Model Polypeptide of Mussel Adhesive Protein. I. Synthesis and Adhesive Studies of Sequential Polypeptides $(X\text{-}Tyr\text{-}Lys)_n$ and $(Y\text{-}Lys)_n$

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ABSTRACT: The sequential polytripeptides and polydipeptides, $(X\text{-}Tyr\text{-}Lys)_n$, (X═Gly, Ala, Pro, Ser, Leu, Ile, Phe), $(Y\text{-}Lys)_n$, (Y═Gly, Tyr), and $(Gly\text{-}Tyr)_n$, which imitate a mussel adhesive protein, have been synthesized. The molecular weights of the polypeptides were estimated to be 7,200 ~ 13,400 (19 ~ 42 repeating units), and the polypeptides were found to have satisfactory amino acid sequences. The polypeptides were crosslinked by tyrosinase, and the optimal pH in the crosslinking reaction was 7.4 in the case of the polytripeptide, $(Gly\text{-}Tyr\text{-}Lys)_n$. The optimal tyrosinase amount for the adhesive strength of $(Gly\text{-}Tyr\text{-}Lys)_n$ was 0.34 unit/mg (polypeptide) at pH 7.4. The shear adhesive strength of the polytripeptide increased with an increase in the polytripeptide concentration, and was not influenced by the third amino acid, X. The shear adhesive strengths of polytripeptides $(X\text{-}Tyr\text{-}Lys)_n$ were equal to one of the synthetic polydecapeptides, $(Ala\text{-}Lys\text{-}Pro\text{-}Ser\text{-}Tyr\text{-}Pro\text{-}Pro\text{-}Thr\text{-}Tyr\text{-}Lys)_n$ and $(Gly\text{-}Pro\text{-}Lys\text{-}Thr\text{-}Tyr\text{-}Pro\text{-}Pro\text{-}Thr\text{-}Tyr\text{-}Lys)_n$ which were the model polydecapeptides for blue mussel and Californian mussel, respectively. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 76: 929–937, 2000

Key words: marine adhesive protein; sequential synthetic polypeptide; enzymatic reaction; adhesive strength

INTRODUCTION

Marine invertebrates, such as mussels, oysters, and barnacles, secrete adhesive proteins and cling to reefs. Several marine adhesive proteins have been successfully purified, and the sequences of their major tryptic peptides have been determined. For example, the structure of California mussel[1] is $(Hyp\text{-}Lys\text{-}Gly\text{-}Thr\text{-}Dopa\text{-}Hyp\text{-}Hyp\text{-}Thr\text{-}Dopa\text{-}Lys)_n$ and that of blue mussel[2–5] is $(Ala\text{-}Lys\text{-}Pro\text{-}Ser\text{-}Dopa\text{-}Hyp\text{-}Hyp\text{-}Thr\text{-}Dopa\text{-}Lys)_n$. It is well known that the marine adhesive proteins include two key amino acids, Lys and Tyr/Dopa (L-β-3,4-dihydroxyphenyl-α-alanine), and that these two amino acids play an important role in the crosslinking of the proteins. The adhesive mechanism of the proteins was found to be autocrosslinking one by Lindner et al.[6–8] The autocrosslinking reaction occurs between dopaquinone residue yielded by the oxidation of tyrosyl (Dopa) residues of the protein and free amino group of Lys residue. Because these proteins can

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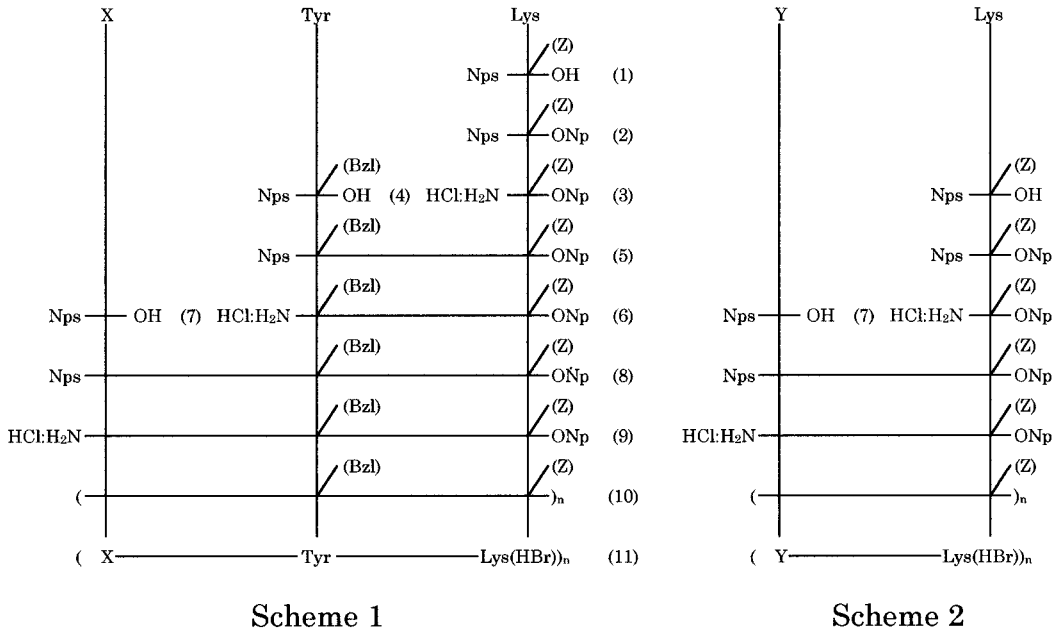

