Synthesis and Characterization of Barnacle Adhesive Mimetic towards Underwater Adhesion

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A polyacrylamide including tetra-alanine units, hydroxy groups, and hexyl groups was synthesized as a mimetic of barnacle underwater adhesive proteins. The synthesized barnacle mimetic polymer was dissolved in water and subjected to a condensation reaction with hexylamine. Gelation through multiple hydrogen bonds of the oligo-alanine units was confirmed. The adhesive strength of the bonded substrate with the gel-forming barnacle mimetic polymer solution was demonstrated to be 402 kPa for poly(methyl methacrylate) (PMMA) plate adhesion by the tensile shear adhesion test.

Underwater adhesion is a fascinating and complex theme for human technology. Marine sessile organisms such as mussels and barnacles easily accomplish underwater adhesion. The welldocumented adhesion mechanism in mussels is based on mussel adhesive proteins that have a high concentration of dihydroxyphenylalanine (DOPA).¹ The catechol group in DOPA is involved in the crosslinking reaction through aerobic oxidation, and the anchor to metal oxide surfaces is accomplished by hydrogen bonding or chelate bond formation.^{2,3} Based on these findings, various artificial mussel adhesive protein mimetics were reported.^{4–8}

Barnacles are another well-known marine sessile organism. Barnacles attach to material surfaces in seawater through the formation of a proteinaceous adhesive cement.⁹ Because the adhesive cement is insoluble, the adhesive materials and adhesion mechanism have not been fully understood. Some interesting features for the barnacle adhesive have been reported: i) Crosslinking points are not covalent bonds.^{10,11} It is expected that amyloid-like aggregates of self-assembled proteins produce the insoluble and tough crosslinking network. ii) Proteins that localize at the adhesive/adherent interface contain hydrophilic and hydrophobic amino acid residues¹² that interact with universal substrates.¹

In the present study, we designed a polyacrylamide as a new artificial barnacle adhesive protein mimetic consisting of two components: i) a self-assembly motif for polymer crosslinking and ii) surface anchoring moieties for attachment to universal substrates (Figure 1). For the self-assembly motif, we adopted oligo-alanine units, which exist in natural materials such as the crystalline phase of spider silk^{13,14} and insoluble amyloid protein complexes.^{15,16} The strong interaction of multiple hydrogen bonds and molecular packing lead to the remarkable selfassembly. There are many reports on the construction of ordered structures from oligo-alanine containing chemicals, based on their superior self-assembling property.¹⁷⁻²⁰ Therefore, the oligoalanine unit is expected to be a good candidate for the self-assembled crosslinking motif. For self-assembling ability enhancement, an alkyl chain was introduced at the end of the alanine unit through condensation reaction of the terminal



Figure 1. Schematic image of adhesion by barnacle mimetic polymer.



Figure 2. Polyacrylamides with oligo-alanine units.

carboxy group of oligo-alanine units and hexylamine. Crosslinking through the oligo-alanine unit assembly is triggered by the reaction induced hydrophobic interaction. For the surface interacting moieties, the hydrophilic hydroxy groups and the hydrophobic hydrocarbon (hexyl) groups are incorporated into the side group. The adhesive strength for the metal or plastic plates with the gel-forming barnacle mimetic adhesive was examined by the tensile shear adhesion test.

The target polymer was synthesized by radical copolymerization of an acrylamide with the oligo-alanine unit and acrylamide monomers. The protected oligo-alanine unit was synthesized through conventional methods, adopting 9-fluorenylmethyloxycarbonyl (Fmoc) and tert-butyl ester (OtBu) protecting groups (Scheme S1, Supporting Information). The Fmoc protecting group at the amine group was removed, and a polymerizable acrylamide group was introduced by condensation reaction with an activated acrylamide derivative (Scheme S2). In the case of di- and tri-alanine type monomers, the protecting group was removed and radical polymerization was performed (Scheme S3). In the case of tetra-alanine type monomers, the protecting group was removed after polymerization (Scheme S4). Polyacrylamides that consist of acrylamides with the oligo-alanine unit and acrylamide (PAAm-diAla, PAAm-triAla, and PAAm-tetAla) are shown in Figure 2.

Gelation tests were carried out with the aqueous polymer solution to confirm the crosslinking network of the alanine unit. The polyacrylamides were dissolved in a basic solution through



Figure 3. Gelation tests for tetra-alanine type polymer (PAAm-tetAla) aqueous solutions. (a) 5 wt % PAAm-tetAla aqueous solution, (b) 5 wt % PAAm-tetAla aqueous solution with hexylamine and condensation reagents (WSC-HONSu), (c) 5 wt % PAAm-tetAla aqueous solution with butylamine and WSC-HONSu.

deprotonation of the carboxy group at the oligo-alanine terminal, and then were protonated by adding hydrochloric acid solution. Under these conditions, no gelation was observed in any type of alanine unit. However, the tetra-alanine type polymer (PAAmtetAla) solution became opaque (Figure 3a). This result suggests that the aggregation of the tetra-alanine units exists in the solution, but the network structure is not enough for gelation.

To enhance the interaction of the tetra-alanine group, we introduced a hydrophobic group at the end of the alanine unit. Dehydration condensation reaction of the carboxy groups in PAAm-tetAla with alkyl amines (butylamine or hexylamine) was performed by means of water-soluble carbodiimide and *N*-hydroxysuccinimide (WSC-HONSu). The PAAm-tetAla solution was gelated by the reaction with hexylamine after 4 h (Figure 3b), but gelation was not observed by the reaction with butylamine even after 24 h (Figure 3c). The complimentary interaction of the amide bonds in oligo-alanine units is not sufficient for self-assembly in aqueous solution, and the hydrophobic part is necessary for assembly. Hydrophobic interaction promotes the aggregation of tetra-alanine units, and the hexyl groups are suitable for the gelation trigger.

