Synthetic Polypeptide Mimics of Marine Adhesives

Miaoer Yu and Timothy J. Deming*

Departments of Materials and Chemistry, University of California, Santa Barbara, Santa Barbara, California 93106

Received February 20, 1998; Revised Manuscript Received June 1, 1998

ABSTRACT: Water soluble copolypeptides containing L-dihydroxyphenylalanine (DOPA) and L-lysine were prepared by ring-opening polymerization of α-amino acid *N*-carboxyanhydride (NCA) monomers. We have prepared a range of different copolymers to probe the effects of functional group composition on adhesive and cross-linking behavior. Aqueous solutions of these copolymers, when mixed with a suitable oxidizing agent (e.g., O_2, mushroom tyrosinase, Fe^{3+}, H_2O_2, or IO_4^-), formed cross-linked networks that were found to form moisture-resistant adhesive bonds to a variety of substrates (e.g., aluminum, steel, glass, and plastics). It was found that successful adhesive formation was dependent on oxidation conditions, with chemical oxidants giving the best results. Optimized systems were found to form adhesive bonds that rival in strength those formed by natural marine adhesive proteins. Our synthetic systems are readily prepared in large quantities and require no enzymes or other biological components.

Introduction

The prolific adhesive capabilities of many marine organisms are well-known. Each year a considerable effort is spent worldwide on the removal of barnacles and mussels from fouled vessels and other man-made structures exposed to the oceans.[1] The adhesives are remarkable in that they function over wide temperature ranges (−40 to 40 °C), fluctuating salinities, and humidities and in the tides, waves, and currents of marine environments.[2] These glues are able to form permanent bonds in a few seconds to a wide variety of substrates with complex and often irregular surface coatings. In contrast, the success of synthetic adhesives in wet environments requires carefully cleaned adherends, which often must also be chemically treated and/or partially dried.[3] Furthermore, preliminary immunological studies on marine adhesive proteins revealed that they are poor antigens and thus excellent candidates for in vivo medical applications.[4] An understanding of the materials and mechanisms used by mussels and barnacles to adhere to underwater surfaces would be valuable for the design and synthesis of superior moisture-resistant adhesives.

This premise has driven much of the research on marine adhesives. Decades of investigation into this field have led to the discovery of many marine organisms that secrete adhesive materials. These organisms include varieties of mussels,[5] barnacles,[6] and tube worms (polychaetes),[7] which have different environmental needs and subsequent uses for the adhesives they produce. However, they are alike in that the materials they use for adhesion and cementing contain many of the same building blocks and apparently operate by similar mechanisms. Adhesive precursor proteins have been isolated and sequenced from most of these organisms; a partial list is given in Figure 1. It is important to note that these consensus repeats are just that and that considerable variation is present in the sequence of each protein. The repetitive polypeptides have basic isoelectric points (due to lysine residues), flexible conformations (due to high percentages of small glycine and serine residues), and high levels of the amino acid 3,4-dihydroxyphenyl-L-alanine, DOPA (Figure 2).[5] The DOPA residues are believed to be primarily responsible for (i) chemisorption of the polymers to surfaces underwater and (ii) covalent cross-linking of the adhesive.[8]

Natural adhesive precursor protein has been extracted from the blue mussel, *Mytilus edilus*, and when this material was spread on culture plates and oxidized with mushroom tyrosinase, it formed an adhesive that could be used for cell attachment and growth.[9] It has also been shown to be effective as an adhesive for the bonding of wet tissue samples.[10] The adhesive protein was found to be nontoxic; however, the toxicity of the enzymatic oxidizing agent was problematic. Alternatively, synthetic DOPA-containing polypeptides have been reported by Yamamoto and co-workers. They synthesized DOPA homopolymer as well as sequence-specific copolymers of DOPA with L-lysine and L-glutamic acid.[11] They have also reported random L-lysine/L-tyrosine copolymers[12] and the synthesis of random copolypeptides that contain as many as 18 different amino acids, including DOPA.[13] Studies on the cross-linking and adhesive properties of these polymers were limited. Initial adhesive studies were performed using iron and Al_2O_3 adherends with no oxidizing agent.[14] More detailed studies focused on L-lysine/L-tyrosine random copolymers and complex random copolymers where tyrosinase enzyme was used as the oxidizing agent.[12] The adhesive systems were studied in water and diluted synthetic seawater and were found to form adhesive bonds, although conversion of tyrosine residues to DOPA was inefficient. Successful oxidation was achieved only when the polymer chains were cleaved with chymotrypsin.

Our efforts in this area are focused on the design and synthesis of simplified adhesive polymers that incorporate only the essential functional components of the marine proteins. We especially wanted to test the premise that functionality, and not amino acid sequence, was the only feature necessary for moisture-resistant adhesion. Using the known compositions of the natural

S0024-9297(98)00268-X CCC: $15.00
Published on Web 07/10/1998

Organism	M_n	Consensus Repeat
Phragmatopoma californicia	35,000	ValGlyGlyDOPAGlyDOPAGlyAlaLys
Mytilus edulis	130,000	AlaLysProSerTyrdiHypHypThrDOPALys
Geukensia demissa	130,000	ThrGlyDOPAGlyProGlyDOPALys
Aulacomya ater	130,000	AlaGlyDOPAGlyGlyLeuLys
Dreissena polymorpha	76,000	GlyProDOPAValProAspGlyProTyrAspLys
Brachidontes exustus	105,000	GlyLysProSerProDOPAAspProGlyDOPALys

Figure 1. Sequences of some naturally occurring marine adhesive protein repeats.

