Synthesis and Adhesive Studies of Marine Polypeptides

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The marine adhesive polydecapeptide (Ala-Lys-Pro-Ser-Tyr-Hyp-Hyp-Thr-Dopa-Lys)$_n$ (*n ca.* 10) has been synthesized by coupling reactions, followed by polycondensation. The *O*-benzyl, *O,O'*-dibenzyl, and *N*-ε-2-chlorobenzyloxycarbonyl groups were used to protect the side chains of Ser, Tyr, Hyp, Thr, Dopa, and Lys. The protecting groups were simultaneously cleaved by hydrogen bromide. The bonding strength of the polydecapeptide and other synthetic polypeptides on metals has been measured in water. The bonding strength of the synthesized polydecapeptide exhibited a tensile strength of 28 kg cm^{-2} on Fe and a compressive shear strength of 3 kg cm^{-2} on Al_2O_3, while high molecular weight poly(Lys)·HBr was found to have the highest tensile strength (123 kg cm^{-2}) on Fe, and gelatin the highest compressive shear strength (21 kg cm^{-2}) on Al_2O_3. The factors important for the effective adhesion on metals are discussed.

Since the early 1950's synthetic polypeptides have been investigated for such medical purposes as antigenicity, antiviral, antibacterial, and antitumour activities, and growth-inhibitory effects,[1,2] and also for uses such as synthetic fibres, water- and gas-permeable membranes, and artificial skins.[3-5] Recently, Waite *et al.*[6] identified some of Nature's most powerful adhesives secreted by marine molluscs such as mussels, oysters, and barnacles, which must routinely cope with the force of surf and tides, as L-β-3,4-dihydroxyphenyl-α-alanine (Dopa) containing proteins.[7-12] The marine adhesive proteins have since been purified and the sequence of the major tryptic peptide was analysed as NH_2-Ala-Lys-Pro-Ser-Try-4-Hyp-4-Hyp-Thr-Dopa-Lys-CO_2H.[6,11,12] This and related sequences are reported to repeat as often as 75 times in the marine adhesive protein.[12,13] The subject of marine animals and their adhesive capabilities has been reviewed by Young and Crisp.[14]

Previously we have reported the synthesis and conformational studies of poly(Dopa) and of a series of random and sequential copolypeptides of Dopa with Glu and Lys.[15-20] The objective of the present article is to report the synthesis of marine adhesive proteins with deca-repeating amino acids containing Dopa and to discuss the quantitative evaluation of the adhesive power of synthetic polypeptides including the polydecapeptide on metals (Fe and Al_2O_3).

Experimental

Materials.—Dopa was purchased from the Tokyo Chemical Industries Co. Ltd. t-Butyl carbazate, Boc-Ala,[6,*] Boc-Lys[Z^2(Cl)], Boc-Ser(CH_2Ph), Boc-Try(CH_2Ph), Boc-4-Hyp-(CH_2Ph), and Pro were purchased from the Protein Research Foundation. Gelatin, *p*-nitrophenol, 25% hydrogen bromide in glacial acetic acid, dicyclohexylcarbodi-imide (DCCI), 1-hydroxybenzotriazole (HOBt), and trifluoroacetic acid (TFA) were purchased from the Wako Pure Chemical Industries Ltd. *N,N*-Dimethylformamide (DMF; dried over potassium hydroxide) and dichloroacetic acid (DCA) both from Wako, were distilled at 40 °C/10 mmHg and at 102 °C/20 mmHg, respectively, and stored in brown bottles. Triethylamine (TEA) and *N*-methylmorpholine from Wako were refluxed over sodium and then fractionally distilled at atmospheric pressure. 6M-Hydrogen chloride in dry dioxane was purchased from the Kokusan Chemical Works Ltd. All other reagents and solvents were reagent grade and were used without further purification.

Water-soluble polypeptides in the Table, except the marine polydecapeptide, were synthesized according to the *N*-carboxy-anhydride method[21] and *p*-nitrophenyl active ester method.[22]

Methods.—Melting points were measured using a Yamato capillary melting points apparatus MP-1 and are uncorrected. Optical rotations were measured with a JASCO DIP-4 polarimeter at 589 nm. Elementary analyses were carried out with a Yanagimoto CHN recorder MT-2. Amino acid analyses were carried out with a Kyowa Seimitsu KLA-101S.

Intrinsic viscosities were measured in DCA at 25 °C using an Ubbelohde viscometer. The molecular weights and degree of polymerization (DP) were estimated from the following empirical equations; $[\eta]^{25}_{DCA} = 3.2 \times 10^{-2}\ M_r^{0.66}$ for poly-[Tyr(Z)][23] and $\log DP = 1.47 \log [\eta]^{25}_{DCA} + 2.99$ for poly[Lys(Z)].[24]

Bonding Strength.—The bonding strength of polypeptides on metals was measured according to the Japanese Industrial Standard (JIS). The tensile strength was measured using iron (Fe) test pieces (12.7 × 12.7 × 38 mm) according to the JIS K6849 method. Each testing piece was sandblasted, degreased with trichloroethane, and then treated with an adhesive (polypeptide). Two iron pieces were stuck together and allowed to stand for 3 days at 23 °C and 60% relative humidity (RH). The adhered iron pieces were examined on a tensile testing machine at a rate of 10 mm min^{-1}. The compressive shear strength was measured using alumina (Al_2O_3) test pieces (10 × 25 × 30 mm) according to the JIS K6852 method which were treated exactly as described for the iron test pieces. After 3 days, the adhered alumina pieces were examined on a compressive testing machine at a rate of 10 mm min^{-1}. The Figure shows the experimental arrangement for the measurement of the two kinds of bonding strengths. Both the tensile and compressive shear strengths reported are the average values of three measurements each.