Figure 1 Synthetic routes of polypeptides. Scheme 1: $(X\text{-}Tyr\text{-}Lys(HBr))_n$ (X; Gly, Ala, Pro, Ser, Leu, Ile, Phe). Scheme 2: $(Y\text{-}Lys(HBr))_n$ (Y; Gly, Tyr).

exhibit large adhesive strength in water, we can anticipate applying them to biological tissue glues. This report describes the synthesis of the sequential polytripeptides and polydipeptides, which imitate mussel adhesive proteins, and their adhesive strength.

EXPERIMENTAL

Materials

ε-Benzyloxycarbonyl lysine (Lys(Z)), *o*-benzyl tyrosine (Tyr(Bzl)), glycine (Gly), prorine (Pro), alanine (Ala), serine (Ser), leucine (Leu), isoleucine (Ile), phenylalanine (Phe), trifluoroacetic acid (TFA), *N,N*-dimethylformamide (DMF), *N,N′*-dicyclohexylcarbodiimide (DCC), and 4N solution of hydrogen chloride (HCl) in dry dioxane were purchased from Kokusan Chemical Works (Japan). *o*-Nitrophenylsulfenyl chloride (Nps-Cl) was purchased from Tokyo Kasei Kogyo (Japan). *p*-Nitrophenol, dichloroacetic acid (DCA), and triethylamine (TEA) were purchased from Kanto Chemical Works (Japan). Thioanisole and 25 wt % hydrogen bromide (HBr) in acetic acid (AcOH) were purchased from Merck (USA). Tyrosinase from mushroom was purchased from Calzyme Lab. Inc. (USA).

DMF and DCA were distilled at 40°C/10 mmHg and 102°C/20 mmHg, respectively, and were stored in brown bottles. All other reagents and solvents were reagent grade and were used without further purification.

General Strategy for Synthesis

The synthetic routes of the sequential polytripeptides and polydipeptides are shown in Figure 1, where all the reactions are the standard coupling method. The reaction in each step was monitored by TLC (Merck Kieselgel G Type 60, Germany). The solvent system used for TLC was chloroform-methanol-acetic acid (95 : 5 : 3, vol/vol). In the coupling reactions, the following protecting groups were chosen. The Nps group was for α-amino group,[9] the Bzl group was for hydroxyl group of Tyr, and the ε-Z and α-Nps groups were for the side chain of Lys. All protecting groups of the polypeptides were finally cleaved by hydrogen bromide.

Characterization

Elementary analysis of each compound and polypeptide was carried out with a CHN recorder MT-3 (Yanagimoto, Japan). Amino acid sequence analysis of the polypeptides was carried out with an ABI 473A protein sequencer (Applied Biosystem Industry, USA).

Intrinsic viscosities, [η], of the polypeptides without the deprotection were measured in DCA at 25°C by use of Ubbelohde viscometer. The viscosity-average molecular weights (Mv) and the degrees of polymerization (DP) of the polypeptides were obtained as follows. The two Mvs and one DP were calculated for one polypeptide by use of eqs. (1), (2), and (3). From these Mvs and DP, two DPs and one Mv were calculated, respectively, on the basis of the molar weight of the repeating unit of the polypeptide. Thus, the three Mvs and DPs were averaged, and the values were defined as Mv and DP for the polypeptide.[10]

$$[\eta] = 3.2 \times 10^{-2}\ \mathrm{Mv}^{0.66} \quad (1)$$

$$[\eta] = 2.78 \times 10^{-5}\ \mathrm{Mv}^{0.87} \quad (2)$$

$$\log \mathrm{DP} = 1.47 \log[\eta] + 2.99 \quad (3)$$

Enzymatic Reaction

The oxidative reaction the of polypeptide by tyrosinase was carried out at 25°C under following condition. The polypeptide was dissolved in phosphate buffer (pH 7.4), and the tyrosinase amount and the polypeptide concentration were 34 unit/mg and 1×10^{-3} mol/L, respectively. The conversion of Tyr to Dopa was monitored for initial 30 min after the addition of tyrosinase to the polypeptide solution by means of the observation of the optical density (OD) at 283 nm (Dopa)[11] using a spectrophotometer (UV-2100S Shimadzu, Japan). The increase rate of OD, which would correspond to the rate of oxidative reaction, was calculated from the initial slope of the tangent to the curve obtained by plotting the OD against the reaction time.

