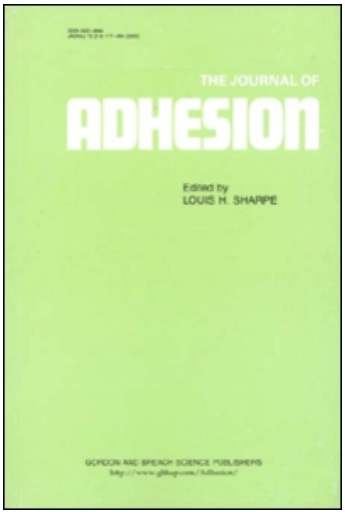

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Hiroyuki Yamamoto[a]; Takahiro Ogawa[a]; Shin-Ichi Ohara[a]; Ayako Nishida[a]; Yuuki Hirata[a]; Hideki Tatehata[a]; Kousaku Ohkawa[a]

[a] Faculty of Textile Science and Technology, Shinshu University, Institute of High Polymer Research, Ueda, Japan

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Adhesion and Wettability of Marine Adhesive Proteins in Aqueous Systems*

HIROYUKI YAMAMOTO**, TAKAHIRO OGAWA, SHIN-ICHI OHARA, AYAKO NISHIDA, YUUKI HIRATA, HIDEKI TATEHATA and KOUSAKU OHKAWA

Institute of High Polymer Research, Faculty of Textile Science and Technology, Shinshu University, Ueda, 386 Japan

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The work of adhesion on substrates with low and high surface energies using a variety of homo, random and sequential polypeptides containing L-lysine has been investigated to evaluate the role of individual amino acids together with the sequences in marine adhesive proteins. The work of adhesion of poly (L-lysine) was lower (34–79 mJ/m^2) on the surfaces of Teflon and polyethylene (PE) with low energy, and was higher (108 mJ/m^2) on the surface of glass with high energy, and the order was glass > nylon > polyethylene > Teflon. The work of adhesion increased with the increasing amount of the Tyr residues, and Lys copolypeptides were always larger than Glu copolypeptides. Among sequential polypeptides, polypeptides containing Gly and Lys exhibited higher work of adhesion on four different substrates, suggesting the important role of Gly and Lys residues in the adhesive proteins. Biological adhesion of a goby fish sperm on the glass plate exhibited marked adhesion activity due to the lysyl residues in the precoated marine adhesive proteins.

KEYWORDS: Work of adhesion; low and high surface energy substrates; homo polypeptides; random polypeptides; sequential polypeptides; synthetic adhesive proteins; mussel adhesives; Teflon; polyethylene; glass; nylon.

INTRODUCTION

We have investigated the polymer chemistry of a variety of adhesive model proteins of sessile animals in sea water, silkworms in the air, and caddis worms in fresh water. Marine adhesive proteins secreted from invertebrates, such as mussels and barnacles, insolubilize and adhere to the surfaces of a variety of substrates, such as rock (granite), glass and plastics, in a watery environment (for example, Fig. 1). The marine adhesive proteins change the contact angles on the surfaces depending on

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**Corresponding author.

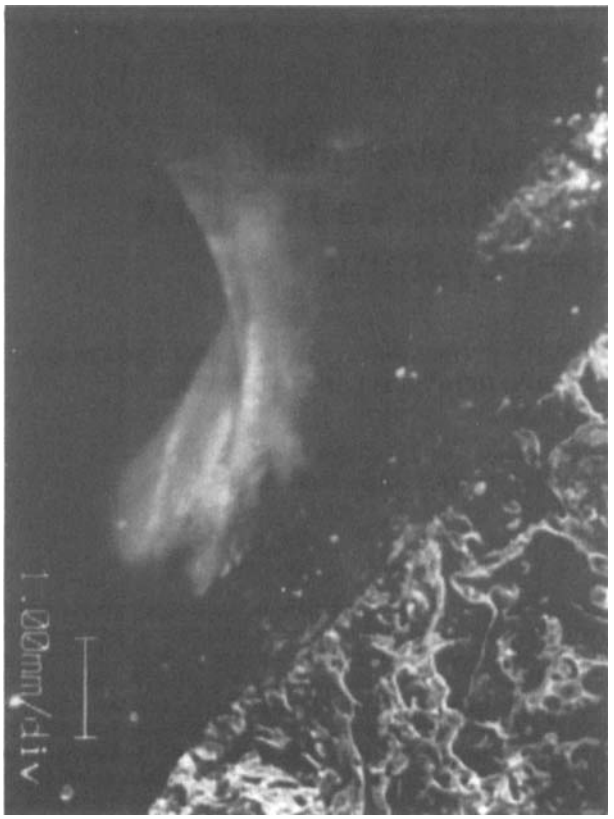

FIGURE 1 The disc of pearl oyster *Pinctada fucata* attached on granite. See Color Plate I.

