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Light-Triggered Adhesion of Water-Soluble Polymers with a Caged Catechol Group

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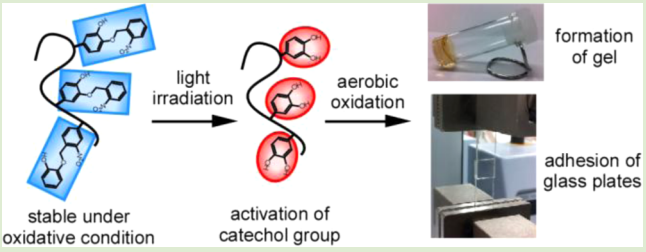
Supporting Information

ABSTRACT: An acrylamide-type copolymer containing hydroxyl, amino, and *ortho*-nitrobenzyl protected catechol groups was synthesized as a functional mussel adhesive protein (MAP) mimetic. The resulting copolymer was stable even in the oxidative condition. Light irradiation to aqueous solution of the copolymer induced deprotection of a caged compound to give a native catechol group and triggered an oxidative cross-linking reaction to afford the adhesive gel. Two glass plates were adhered through light-activated gelation of the polymer solution in a humid air atmosphere. A novel type of light-activated adhesive with excellent stability and light controllable activation properties was successfully fabricated by modification of the MAP mimetic copolymer.



Because of the fascinating adhesion properties of mussels in water, mimicking mussel adhesion is an interesting theme for biomimetic chemistry.[1] Mussels are able to anchor to underwater surfaces by secreting an adhesive plaque composed of mussel adhesive proteins (MAPs).[2] Each of these proteins has a high content of lysine and a "non-standard" amino acid, dihydroxyphenylalanine (DOPA).[3,4] Especially, the catechol moiety in DOPA plays two roles in adhesion: tethering to surfaces through stable coordination bonds with metal oxides[5,6] and the formation of insoluble poly(DOPA) derivatives by cross-linking. Catechol units are easily oxidized by the oxygen in water, environmental oxidant, and metal ions to form catechol quinone, which undergoes a cross-linking reaction through radical coupling or nucleophilic attack by amine.[7,8]

Many research groups have already synthesized various catechol-containing polypeptides,[9,10] polystyrene,[11−13] polymethacrylates,[14] polyacrylamides,[15] and polysaccharides,[16,17] to mimic the functions of MAPs. These mussel-inspired adhesives are expected as not only general glues for the bonding metal oxide materials, but also biological adhesives for wet tissue surfaces under the clinical environments.[18] The catechol units of the polymers are oxidized immediately under air or in a basic solution to form cross-linked gels, which enhance the adhesion strength. The gelation reaction is controllable by pH or oxidant concentration of the polymer solution. However, it is still difficult to control the gelation at desired time and position under wet conditions and stable storage of the adhesive polymers before use.

Messersmith et al. synthesized the thermosensitive adhesive hydrogel by poly(ethylene oxide)−poly(propylene oxide)−poly(ethylene oxide) triblock copolymers having the catecol unit at their chain ends[19] and rapid curing adhesive gel by photopolymerization of hydrophilic methacrylate monomers containing catechol groups.[14] Biocompatible hydrogel tissue adhesives have also been prepared by the mixing of the catechol-containing Chitosan and thiol-terminated Pluronic copolymer.[18] The gelation rate and activation of these polymers were well-controlled by temperature and light. Most of them used native catechol units as the mimicking MAP functions.

We propose here novel functional MAP mimetics with chemically modified catechol units by using "caged compounds", which contain photocleavable protecting groups. The activation of these compounds can be controlled by light irradiation at the desired time and place.[20,21] Because *ortho*-nitrobenzyl protecting groups can be easily deprotected by light irradiation, various derivatives containing these groups are widely used as cage biologically active molecules.[22−24] A caged catechol unit is also used as a light-activated hydrogen peroxide generator.[24]

In this study, MAP mimetics combined with the caged compound was designed. Caged MAP mimetics will be stable even under oxidative conditions, and can be activated at a suitable time and position by light irradiation (Figure 1). This approach not only mimics MAP but can also surpass natural systems by adding novel artificial chemical functions.