Adhesive Strength

The shear adhesive strengths of the polypeptides were measured using a pigskin piece (18×18 mm). The schematic figure of the equipment to measure the shear adhesive strength is shown in Figure 2. The equipment was composed of two parts. The hair side of the pig skin was adhered onto each part of the equipment with cyanoacrylate glue. The polypeptide solution of phosphate buffer was applied on the pigskin test pieces, and the two pieces were combined. After the equipment was allowed to stay at 37°C for 30 min in air, the adhesive strengths of the polypeptides were measured with a universal testing machine (Auto-graph AGS-100A, Shimadzu, Japan) at the crosshead speed of 10 mm/min. The tests were carried out five times for each polypeptide, and the values were averaged. Statistical analysis was performed by using a Student's *t*-test. For all tests, a p value of .05 was used to determine significant differences.

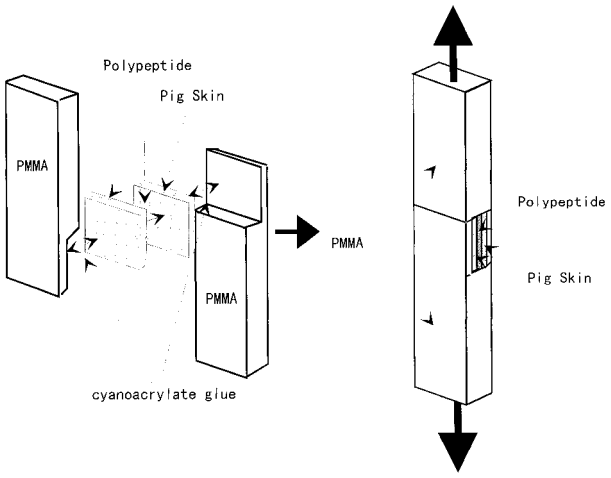

Figure 2 Schematic equipment for shear adhesive strength test.

Synthesis of Each Compound

Nps-Lys(Z)-OH (1)

Lys(Z) (60 mmol, 16.8 g) was dissolved in a mixture of 2N NaOH (36 mL) aqueous solution and dioxane (100 mL). Nps-Cl (72 mmol, 13.7 g) and 2N NaOH (30 mL) were added to the solution with vigorous stirring. The solution thus obtained was diluted with water (300 mL) and was filtered. To the filtrated solution, 1N sulfuric acid (60 mL) was added, and the acidic solution was extracted with ethyl acetate. The extract was washed with distilled water until the aqueous layer became neutral and then dried over anhydrous sodium sulfate (Na_2SO_4). The solvent in the extract was evaporated, and the Nps derivative was obtained as an oily substance.

Yield: 26.0 g (100%), and Rf = 0.74. This oily substance was used as compound (1) without further purification.

Nps-Lys(Z)-ONp (2)

To 50 mL of ethyl acetate solution of Nps-Lys(Z)-OH (1), DCC (72 mmol, 14.9 g) was added at 0°C. After 20 min, *p*-nitrophenol (60 mmol, 8.4 g) was added to the solution, and the reaction mixture

was stirred at 0°C for 2 h and allowed to stand overnight at room temperature. After a few drops of AcOH were added to the reaction solution, the insoluble dicyclohexylurea in the solution was filtered. The filtrate was washed with 1% aqueous sodium hydrogen carbonate (three times), water (three times), 10% aqueous citric acid (three times), and water (three times), and then was dried over Na_2SO_4. After the removal of the solvent from the filtrate, the residue was repeatedly triturated with n-hexane, and an oily substance was obtained.

Yield: 30.7 g (92%), and Rf = 0.88. The oily product was used as compound (2) without further purification.

Lys(Z)-ONp Hydrochloride (3)

After the dissolution of the compound (2) (30.7 g, 55 mmol) in dioxane (70 mL), 2 equivalent 4N HCl/dioxane solution (110 mmol, 27.5 mL) was added to the solution, and the solution was stirred at room temperature for 2 h. After evaporation of the solvent from the reaction solution, the residue was repeatedly triturated with ether, and the recrystallization from methanol/ether mixture (1 : 8 vol/vol) yielded compound (3).

Yield: 19.5 g (81%), m.p. = 156°C ~ 157°C, Rf = 0.12, and Elemental Analysis; Found: C, 54.67; H, 5.34; N, 9.59. Calcd C, 54.86; H, 5.52; N, 9.60.

Nps-Tyr(Bzl)-OH (4)

The Nps derivative (4) was prepared from Tyr(Bzl) and Nps-Cl in a mixture of 2N NaOH aqueous solution and dioxane in the same way as described for the synthesis of compound (1). The product was recrystallized from ethyl acetate/n-hexane mixture (3 : 25 vol/vol), and compound (4) was obtained.

Yield: 21.5 g (92%), m.p. = 138°C, Rf = 0.48, and Elemental Analysis; Found: C, 61.98; H, 4.71; N, 6.55. Calcd C, 62.25; H, 4.75; N, 6.60.

Nps-Tyr(Bzl)-Lys(Z)-ONp (5)

Compound (4) (18.7 g, 44 mmol) was coupled with compound (3) (19.3 g, 44 mmol) in chloroform by using TEA (6.12 mL, 44 mmol) and DCC (10.89 g, 52.8 mmol). The recrystallization of the reaction product from chloroform/ether mixture (1 : 8 vol/vol) yielded compound (5).