the surface energy of the substrates. In sea water, a characteristic finding of the contact angles and the bonding strengths on the substances first described by Crisp *et al.*[1], and a hypothetical explanation proposed later[2], are completely opposite to the relationship between the contact angles and the bonding strengths on the substrates with different surface energies in the air. In order to clarify the discrepancy between an explanation for the marine adhesive proteins in sea water and the observation from petrochemical adhesives in the air, we started the present investigation.

During the course of our polymer chemical studies on marine adhesive proteins as bioadhesives[3-9], the role of Lys and Tyr residues in adhesive proteins was found to be more important to adhesion than previously thought. So we investigated the basic surface chemistry using simplified adhesive model proteins containing lysine residues and synthetic marine adhesive proteins on a variety of substrates with high and low surface energy. The objective of the present report is to discuss the wettability and adhesion of the synthetic adhesive proteins in aqueous solution on high and low energy surfaces by surface chemical approaches.

MATERIALS AND METHODS

The model proteins used were poly(L-lysine) (PLL) with different degrees of polymerization (DP), two Tyr-containing copolypeptides, copoly(Tyr^1 Lys^x) ($x = 1 - 10$) and copoly(Tyr^1Glu^y) ($y = 1 - 9$), Lys containing sequential polypeptides, poly(Lys-Gly), poly(Lys-Lys-Gly), poly(Lys-Lys-Ala) and poly(Lys-Lys-Ser), and synthetic mussel adhesive proteins, poly(Ala-Gly-Tyr-Gly-Gly-Ala-Lys) (Chilean mussel *Aulacomya ater*), poly(Ala-Lys-Pro-Ser-Tyr-Pro-Pro-Thr-Tyr-Lys) (blue mussel *Mytilus edulis*) and poly(Pro-Lys-Gly-Thr-Tyr-Pro-Pro-Thr-Tyr-Lys) (Californian mussel *Mytilus californianus*). All these samples are water soluble and the concentrations of the aqueous solutions were 1–50% (w/v).

The substrates used were polytetrafluoroethylene (Teflon), polyethylene (PE), nylon, soda-lime glass, iron annd alumina. The advancing contact angles ($\theta°$) on the substrates were measured by the sessile drop method using a contact angle goniometer at 20°C. The surface tensions (surface free energy, γ_{LV}) of the aqueous solutions were measured by Wilhelmy method using a surface tension balance. The work of the adhesion (W_A) was calculated by the following Young-Dupre equation, using the average contact angle and the average surface free energy: $W_A \doteqdot \gamma_{LV}(1 + \cos\theta)$. Here, the surface pressure (π) can be considered negligible in the case of low energy surfaces.

Sperm cells of the goby fish *Tridentiger kuroiwae brevispinis* were used to examine the adhesive activity on a soda-lime glass plate.

RESULTS AND DISCUSSION

Low energy surfaces ($\gamma_s < 33$ mJ/m^2) and high energy surfaces ($\gamma_s > 56$ mJ/m^2) were used. Table I summarizes the surface tensions, the contact angles, and the work of adhesion of PLL, sequential polypeptides containing Lys residues, and synthetic marine adhesive proteins. Both the surface tension and contact angles of the PLL solution decreased when the DP was increased from DP2 to 32; these reached the minimum values at DP32, and then actually increased up to DP3260. These PLL samples also exhibited a clear tendency that increasing the concentration tends to decrease the surface tension. The contact angle of the PLL (DP640 sample) solution at 10% concentration is 36° on glass (a high energy surface) and is 92° on Teflon (a low energy surface). This tendency of the contact angle on the surfaces corresponds well with the contact angle-surface energy relationship for the organic liquids. The contact angles on Teflon and PE decreased when the PLL concentrations were increased from 0% to 50%, while they changed a little on nylon and on glass (not shown here). The work of adhesion of the PLL solutions was lower (34–59 mJ/m^2) on the surface of Teflon, with low energy, and was higher (63–108 mJ/m^2) on the surface of glass, with high energy.