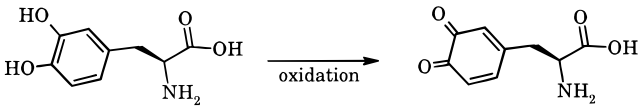


Figure 2. Oxidation of L-DOPA to DOPA quinone.

adhesive proteins, we prepared sequentially random copolypeptides through copolymerization of a few select α-amino acid *N*-carboxyanhydrides (NCAs) (Scheme 1). NCAs are readily prepared from amino acids by phosgenation and can be polymerized into high molecular weight polypeptides via successive ring-opening addition reactions that liberate carbon dioxide.[15] NCA polymerizations allow the preparation of multigram quantities of polymer, and the monomers readily copolymerize, allowing copolymer composition to be easily varied. We also wanted to study the oxidation of these polymers in detail since this chemistry is a key feature of the DOPA functionality. Thus we oxidized our copolymers under a variety of conditions and evaluated the effect of oxidant on both cross-linking and adhesive capabilities. The role of oxidation in these adhesives has not received much attention in the past: the complexity of the full-length proteins and ambiguities associated with oxidation of tyrosine residues to DOPA and/or quinone has hindered characterization of the oxidized polymers. Our functionally simple copolymers allowed us to make strong correlations between oxidation and physical behavior.

Experimental Section

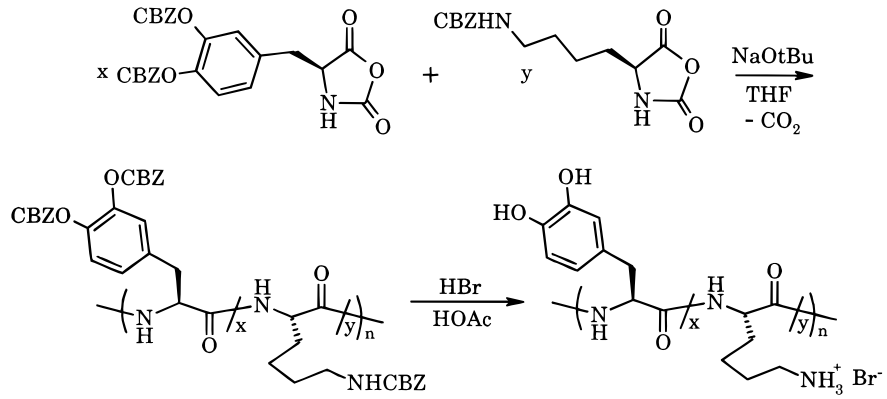
General Information. Tandem gel permeation chromatography/light scattering (GPC/LS) was performed on a Spectra Physics Isochrom liquid chromatograph pump equipped with a Wyatt DAWN DSP light-scattering detector and Wyatt Optilab DSP. Separations were effected by 10^5, 10^4, and 10^3 Å Phenomenex 5 μm columns using 0.10 M LiBr in DMF as eluent. NMR spectra were recorded on a Varian Gemini 200 MHz spectrometer. A Mettler TA3000 thermal analysis system was used for TGA measurements on the polymers. All IR samples were prepared on NaCl plates, and IR spectra were recorded on a Perkin-Elmer model 1615 FTIR. Optical rotations were measured using a JASCO P1020 digital polarimeter equipped with a sodium lamp source. Elemental analyses were performed by the Marine Science Institute Laboratory at the University of California, Santa Barbara. L-DOPA (99%), benzyl chloroformate (97%), N_ϵ-carbobenzyloxy-L-lysine (98%), sodium hydride, *tert*-butyl alcohol, and 33 wt % hydrobromic acid in glacial acetic acid (Technical grade) were obtained from Acros Organics. Mushroom tyrosinase was obtained from Sigma Chemical Co. Phosgene was purchased from Fluka. Phosphorus pentachloride (95%) was obtained from Aldrich Chemical Co. Hexanes and THF were distilled from sodium/benzophenone in an inert atmosphere and stored under nitrogen. Sodium *tert*-butoxide was prepared from the reaction of NaH and *tert*-butyl alcohol in THF. The product was dried under vacuum and stored in a glovebox.

Amino Acid *N*-Carboxyanhydrides and Polypeptides. N_ϵ-Carbobenzyloxy-L-lysine *N*-carboxyanhydride, **1**, was prepared by using phosgene following literature procedures.[16] *O*,*O*′-Dicarbobenzyloxy-L-DOPA *N*-carboxyanhydride, **2**, was prepared from *N*,*O*,*O*′-tricarbobenzyloxy-L-DOPA and phosphorus pentachloride.[17] Poly(N_ϵ-carbobenzyloxy-L-lysine), **3**, and Poly(L-lysine•HBr), **4**, were prepared according to literature procedures.[16]

Poly(N_ϵ-carbobenzyloxy-L-lysine$_9$−*O*,*O*′-dicarbobenzyloxy-L-DOPA$_1$), 5. To a mixture of **1** (1.3 g, 4.3 mmol) and **2** (0.23 g, 0.48 mmol) in THF (15 mL) in a Schlenk tube was added 0.1 N sodium *tert*-butoxide (0.95 mL, 0.096 mmol) with stirring. The mixture was stirred for 1 day at room temperature, then 2 days at 40 °C, and finally 4 h at 80 °C. The product was precipitated by addition of ether and then dried in vacuo at room temperature overnight to give a white polymer (1.13 g, 85%). GPC: M_n = 167 000, M_w/M_n = 1.48. ^{1}H NMR (TFA-*d*): δ 7.60−7.20 (br m, −(NHCH-((CH$_2$)$_3$CH$_2$NHCOOCH$_2$C$_6$*H*$_5$)C(O))$_9$−(NHCH(CH$_2$C$_6$*H*$_3$-(OCOOCH$_2$C$_6$*H*$_5$)$_2$)−C(O))$_1$−, 5.8H), 5.28 (br s, −(NHCH-((CH$_2$)$_3$CH$_2$NHCOOC*H*$_2$C$_6$H$_5$)C(O))$_9$−(NHCH(CH$_2$C$_6$H$_3$-(OCOOC*H*$_2$C$_6$H$_5$)$_2$)C(O))$_1$−, 2.2H), 4.68 (br s, −(NHC*H*-((CH$_2$)$_3$CH$_2$NHCOOCH$_2$C$_6$H$_5$)C(O))$_9$−(NHC*H*(CH$_2$C$_6$H$_3$-(OCOOCH$_2$C$_6$H$_5$)$_2$)C(O))$_1$−, 1H), 3.30 (br s, −(NHCH-((CH$_2$)$_3$C*H*$_2$NHCOOCH$_2$C$_6$H$_5$)C(O))$_9$−(NHCH(C*H*$_2$C$_6$H$_3$-(OCOOCH$_2$C$_6$H$_5$)$_2$)C(O))$_1$−, 2H), 2.06−1.38 (br d, −(NHCH-((C*H*$_2$)$_3$CH$_2$NHCOOCH$_2$C$_6$H$_5$)C(O))$_9$−(NHCH(CH$_2$C$_6$H$_3$-(OCOOCH$_2$C$_6$H$_5$)$_2$)C(O))$_1$−, 5.4H). FTIR(THF): 1772 cm^{-1} (ν_{CO}, vs), 1723 cm^{-1} (ν_{CO}, vs), 1654 cm^{-1} (amide I, s br), 1544 cm^{-1} (amide II, s br). Anal. Calcd for $C_{13.5}O_3N_{1.9}H_{17.1}$: C, 64.58; H, 6.57; N, 9.48. Found: C, 63.94; H, 6.88; N, 9.55. $[\alpha]_D^{20}$ (DMF, c = 0.005) = +7.7.