Yield: 31.7 g (89%), m.p. = 121°C, Rf = 0.62, and Elemental Analysis; Found: C, 62.70; H, 5.37; N, 8.86. Calcd C, 62.44; H, 5.12; N, 8.67.

Tyr(Bzl)-Lys(Z)-ONp Hydrochloride (6)

Compound (5) (31.5 g, 39 mmol) was dissolved in dioxane (120 mL) and treated with 2 equivalent 4N HCl/dioxane solution (78 mmol, 19.5 mL) in the same way as described in the synthesis of compound (3). The recrystallization of the reaction product from chloroform/ether mixture (3 : 20 vol/vol) yielded compound (6).

Yield: 19.1 g (71%), m.p. = 218°C, Rf = 0.17, and Elemental Analysis; Found: C, 62.35; H, 5.78; N, 8.14. Calcd C, 62.56; H, 5.69; N, 8.11.

Nps-X-OH (7) (X═Gly, Ala, Pro, Ser, Leu, Ile, Phe)

The Nps derivatives (7), X (X═Gly, Ala, Pro, Ser, Leu, Ile, Phe) were prepared in the same way as described in the synthesis of compound (1). The recrystallization of the reaction product from chloroform/ether mixture (1 : 10 vol/vol) yielded compounds (7). The experimental results for X═Gly were as follows.

Yield: 3.4 g (88%), Rf = 0.33, m.p. = 127°C, and Elemental Analysis; Found: C, 42.37; H, 3.39; N, 12.01. Calcd C, 42.10; H, 3.53; N, 12.27.

The other compounds (7) were also characterized in terms of melting point and elemental analysis.

Nps-X-Tyr(Bzl)-Lys(Z)-ONp (8) (X═Gly, Ala, Pro, Ser, Leu, Ile, Phe)

Compound (7) was coupled with compound (6) (Tyr(Bzl)-Lys(Z)-ONp) in chloroform by using TEA (1 equivalent) and DCC (1.2 equivalent). The recrystallization of the reaction product from chloroform/ether mixture (3 : 25 vol/vol) yielded compound (8). The experimental results for X═Gly were as follows.

Yield: 11.5 g (95%), Rf = 0.64, m.p. = 107°C, and Elemental Analysis; Found: C, 60.92; H, 5.25; N, 9.91. Calcd C, 61.10; H, 5.13; N, 9.72.

The other compounds (8) were also characterized in terms of melting point and elemental analysis.

X-Tyr(Bzl)-Lys(Z)-ONp Hydrochloride (9) (X═Gly, Ala, Pro, Ser, Leu, Ile, Phe)

Compound (8) was dissolved in dioxane, and treated with 2 equivalent 4N HCl/dioxane solu-

tion in the same way as described in the synthesis of compound (3). The recrystallization of the reaction product from chloroform/ether mixture (1 : 10 vol/vol) yielded compound (9). The experimental results for X═Gly were as follows.

Yield: 4.2 g (80%), Rf = 0.10, m.p. = 128°C, and Elemental Analysis; Found: C, 60.82; H, 5.39; N, 9.47. Calcd C, 61.00; H, 5.66; N, 9.36.

The other compounds (9) were also characterized in terms of melting point and elemental analysis.

Poly(X-Tyr(Bzl)-Lys(Z))$_n$ (10) (X═Gly, Ala, Pro, Ser, Leu, Ile, Phe)

Compound (9) was dissolved in DMF. TEA (1 equivalent) was added to the solution at 0°C and the reaction solution was stirred for 1 h, and then the solution was kept at room temperature for 7 days. The reaction solution was then treated with water to yield a pale yellow precipitate (compound (10)). The precipitate was washed thoroughly with water and ether until the yellow color disappeared and then dried in vacuo. The experimental results for X═Gly were as follows.

Yield: 2.5 g (87%), and Elemental Analysis; Found: C, 67.55; H, 6.54; N, 10.13. Calcd C, 67.12; H, 6.34; N, 9.78.

The other compounds (10) were also characterized in terms of elemental analysis.

Poly(X-Tyr-Lys(HBr))$_n$ (11) (X═Gly, Ala, Pro, Ser, Leu, Ile, Phe)

The TFA solution of compounds (10) was treated with 25% HBr/AcOH (6 equivalent) and thioanisole (2 equivalent) were added to the solution. The reaction solution was stirred for 5 h at 50°C, and the stirring was continued for 5 h at room temperature. After evaporation of the solvent, the residue was repeatedly washed with ether. After the removal of thioanisole from the residue was confirmed by spectrophotometry with the characteristic absorbance at 250 nm, the product was dried in vacuo. Thus, compound (11) was obtained. The experimental results for X═Gly were as follows.

Yield: 1.3 g (65%), and Elemental Analysis; Found: C, 48.15; H, 6.42; N, 12.44. Calcd C, 47.56; H, 5.87; N, 13.05.