Synthesis: General Strategy for Synthesis.—T.l.c. was carried out on precoated silica gel plates (Merck Kieselgel G Type 60). The following solvent systems were used: A, chloroform–methanol–acetic acid (95:5:3, v/v); B, chloroform–acetic acid (95:5, v/v); C, butanol–acetic acid–water (4:1:1, v/v). Spots were located on the plates by spraying with a 1% solution of ninhydrin in water-saturated butanol and heating (free amine)

* Abbreviations used for amino acid protecting groups: Et = ethyl, Boc = *N*-α-t-butyloxycarbonyl, Z = benzyloxycarbonyl, Z^2(Cl) = *N*-ε-2-chlorobenzyloxycarbonyl, CH_2Ph = *O*-benzyl, diCH_2Ph = *O,O'*-dibenzyl, and Np = *p*-nitrophenyl.

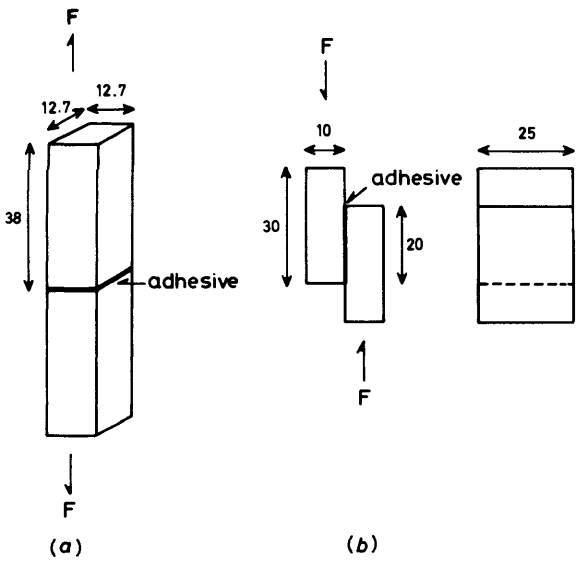


Figure. Experimental arrangements for the bonding strengths; (*a*) tensile strength and (*b*) compressive shear strength. Length in mm

or by heating for 10 min, spraying with ninhydrin, and heating (Boc-amine).

The synthesis of the marine adhesive polydecapeptide is outlined in the Scheme in which Boc α-amino and Et carboxy protecting groups were used. To protect five different kinds of hydroxy group (Ser, Tyr, Hyp, Thr, and Dopa) in the side chains, the CH_2Ph groups where chosen whilst $Z^2(Cl)$ groups were chosen to protect the ε-amino groups of Lys, since Schnable *et al.*[25] recommends the use of chloro- and nitro-substituted Z groups for the selective removal of *N*-α-Boc and *N*-ε-Z protecting groups. *p*-Nitrophenyl active ester was chosen to polymerize the protected decapeptide[22] and hydrogen bromide was used for the simultaneous cleavage of OCH_2Ph and *N*-ε-amine protecting groups.[26–28] All the reactions were monitored by t.l.c.

Lys[Z^2(Cl)]-Pro *Ethyl Ester Hydrochloride* (**4**).—Pro ethyl ester hydrochloride (**2**) was prepared from Pro and ethanol containing thionyl chloride as described by Greenstein and Winitz.[29] To a solution of Boc-Lys[Z^2(Cl)] (**1**) [isolated from Boc-Lys[Z^2(Cl)]·t-butylamine (24.4 g, 50 mmol) using dilute sulphuric acid] in chloroform (30 ml), was added DCCI (12.4 g, 55 mmol) at 0 °C. After 20 min, Pro ethyl ester [isolated from Pro ethyl ester hydrochloride (9.7 g, 50 mmol) using *N*-methylmorpholine] was added to the cold solution, and the reaction mixture was stirred at 0 °C for 2 h and allowed to stand overnight at 5 °C. A few drops of acetic acid were then added, and the insoluble dicyclohexylurea was filtered off and the solvent was evaporated under reduced pressure. The residue was redissolved in chloroform and further dicyclohexylurea was filtered off. The filtrate was washed successively with 4% aqueous sodium hydrogen carbonate (×3), water (×3), 0.5M-hydrochloric acid (×3), and water, and then dried (Na_2SO_4). After the solvent had been removed, the residue was repeatedly triturated with hexane to yield the oily Boc-Lys[Z^2(Cl)]-Pro ethyl ester (**3**) (27.0 g, 100%). This was dissolved in dry dioxane (100 ml), and a solution of hydrogen chloride in dioxane (6M; 83 ml) was added, after which the solution was stirred at room temperature for 1 h. Dry ether (150 ml) was then added to the solution, and the mixture was stirred for an additional 2 h. Finally, further dry ether (250 ml) was added and the solution was kept overnight in a refrigerator. After the solvent had been removed, the residue was triturated with ether and hexane. The product (**4**) solidified as an amorphous foam (19.2 g, 81%), m.p. 60—62 °C (Found: C, 52.9; H, 6.6; N, 8.7. $C_{21}H_{31}Cl_2N_3O_5$ requires C, 52.9; H, 6.6; N, 8.8%); $[\alpha]_D^{23}$ −17.3° (*c* 1, chloroform); R_F 0.18 (A), 0.06 (B), and 0.63 (C).

