Ultrathin Polymer Networks of Itaconic Acid Copolymers and Poly(allylamine) by the Langmuir-Blodgett Technique

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Ultrathin networks of itaconic acid copolymers and poly(allylamine) were produced by a Langmuir-Blodgett (LB) technique employing a double-chain amine as a monolayer template which was subsequently removed by extraction after thermal crosslinking. Itaconic acid copolymers used were copoly (itaconic acid-ethyl vinyl ether) and copoly (itaconic acid-n-butyl vinyl ether). The polyion-complexed monolayers of three components consisting of template amine, itaconic acid copolymer and poly (allylamine) were formed at the air-water interface. The Langmuir film properties have been studied by the surface pressure-area isotherm and fluorescence microscopy. The monolayers were transferred on solid substrates and were characterized by FT-IR spectroscopy, X-ray photoelectron spectroscopy, and scanning electron microscopy (SEM). Two-dimensional polymer networks were formed through imide or amide linkages by heat treatment under vacuum. The heat-treated films were extracted with chloroform after immersion in aq. sodium chloride to remove the template amines. SEM observation of a LB film on a porous fluorocarbon membrane filter with pore diameter of 0.1 μm showed covering of the pores by six layers in the polyion complex state.

Introduction

The formations of polyion-complexed monolayers at the air-water interface have broadened the scope of Langmuir-Blodgett (LB) film fabrications and made the LB films with special functionalities possible[1]. For example, Kunitake *et al.*[2] succeeded in forming LB film of a fluorocarbon amphiphile by complexing with potassium poly(styrenesulfonate), which is otherwise intractable to transfer the monolayer onto a substrate. The polyion-complexed LB films of azobenzene amphiphiles with ionic head groups were prepared by spreading monolayers on subphases containing anionic[1] or cationic[3] polymers. The domain formation of fatty acid monolayers by polyion interaction with poly(ethyleneimine) was observed at the air-water interface.[4] Thin films of poly(imide)[5,6] and poly(arylenevinylene)[7] were produced by the LB technique by attaching electrostatically hydrophobic chains to the precursor polymers which, by themselves, are incapable of monolayer formation.

The polyion complexes were extended to polymer-polymer systems of two kinds of different polymers. The formations of salt bridges between a polymerized ammonium monolayer and an anionic polymer at the air-water interface[8] and of two-dimensional polymer network of LB films of a polyamine-polycarboxylate salt[9,10] have been reported. Very recently, we described the formations of monolayers and resulting LB films composed of three components which are electrostatically interacting.[11] In those systems, the maleic acid copolymers form polyion salts both with a template amine and subphase polymer poly(allylamine) (PAA).

In this paper, we applied the template LB technique to water-soluble itaconic acid copolymers and PAA for ultrathin network formations. Double-chain tertiary amine $2C_{18}DAE$ was used as a monolayer template for enhancing the monolayer stability and deposition efficiency, and was eventually

removed from the LB film.
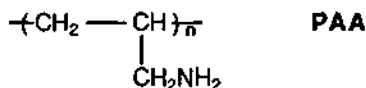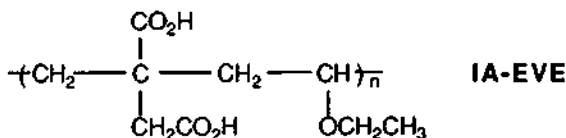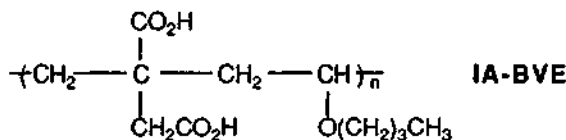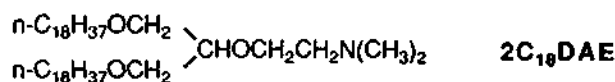
Experimental

Material. The template amine $2C_{18}DAE$, 1,3-dioctadecyl-2-(2-(N,N-dimethyl amino) ethyl)glyceryl triether, was prepared as described previously.[11] Copoly(itaconic acid-n-butyl vinyl ether) (IA-BVE) was synthesized as follows. Methyl ethyl ketone solution (10 mL) of itaconic anhydride (1.00 g, 8.9×10^{-3} mol), n-butyl vinyl ether (2.70 g, 2.7×10^{-2} mol), and AIBN (0.07 g, 4.0×10^{-4} mol) was degassed by the freeze-thaw-cycle, and the polymerization tube was sealed under vacuum with Teflon screw. Polymerization was continued for 15 h at 60 °C. The reaction solution was poured

into 200 mL of aq. NaOH (0.05 N), and stirred for 5 h at 50 °C. After cooling the solution in ice bath, the solution was acidified to pH 3 with aq. HCl (0.1 N). The precipitate was washed with pure water and dried. The structure was confirmed by ^1H NMR and IR spectra. The weight-average molecular weight was 19.6×10^4 (Mw/Mn=2.05) as determined by gel permeation chromatography (polystyrene calibration) and the composition was determined to be 50% of itaconic acid unit by elemental analysis: ^1H NMR (DMSO-d_6) δ (ppm); 0.8-1.2 (broad s, 3H), 1.2-1.9 (broad s, 8H), 2.4-2.6 (broad s, 2H), 3.0-3.7 (broad s, 3H), 12.0 (broad s, 2H). IR (KBr) v (cm^{-1}); 3490 (O-H), 3200 (O-H), 2956 (C-H), 2870 (C-H), 1707 (C=O), 1408 (C-O-H, bending), 1203 (C-O), 1087 (C-O). Anal. Calcd for $C_{11}H_{18}O_5$ (as 50% composition of acid unit); C, 57.37; H, 7.88. Found; C, 57.37; H, 7.64.