Poly(L-lysine$_9$•HBr-L-DOPA$_1$), 6. To a solution of **5** (1.1 g, 4.0 mmol) in TFA (~10 mL) was added 4 equiv of 33% HBr in acetic acid (w/w) with stirring. The mixture was stirred for 1 h. The product was precipitated by addition of ether and then dried under a flow of nitrogen. The crude polymer was dissolved in a small amount of water/methanol (1:4), precipitated out by addition of ether, and then dried in vacuo at room temperature overnight. The polymer was dissolved in water and isolated by freeze-drying as a white fluffy solid (0.72 g, 88%). ^{1}H NMR (D$_2$O): δ 7.58 (br s, −(NHCH((CH$_2$)$_3$CH$_2$-NH$_2$•HBr)C(O))$_9$−(NHCH(CH$_2$C$_6$H$_2$*H*(OH)$_2$)C(O))$_1$−, 0.1H), 6.90−6.70 (br m, −(NHCH((CH$_2$)$_3$CH$_2$NH$_2$•HBr)C(O))$_9$−(NHCH-(CH$_2$C$_6$*H*$_2$H(OH)$_2$)C(O))$_1$−, 0.2H), 4.40 (br s, −(NHC*H*((CH$_2$)$_3$-CH$_2$NH$_2$•HBr)C(O))$_9$−(NHC*H*(CH$_2$C$_6$H$_2$H(OH)$_2$)C(O))$_1$−, 1H), 3.10(br s, −(NHCH((CH$_2$)$_3$C*H*$_2$NH$_2$•HBr)C(O))$_9$−(NHCH-(C*H*$_2$C$_6$H$_2$H(OH)$_2$)C(O))$_1$−, 2H), 2.18−1.40 (br d, −(NHCH-((C*H*$_2$)$_3$CH$_2$NH$_2$•HBr)C(O))$_9$−(NHCH(CH$_2$C$_6$H$_2$H(OH)$_2$)C-(O))$_1$−, 5.4H). Anal. Calcd for $C_{6.3}O_{1.2}N_{1.9}H_{12.6}Br_{0.9}$: C,

Scheme 1. Synthesis of Adhesive Copolypeptides Using NCA Monomers



36.72; H, 6.16; N, 12.91. Found: C, 35.18; H, 5.69; N, 11.43. The composition of the polymer was estimated to be 11 mol % DOPA by measurement of the UV/vis absorption of a polymer solution at 280 nm and comparison of the data to a standard absorption curve of **8**. $[\alpha]_D^{20}$ (H_2O, c = 0.005) = −61.7.

Recarbobenzyloxylation of 6. 6 (M_n = 182 000, 50 mg, 0.24 mmol) was dissolved in 1 mL of water in a 25 mL Schlenk flask under N_2. The polymer solution was stirred in an ice bath. (4 N, NaOH 0.34 mL) and benzyl chloroformate (77 μL, 0.55 mmol) were added to the polymer solution in three portions over a 30 min period. A white precipitate formed after addition of the first portion of benzyl chloroformate. After the final addition, the mixture was stirred for an additional hour. The polymer was isolated by filtration, washed with water, methanol, and ether, and then dried in vacuo at room temperature overnight (46 mg, 68%). The ^{1}H NMR spectrum of the product was identical to an unmodified sample of **5**. The molecular weight of the reprotected polymer was determined by GPC: M_n = 252 000, M_w/M_n = 1.54. Original M_n of protected polymer = 330 000.