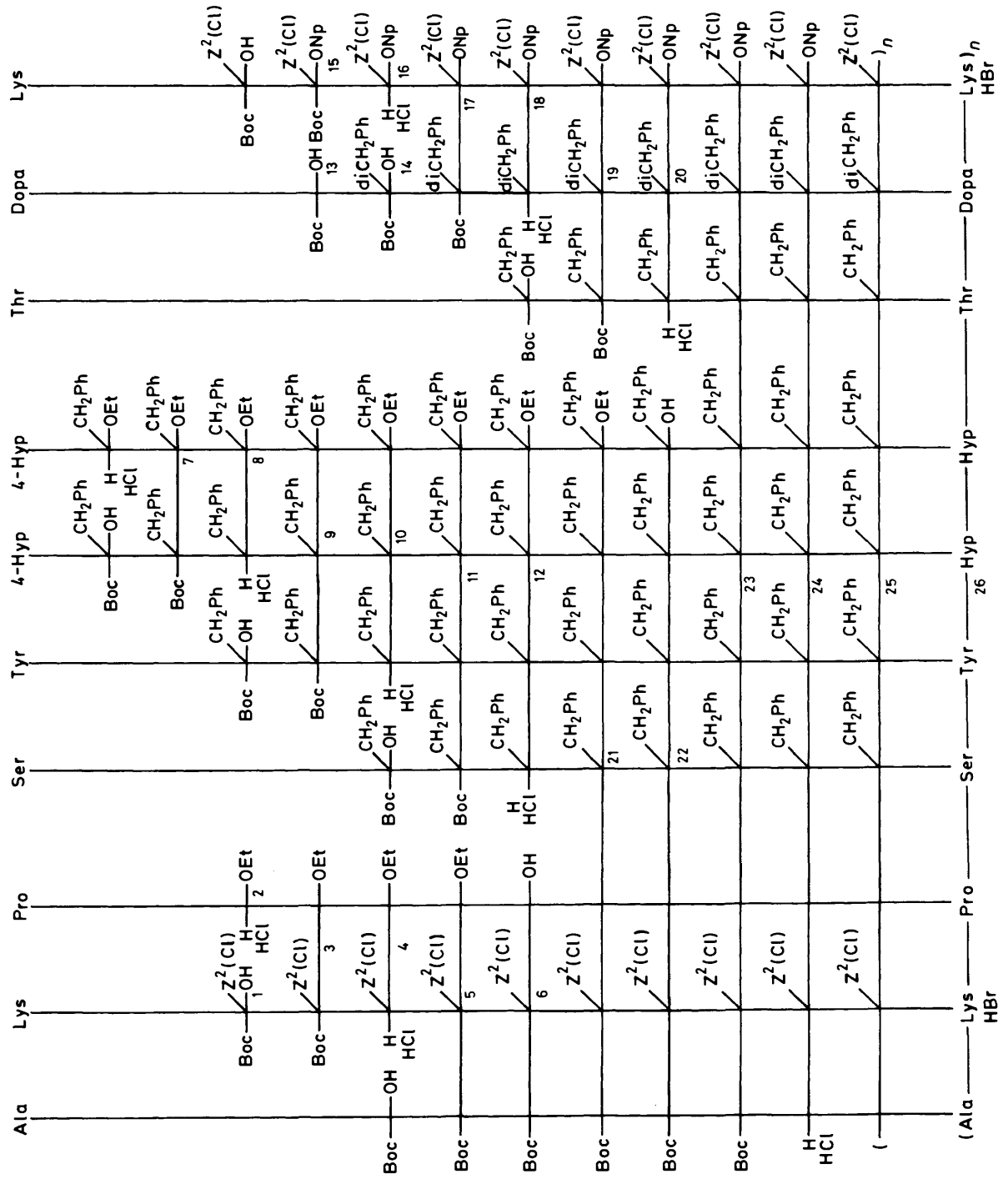
Boc-Ala-Lys[Z^2(Cl)]-Pro *Ethyl Ester* (**5**).—To a solution of Boc-Ala (5 g, 26.4 mmol) in chloroform (30 ml) was added DCCI (6.5 g, 31.7 mmol) at 0 °C. After 20 min, Lys[Z^2(Cl)]-Pro ethyl ester [isolated from compound (**4**) (12.6 g, 26.4 mmol) using *N*-methylmorpholine] was added to the cold solution. The product was worked up in a manner similar to that described for compound (**3**) and solidified as an amorphous foam (15.1 g, 94%), m.p. 59—61 °C (Found: C, 57.1; H, 7.1; N, 9.3. $C_{29}H_{43}ClN_4O_8$ requires C, 57.0; H, 7.1; N, 9.2%); $[\alpha]_D^{23}$ −21.8° (*c* 1, chloroform); R_F 0.62 (A), 0.50 (B), and 0.93 (C).