To clarify the degree of substitution, the ¹H NMR spectrum of the hydrophobized polymer was measured. The formed gel was purified by dialysis. After lyophilization, the obtained polymer was dissolved in DMSO- d_6 , and the ¹H NMR spectrum was measured (Figure 4). The degree of substitution was estimated from the ratio of methylene groups in the alkyl chain signal (0.8 ppm) and the methine groups in the oligo-alanine (4.2 ppm). The integration ratio of methylene to methine was 2.7/4, and the degree of substitution was calculated to be 0.9.

A gelation test was carried out with a polyacrylamide with shorter oligo-alanine units for the confirmation of the effect of oligo-alanine groups. The tri-alanine type polymer (PAAmtriAla) was subjected to a condensation reaction with hexylamine under the same conditions as the PAAm-tetAla system. In this case, no gelation was observed even after 24 h (Figure S5). The 10 wt % aqueous solution of the PAAm-triAla system was more viscous than the 5 wt % solution as a result of the increase in the association constant; however, the solution could not accomplish the gelation. Both the hydrophobic interaction by the hexylamide terminal and the coherent interaction through



Figure 4. ¹H NMR spectra of PAAm-tetAla before and after reaction with hexylamine. ¹H NMR measurements were carried out at 298 K in DMSO- d_6 .



Figure 5. Barnacle adhesive protein mimetic polymers.

complementally multiple hydrogen bonds in tetra-alanine units are required for gelation. The repeating number of the oligoalanine unit and terminal alkyl chain length would correlate with the adhesive strength of the adhesives.

Another function of barnacle cement proteins, the surface anchoring moiety, was introduced through the adoption of hydroxy and hexyl groups in the polyacrylamide. The tetraalanine-containing monomer was copolymerized with hydroxyethylacrylamide and hexylacrylamide. We obtained the tetraalanine type polymer with the hydroxy group (Figure 5, PHydAAm-tetAla) or hydroxy and hexyl groups (Figure 5, PHydAAm-Hex-tetAla). Gelation and the adhesive properties of these copolymers were confirmed by the gelation test and the tensile shear adhesion test, respectively. Aqueous solutions of PHydAAm-tetAla or PHydAAm-Hex-tetAla were prepared with condensation reagents (WSC-HONSu) and a curing reagent (hexylamine). The solution viscosity gradually increased, and a gel was obtained after 4 h (Figure S6). The induction time for

Table 1. Tensile shear adhesion strength of adhered stainless

 steel or PMMA plates by gelation of barnacle mimetic polymer

 aqueous solution

	Tensile shear adhesion strength/kPa	
	PHydAAm-tetAla gel	PHydAAm-Hex-tetAla gel
Stainless steel	24	21
PMMA	91	402

gelation was almost the same as an adhesive without anchoring groups. Adhesion tests were carried out with stainless steel or poly(methyl methacrylate) (PMMA) plates. The adhesive solution including polyacrylamide (PHydAAm-tetAla or PHydAAm-Hex-tetAla), condensation reagents, and curing reagent was sandwiched between the plates. Samples were kept at room temperature, under high relative humidity (more than 90%). The adhesion strength of the stainless steel plates or PMMA plates was measured using a tensile shear adhesion tester (Table 1). In the case of stainless steel, the adhesion strength through the gelation of PHydAAm-tetAla or PHydAAm-Hex-tetAla solution was almost equal, whereas there was a large difference in the adhesion strength of PMMA plates. For PMMA plate adhesion, PHydAAm-tetAla shows adhesion strength similar to that of stainless steel. On the other hand, PHydAAm-Hex-tetAla exhibits adhesion strength for PMMA plates 20 times higher than that of stainless steel. In the case of stainless steel, partial dewetting of the polymer gel was observed at the failure faces both in PHydAAm-tetAla and PHydAAm-Hex-tetAla systems, whereas the polymer gel of PHydAAm-Hex-tetAla entirely wetted and remained the failure faces of PMMA plates without dewetting. The hydrophobic hexyl groups contribute to the cohesive interaction with PMMA surfaces and adhesive polymers, and prevent dewetting during gel formation. The difference in adhesion strength is attributed to the homogeneity and defects in the adhesive interfaces.

In conclusion, as a barnacle adhesive protein mimetic, a new polymer for underwater adhesion was designed and synthesized, and the adhesive property was demonstrated. The polymer includes two essential functions of barnacle adhesive proteins. One is crosslinking ability based on self-assembly though complementary hydrogen bonds (not covalent bonds). The other is affinity to the adherent through both polar and van der Waals interactions. We confirmed that the polymer aqueous solution causes gelation by aggregation of oligo-alanine units through hydrogen bonds with the assistance of the hydrophobic terminal group. Adhesion of stainless steel plates and PMMA plates was accomplished through the gelation of the aqueous polymer solution. We believe that very efficient adhesives can be designed by tuning the polymer structure, including the molecular weight and composition of the monomer units based on our barnacle mimetic molecular design. This barnacle adhesive protein mimetic is useful for aqueous underwater adhesives, and it would provide insights for barnacle adhesive cement.

Supporting Information is available electronically on J-STAGE.

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