Table I Molecular Weights of Polypeptides, (X-Tyr-Lys)$_n$ and (Y-Lys)$_n$

Polypeptide	[η][a]	Mv[b]	DP[c]
X			
Gly	0.140	7,660	22
Ala	0.208	134,00	37
Pro	0.175	11,300	29
Ser	0.136	7,200	19
Leu	0.144	8,900	22
Ile	0.178	11,700	29
Phe	0.183	12,700	29
Y			
Tyr	0.144	8,160	28
Gly	0.149	7,780	42

[a] Intrinsic viscosity of polypeptide, at 25°C, in DCA.
[b] Viscosity-average molecular weight of polypeptide without deprotection.
[c] Degree of polymerization.

The other compounds (11) were also characterized in terms of elemental analysis.

Polydecapeptides

The polydecapeptides, model precursors of blue mussel and California mussel proteins, with the structure of (Ala-Lys-Pro-Ser-Tyr-Pro-Pro-Thr-Tyr-Lys)$_n$ and (Gly-Pro-Lys-Thr-Tyr-Pro-Pro-Thr-Tyr-Lys)$_n$, respectively, were prepared according to the method reported by Yamamoto et al.[11] Their molecular weight of the former was 10,300, and that of the latter was 10,600.

RESULTS AND DISCUSSION

Characterization of Polypeptides

The molecular weights of the polytripeptides and polydipeptides with the protecting groups, poly(X-Tyr(Bzl)-Lys(Z))$_n$, poly(Y-Tyr(Bzl))$_n$, and poly(Y-Lys(Z))$_n$ were in the range of 7,200 ~ 13,400 (DP = 19 ~ 42), which were calculated from the intrinsic viscosities (0.140 ~ 0.208) of the polypeptides in DCA at 25°C (Table I). These molecular weights are considerably lower than that of natural mussel adhesive protein. For example, the protein of blue mussel has the molecular weight of 130,000.[2] However, as a synthetic sequential polypeptide, it is considered that they have sufficient molecular weights to investigate their adhesive characteristics.[12,13] It was proved that the

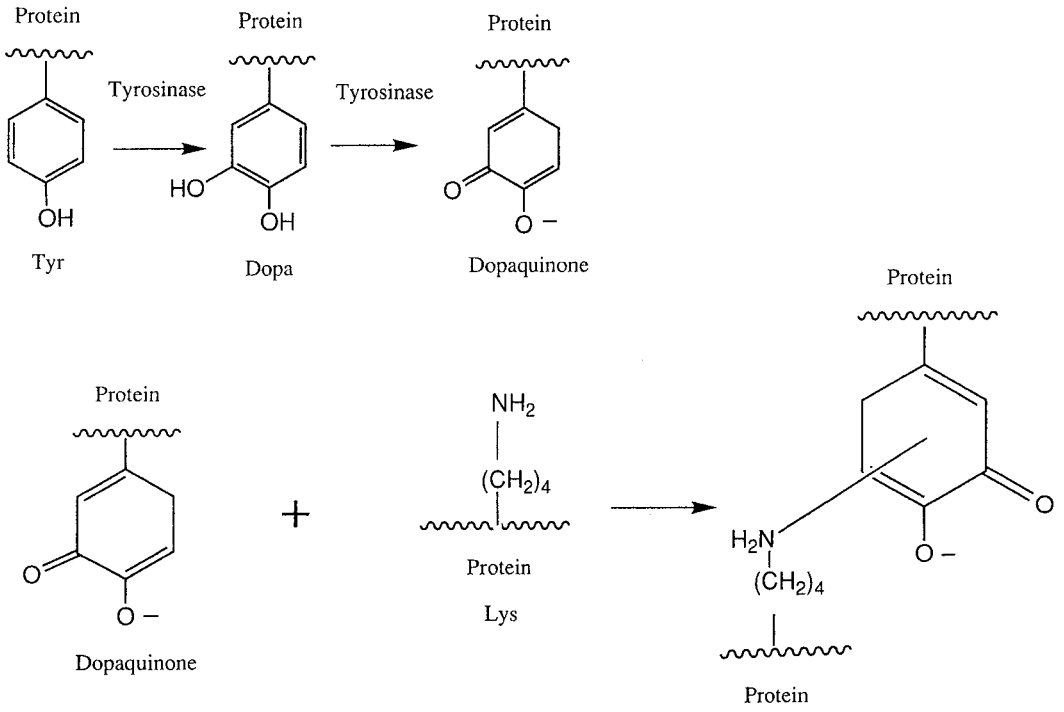

Figure 3 Crosslinking mechanism of polypeptide.

polytripeptides and polydipeptides obtained had good amino acid sequences on the basis of the results of the amino acid sequence analysis.

Enzymatic Reaction

The insolubilization and adhesion of mussel adhesive proteins have been explained by autocrosslinking mechanism,[6–8] as shown in Figure 3, and it has been said that the Tyr/Dopa and Lys residues in them are key amino acids in the adhesion. As shown in Figure 3, the first reaction is the oxidation of Tyr/Dopa residue to dopaquinone catalyzed by tyrosinase. Consecutively, the autocrosslinking occurs between the dopaquinone residue and free amino group of Lys residue in the polypeptides.