Boc-Ala-Lys[Z^2(Cl)]-Pro (**6**).—Compound (**5**) (16.0 g, 26.2 mmol) was dissolved in ethanol (150 ml). 1M-Sodium hydroxide (35 mmol) was then added to the solution at 0 °C. After the reaction had been stirred for 4 h at room temperature, the solvent was removed under reduced pressure. The residue was washed with ether to remove the unchanged ester, acidified to pH 3 with 1M-hydrochloric acid, and extracted with ethyl acetate. The organic layer was washed with water and dried (Na_2SO_4). After the solvent had been removed, the residue was triturated with hexane and kept overnight in a refrigerator. The crystalline product obtained was recrystallized from ethyl acetate–hexane (14.3 g, 93%), m.p. 85—90 °C (Found: C, 55.8; H, 6.9; N, 9.7. $C_{27}H_{39}ClN_4O_8$ requires C, 55.6; H, 6.7; N, 9.6%); $[\alpha]_D^{24}$ −15.7° (*c* 1, ethanol); R_F 0.43 (A), 0.19, (B), and 0.92 (C).

4-Hyp(CH_2Ph)-4-Hyp(CH_2Ph] *Ethyl Ester Hydrochloride* (**8**).—To a solution of Boc-4-Hyp(CH_2Ph) (6.4 g, 20 mmol) in chloroform (30 ml), was added DCCI (5 g, 24 mmol) at 0 °C. After 20 min, oily 4-Hyp(CH_2Ph) ethyl ester (5.7 g, 10 mmol) [prepared from 4-Hyp(CH_2Ph) in a manner similar to that described for compound (**2**) and isolated using *N*-methylmorpholine] was added to the cold solution. The product (**7**) was worked up as for compound (**3**) and was obtained as an oil (9.9 g, 90%). The oily Boc-4-Hyp(CH_2Ph)-4-Hyp(CH_2Ph) ethyl ester (**7**) (9.5 g, 17.2 mmol) was treated with hydrogen chloride in dioxane (2.2M; 80 ml) by the same procedure described for the synthesis of compound (**4**). The product (**8**) was recrystallized from dioxane–ether (7.5 g, 89%), m.p. 88—91 °C (Found: C, 63.7; H, 6.8; N, 5.7. $C_{26}H_{33}ClN_2O_5$ requires C, 63.9; H, 6.8; N, 5.7%); $[\alpha]_D^{24}$ −21.4° (*c* 1, dioxane); R_F 0.02 (A), 0.02 (B), and 0.73 (C).

Boc-Tyr(CH_2Ph)-4-Hyp(CH_2Ph)-4-Hyp(CH_2Ph) *Ethyl Ester* (**9**).—To a solution of Boc-Tyr(CH_2Ph) (5 g, 13.5 mmol) in chloroform (30 ml), was added DCCI (3.3 g, 16.2 mmol) at 0 °C. After 20 min, 4-Hyp(CH_2Ph)-4-Hpy(CH_2Ph) ethyl ester [isolated from compound (**8**) (6.6 g, 13.5 mmol) using *N*-methylmorpholine] was added to the cold solution. The product was worked up in a manner similar to that for compound (**3**) and was obtained as an amorphous foam (10.0 g, 92%), m.p. 45—48 °C (Found: C, 70.0; H, 7.1; N, 5.5. $C_{47}H_{55}N_3O_9$ requires C, 70.0; H, 6.9; N, 5.2%); $[\alpha]_D^{27}$ 14.0° (*c* 1, chloroform); R_F 0.93 (A), 0.55 (B), and 0.97 (C).

Tyr(CH_2Ph)-4-Hyp(CH_2Ph)-4-Hyp(CH_2Ph) *Ethyl Ester Hydrochloride* (**10**).—Compound (**9**) (9.9 g, 12.3 mmol) was treated with 1.5M-hydrogen chloride in dioxane (81 ml) by the same procedure as that described for the synthesis of compound (**4**). The product was recrystallized from dioxane–hexane (8.5 g, 93%), m.p. 80—85 °C (Found: C, 67.8; H, 6.6; N, 5.7. $C_{42}H_{48}ClN_3O_7$ requires C, 68.0; H, 6.5; N, 5.7%); $[\alpha]_D^{26}$ 18.1° (*c* 1, dioxane); R_F 0.59 (A), 0.29 (B), and 0.81 (C).



Scheme.

Boc-Ser(CH_2Ph)-Tyr(CH_2Ph)-4-Hyp(CH_2Ph)-4-Hyp-(CH_2Ph) *Ethyl Ester* (**11**).—Boc-Ser(CH_2Ph) (3.6 g, 12.1 mmol) in chloroform (30 ml) was coupled with Tyr(CH_2Ph)-4-Hyp(CH_2Ph)-4-Hyp(CH_2Ph) ethyl ester [isolated from compound (**10**) (9.0 g, 12.1 mmol) using *N*-methylmorpholine] using DCCI (3.0 g, 14.5 mmol). The product was worked up in a manner similar to that for compound (**3**) and recrystallized from ethyl acetate–hexane (10.4 g, 87%), m.p. 68—70 °C (Found: C, 69.5; H, 6.9; N, 5.6. $C_{57}H_{66}N_4O_{11}$ requires C, 69.6; H, 6.8; N, 5.7%); $[\alpha]_D^{27}$ 16.6° (*c* 1, chloroform); R_F 0.68 (A), 0.40 (B), and 0.97 (C).