Poly(N_ϵ-carbobenzyloxy-L-lysine$_4$−*O, O′*-dicarbobenzyloxy-L-DOPA$_1$), 7. To a mixture of **1** (1.1 g, 3.7 mmol) and **2** (0.46 g, 0.93 mmol) in THF (15 mL) in a Schlenk tube was added 0.1 N sodium *tert*-butoxide in THF (0.11 mL, 0.019 mmol) with stirring. The mixture was stirred for 1 day at room temperature, then 2 days at 40 °C, and finally 4 h at 80 °C. The product was precipitated by addition of ether and then dried in vacuo at room temperature overnight to give a white polymer (1.22 g, 87%). GPC: M_n = 186 000, M_w/M_n = 1.35. ^{1}H NMR (TFA-*d*): δ 7.58−7.30 (br m, −(NHCH((CH$_2$)$_3$CH$_2$NHCOOCH$_2$C$_6$H$_5$)C(O))$_4$−(NHCH-(CH$_2$C$_6$H$_3$(OCOOCH$_2$C$_6$H$_5$)$_2$)C(O))$_1$−, 6.6H), 5.26 (br s, −(NHCH((CH$_2$)$_3$CH$_2$NHCOOC*H*$_2$C$_6$H$_5$)C(O))$_4$−(NHCH(CH$_2$C$_6$H$_3$-(OCOOC*H*$_2$C$_6$H$_5$)$_2$)C(O))$_1$−, 2.4H), 4.70 (br s, −(NHC*H*((CH$_2$)$_3$-CH$_2$NHCOOCH$_2$C$_6$H$_5$)C(O))$_4$−(NHC*H*(CH$_2$C$_6$H$_3$(OCOOCH$_2$-C$_6$H$_5$)$_2$)C(O))$_1$−, 1H), 3.28 (br s, −(NHCH((CH$_2$)$_3$C*H*$_2$NHCOO-CH$_2$C$_6$H$_5$)C(O))$_4$−(NHCH(C*H*$_2$C$_6$H$_3$(OCOOCH$_2$C$_6$H$_5$)$_2$)C-(O))$_1$−, 2H), 2.10−1.36 (br d, −(NHCH((C*H*$_2$)$_3$CH$_2$NHCOO-CH$_2$C$_6$H$_5$)C(O))$_4$−(NHCH(CH$_2$C$_6$H$_3$(OCOOCH$_2$C$_6$H$_5$)$_2$)-C(O))$_1$−, 4.8H). FTIR (THF): 1772 cm^{-1} (ν_{CO}, vs), 1722 cm^{-1} (ν_{CO}, vs), 1652 cm^{-1} (amide I, s br), 1543 cm^{-1} (amide II, s br). Anal. Calcd for $C_{16.2}O_{3.8}N_{1.8}H_{18.6}$: C, 65.00; H, 6.26; N, 9.62. Found: C, 64.31; H, 6.46; N, 8.32. $[\alpha]_D^{20}$ (DMF, c = 0.005) = +13.2.

Poly(L-lysine$_4$•HBr-L-DOPA$_1$), 8. To a solution of **7** (1.1 g, 3.7 mmol) in TFA (∼10 mL) was added 4 equiv of 33% HBr in acetic acid (w/w) with stirring. The mixture was stirred for 1 h. The product was precipitated by addition of ether and then dried. The crude polymer was dissolved in a small amount of water/methanol (1:4), precipitated out by addition of ether, and dried in vacuo at room temperature overnight. The polymer was dissolved in water and isolated by freeze-drying as a white fluffy solid (0.74 g, 3.6 mmol 97%). ^{1}H NMR (D_2O): δ 7.52 (br s, −(NHCH((CH$_2$)$_3$CH$_2$NH$_2$•HBr)C(O))$_4$−(NHCH(CH$_2$C$_6$H$_2$*H*(OH)$_2$)C(O))$_1$−, 0.2H), 6.96−6.64 (br m, −(NHCH((CH$_2$)$_3$CH$_2$NH$_2$•HBr)C(O))$_4$−(NHCH(CH$_2$C$_6$*H*$_2$H-(OH)$_2$)C(O))$_1$−, 0.4H), 4.36 (br s, −(NHC*H*((CH$_2$)$_3$CH$_2$-NH$_2$•HBr)C(O))$_4$−(NHC*H*(CH$_2$C$_6$H$_2$H(OH)$_2$)C(O))$_1$−, 1H), 3.05 (br s, −(NHCH((CH$_2$)$_3$C*H*$_2$NH$_2$•HBr)C(O))$_4$−(NHCH(C*H*$_2$C$_6$H$_2$H-(OH)$_2$)C(O))$_1$−, 2H), 2.05−1.30 (br d, −(NHCH((C*H*$_2$)$_3$CH$_2$-NH$_2$•HBr)C(O))$_4$−(NHCH(CH$_2$C$_6$H$_2$H(OH)$_2$)C(O))$_1$−, 4.8H). Anal. Calcd for 19% DOPA copolymer, 85% TFA salt, 15% HBr salt: C, 42.86; H, 5.52; N, 11.32. Found: C, 42.86; H, 5.92; N, 10.08. The composition of the polymer was estimated by ^{1}H NMR: DOPA content found = 19 mol % [mol % DOPA = 100 − mol % lysine. Mol % lysine = {100(integration of peaks at 2.05−1.30 ppm)/3}/(integration of peak at 3.05 ppm)]. $[\alpha]_D^{20}$ (H_2O, c = 0.005) = −39.7.

Thermal Analysis. The polymers were heated at a rate of 15 °C/min from 50 to 500 °C under N_2. The decomposition temperature (T_d) was determined as the temperature where each sample showed 5% weight loss.

	polymer					
	3	**4**	**5**	**6**	**7**	**8**
T_d(K)	247	306	206	288	206	290

Rheology. The rheological behavior of solutions of **8** under different oxidation conditions was studied using a rheometrics ARES Rheometer at room temperature. A cone and plate geometry was used in a dynamic time sweep experiment at a frequency of 2.0 rad/s and at 100% strain. The concentration of each polymer solution was 2.5 mg/mL. Solutions were prepared by mixing a 5.0 mg/mL solution of the copolymer in deionized water with an equal volume of oxidant solution. The viscosity of each adhesive is reported at the beginning of curing and at the onset of gelation. The gel point was determined by the time at which G' and G'' became parallel as functions of frequency.[18]

Tensile Shear Measurements. Adherend Preparation. Aluminum adherends (5052-H32) were treated with a mixture of water, H_2SO_4 (concentrated), and $Na_2Cr_2O_7$ (40:20:4) at 65−70 °C for 20 min. They were then rinsed with deionized water and air-dried. Steel adherends (A366) were polished with sandpaper (220 grit) and then rinsed with hexane followed by acetone. Poly(methyl methacrylate), polystyrene, and polyethylene adherends were sonicated in aqueous Alconox detergent (2% w/v) for 30 min and rinsed with deionized water. Glass adherends were prepared by attaching glass slides to stainless steel test pieces using epoxy resin, and the exposed glass surfaces were cleaned by soaking in an 2-propanol/KOH bath for 30 min followed by rinsing with deionized water (18 MΩ resistivity).