Because this oxidative reaction is enzymatic reaction involved with tyrosinase, the effect of the pH of the solution on the reaction was investigated by use of $(Gly\text{-}Tyr\text{-}Lys)_n$ as a representative of the polytripeptides. The result is shown in Figure 4, where the *y*-axis presents the increase rate of the optical density due to the absorption of Dopa residue at 283 nm, and the pH is varied from 4 to 8 by use of buffer solution. In this evaluation, we observed the absorption change of Dopa instead of that of dopaquinone, because

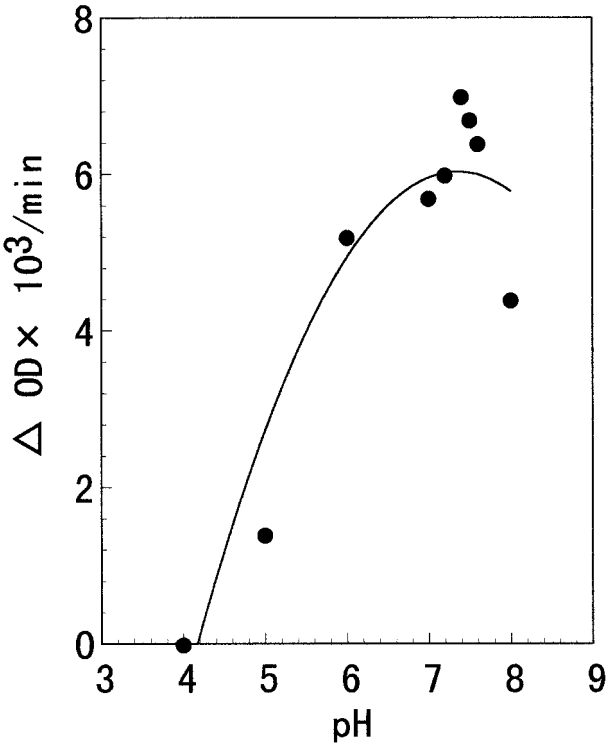

Figure 4 pH dependence profile of oxidative reaction of polytripeptide $(Gly\text{-}Tyr\text{-}Lys)_n$. Δ OD/min: the increase rate of the optical density at 283 nm that corresponds to the absorption band of Dopa. Polypeptide concentration: 1×10^{-3} mol/L, 25°C, pH 7.4 phosphate buffer solution.

dopaquinone is very reactive and unstable. The optical density increases from 0 to 7.0×10^{-3} with the increase of pH from 4 to 7.4 and then drops to 4.4×10^{-3} when the pH of the reaction solution is 8.0. These results indicate that the optimal pH for the enzyme activity is 7.4. It is known that the enzyme activities of tyrosinase for the oxidations of synthetic adhesive proteins at 25°C in the simulated sea water have optimal pH at 7.0 in the model of California mussel and at 8.0 in the model of blue mussel.[11] These facts agree with our result for $(Gly\text{-}Tyr\text{-}Lys)_n$; therefore, it is anticipated that the optimal pH of our synthetic polytripeptides and polydipeptides for the oxidative reactions by tyrosinase are in the range of 7–8. Hence, the experiments described below were carried out at pH 7.4.

Adhesive Strength

The effect of the tyrosinase concentration on the shear adhesive strength of polytripeptide was investigated by use of $(Gly\text{-}Tyr\text{-}Lys)_n$ as a representative of the polypeptides. The concentration was varied from 0.034 to 34 units/mg at pH = 7.4. The adhesive strength was measured after the test piece was allowed to stand in the air at 37°C for 30 min. The results shown in Figure 5 indicate clearly that the strength depends on the enzyme concentration and that it has a maximum point,

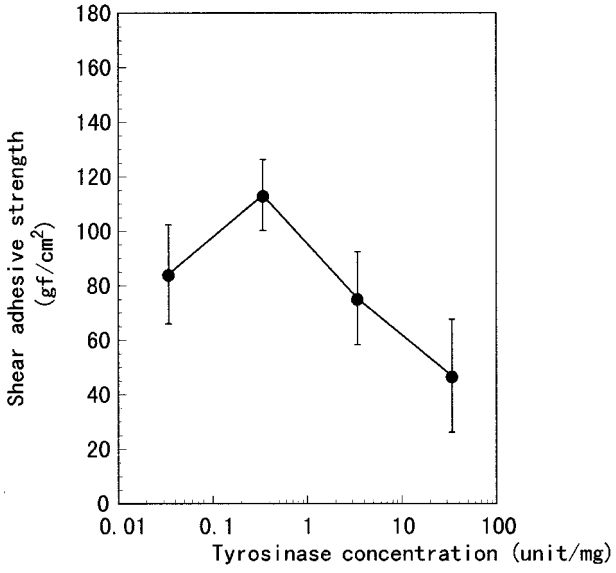

Figure 5 Effect of tyrosinase concentration on shear adhesive strength. Polytripeptide: $(Gly\text{-}Tyr\text{-}Lys)_n$, polytripeptide concentration: 20 wt %. 37°C, pH 7.4 phosphate buffer solution.