Ser(CH_2Ph)-Tyr(CH_2Ph)-4-Hyp(CH_2Ph)-4-Hyp(CH_2Ph) *Ethyl Ester Hydrochloride* (**12**).—Compound (**11**) (10.8 g, 11.0 mmol) was treated with 1.4M-hydrogen chloride in dioxane (78 ml) by the same procedure as that described for the synthesis of compound (**4**). This was recrystallized from dioxane and ether–hexane (1:1, v/v) (9.0 g, 89%), m.p. 65—68 °C (Found: C, 67.8; H, 6.4; N, 6.0. $C_{52}H_{59}ClN_4O_9$ requires C, 67.9; H, 6.5; N, 6.1%); $[\alpha]_D^{26}$ 16.0° (*c* 1, dioxane); R_F 0.37 (A), 0.16 (B), and 0.92 (C).

Boc-Dopa(diCH_2Ph) (**14**).—Boc-Dopa (**13**) was prepared from Dopa by the direct method described by Kaiser *et al.*;[30] $[\alpha]_D^{25}$ 16.1° (*c* 1, methanol). Boc-Dopa(diCH_2Ph) was prepared from Boc-Dopa and benzyl chloride in the presence of potassium carbonate and sodium iodide as described by Banerjee and Ressler[31] (51%); m.p. 105 °C (Found: C, 70.4; H, 6.6; N, 2.9. $C_{28}H_{31}NO_6$ requires C, 70.4; H, 6.5; N, 2.9%); $[\alpha]_D^{25}$ 14.2° (*c* 1, methanol); R_F 0.61 (A), 0.50 (B), and 0.92 (C).

Boc-Dopa(diCH_2Ph)-Lys[Z^2(Cl)] p-*Nitrophenyl Ester* (**17**).—Lys[Z^2(Cl)] *p*-nitrophenyl ester hydrochloride (**16**) was prepared from Boc-Lys[Z^2(Cl)] *p*-nitrophenyl ester (**15**) using the same procedure as that described in our previous paper.[19] Boc-Dopa(diCH_2Ph) (4.0 g, 8.4 mmol) in chloroform (20 ml) was coupled with Lys[Z^2(Cl)] *p*-nitrophenyl ester [isolated from compound (**16**) (4.0 g, 8.4 mmol) using *N*-methylmorpholine] using DCCI (2.1 g, 10.1 mmol). The product was worked up in a manner similar to compound (**3**) and recrystallized from dioxane–hexane (6.4 g, 85%), m.p. 159 °C (Found: C, 64.3; H, 5.9; N, 6.3. $C_{48}H_{51}ClN_4O_{11}$ requires C, 64.4; H, 5.7; N, 6.3%); $[\alpha]_D^{24}$ 6.0° (*c* 1 chloroform), R_F 0.72 (A), 0.51 (B), and 0.89 (C).

Dopa(diCH_2Ph)-Lys[Z^2(Cl)] p-*Nitrophenyl Ester Hydrochloride* (**18**).—Compound (**17**) (6.2 g, 6.9 mmol) was treated with 1.1M-hydrogen chloride in dioxane (62 ml) by the same procedure described for the synthesis of compound (**4**). This was recrystallized from dioxane–ether (4.7 g, 81%), m.p. 153 °C (Found: C, 62.3; H, 5.4; N, 6.6. $C_{43}H_{44}Cl_2N_4O_9$ requires C, 62.1; H, 5.3; N, 6.7%); $[\alpha]_D^{25}$ 17.1° (*c* 1, dioxane); R_F 0.15 (A), 0.65 (B), and 0.90 (C).

Boc-Thr(CH_2Ph)-Dopa(diCH_2Ph)-Lys[Z^2(Cl)] p-*Nitrophenyl Ester* (**19**).—Boc-Thr(CH_2Ph) (1.67 g, 5.4 mmol) in chloroform (20 ml) was coupled with Dopa(diCH_2Ph)-Lys-[Z^2(Cl)] *p*-nitrophenyl ester [isolated from compound (**18**) (4.5 g, 5.4 mmol) using *N*-methylmorpholine] using DCCI (1.34 g, 6.5 mmol). The product was worked up in a manner similar to that described for compound (**3**) and recrystallized from chloroform–hexane (4.6 g, 78%), m.p. 144—146 °C (Found: C, 65.0; H, 6.0; N, 6.5. $C_{59}H_{64}ClN_5O_{13}$ requires C, 65.2; H, 5.9; N, 6.5%); $[\alpha]_D^{25}$ 17.0° (*c* 1, chloroform); R_F 0.86 (A), 0.58 (B), and 0 (C).

Thr(CH_2Ph)-Dopa(diCH_2Ph)-Lys[Z^2(Cl)] p-*Nitrophenyl Ester Hydrochloride* (**20**).—Compound (**19**) (4.5 g, 4.1 mmol) was treated with 0.7M-hydrogen chloride in dioxane (57 ml) by the same procedure as that described for the synthesis of compound (**4**). This was recrystallized from dioxane–ether (3.6 g, 85%), m.p. 119 °C (Found: C, 63.2; H, 5.7; N, 6.7. $C_{54}H_{57}Cl_2N_5O_{11}$ requires C, 63.4; H, 5.6; N, 6.9%); $[\alpha]_D^{25}$ 15.0° (*c* 1, dioxane); R_F 0.13 (A), 0.02 (B), and 0.86 (C).