Test Sample Preparation. The adhesive polymer solution (40 mg/100 μL of solvent, unless otherwise noted) was spread on both adherend slides, which were then overlapped together with two Cu wires (0.06 mm diameter) placed as spacers between the adhesive joint. The overlapped samples were clamped together for 3 h to prevent motion and kept in a temperature-controlled oven for the specified time period. No attempt was made to maintain hydration of the samples.

Table 1. Synthetic Adhesive Copolymers and Control Polymers[a]

Composition	yield (%)	M_n	M_w/M_n	H_2O soluble
poly(CBZ-lysine), **3**	94	147 000	1.42	−
poly(lysine•HBr), **4**	97	103 000	1.45	+
poly(CBZ-lysine$_9$−DiCBZ-DOPA$_1$), **5**	85	167 000	1.48	−
poly(lysine•HBr$_9$-DOPA$_1$), **6**	88	120 000	1.52	+
poly(CBZ-lysine$_4$−DiCBZ-DOPA$_1$), **7**	87	186 000	1.35	−
poly(lysine•HBr$_4$-DOPA$_1$), **8**	97	113 000	1.39	+

[a] Molecular weights of protected polymers were determined by GPC in 0.1 M LiBr in DMF at 60 °C.

Tensile Shear Strength Measurement. The tensile strength was measured at room temperature with an Instron 1123 mechanical testing apparatus according to the ASTM D1002 method.[19] An Instron 5000 lb reversible load cell was used for the measurements. *Data acquisition & control version 3.00* (1994 University of California, Santa Barbara) was used to monitor the data output. The adherend size was 4 in. × 1 in. the loading rate was 0.05 in./min, the bond line thickness was 0.06 mm, and the area of the bond was 0.39 in.^{2}. Three samples were measured for each experiment, and the average of these values is reported. The ranges of the data points are plotted as error bars on the graphs.

Results

We have prepared simple copolypeptides of L-lysine and DOPA containing different compositions of the two monomers. We wanted to incorporate DOPA directly into the polymer to avoid complications associated with the oxidation of precursor tyrosine residues. L-Lysine was the other major component of our copolymers since it (i) is present in large quantities in marine adhesive proteins, (ii) is thought to be involved in protein cross-linking reactions,[20] and (iii) should provide good water solubility to the copolymers when the side chain amine is ionized. We prepared homopolymers of L-lysine and DOPA as well as a series of copolymers of varying compositions and molecular weights. Data for the side-chain protected and deprotected polymers are given in Table 1.

Di-carbobenzyloxy-DOPA NCA (di-CBZ-DOPA NCA) was found to only form short chains in low yield when polymerized using sodium *tert*-butoxide initiator. As carbobenzyloxy-L-lysine NCA (CBZ-L-lysine NCA) polymerized very efficiently, this suggested that the steric crowding around the DOPA monomer resulted in poor homopolymerization. DOPA, however, was found to incorporate well in copolymerization reactions: yields were near quantitative, and no short homopoly(DOPA) oligomers were formed. ^{1}H NMR and UV/vis quantification of the DOPA catechol functional groups confirmed that the bulk copolymer compositions were essentially equal to the comonomer feed compositions. Deprotection of the copolypeptides was accomplished using HBr in acetic acid. Reprotection of a deprotected lysine/DOPA copolymer with excess benzyl chloroformate followed by GPC analysis showed that there was only limited peptide chain cleavage in the deprotection step: molecular weight of the polymer after reprotection with benzyl chloroformate was ca. 75% of the original value. The white copolymers were all found to be soluble in aqueous buffers over a wide pH range (ca. 2−12). The DOPA-containing copolymers were stable in air for days under acidic and neutral conditions, but discolored rapidly in basic environments. Thermal analysis of the polymers showed negligible weight loss under N_2 up to ca. 200 °C. The polypeptides could be stored in a −40 °C freezer indefinitely with no discoloration or change in properties.

The first goal of this project was to verify that these simple copolypeptides would form cross-linked networks in aqueous environments, analogous to the natural adhesive proteins. Specifically, we wanted to evaluate the ability of copolymer solutions to form networks as functions of solution pH and oxidizing agent. The oxidizing agents utilized were air (O_2), $NaIO_4$, H_2O_2, and mushroom tyrosinase. The oxidizing agents were found to darken solutions of the DOPA-containing copolymers, resulting in formation of cross-linked networks. By variation of the oxidizing agent and pH, we were able to obtain systems where gelation times could be adjusted from seconds to days. The kinetics of gel formation and gel strength were followed by viscosity measurements (Table 2). Periodate, hydrogen peroxide, and base (pH = 12) formed gels fastest, with tyrosinase and aerobic oxidation being much slower. Hydrogen peroxide and base also gave the highest cross-link densities, as estimated by solution viscosities. From these experiments, it appeared that either basic aqueous solution or H_2O_2 would be the most efficient oxidants for our synthetic adhesive polymers.

Lap shear tensile adhesive measurements were also performed on the L-lysine/DOPA binary copolymers. The copolymers were tested under a variety of oxidizing conditions and were found to form moisture-resistant adhesive bonds to aluminum under all conditions (Table 3). If allowed to cure for a sufficient period of time (1 day), all oxidizing conditions gave adhesive bonds of nearly equivalent strength, which was proportional to the amount of DOPA in the copolymer. Copolymer containing 20% DOPA formed adhesive bonds nearly 10 times stronger than those formed with the control, pure poly-L-lysine. When the adhesives were allowed to cure for shorter periods of time, the choice of oxidant became significant. A comparison between aerobic and peroxide oxidation is given in Figure 3. As the peroxide concentration was increased, oxidation of the polymer was accelerated, resulting in strong bonds after only 3 h of curing. With aerobic oxidation, only very weak bonds were formed in this time period.

