 2) A solution of P(10H-1C-1A) with tyrosinase was placed between two aluminum plates and stored under humid atmosphere for 24 h. The adhered aluminum plates were immersed in water for 24 h, and then the lap shear strength was measured under air without any drying. In the case of Procedure 1, the aluminum plates were adhered but significantly low adhesion strength to be 0.036 MPa. This value was almost one-tenth the adhesion strength compared with gelation under humid air. In the case of Procedure 2, the lap shear adhesion strength was 0.22 MPa, maintaining half the value compared with that before water immersion. In each case, polymer gel remained at the face of the aluminum plates after the lap shear test. This means cohesive failure, and rupture was caused by the collapse of the hydrogel. The difference in adhesion strength between the two procedures was attributed to the mechanical strength of the gel. The hydrogel prepared in water was more fragile than the gel prepared in humid air.

Strength of the gel seems to be related with degree of swelling (water contents) of the gel. Water contents of adhesive gel layers formed in humid air (Sample a), formed in water (Sample b, Procedure 1), formed in humid air then immersed in water (Sample c, Procedure 2) were compared. Water content of the hydrogels was valuated after the adhesion test. The P(10H-1C-1A) gel layer was peeled away from the aluminum surfaces. This sample was heated from room temperature to 600 °C at the rate of 10 °C/min, and weight loss was recorded by thermogravimetric analyzer (TGA). Water mass was determined from the weight loss during heating from room temperature to 150 °C, and polymer mass was determined during heating from 150 to 600 °C. The water content of the gel formed under humid air (Sample a) was 10.5%, which is much lower value compared with initial solution of MAP mimetic polymer containing 90% of water. The water evaporated from the polymer solution during gelation process even under humid air conditions. Conversely, when adhesion was conducted in water by Procedure 1, water content in the gel (Sample b) was 83.1%. In addition, when adhesion was conducted in humid air then



FIGURE 6 Homogeneous gel prepared from aqueous solution MAP mimetic P(10H-1C-1A) containing tyrosynase and buffers (pH = 8) by oxidation with 1 mol % of H_2O_2 and Fe^{3+} for 5 min at 25 °C.

immersed in water for 24 h (Sample c, Procedure 2), the water content was 13.8%. The water content was increased by comparing with Sample a, but still lower than Sample c.

Relationship between lap shear adhesion strength and water contents in the gel is shown in Table 4. When the substrates were adhered in water (Sample b), the MAP mimetic polymer formed a gel with 83% content of water and low mechanical strength leading to low adhesion strength of 0.036 MPa. When the substrates were adhered under humid air for 24 h (Sample a), the hydrogel having relatively high strength was formed to show 0.46 MPa. Dipping the adhered substrates into water increased the water content in the gel (Sample c) to reduce the adhesion strength to 0.22 MPa. Adhesion strength closely related with water content in the MAP mimetic polymer gel. However, we should consider why water content of the gel formed in water (Sample b, Procedure 1) was much higher than that obtained under humid air (Sample c, Procedure 2).

We carefully observed the gel prepared by aerobic oxidation again. The color distribution in the gel formed by P(10H-1C-1A) was not uniform, which can be seen in Figures 1(a), 1(b), and 5(a). In general, the brown color appears by the oxidation and cross-linking reaction of catechol groups.²⁴ The characteristic deep brown color appeared near air–water interfaces, but inside of the hydrogel apart from the air interface were still pale yellow [Fig. 5(a)]. This gradient distribution of brown color suggested that cross-linking

densities were high near the air interface and low at inside of the hydrogel. The gradient of cross-linking density was also confirmed by the swelling test. When the gel was pulled out from the bottle and placed in the water, the faint color region swelled more than the deeper color region, as shown in Figure 5(b). These results suggest that the oxidation of catechol groups near the air interface region efficiently took place to give densely cross-linked gel. Conversely, hydrogel with low cross-linking density was formed inside the solution. This heterogeneous gel structure might be formed in the gap between the aluminum substrates to result in the low adhesive strength of MAP mimetic polymer. To improve the gel formation, we use reagent oxidization instead of aerobic oxidation.

Adhesion of Aluminum Plates by Gelation of MAP Mimetic Polymer Solution Triggered by Reagent Oxidation

For oxidizing the catechol groups in the polymer, a combination of hydrogen peroxide (oxidant) and Fe^{3+} (catalyst) was selected. The mixture of 5 wt % aqueous solution of MAP mimetic polymer, equimolar hydrogen peroxide, and 1 mol % $Fe(NO_3)_3$ became viscose rapidly to form hydrogel within few minutes, as shown in Figure 6. As we expected, the brown color of the gel was uniform. Rapid gel formation without oxygen was achieved.

A portion of 10 wt % aqueous solution of MAP mimetic P(10H-1C-1A) containing hydrogen peroxide and Fe(NO₃)₃ was sandwiched between two aluminum plates and then stored under humid atmosphere at room temperature for 24 h. Lap shear strength of the adhered sample was 0.48 MPa, as shown in Table 4, which is almost the same value as in the case of aerobic oxidation under humid air. However, the adhesion in water was improved as follows. Aqueous P(10H-1C-1A) solution containing hydrogen peroxide and $Fe(NO_3)_3$ as oxidant was placed between two aluminum plates, then they were immediately immersed in water and allowed to stand for 24 h at room temperature. The lap shear strength under air was 0.15 MPa, which is higher than the adhesion strength of 0.036 MPa observed by the sample previously prepared by Procedure 1. Water content in the gel formed with H_2O_2/Fe^{3+} system in water was 14–16%, which was greatly lower than the gel formed by aerobic oxidation in water (83%). This result suggests that the oxidizing agent markedly promoted homogeneous cross-linking reaction along with oxidation of catechol in the polymer solution. The resulting gel had high cross-linking density as an adhesive layer. As the result, adhesive strength was improved even in the water.

CONCLUSIONS

We synthesized a simple water soluble acrylamide copolymer, having hydroxyl, catechol, and amino groups, as a mimetic of the natural mussel adhesion proteins. The resulting polymer formed an adhesive hydrogel by oxidation of catechol groups triggered by oxygen in air or oxidant

reagents. Aluminum, stainless steel, and glass plates were adhered through the gelation of the polymer solution. Cohesive failure took place at the gel layer because of higher adhesive interaction between gel and substrate surface than the mechanical strength of the gel. The copolymer with higher content of catechol and amino groups showed higher adhesion strength. These results indicate that adhesion strength was mainly dominated by the mechanical strength of the gel. Adhesion in water was also achieved by addition of the oxidizing agent $Fe(NO_3)_3$ in the copolymer solution. These results clearly show that the synthesized polymer acts like the adhesive proteins. By mimicking nature's system, unique gel formation and adhesion properties were successfully introduced in an artificial acrylamide polymer. We expect that this polymer system will become a useful platform for constructing functional materials that work in water.

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