Boc-Ala-Lys[Z^2(Cl)]-Pro-Ser(CH_2Ph)-Tyr(CH_2Ph)-4-Hyp(CH_2Ph)-4-Hyp(CH_2Ph) *Ethyl Ester* (**21**).—Boc-Ala-Lys[Z^2(Cl)]-Pro (6.3 g, 10.8 mmol) in chloroform (30 ml) was coupled with 4-Hpy(CH_2Ph)-4-Hyp(CH_2Ph) ethyl ester [isolated from compound (**12**) (10.0 g, 10.8 mmol) using *N*-methylmorpholine] using DCCI (2.67 g, 13.0 mmol). The product was worked up in a manner similar to that described for compound (**3**) and recrystallized from ethyl acetate–hexane (13.9 g, 89%), m.p. 68—71 °C (Found: C, 65.2; H, 6.8; N, 7.7. $C_{79}H_{95}ClN_8O_{16}$ requires C, 65.5; H, 6.6; N, 7.7%); $[\alpha]_D^{25}$ −3.8° (*c* 1, chloroform); R_F 0.52 (A), 0.18 (B), and 0.85 (C).

Boc-Ala-Lys[Z^2(Cl)]-Pro-Ser(CH_2Ph)-Tyr(CH_2*Ph*)-4-Hyp(CH_2Ph)-4-Hyp(CH_2Ph) (**22**).—To a solution of Boc-heptapeptide ethyl ester (**21**), (14.6 g, 10.1 mmol) in ethanol (100 ml), was added 1M-sodium hydroxide (15 ml; 15 mmol) at 0 °C. After the solution had been stirred for 4 h at room temperature, complete saponification was confirmed by t.l.c. The product was worked up in a manner similar to that described for compound (**6**) and recrystallized from ethyl acetate–hexane (13.3 g, 93%), m.p. 75—80 °C (Found: C, 64.8; H, 6.5; N, 7.9. $C_{77}H_{91}ClN_8O_{16}$ requires C, 65.1; H, 6.5; N, 7.9%); $[\alpha]_D^{25}$ −7.4° (*c* 1, chloroform); R_F 0.33 (A), 0.06 (B), and 0.84 (C); amino acid analysis $Ala_{1.00}Lys_{1.10}Pro_{1.05}Ser_{0.96}Tyr_{0.93}Hyp_{1.89}$.

Boc-Ala-Lys[Z^2(Cl)]-Pro-Ser(CH_2Ph)-Tyr(CH_2Ph)-4-Hyp(CH_2Ph)-4-Hyp(CH_2Ph)-Thr(CH_2Ph)-Dopa(diCH_2Ph)-Lys[Z^2(Cl)] p-*Nitrophenyl Ester* (**23**).—Boc-heptapeptide (**22**) (4.86 g, 3.4 mmol) in chloroform (30 ml) was coupled with Thr(CH_2Ph)-Dopa(diCH_2Ph)-Lys[Z^2(Cl)] *p*-nitrophenyl ester [isolated from compound (**20**) (3.5 g, 3.4 mmol) using *N*-methylmorpholine] using DCCI (0.85 g, 4.1 mmol). The product was worked up in a manner similar to that described for compound (**3**) and recrystallized from ethyl acetate–hexane (7.2 g, 88%), m.p. 88—93 °C (Found: C, 65.9; H, 6.3; N, 7.6. $C_{131}H_{145}Cl_2N_{13}O_{26}$ requires C, 65.9; H, 6.1; N, 7.6%); $[\alpha]_D^{25}$ −4.8° (*c* 1, chloroform); R_F 0.42 (A), 0.07 (B), and 0.86 (C).

Ala-Lys[Z^2(Cl)]-Pro-Ser(CH_2Ph)-Tyr(CH_2Ph)-4-Hyp-(CH_2Ph)-4-Hyp(CH_2Ph)-Thr(CH_2Ph)-Dopa(diCH_2Ph)-Lys-[Z^2(Cl)] p-*Nitrophenyl Ester Hydrochloride* (**24**).—Boc-decapeptide (**23**) (8.0 g, 3.4 mmol) was treated with 0.75M-hydrogen chloride in dioxane (46 ml) by the same procedure as that described for the synthesis of compound (**4**). The product was recrystallized from dioxane–ether (6.9 g, 89%), m.p. 98—103 °C (Found: C, 64.8; H, 6.1; N, 7.9. $C_{126}H_{138}Cl_3N_{13}O_{24}$ requires C, 65.1; H, 6.0; N, 7.8%); $[\alpha]_D^{25}$ 2.0° (*c* 1, dioxane); R_F 0.02 (A), 0 (B), and 0.91 (C); amino acid analysis $Ala_{1.00}Lys_{1.80}Pro_{0.95}Ser_{1.06}Hyp_{2.13}Thr_{0.88}$. Dopa decomposed during hydrolysis with 6M-hydrochloric acid.