We also evaluated the ability of our copolymers to adhere to substrates besides aluminum. The survey of adherends included steel (A366), plastic (PMMA, PS, and PE), and borosilicate glass (Figure 4). The plastic adherends formed the weakest adhesive bonds of all adherends studied. It is likely the relatively nonpolar surfaces displayed by the plastics provided a poor substrate for chemisorption of the polar functionalities of the polymer. The polar surface of glass provided a better substrate, nearly equivalent to the metals. Steel adherends gave adhesive bond strengths that were comparable to those formed on aluminum (Table 3). The major difference from aluminum was that adventitious iron oxide present on the steel surface was able to act as an efficient polymer oxidant. Consequently, no additional oxidant was necessary to obtain fast oxidation on steel adherends. In particular, it was found to be unnecessary to scrupulously clean the steel surface since a thin coating of iron oxide promoted rapid curing of the adhesive. The rate of adhesive bond formation on steel can be seen in Figure 5, where reasonable bonds were obtained after ca. 6 h. Bond strengths on steel were also strongly influenced by the copolymer composition, with the 20% DOPA copolymer forming bonds an

Table 2. Viscosity Measurements[a]

oxidation conditions	tyrosinase[b]		pH = 12[c]		$NaIO_4$[d]		H_2O_2[e]		air (O_2)	
time (min)	0	48	0	4	0	3	0	6	0	180
η^* (Pa s)	0.004	0.047	0.118	0.196	0.082	0.085	0.005	0.144	0.008	0.198

[a] The copolymer used was **8**, M_n = 126 000. [b] 300 units of enzyme were added to a pH = 7 phosphate buffer solution (0.01 N phosphate). [c] 0.01 N phosphate buffer. [d] 0.04 N solution. [e] 1.5 wt % solution. The two time points represent the beginning of the experiment and the onset of gelation.

Table 3. Adhesive Strengths (MPa) of Polymers as a Function of Composition under Different Oxidizing Conditions at 35 °C for 1 Day[a]

oxidation conditions	aluminum					
	air	H_2O_2[b]	pH = 7[c]	pH = 12[c]	tyrosinase/pH = 7[d]	steel air
4 [144k]	0.58 (0.25)	0.61 (0.23)	0.49 (0.03)	0.65 (0.15)	0.56 (0.18)	0.44 (0.16)
6 [242k]	2.52 (0.78)	2.39 (0.49)	2.24 (0.38)	3.45 (0.48)	2.81 (0.73)	3.36 (0.63)
8 [255k]	4.32 (0.55)	4.29 (0.81)	4.02 (0.73)	3.75 (0.39)	4.70 (0.87)	4.00 (0.60)

[a] The ranges of the data points are given in parentheses. Polymer concentration = 40 mg/100 μL. The polymer molecular weight is given in brackets. [b] The amount of H_2O_2 (0.3 wt %) used was 0.5 mL/g polymer. [c] Final concentration of phosphate buffer was 0.025 M. [d] The concentration of enzyme was 5 units/mg polymer.

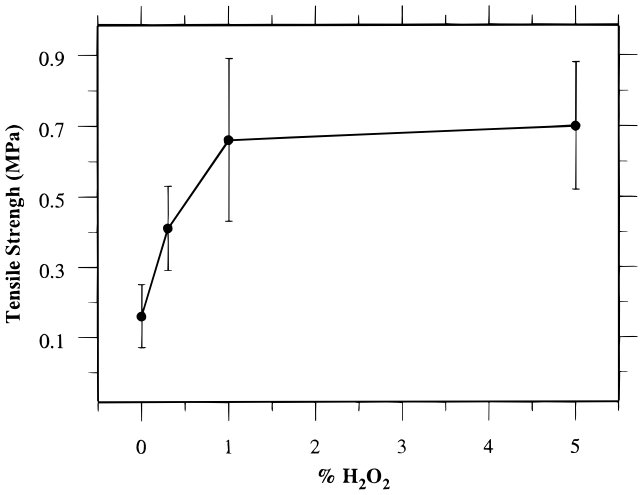


Figure 3. Adhesive strength as a function of H_2O_2 concentration for **8** (M_n = 98 000) on aluminum at 35 °C after 3 h cure time. The polymer concentration was 400 mg/mL of H_2O solution.

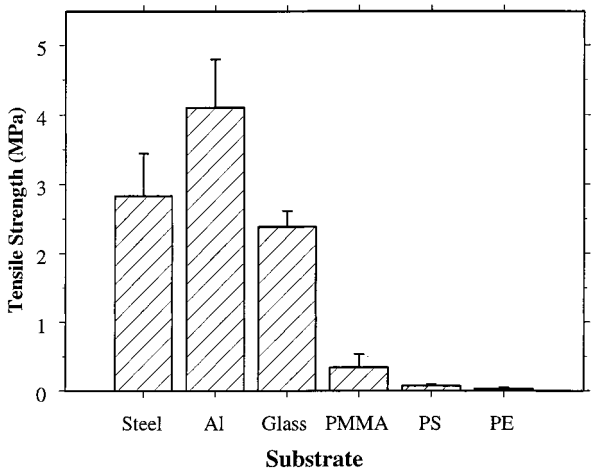


Figure 4. Adhesive strength of **8** (M_n = 98 000) on various substrates at 40 °C after 1 day cure time. The polymer concentration was 400 mg/mL of H_2O solution. The samples were cured in air. PS = polystyrene. PE = polyethylene. PMMA = poly(methyl methacrylate).

order of magnitude stronger than those formed with poly(L-lysine). These results correlate well with the measurements on aluminum in showing the importance of DOPA in adhesive formation.