Mussel adhesive proteins 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 coordination bonds with metal oxide surfaces to anchor the proteins. Catechol and amino groups are used for cross-

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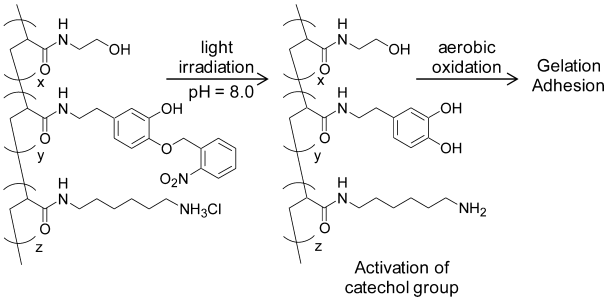
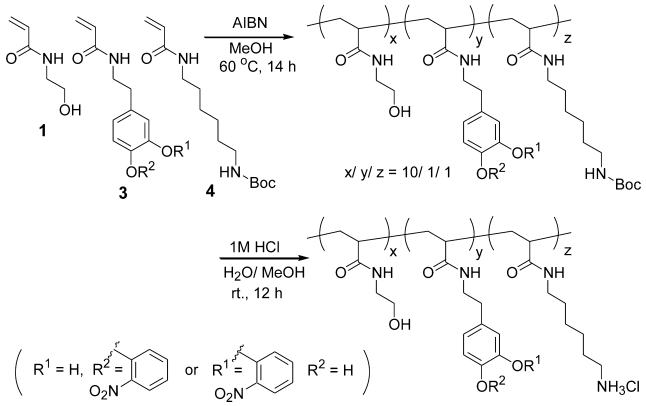


Figure 1. Light-activation of caged MAP mimetic polymer.

linking reaction of proteins after aerobic oxidation, while hydroxyl groups are useful for improving water solubility of the proteins.

A cross-linking reaction of catechol group is triggered by oxidation of the catechol group to result in the gelation and fixation of protein solution. In this study, the light-activated control of oxidation was investigated by using catechol with a photolabile protecting group in MAP mimetic polymer, as shown in Scheme 1. It was previously reported that oxidation of catechol was prevented by *ortho*-nitrobenzyl protecting groups, and deprotection by light irradiation was possible.[23]

Scheme 1. Synthesis of Caged MAP Mimetic Polymer



The synthesis procedures for acrylamide derivatives and model compound were described in Supporting Information. Briefly, commercially available *N*-(2-hydroxyethyl)acrylamide (**1**) was purified by flash column chromatography before use. *N*-(2-[3,4-Dihydroxyphenyl]ethyl)acrylamide (**2**) was obtained by the reaction of 3-hydroxytyramine hydrochloride and acryloyl chloride in MeOH/THF mixture at 0 °C. Acetone solution of **2** was refluxed with 2-nitrobenzyl bromide and potassium carbonate for 12 h to give a caged catechol monomer (**3**). Boc-protected monomer, *N*-[*N*′-(*tert*-butoxycarbonyl)-6-aminohexyl] acrylamide (**4**) was synthesized by *N*-(*tert*-butoxycarbonyl)-1,6-diaminohexane and acryloyl chloride in THF at 0 °C for 1 h.

Free radical copolymerization of monomers **1**, **3**, and **4** were carried out by AIBN in MeOH 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 ^{1}H NMR spectrum of the polymer (Figure 2a) showed characteristic peaks attributed to each monomer unit, such as aromatic (7.4−8.1 ppm) and

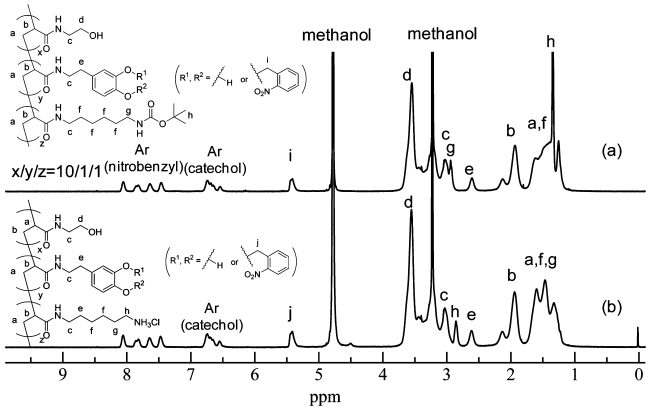


Figure 2. ^{1}H NMR spectra of (a) protected polymer and (b) target polymer (in CD_3OD).