Poly{Ala-Lys[Z^2(Cl)]-Pro-Ser(CH_2Ph)-Tyr(CH_2Ph)-4-Hyp(CH_2Ph)-4-Hyp(CH_2Ph)-Thr(CH_2Ph)-Dopa(diCH_2Ph)-Lys[Z^2(Cl)]} (**25**).—Compound (**24**) (3.3 g, 1.4 mmol) was dissolved in dry DMF (2.5 ml). With cooling, were added (*a*) TEA (0.218 ml; 1.56 mmol) or (*b*) TEA (0.218 ml) and HOBt (38 mg, 0.28 mmol) with stirring. This was kept at room temperature for 2 weeks and then treated with water, to yield a pale yellow precipitate. The precipitated polydecapeptide was washed throughly with water and alcohol until the yellow colour disappeared, then centrifuged, washed with ether, and

Table. Bonding strength of polypeptides on metals in water systems[a]

Adhesive	DP[b]	Solvent	Concentration weight % (g 100 g^{-1})	Tensile strength Fe (kg cm^{-2})[c]	Compressive shear strength Al_2O_3 (kg cm^{-2})[c]
Byssus thread[d]				8	
Whole thread[d]				200—2 000	
Adhesive disc[f]				4—8	
Polydecapeptide[g]	100	Water	50	28	3
Gelatin		Water	20	79	21
Poly(Lys)·HBr	3 260	Water	36	123	7
	980	Water	5	21	3
	460	Water	10	22	2
Poly(Lys)·1/2H_2SO_4	3 260	Water	25	60	9
Poly(Lys)·HCl	3 260	Water	25	46	17
Poly(Orn)·HBr	720	Water	7	10	7
Poly(Glu)Na	75	Water	44	7	2
Poly(D-Glu)Na	400	Water	15	11	3
Poly(3-hydroxypropyl-Gln)	215	Water	13	31	2
Poly(Cys)Na		Water	20	3	4
Poly(DL-Ala)		Water	29	38	8
Poly(CysNa^{1}Lys·HBr^3)		Buffer[h]	10	51	2
Poly(Glu^{1}Lys·HBr^1)		Water	33	25	2
Poly(TyrNa^{1}GluNa^{1}Ala$^{1.1}$)	110	Water	23	6	1
Poly(Dopa-Lys·HBr)	44	Water	33	37	1

[a] Partly taken from ref 38. [b] Total amino acid residues. [c] Average of three measurements. [d–f] *Mytilus edulis* ([d] ref 39, [e] ref 40, and [f] to periostracum and shell, ref 14). [g] Compound (**26**). [h] pH 6.9 phosphate buffer.

dried, to yield, (*a*) (2.4 g, 79%), $[\alpha]_D^{25}$ −9.8° (*c* 1, chloroform) and (*b*) (2.3 g, 75%) [Found: (*a*) C, 66.8; H, 6.1; N, 7.6% and (*b*) C, 66.7; H, 6.1; N, 7.6%. $(C_{120}H_{132}Cl_2N_{12}O_{21})_n$ requires C, 67.1; H, 6.2; N, 7.8%]. The intrinsic viscosities, $[\eta]_{DCA}^{25}$, were (*a*) 0.20 and (*b*) 0.14. The estimated molecular weights are shown in the discussion below.

Poly(Ala-Lys-Pro-Ser-Tyr-4-Hpy-4-Hyp-Thr-Dopa-Lys) *Dihydrobromide Dihydrate* (**26**).—The protected polydecapeptide compound (**25**) (1.7 g, 0.79 mmol) with $[\eta]_{DCA}^{25}$ 0.20 was treated with TFA (10 ml) and 25% HBr–AcOH (200 ml) for 10 h at 50 °C and for a further 1 day at room temperature with stirring. After the solvent had been removed under reduced pressure, the residue was repeatedly triturated with dry ether and ether–alcohol, and dried. The resulting crude product was dissolved in water and treated with activated charcoal. The filtrate was dialyzed for 2 days against distilled water using wet cellulose dialysis tubing (molecular weight cut off 1 000) from Spectrum Medical Industries Inc, and lyophilized (0.68 g, 62% as dihydrobromide dihydrate) [Found: C, 47.4; H, 6.2; N, 12.0. $(C_{55}H_{80}N_{12}O_{17}\cdot 2HBr\cdot 2H_2O)_n$ requires C, 47.9; H, 6.3; N, 12.2%].

Results and Discussion

Since it is known that the addition of HOBt affords peptides in excellent yield and a high purity, and accelerated the aminolysis reactions of active esters to form amide bonds,[32,33] HOBt was added to polymerize the protected decapeptide active ester (**24**). Contrary to expectation, however, an increase in viscosity was not observed and the predicted effect of HOBt was not realised for reasons which are unclear. The molecular weights of protected polydecapeptide {$[\eta]_{DCA}^{25}$ 0.20} were estimated to be 16 600 (7.7 repeating unit as decapeptide),[23] 18 900 (8.8 repeating unit),[24] and 26 300 (12.2 repeating unit) from $[\eta]_{DCA}^{25}$ 2.78×10^{-5} $M_r^{0.87}$ for poly[Glu(CH_2Ph)].[34] The estimated repeating units (8—12) are considerably lower than that of natural marine adhesive protein which has 75 repeating units.

In order to remove the CH_2Ph groups from the protected polypeptide (**25**) to give the final polypeptide (**26**), methanesulphonic acid (MSA) was used. Unfortunately the Tyr residue only underwent *ca.* 50% debenzylation even after treatment with MSA at 50 °C for 2 days. T.l.c. also indicated that parent amino acids were not in fact regenerated from OCH_2Ph (and diCH_2Ph) amino acids using this reagent as claimed by Yajima *et al.*[35–37] However when HBr–AcOH was used, all OCH_2Ph and *N*-ε-Z^2(Cl) protecting groups were cleaved simultaneously. It was thought that partial *O*-acetylation might be a competing process with this reagent, however, analytical data (elemental and amino acid analyses) showed sufficient purity for the study of the adhesive power of the synthetic marine polypeptides. With respect to amino acid analysis, authentic Dopa and 9-Dopa in compound (**24**) decomposed during hydrolysis using 6M-hydrochloric acid for 40 h at 110 °C.