In addition to copolymer composition and oxidant, we also studied the effects of cure temperature, copolymer molecular weight, and copolymer concentration on adhesive strength. We found that increasing the solution concentration of copolymer in adhesive formulations resulted in increased bond strengths on steel adherends (Figure 6). Presumably, this result was simply due to the increase in quantity of polymer within the adhesive joint. When the adhesives were cured at different temperatures, a large effect on ultimate bond strength was observed (Figure 7). Highest strengths were observed when the adhesives were cured at temperatures greater than 40 °C; shear strengths being more than double the values obtained at 20 °C. Oxidation occurred at all temperatures; however, curing of the adhesive may be more extensive at elevated temperatures. Finally, we found that adhesive bond strengths were also highly dependent on the molecular weight of the copolymer. Tensile strengths increased almost linearly with copolypeptide molecular weight (Figure 8), likely due to increased chain entanglements and cross-links.

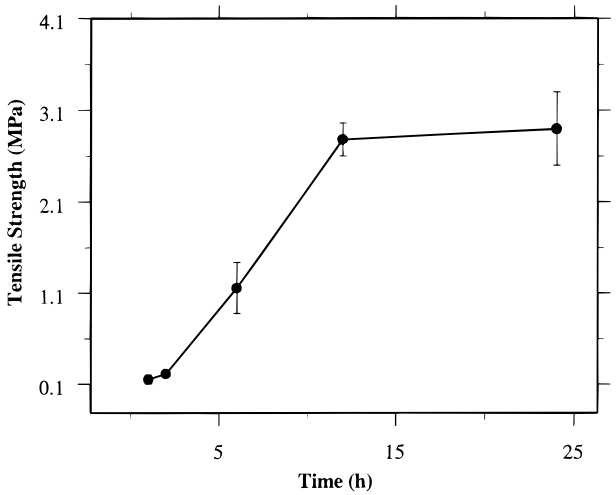


Figure 5. Adhesive strength as a function of cure time for **8** (M_n = 186 000) on steel at 40 °C. The polymer concentration was 400 mg/mL of H_2O solution.

Summary

We have reported the synthesis of copolymers of DOPA and L-lysine that display moisture-resistant

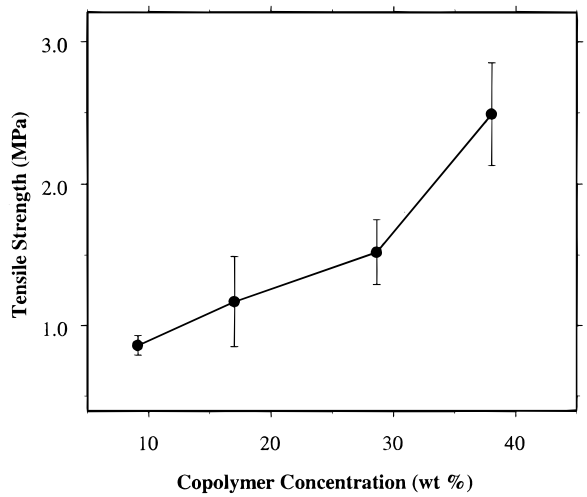


Figure 6. Adhesive strength as a function of copolymer concentration for **8** (M_n = 98 000) on steel at 40 °C after 1 day cure time.

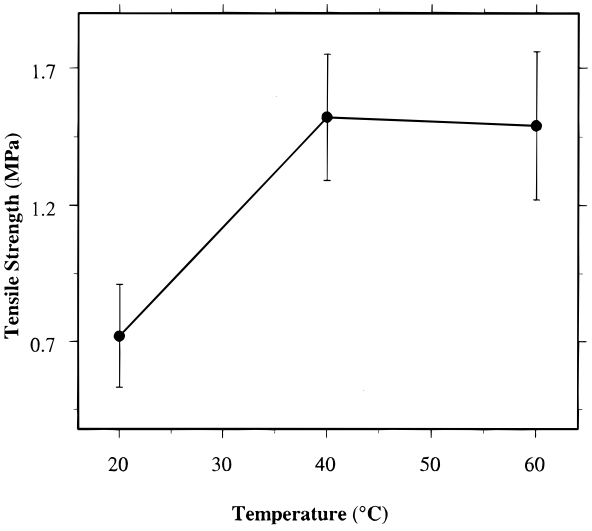


Figure 7. Adhesive strength as a function of cure temperature for **8** (M_n = 98 000) on steel after 1 day cure time. The polymer concentration was 400 mg/mL of H_2O solution.

adhesive properties when suitably oxidized. The adhesive bonds were all able to form when aqueous solutions of the polymers were applied to the substrates. Most of the water was found to evaporate from the bond during curing. However, since no effort was made to drive off all of the water, the adhesives likely retained some moisture of hydration. The choice of oxidant was partially substrate specific, since in some cases, as with steel adherends, no external oxidant is required. On other substrates, different oxidants can be chosen to satisfy particular requirements for curing time. Generally, hydrogen peroxide was found to be the most versatile oxidant when fast curing is required. Our system consisting of synthetic polypeptides and chemical oxidants thus provides an alternative to natural marine adhesive proteins and oxidizing enzymes for formation of adhesive bonds in water.[21] The advantages of our system include the incorporation of inexpensive oxidizing agents and the ready availability of large quantities of adhesive polymer of consistent quality. Furthermore, through adjustment of copolymer composition, molecular weight, or curing conditions, the adhesive properties of these synthetic copolypeptides can be readily tuned for specific applications.

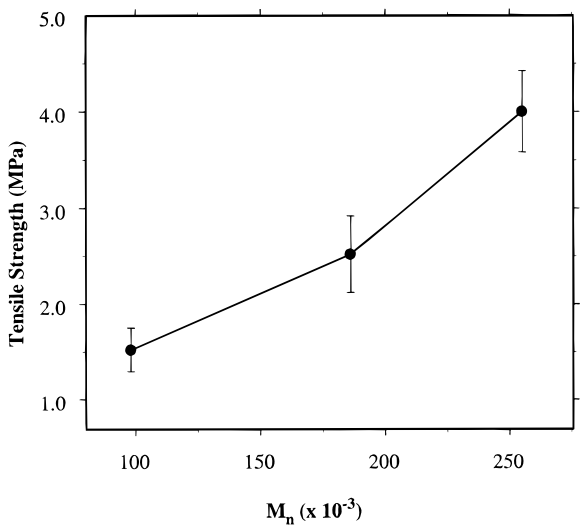


Figure 8. Adhesive strength as a function of copolymer molecular weight for **8** on steel at 40 °C after 1 day cure time. The polymer concentrations were 400 mg/mL of H_2O solution.