The discussion will now focus on the work of adhesion as a function of Tyr content of two different tyrosine copolypeptides containing cationic Lys or anionic Glu. This work of adhesion depended on the substrate, and the order was glass > nylon > PE > Teflon. When the molar ratios of the Tyr residues in copoly(Tyr^1 LYs^x) and

TABLE I
The surface tension, the contact angle, and the work of adhesion of synthetic adhesive proteins

Sample	Conc. (%)	γ_L (mJ/m^2)	Contact angle (θ°)					Work of adhesion (mJ/m^2)				
			Teflon	PE	Nylon	Iron	Glass	Teflon	PE	Nylon	Iron	Glass
Water		72.8	108	90	57	44	21	50	73	112	125	141
$(Lys)_n$ n = 2	10	54.9	100	76		51	40	45	68		89	97
4	10	43.5	97	74		51	30	38	55		71	81
8	10	42.6	94	74		50	28	40	54		70	80
16	10	35.2	90	67		46	26	35	49		60	67
32	10	32.5	88	64		46	22	34	47		55	63
84	10	47.2	90	72		68	34	47	62		65	86
640	10	57.4	92	77		70	36	56	70		77	104
1310	10	59.0	92	77		70	37	57	72		79	106
3260	10	64.2	95	77		72	47	59	79		84	108
$(Lys\text{-}Gly)_{39}$	1	72.1	116	71	19		19	41	96	140		141
$(Lys\text{-}Lys\text{-}Gly)_{21}$	1	67.1	114	69	29		21	40	91	126		130
$(Lys\text{-}Lys\text{-}Ala)_{18}$	1	54.7	96	62	33		20	49	80	103		106
$(Lys\text{-}Lys\text{-}Ser)_{14}$	1	50.9	108	67	21		23	35	71	98		98
Blue mussel	1	51.4	100	65		52	16	42	73		83	101
Californian mussel	1	48.9	103	64		37	15	38	71		88	96
Chilean mussel	1	61.7	133	55	51		25	20	97	100		117

copoly(Tyr^1 Glu^y) were increased from zero to 50 mol%, the work of adhesion increased proportionately with the increasing amount of the Tyr residues (Fig. 2). Furthermore, when the work of adhesion of two series of copolypeptides on four different substrates were compared, Lys copolypeptides were always larger than Glu copolypeptides. These results clearly revealed a new, additional importance for Lys residues in the adhesive proteins secreted by marine invertebrates, since until now the Lys residues in marine adhesive proteins have been considered to act as the crosslinking bridgehead by reacting with dopa quinone in order to insolubilize the proteins. Additionally, the β-structural conformation and cross-linking caused by tyrosinase enhanced the adhesability of the surfaces (the results are not shown here; refer to Ref. 8).

Among these sequential polypeptides, polypeptides containing Gly exhibited higher work of adhesion in four different substrates with four different surface energeis (Table I). This effect could be attributed to the free rotation ability of the Gly residues with the smallest side chains (proton only). Furthermore, when we compared poly(Lys-Gly) with poly(Lys-Lys-Gly), the works of adhesion of polydipeptide were always larger than those of polytripeptides on the four substrates. These findings from sequential polypeptides suggest the important role of Gly residues in the adhesive proteins. Figure 3 shows the Lys and Gly effects in the adhesion of synthetic marine adhesive proteins and related model polypeptides. We extended the surface chemistry to the synthetic marine adhesive proteins of the mussels whose