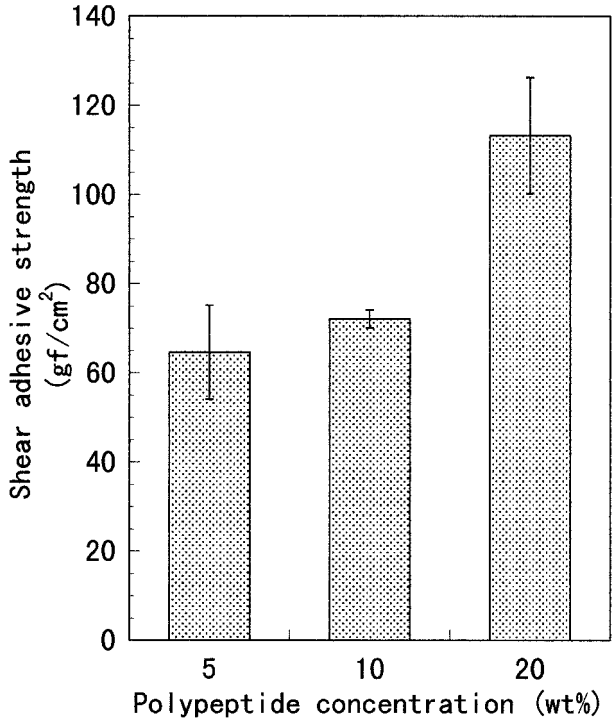

Figure 6 Effect of polypeptide concentration on shear adhesive strength. Polytripeptide: $(Gly\text{-}Tyr\text{-}Lys)_n$, 37°C, pH 7.4 phosphate buffer solution. Tyrosinase amount: 0.34 unit/mg (polytripeptide).

113.2 gf/cm^2 at 0.34 unit/mg of the tyrosinase concentration. This concentration will be the optimal enzyme concentration that gives the maximum adhesive strength. The reason for the low adhesive strength in the low enzyme concentration region, < 0.1 unit/mg, can be that the first conversion of Tyr residue to Dopa and the second conversion to dopaquinone by tyrosinase is too slow because of the low enzyme concentration. On the other hand, in the high concentration region, > 1 unit/mg, the high enzyme concentration causes rapid conversion of Tyr to dopaquinone, so the dopaquinone concentration becomes high enough to react with the free amino group of Lys in the intramolecule. This intramolecular reaction brings about an insolubilization and a precipitation of the polypeptide, thus, the adhesive strength is decreased from 113.2 to 52.4 gf/cm^2 with an increase in the enzyme concentration.

The relationship between the shear adhesive strength of the polytripeptide, $(Gly\text{-}Tyr\text{-}Lys)_n$ and the polytripeptide concentration is discussed. The results are shown in Figure 6, where the concentration is varied from 5 to 20 wt %. The strength rises from 65 to 113 gf/cm^2 with an increase in the

polypeptide concentration from 5 to 20 wt %. This is because the increase of the polypeptide concentration causes the rises of the mechanical strength of the gel prepared by the crosslinking of the polypeptide, thus, the increase of the adhesive strength can be achieved.

Next, to confirm the effect of the third amino acid residue, X, on the adhesive strength of the polytripeptides, $(X\text{-}Tyr\text{-}Lys)_n$, adhesive performances were investigated, where residues, X, are Ala, Pro, Ser, Leu, Ile, and Phe. The condition of the adhesion test were the same as that was adopted for $(Gly\text{-}Tyr\text{-}Lys)_n$; that is, the tyrosinase and polytripeptide concentrations were 0.34 unit/mg and 20 wt %, respectively, in pH 7.4 phosphate buffer. The results are shown in Figure 7. The adhesive strength of each polypeptide is in the range of 85 to 118 gf/cm^2, and the statistical treatment for these strengths indicates that there is no difference among them with the probability of $p < .05$. Therefore, it is concluded that the third amino acid residue, X, has little or no effect on the adhesive strength of the polytripeptide. Moreover, all of the polytripeptides have adhesive strength close to those of the synthetic polydecapeptides that are the models of California and blue mussel adhesive proteins. These results strongly indicate that Lys and Tyr residues play an important role in adhesion of the polytripeptide; that is, the adhesion will be not affected by the primary structure and the higher order structure of the polytripeptides and will depend on the existence of both the amino acid residues, Tyr and

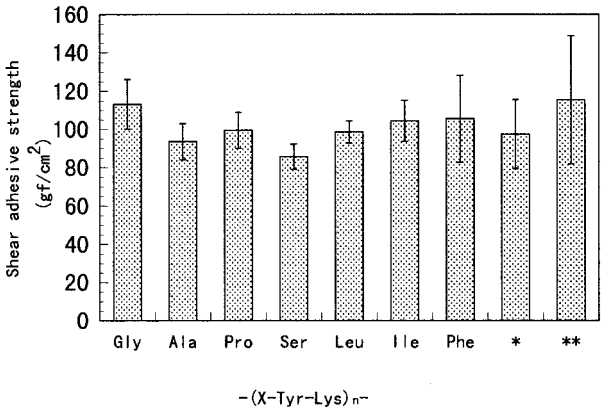

Figure 7 Shear adhesive strength of various polypeptides. Polypeptide concentration: 20 wt %. 37°C, pH 7.4 phosphate buffer solution. Tyrosinase amount: 0.34 unit/mg (polypeptide) *Model polypeptide of blue mussel adhesive protein. **Model polypeptide of California mussel adhesive protein.