benzyl (5.4 ppm) proton in *ortho*-nitrobenzyl group and aromatic proton (6.5−6.8 ppm) in caged catechol monomer **3**, *tert*-butyl group (1.4 ppm) in amino monomer **4**, and oxymethylene group (3.6 ppm) in hydroxyl monomer.

Aqueous hydrochloric acid solution (2 M, 3.5 mL) was added to the MeOH solution (3.5 mL) of precopolymer (348 mg) at 0 °C and stirred at room temperature for 14 h to remove Boc groups from amino groups, which was confirmed by ^{1}H NMR spectrum (Figure 2b). Signals due to the *tert*-butyl group completely disappeared, whereas peaks attributed to the *ortho*-nitrobenzyl group (7.4−8.1 and 5.4 ppm) were preserved. The ratio of peak area attributed to aromatic proton of nitrobenzyl to catechol was 4.0/3.0, which agrees well with the caged catechol unit. Therefore, the caged catechol group was not affected by acid treatment. The monomer ratio in the obtained polymer was estimated from integration ratio of the peaks at 3.6 ppm ($-CH_2-OH$) for the hydroxyl group, at 6.5−6.8 ppm (Ar-3H) for the catechol group, and at 2.9 ppm ($-CH_2-NH_2$) for the amino group. The ratio of each peak area was determined as 20/3.0/2.0, indicating that the comonomer ratio of **1**, **3**, and **4** was $x/y/z$ = 10/1/1, which agreed well with the feed ratio.

A model compound **5** was synthesized to evaluate the deprotection rate by light irradiation. Caged catechol **5** in a MeOH/$CHCl_3$ (3/1, v/v) solution, caged catechol features a moderate-intensity UV absorbance band centered at 278 nm (ε = 75700 M^{-1} cm^{-1}, Figure S1). Therefore, deprotection of caged catechol was conducted by visible light. The solution of **5** was irradiated under a mercury xenon lamp through a glass slide to filter out UV light. The time-course change of the concentration of caged catechol and catechol was determined by GC/MS (Figure 3). Almost all caged catechol disappeared within 30 min, and a concurrent increase of free catechol concentration was detected. Based on this result, the synthesized caged MAP mimetic polymer was irradiated for 30 min.

The synthesized caged MAP mimetic polymer was soluble in aqueous solution regardless of the hydrophobic *ortho*-nitrobenzyl protection group on the catechol group. The polymer dissolved in basic phosphate buffer solution (0.1 M, pH = 8.0) maintained fluidity without gelation even after 1 week. In contrast, when MAP mimetic polymer without protecting group was dissolved in this condition, gel was formed within 2 h.[15] The stability of the caged MAP mimetic copolymer against the oxidation was increased. Contrast between these gelation

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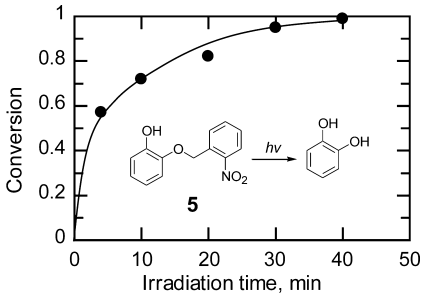

Figure 3. Time−conversion plot for the deprotection of model compound **5** by visible light. The conversion of the reaction was determined by GC/MS detection of **5** and catechol.

behavior clearly indicated that cross-linking reaction was inhibited by the nitrobenzyl ether protecting group through prevented the oxidation reaction of the catechol moiety as well as the transformation to the quinone structure.