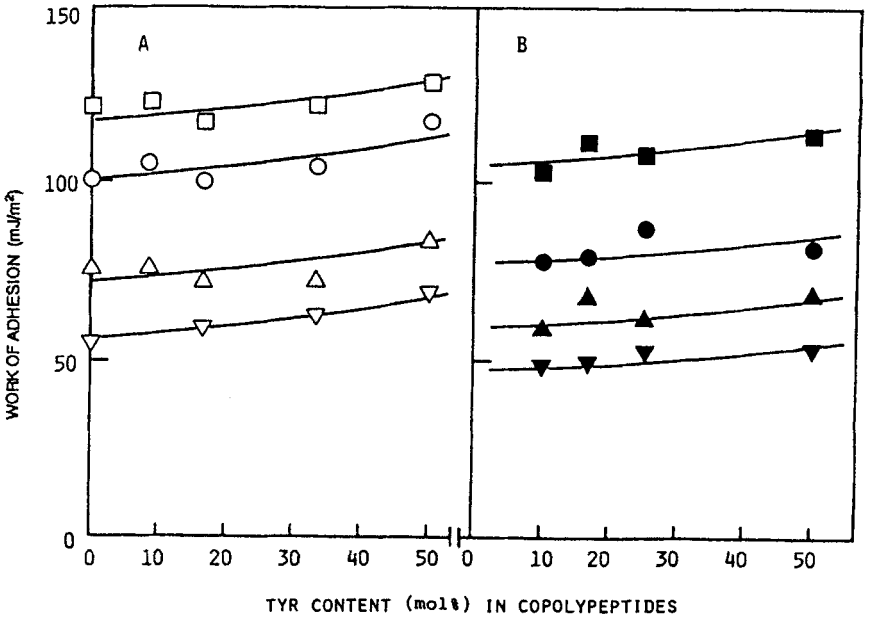

FIGURE 2 The effects of the amino acid residues and the molar ratios in Tyr polypeptides for the work of adhesion on the substrates; (A) copoly (Tyr^1 Lys^x) solutions and (B) copoly (Tyr^1 Glu^y) solutions. The substrates used are Teflon (▽, ▼), polyethylene (△, ▲), nylon (○, ●) and glass (□, ■).

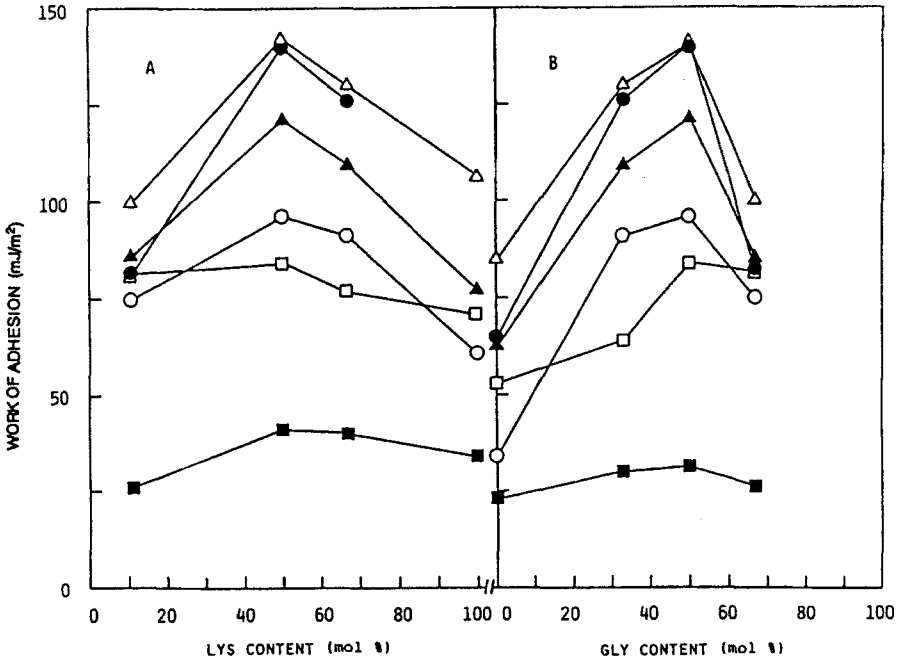

FIGURE 3 The effects of the Lys and Gly residues in synthetic adhesive model polypeptides and proteins for the work of adhesion on the substrates; (A) Lys effect and (B) Gly effect. The substrates used are glass (Δ), nylon (●), iron (▲), polyethylene (○), alumina (□) and Teflon (■).

sequences contain Gly and Lys residues in their primary structures. The works of adhesion of the synthetic mussel proteins generally supported the findings from the much simpler model polypeptide structures described above. In this connection, two additional important interactions, the interaction between protein and water (stability of protein molecule in aqueous solution) and the interaction between substrate surface and water (freezable bound and nonfreezing water at the surface), can not be ignored[10]. These interactions relate closely with the surface adhesion of protein molecules and will be the next target of the present study.

