Specific Binding of L-Alanine onto a Monolayer Composed of Polyallylamine Containing Poly(L-alanine) Graft Chains

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Polyallylamine having poly(L-alanine) as hydrophobic graft chains, (PAAgPAla), formed a monolayer at an air-water interface. The morphology of the monolayer was found to be strongly dependent on the aqueous pH. In the alkaline condition, the PAAgPAla formed a stable monolayer in which the graft polypeptide chains self-assembled at the interface to yield active domains specifically interact with aqueous L-alanine compared with its D-isomer.

Binding of a signaling molecule onto the surface of the target cell is the initial event for the signal transduction in living systems. In this case, the signaling molecule binds to a specific cell-surface protein called a receptor. It has been recognized that the structure of binding site in the receptor closely related to the molecular recognition and signal transfer on and through the biological membrane. Investigation on the specific interactions on the artificial membranes[1] may give a simple and/or essential mechanism of a signal reception and transduction through the biological interfaces. For example, Kunitake et. al., reported[2a] specific binding of aqueous dipeptides onto peptide-functionalized monolayer. They suggested that the recognition site is self-assembled on the surface of the monolayer *via* the interaction with guest dipeptides.

In this study, we reported morphology of a monolayer of polyallylamine containing poly(L-alanine) graft chains (PAAgPAla) and molecular recognition of aqueous amino acids on the PAAgPAla monolayer at an air-water interface. The specific binding of L-alanine onto the monolayer occurred depending on the morphology of the monolayer, which could be regulated by aqueous pH.

PAAgPAla (Figure 1) was obtained by polymerization of N-carboxy L-alanine anhydride with polyallylamine (Mw = 10000) as an initiator.[3] The graft chain, poly(L-alanine), content of PAAgPAla obtained was determined to be 37 mol% by potentiometric counting of the unreacted allylamine groups. The average degree of polymerization of poly(L-alanine) was determined to be 13 by elemental analysis.

PAAgPAla formed a monolayer at the air-water interface by

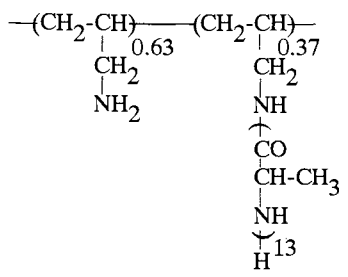

Figure 1. Chemical structure of PAAgPAla.

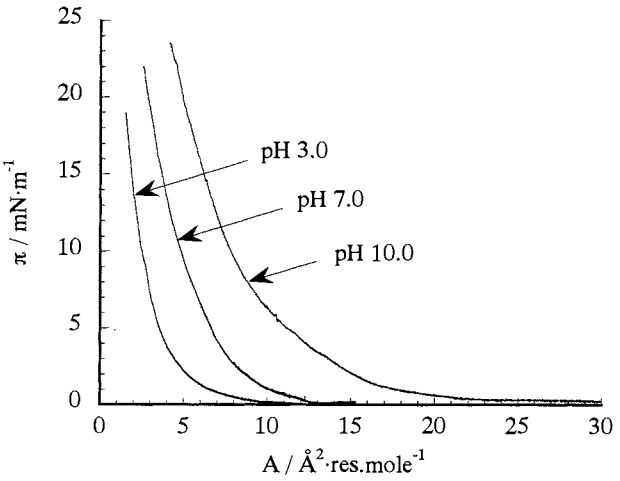

Figure 2. π-A isotherms of PAAgPAla monolayer at pH 3.0, pH 7.0 and pH 10.0 at 21.0±0.5 °C on 0.1 M NaCl.