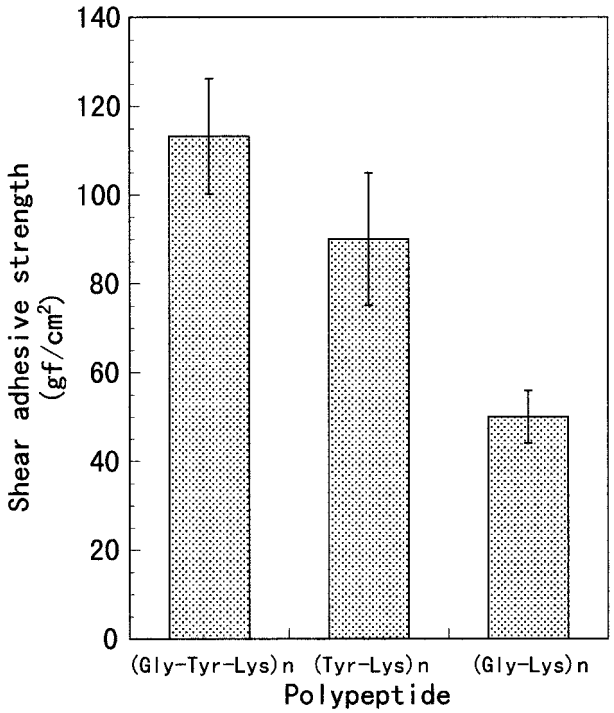

Figure 8 Shear adhesive strength of polypeptide. Polypeptide concentration: 20 wt %. 37°C, pH 7.4 phosphate buffer solution. Tyrosinase amount: 0.34 unit/mg (polypeptide).

Lys. This conclusion was supported by the following experiment. Three kinds of polydipeptides, $(Tyr\text{-}Lys)_n$, $(Gly\text{-}Lys)_n$, and $(Gly\text{-}Tyr)_n$, were synthesized, but, unfortunately, $(Gly\text{-}Tyr)_n$ did not dissolve in phosphate buffer at pH 7.4 because of its hydrophobicity (Table I); thus, investigation of the adhesive strength was carried out for two polydipeptides, $(Tyr\text{-}Lys)_n$ and $(Gly\text{-}Lys)_n$. The results are shown in Figure 8. The adhesive strength of $(Tyr\text{-}Lys)_n$ is close to that of $(Gly\text{-}Tyr\text{-}Lys)_n$. The statistical treatment for both the strengths reveals that there is no difference between them with the probability of $p < .05$. On the other hand, the strength of $(Gly\text{-}Lys)_n$ is very low and is 50 gf/cm^2, which is about half of that of the $(Gly\text{-}Tyr\text{-}Lys)_n$. In this case, the statistical treatment shows the difference between them with the probability of $p > .05$. These results support the conclusion that Lys and Tyr contribute to the adhesion.

CONCLUSION

Several sequential polytripeptides and polydipeptides that imitate mussel adhesive proteins were

synthesized, and the adhesive properties of them were investigated. The sequence of the amino acid residues of the polypeptides were $(X\text{-Tyr-Lys})_n$, (X═Gly, Ala, Pro, Ser, Leu, Ile, and Phe), $(Tyr\text{-}Lys)_n$, $(Gly\text{-}Lys)_n$, and $(Gly\text{-}Tyr)_n$. The molecular weights of the polypeptides with the protecting groups were estimated to be in the range of 7,200–13,400 (19–42 repeating units). Tyrosinase had a optimal pH at 7.4 for the oxidative reaction of $(Gly\text{-}Tyr\text{-}Lys)_n$, and the optimal tyrosinase concentration for the adhesive strength $(Gly\text{-}Tyr\text{-}Lys)_n$ was 0.34 unit/mg (polypeptide). The adhesive strengths of the polytripeptides, $(X\text{-}Tyr\text{-}Lys)_n$ were in the range of 85 to 118 gf/cm^2, and the statistic treatment for them indicated that there was no difference of the adhesive strength among the seven polytripeptides. It was concluded that the third amino acid residue, X, did not affect the shear adhesive strength. Moreover, these polytripeptides had adhesive strength equal to that of the synthetic model polypeptides of blue and California mussels. These facts indicated that Lys and Tyr residue in the polytripeptide were key amino acids for such adhesion as the mussel adhesive polypeptides, and that the adhesion was a nonspecific one. The results that the adhesion strength of $(Gly\text{-}Lys)_n$ was lower, and that of $(Tyr\text{-}Lys)_n$ was equal to that of polytripeptides supported the conclusion mentioned above.

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