Biological adhesion on the glass surface which was precoated with synthetic adhesive proteins has also been examined. Table II summarizes the results of the sperm cell adhesion of a goby fish on soda-lime glass plates. When the adhesion results between poly(Glu) and poly(Lys) were compared, cationic Lys residues were responsible for the sperm cell adhesion. Synthetic adhesive proteins exhibited 7–33% of the adhesion activity of the goby fish sperm cell on the glass plate. Among these, Chilean mussel adhesive proteins exhibited the strongest adhesive ability and the second strongest was the Liver fluke (*Fasciola hepatica*) egg-shell-hardening protein. When we compared the sperm cell adhesion among three analogues of the Liver fluke adhesive proteins containing 9-Lys, 9-Arg and 9-His, Lys adhesive protein was the highest, with 17% adhesion activity. The sperm cell adhesion activity of the synthetic adhesive proteins was higher than that of the

TABLE II
Adhesion activity on galss surface of synthetic adhesive proteins in a goby fish (*Tridentiger kuroiwae brevispinis*) sperm cell.

Sample	Cells				Adhesion (%)[1)]
	Motile	Immotile	Adhered	Total	
Control[2)]	20	2	0	22	0
Poly(L-Lys)	0	9	1	10	10
Poly(L-Glu)	18	2	0	20	0
Copoly(Lys^1Tyr^1)	0	17	0	17	0
Chilean mussel	2	6	4	12	33
Blue mussel	5	9	5	19	26
Liver fluke[3)]					
9-Lys	7	3	2	12	17
9-Arg	10	4	1	15	7
9-His	7	11	3	21	14

1) the ratio of (adhered cells)/(total cells)
2) adhesion of sperm cells on glass in 0.2M Tris HCl buffer at pH 7
3) $(Gly\text{-}Gly\text{-}Gly\text{-}Tyr\text{-}Gly\text{-}Gly\text{-}Tyr\text{-}Gly\text{-}X)_n$

simpler model polypeptides, exhibiting the importance of the primary structures containing Gly and Lys in the marine and related adhesive proteins.

CONCLUSIONS

We have reported here some data on adhesion in watery environments using marine and related adhesive proteins. Based on the experimental results, we propose the following requirements for biological adhesion: (1) high molecular weight PLL is effective for wettability and adhesion; (2) cationic Lys residues are more effective than anionic Glu residues; (3) Lys and Gly residues in adhesive proteins are effective for adhesion; (4) the primary structures of the adhesive proteins do have significance; (5) the Lys residues are important for fish sperm cell adhesion. The findings suggest that mussel adhesives have a rational primary structure which adheres to both the high and low energy surfaces in a watery environment.

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References

1. D. Crisp, G. Walker G. Young and A. Yule, *J. Colloid Interface Sci.*, **104**, 40 (1985).
2. J. H. Waite, *Int. J. Adhesion and Adhesives*, **7**, 9 (1987).
3. H. Yamamoto, *J. Chem. Soc. Perkin Trans. I*, 613 (1987).

4. H. Yamamoto, *J. Adhesion Sci. Tech.*, **1**, 177 (1987).
5. H. Yamamoto, S. Ohara, H. Tanisho, K. Ohkawa and A. Nishida, *J. Colloid Interface Sci.*, **156**, 515 (1993).
6. H. Yamamoto, S. Yamauchi and S. Ohara, *Biomimetics*, **1**, 219 (1993).
7. H. Yamamoto and H. Tanisho, *Materials Sci. Eng.*, **1**, 45 (1993).
8. S. Ohara, K. Ohkawa and H. Yamamoto, *J. Adhesion Soc. Japan*, **29**, 345 (1993).
9. K. Ohkawa, H. Tatehata, T. Ogawa, A. Nishida and H. Yamamoto, *Polymer Preprints Jpn.*, **43**, 1583 (1994).
10. A. Higuchi and T. Iijima, *Polymer*, **26**, 1207 (1985).