The caged MAP mimetic polymer solution was irradiated with visible light for 30 min by using a mercury xenon lamp light source. UV light was filtered out by a glass plate placed between the lamp and solution. Removal of *ortho*-nitrobenzyl protecting group in the polymer might be expected under this reaction condition because the deprotection of the model compound **5** by light irradiation was completed in the same condition. After irradiation, a gel was formed apparently (Figure 4a), whereas no gelation was observed without light

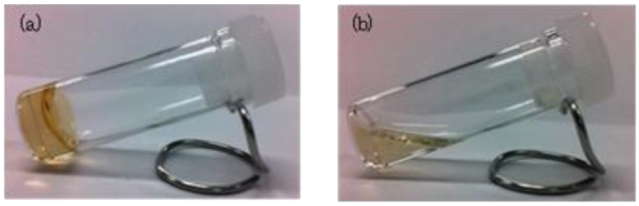

Figure 4. Caged MAP mimetic polymer in phosphate buffer solution (a) after light irradiation for 30 min and (b) without light irradiation.

irradiation (Figure 4b). This result clearly indicates that the protecting group on catechol was removed by light irradiation and cross-linking points were formed by subsequent oxidation reaction with molecular oxygen. Thus, we successfully obtained a novel functional biomimetic MAP polymer, which has good stability compared with native catechol compounds, and useful activity to light irradiation.

A phosphate buffer (0.1 M, pH = 8.0) solution of caged MAP mimetic polymer was put on a glass plate and sandwiched between two glass plates. The sample was fixed by clamps, and light was irradiated in air. Then, the sample was stored in a humid atmosphere at room temperature for 24 h to allow the oxidation reaction to proceed. The two substrates adhered through gelation of the polymer solution. The sample was set on a tensile tester and the lap shear adhesion strength was measured (Figure 5a). The glass plates adhered by caged MAP mimetic polymer broke at a lap shear adhesion strength of 0.061 ± 0.006 MPa (Figure 5b). Broken hydrogel remained on the failed area of each glass plate surface, indicating that cohesive failure took place at the polymer gel.

As a reference experiment, adhesion test without light irradiation was also conducted by using same caged MAP mimetic polymer solution. However, the polymer solution remained as a viscous solution between the glass plates with no

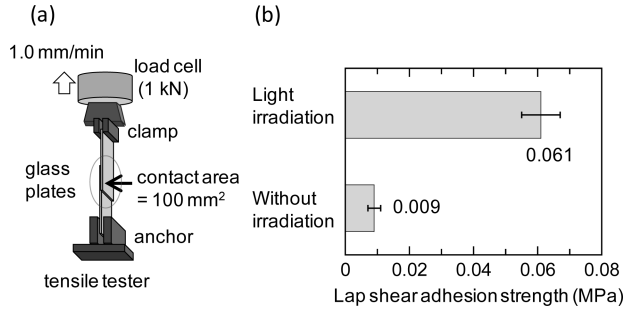

Figure 5. (a) Setup for measuring lap shear adhesion strength and (b) lap shear adhesion strength of glass plates adhered by caged MAP mimetic polymer after light irradiation or without irradiation (standard deviation was calculated based on five measurements).

adhesion. The lap shear adhesion strength of the sample was 0.0090 ± 0.002 MPa (Figure 5b). These results show that light-activated adhesion process was achieved by using the caged catechol containing polymer.

On the other hand, we already reported adhesion by MAP mimetic polymer (activated form of caged MAP mimetic polymer). In that case, the lap shear adhesion strength was 0.46 MPa.[15] The adhesion strength of caged mimetic polymer was about 13% of the previously reported value. To clarify a reason for low adhesion strength, the ^{1}H NMR spectrum of caged MAP mimetic polymer was measured after light irradiation under an argon atmosphere (Figure S4). We found that the protecting group was reduced but still remaining. The integration ratio of the peak of the nitrobenzyl group (7.4−8.1 ppm) and the catechol group (6.5−6.8 ppm) was 2.1/3.0. That means 54% of nitrobenzyl groups were remaining. In the case of light irradiation to model compound solution, almost all the protecting group was removed. Recombination of the cleaved protecting group might occur in aggregation of hydrophobic nitrobenzyl-catechol group in aqueous media. This incomplete deprotection might induce the lower adhesion strength of 0.1 MPa even after light irradiation.