spreading a trifluoroethanol solution onto the aqueous phase. Figure 2 showed surface pressure-area (π-A) isotherms of PAAgPAla on 0.1 mol dm^{-3} NaCl solution at pH 3.0, pH 7.0, and pH 10.0 respectively, at 21°C (Figure 2). Compression of the monolayer was carried out successively at 5 mm min^{-1}. The surface area per monomer residue of PAAgPAla decreased by decreasing the pH of the aqueous phase. The conformation of poly(L-alanine) graft chain in the monolayer was estimated from reflection-absorption Fourier transform infrared spectroscopy[4] with a PAAgPAla monolayer transferred onto a gold-deposited glass plate (Figure 3). The transfer was carried out by using a vertical dipping method at up-stroke motion at a surface pressure of 10 mN m^{-1}. It was found, as a result, that poly(L-alanine) in the monolayer was in α-helix conformation with considerable amounts of random coil at pH3.0. The exact content is not clear at present. On the other hand, the formation of the β-sheet structure of the graft chains was confirmed in the FT-IR spectra at pH 7.0 and pH 10.0.

The morphological difference of the monolayer at pH 3.0 and pH 10.0 could be also clarified with an atomic force microscope (AFM) image in the contact mode. The monolayers were transferred onto freshly cleaved mica by a horizontal lifting method at π = 10 mN m^{-1}. AFM images were done in "height " mode using oxide-sharpened silicon nitride cantilevers which were V-shaped and 120 μm long, with a spring constant of 0.58 N m^{-1}. A 10 μm by 10 μm scanner was used for imaging. Figure 4a showed the AFM image at pH 3.0. The small domains having several nm height were clearly seen in the image. On the other hand, at pH 10.0 the domains were disappeared and an uniform monolayer structure was confirmed (Figure 4b).

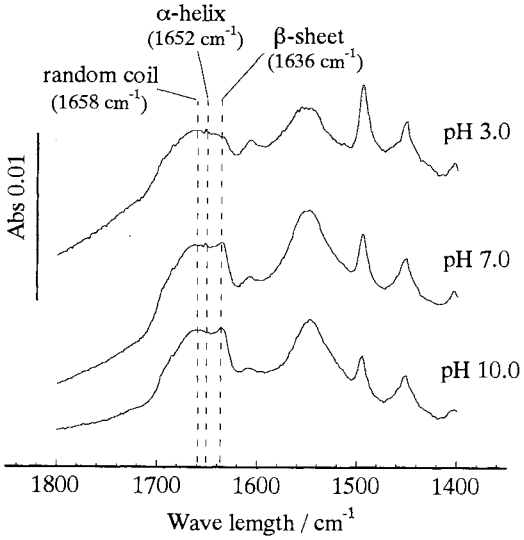

Figure3. FT-IR/RAS spectra of PAAgPAla monolayer on gold-deposited glass.

Similar AFM images could be observed in the LB films transferred onto gold-deposited mica (Pico Substrate, Molecular Imaging Co.). These results suggested that in the acidic condition, a part of the PAAgPAla chains could be dissolved into the aqueous phase owing to the protonation of the allylamine moieties, which resulted in the decrease in the monolayer area at pH 3.0. In other words, the hydrophobic graft chains, poly(L-alanine), kept the monolayer at the interface by their anchoring in the air phase. The hydrated parts of the monolayer beneath the interface may be observed as the domains in the AFM image in Figure 4a. On the other hand, in the alkaline condition, the dehydration of the allylamine moieties were occurred, as a result, PAAgPAla chains entirely located at the air-water interface. This was reflected as the uniform monolayer structure in Figure 4b. In the monolayer, poly(L-alanine) graft chains could self-assemble, owing to the loss of the electrostatic repulsion among the neighboring allylamine moieties, to yield their β-sheet structure domains at the interface.