Adhesive Power of Polypeptides.—The bonding strengths of synthetic polypeptides in water have previously been measured.[38] The bonding strengths of polypeptides, including synthesized marine polydecapeptide, on metals are listed in the Table the determination of which led us to the following findings. Gelatin, which has been used as a traditional glue, exhibited a tensile strength of 79 kg cm^{-2} on Fe and a compressive shear strength of 21 kg cm^{-2} on Al_2O_3. Among the fifteen polypeptides listed in the Table, gelatin exhibited the second highest tensile strength on Fe and the highest compressive shear strength on Al_2O_3. Poly(Lys)·HBr (DP 3260 sample) exhibited the highest tensile strength of 123 kg cm^{-2} on Fe. The poly(Lys)·HBr sample with a higher DP exhibited the higher bonding strengths on metals. When the counter anion of the ε-quaternary amino group of poly(Lys) was changed from bromide to chloride or sulphate, the tensile strength on Fe was decreased to *ca.* one-half and one-third respectively, but the compressive shear strength on Al_2O_3 was increased 1.3—2.4-fold. The bonding strengths of copolypeptides of Lys with other

amino acids were lower than poly(Lys). Poly(Orn), which is a lower homologue of poly(Lys), exhibited a low tensile strength (10 kg cm^{-2}) on Fe. The bonding strengths of poly(Glu)Na and poly (D-Glu)Na were low. The poly(Gln) derivative, however, exhibited a tensile strength of 31 kg cm^{-2}. Poly(Cys)Na showed little adhesion on Fe. Sticky poly(DL-Ala) exhibited a tensile strength of 38 kg cm^{-2} on Fe, which was one-third of that shown by poly(Lys)·HBr (DP 3260 sample).

The synthesized marine polydecapeptide is water-soluble and exhibited a tensile strength of 28 kg cm^{-2} on Fe and a compressive shear strength of 3 kg cm^{-2} on Al_2O_3. It may be expected that very high DP samples with the same decasequence should exhibit higher bonding strengths. In this connection, the *C*-terminal sequence polydipeptide of the marine adhesive protein, poly(Dopa-Lys), exhibited a tensile strength of 37 kg cm^{-2} on Fe. It is not possible from the results of the bonding strengths given in the Table to demonstrate the function of Dopa, which Waite *et al.*[6-12] have speculated confers unique adhesive properties on the adhesive disc protein. However, the oxidizability of the 3,4-dihydroxy groups of Dopa to an *o*-quinone might contribute to the enhanced interaction of the Dopa-polypeptides on the surfaces of metals and/or shells and rocks.

Young and Crisp have summarized the information of the bonding strength of marine adhesive proteins.[14] The adhesive proteins were reported to exhibit a high bonding strength[39] (8 kg cm^{-2}) and a tensile strength of *ca.* 10^6 Pa (10 kg cm^{-2}) on glass,[14] although maximal values are often 2×10^7—2×10^8 Pa (200—2 000 kg cm^{-2}).[40] The bonding strength of natural mussel adhesive proteins varies according to many factors such as the region which the animal inhabits, whether the mussels are whole or in threads, the conditions of measurements (in the sea or dry) the extension rate, seasonal cycle and wave impact, and foreign objects and surfaces (rocks, glass, slate, and paraffin wax).[39-42] The quantitative bonding strengths of some of synthetic polypeptides in the Table are almost comparable to those of natural marine proteins. From this standpoint gelatin, high DP poly(Lys)·HBr, and lysine copolypeptides are powerful adhesives. However, these polypeptide adhesives are water-soluble after adhesion, while the common sea mussel attaches itself to a foreign substrate through an array of disc-tipped threads and the adhesive is water-insoluble. To overcome this significant difference between the two, the synthetic polypeptides must be treated in some way which renders them water-insoluble: for example, by co-ordination with metals or oxidation of the amino acid functional groups. When the synthesis of the polydecapeptide (**26**), had been completed in this laboratory five related peptides (Ala-Lys-Pro-Thr-Dopa-Lys, Ala-Lys-Hyp-Ser-Dopa-Hyp-Hyp-Thr-Dopa-Lys, Ala-Lys-Pro-Thr-Tyr-Lys, Ala-Lys-Hyp-Ser-Try-Hyp-Hyp-Thr-Dopa-Lys, and Ala-Lys-Pro-Ser-Dopa-Hyp-Hyp-Thr-Dopa-Lys) were also reported as components of the marine adhesive protein from *Mytilus edulis*.[13] These compounds are the next synthetic targets of this continuing study.

Acknowledgements

The author acknowledges helpful discussions with Dr. K. Inouye of this Institute and Dr. N. Nishi of Hokkaido University concerning the synthetic strategy. He is very grateful to Mr. K. Kimura of Toagosei Chemical Industries for measuring the bonding strengths. He is also indebted to Professor T. Hayakawa of this Institute and Mr. M. Miyazaki of Toagosei Chemical Industries for their encouragement and Mrs. A. Nishida for her skilful assistance.

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Received 2nd *April* 1986; *Paper* 6/649