In conclusion, we successfully synthesized a novel light-controllable functional MAP mimetic polymer by combining an artificial photolabile protection technique and naturally inspired catechol chemistry. The synthesized polymer was stable even in the oxidative condition, while they generated native catechol group by light irradiation to afford the adhesive gel. The excellent stability and light controllable activation properties were not found in nature systems. We are investigating artificial MAP mimetic polymers having novel properties and functions superior to nature systems by the molecular design.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and additional supporting figures and schemes. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Waite, J. H. *Ann. N.Y. Acad. Sci.* **1999**, *875*, 301−309.
(2) Waite, J. H. *Int. J. Adhes. Adhes.* **1987**, *7*, 9−14.
(3) Waite, J. H. *Integr. Comp. Biol.* **2002**, *42*, 1172−1180.
(4) Zhao, H.; Waite, J. H. *J. Biol. Chem.* **2006**, *281*, 26150−26158.
(5) Lee, H. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 12999−13003.
(6) Ooka, A.; Garrell, R. L. *Biopolymers* **2000**, *57*, 92−102.
(7) Yamamoto, H.; Kuno, S.; Nagai, A.; Nishida, A.; Yamauchi, S.; Ikeda, K. *Int. J. Biol. Macromol.* **1990**, *12*, 305−310.
(8) Yu, M.; Hwang, J.; Deming, T. J. *J. Am. Chem. Soc.* **1999**, *121*, 5825−5826.
(9) Yamamoto, H. *J. Chem. Soc., Perkin Trans. I* **1987**, 613.
(10) Yu, M.; Deming, T. J. *Macromolecules* **1998**, *31*, 4739−4745.
(11) Westwood, G.; Horton, T. N.; Wilker, J. J. *Macromolecules* **2007**, *40*, 3960−3964.
(12) White, J. D.; Wilker, J. J. *Macromolecules* **2011**, *44*, 5085−5088.
(13) Xu, H.; Nishida, J.; Ma, W.; Wu, H.; Kobayashi, M.; Otsuka, H.; Takahara, A. *ACS Macro Lett.* **2012**, *1*, 457−460.
(14) Lee, B. P.; Chao, C.-Y.; Nunalee, F. N.; Motan, E.; Shull, K. R.; Messersmith, P. B. *Macromolecules* **2006**, *39*, 1740−1748.
(15) Nishida, J.; Kobayashi, M.; Takahara, A. *J. Polym. Sci., Part A: Polym. Chem.* **2013**, DOI: 10.1002/pola.26487.
(16) Lee, Y.; Chung, H. J.; Ahn, C. -H.; Lee, H.; Messersmith, P. B.; Park, T. G. *Soft Matter* **2010**, *6*, 977−983.
(17) You, I.; Kang, S. M.; Byun, Y.; Lee, H. *Bioconjugate Chem.* **2011**, *22*, 1264−1269.
(18) Ryu, J. H.; Lee, Y.; Kong, W. H.; Kim, T. G.; Park, T. G.; Lee, H. *Biomacromolecules* **2011**, *12*, 2653−2659.
(19) Huang, K.; Lee, B. P.; Ingram, D. R.; Messersmith, P. B. *Biomacromolecules* **2002**, *3*, 397−406.
(20) Ellis-Davies, G. C. R. *Nat. Methods* **2007**, *4*, 619−628.
(21) Mayer, G.; Heckel, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4900−4921.
(22) Wood, J. S.; Koszelak, M.; Liu, J.; Lawrence, D. S. *J. Am. Chem. Soc.* **1998**, *120*, 7145−7146.
(23) Curley, K.; Lawrence, D. S. *Curr. Opin. Chem. Biol.* **1999**, *3*, 84−88.
(24) Miller, E. W.; Taulet, N.; Onak, C. S.; New, E. J.; Lanselle, J. K.; Smelick, G. S.; Chang, C. J. *J. Am. Chem. Soc.* **2010**, *132*, 17071−17073.