We also investigated the selective binding of aqueous D- and L-alanine onto PAAgPAla monolayer by decrease of the aqueous alanine concentration.[5] Table 1 shows binding ratio of aqueous alanine toward PAAgPAla monolayer from the 1 mmol

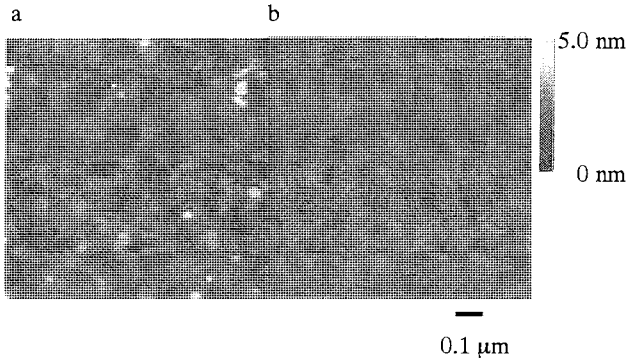

Figure 4. AFM images of PAAgPAla monolayer on mica at pH 3.0 (a) and at pH 10.0 (b).

dm^{-3} aqueous alanine subphase containing 0.1 mol dm^{-3} NaCl at pH 3.0 and pH 10.0, respectively. The binding selectivity was calculated by the ratio of the amount of L-alanine to that of D-isomer onto PAAgPAla monolayer. PAAgPAla monolayer at pH 3.0 could bind both aqueous D- and L-alanine. The binding selectivity of L-alanine to D-isomer was 1.22. This result implied that the aqueous alanine molecules were non-selectively incorporated into the monolayer. In contrast, the binding ratio of L-alanine toward PAAgPAla monolayer was larger than that of D-isomer at pH 10.0. The binding selectivity was 8.56. This suggested that the self-assembled poly(L-alanine) domain in the PAAgPAla monolayer at pH 10.0 can specifically bind aqueous L-alanine.

Table 1. Binding ratio of Ala toward PAAgPAla monolayer.

	L-Ala	D-Ala	Binding Selectivity (L/D)
pH 3.0	1.92	1.57	1.22
pH 10.0	3.34	0.39	8.56

g / g (Ala / PAAgPAla)

In this study, we showed a possibility for the recognition of aqueous amino acids by the monolayer membrane system composed of the polyallylamine containing poly(L-alanine) graft chains, PAAgPAla. In the alkaline condition, PAAgPAla formed the stable and uniform monolayer in which the polypeptide graft chains self-assembled at the interface to yield the recognition site of L-amino acid.

References and Notes

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2 a) X. Cha, K. Ariga, and T. Kunitake, *J. Am. Chem. Soc.*, **118**, 9545 (1996). b) K. Taguchi, K. Ariga, and T. Kunitake, *Chem. Lett.*, **1995**, 701. c) Y. Oishi, Y. Torii, M. Kuramori, K. Suehiro, K. Ariga, K. Taguchi, A. Kamino, and T. Kunitake, *Chem. Lett.*, **1996**, 411.

3 Polyallylamine (0.1 g) was dissolved in water (100 mL). The pH of the solution was adjusted to pH 12.0. Ethyl acetate (100 mL) containing N-carboxy L-alanine anhydride (0.1 g) was gradually added to the vigorously stirred polyallylamine aqueous solution. After 24 h, the solution was filtered and dialyzed overnight. After the dialysis, the solution was lyophilized to obtain PAAgPAla.

4 The conformation of grafted poly(L-alanine) chains of PAAgPAla was confirmed by Amide I (α-helix: 1652 cm^{-1}, β-sheet: 1636 cm^{-1}, random coil: 1658 cm^{-1}) in the RAS spectra of the LB film on gold-deposited glass plate.

5 A Teflon trough (50 mmϕ × 10 mm) was filled with 10 mL of 0.1 mol dm^{-3} NaCl aqueous solution containing 1 mmol dm^{-3} alanine at pH 3.0 and pH 10.0, respectively. PAAgPAla monolayer (surface area: 6.0 $Å^2$ res.$mole^{-1}$) was formed at the air-water interface by spreading 20 μL of a trifluoroethanol solution (1.1 mg mL^{-1}) of PAAgPAla. After 1 h, the concentration of aqueous alanine was estimated from the ellipticity of the aqueous phase.