Acknowledgment. We thank Dr. Yuntao Hu and Professor David Pine for assistance with the viscosity measurements. This work was supported by an Office of Naval Research Young Investigator Award (No. N00014-96-1-0729, awarded to T.J.D.), by University of California Santa Barbara Materials Department start up funds, and partially by the MRSEC program of the National Science Foundation under award No. DMR-9632716.

References and Notes

(1) *Marine Fouling and Its Prevention*; Woods Hole Oceanographic Institute, United States Naval Institute: Annapolis, MD, 1952.
(2) (a) Waite, J. H. *Polym. Prepr.* **1990**, *30* (1), 181−182. (b) Waite, J. H. *Biol. Rev.* **1983**, *58*, 209−231.
(3) (a) Hull, D. *Introduction to Composite Materials*; Cambridge Solid State Series; Cambridge University Press: Cambridge, 1981. (b) Eagland, D. *Endeavour* **1988**, *12*, 179−184. (c) Houwink, R.; Salomon, G. *Adhesion and Adhesives*; Elsevier: New York, 1967.
(4) Green, K. Mussel Adhesive Proteins In *Surgical Adhesives and Sealants*; Sierra, D. H., Saltz, R., Eds.; Technomic: Lancaster, PA, 1997.
(5) (a) Waite, J. H.; Tanzer, M. L. *Science* **1981**, *212*, 1038−1040. (b) Waite, J. H. *J. Biol. Chem.* **1983**, *258*, 2911−2915. (c) Waite, J. H.; Housley, T. J.; Tanzer, M. L. *Biochemistry* **1985**, *24*, 5010−5014. (d) Waite, J. H.; Hansen, D. C.; Little, K. T. *J. Comput. Physiol. B* **1989**, *159*, 517−525. (e) Rzepecki, L. M.; Chin, S.-S.; Waite, J. H.; Lavin, M. F. *Mol. Mar. Biol. Biotechnol.* **1991**, *1*, 78−88. (f) Rzepecki, L. M.; Hansen, K. M.; Waite, J. H.; *Biol. Bull.* **1992**, *183*, 123−37. (g) Rzepecki, L. M.; Waite, J. H. *Mol. Mar. Biol. Biotechnol.* **1993**, *2*, 255−266. (h) *Ibid*., pp 267−79.
(6) (a) Carderelli, N. F. *Barnacle Cement as a Dental Restorative Material*; National Institutes of Health: Bethesda, MD, 1968. (b) Otness, J. S.; Medcalf, D. G. *Comp. Biochem. Physiol.* **1972**, *43B*, 443−449.
(7) (a) Jensen, R. A.; Morse, D. E. *J. Comput. Physiol. B* **1988**, *158*, 317. (b) Waite, J. H.; Jensen, R. A.; Morse, D. E. *Biochemistry* **1992**, *31*, 5733−5738.
(8) (a) Waite, J. H. *Comp. Biochem. Physiol.* **1990**, *97B*, 19−29. (b) Waite, J. H. *Biol. Bull.* **1992**, *183*, 178−184.
(9) Benedict, C. V.; Picciano, P. T. *ACS Symp. Ser.* 1989, No. 385, 465−483.
(10) Saez, C.; Pardo, J.; Gutierrez, E.; Brito, M.; Burzio, L. O. *Comp. Biochem. Physiol.* **1991**, *98B*, 569−572.
(11) (a) Yamamoto, H.; Hayakawa, T. *Polymer* **1978**, *19*, 1115−1117. (b) Yamamoto, H.; Hayakawa, T. *Macromolecules* **1983**, *16*, 1058−1063. (c) Yamamoto, H.; Hayakawa, T. *Biopolymers* **1982**, *21*, 1137−1151. (d) Yamamoto, H.; Hayakawa, T. *Biopolymers* **1979**, *18*, 3067−3076.

(12) (a) Yamamoto, H.; Kuno, S.; Nagai, A.; Nishida, A.; Yamauchi, S.; Ikeda, K. *Int. J. Biol. Macromol.* **1990**, *12*, 305−310. (b) Nagai, A.; Yamamoto, H. *Bull. Chem. Soc. Japn.* **1989**, *62*, 2410−2412.
(13) (a) Yamamoto, H.; Nagai, A. *Mar. Chem.* **1992**, *37*, 131−143. (b) Yamamoto, H.; Nagai, A.; Okada, T.; Nishida, A. *Mar. Chem.* **1989**, *26*, 331−338.
(14) Yamamoto, H. *J. Chem. Soc., Perkin Trans. 1* **1987**, 613−618.
(15) Kricheldorf, H. R. α-*Amino acid-N-Carboxyanhydrides and Related Heterocycles*; Springer-Verlag: New York, 1987.
(16) Bodanszky, M.; Bodanszky, A. *The Practice of Peptide Synthesis*, 2nd ed.; Springer: Berlin, Heidelberg, 1994.
(17) Yamamoto, H.; Hayakawa, T. *Biopolymers* **1977**, *16*, 1593−1607.
(18) Schwittay, C.; Mours, M.; Winter, H. H. *Faraday Discuss.* **1995**, *101*, 93−104.
(19) *ASTM Standards on Adhesives*, 5th ed.; ASTM: Philadelphia, PA, 1961.
(20) Holl, S. M.; Hansen, D.; Waite, J. H.; Schaefer, J. *Arch. Biochem. Biophys.* **1993**, *302*, 255−258.
(21) A polydecapeptide having the sequence of the adhesive protein of *Mytilus edulis* (MW ca. 140 000) was reported to give a tensile bond strength of 2.75 MPa when cured in H_2O on steel adherends.[14]

MA980268Z