Copoly(itaconic acid-ethyl vinyl ether) (IA-EVE) was prepared as same procedure as IA-BVE. Reaction recipe was as follows; Itaconic anhydride (1.00 g, 8.9×10^{-3} mol), ethyl vinyl ether (2.25 g, 3.1×10^{-2} mol), AIBN (0.017 g, 1.1×10^{-4} mol), and 10 mL of methyl ethyl ketone. In this case, precipitation did not occur when the solution was acidified in ice bath. Thus, the solution was concentrated with rotary vacuum evaporator and the concentrated residue (20 mL) was poured into acetone (400 mL). The precipitate was washed with pure water and acetone, and dried under vacuum. The structure was confirmed by ^1H NMR and IR spectra. The molecular weight of this polymer could not be measured because this polymer is not soluble in tetrahydrofuran (THF). The composition was determined to be 50% of itaconic acid unit by elemental analysis: ^1H NMR (DMSO-d_6) δ (ppm); 0.8-1.1 (broad s, 3H), 1.1-1.8 (broad s, 4H), 2.4-2.6 (broad s, 2H), 3.0-3.6 (broad s, 3H), 12.0 (broad s, 2H). IR (KBr) v (cm^{-1}); 3434 (O-H), 2978 (C-H), 2870 (C-H), 1711 (C=O), 1396 (C-O-H, bending), 1200 (C-O), 1066 (C-O). Anal. Calcd for $C_9H_{14}O_5$ (as 50% composition of acid unit); C, 53.46; H, 6.93. Found; C, 53.43; H, 6.85.

n-Butyl vinyl ether and ethyl vinyl ether was purchased from Wako Pure Chemicals and distilled under potassium hydroxide before use. PAA hydrochloride (Mw=1.0×10^4) was purchased from Nitto Boseki Co. and treated with a strong anion exchange resin (Amberlite IRA-402) to obtain free PAA. The concentration of amino group in aqueous solution was determined by colloid titration with standard poly (vinylsulfate) solution using Toluidine Blue O as indicator.

Surface pressure-area (π-A) isotherm. A computer-controlled film balance system FSD-50 (San-esu Keisoku) was used for measuring surface pressure as a function of molecular area (trough size, 150×600 mm). Isotherms were taken at a compression rate of 0.4 mm/sec, and the temperature of the aqueous subphase was maintained at 30.0±0.1 °C. A THF/DMSO (6/4, by volume) mixture was employed as spreading solvent. Monolayers were spread on pure water or on aqueous PAA (2×10^{-4} mol/-NH$_2$) and incubated for 10 min and 30 min, respectively, before starting the compression.

LB deposition of monolayer onto solid substrates.
The deposition of monolayer was performed in the vertical mode by using a computer-controlled film balance (FSD-50) and lifter (FSD-51) system (San-esu Keisoku). The transfer onto solid substrates was carried out at a surface pressure

of 30.0±0.2 mN/m and a deposition rate of 4 mm/min. The temperature of the subphase was kept at 30±0.1 °C. The employed substrates were fluorocarbon membrane filters (FP-010, Sumitomo Electric Co.) for SEM observation and XPS measurement, and CaF$_2$ plates (GL Sciences) for FT-IR measurement.

Measurements. FT-IR measurements were carried out on a Nicolet 710 FT-IR spectrometer by the transmission method. XPS spectra were obtained on an ESCA 5300 X-ray photoelectron spectrometer (Perkin-Elmer). Scanning electron microscopy (SEM; Hitachi S-900) was used to observe the surface morphology of FP-010 membrane filter (pore diameter, 0.1 μm). The monolayer structure on subphase was monitored with a fluorescence microscope (Zeiss, Axiophot) equipped with a SIT TV camera (Hamamatsu, model C-2400). Fluorescein-PE (Molecular Probes, Inc.) was used as a fluorescence probe. Other experimental details were described previously.[11]

Results and Discussion

Monolayer and its transfer on solid substrates.
The copolymers codissolved with equimolar $2C_{18}$DAE (with respect to repeat unit of the copolymers) in a THF/DMSO (6/4, by volume) mixture were spread on pure water or aq. PAA (2×10^{-4} M) subphase at 30 °C. The π-A isotherms are shown in Figure 1. The isotherms show that the amine $2C_{18}$DAE fixed electrostatically with the itaconic acid copolymers forms stable monolayers. The monolayers of $2C_{18}$DAE/IA-EVE give condensed phases, while those of $2C_{18}$DAE/IA-BVE show expanded phases. The bulky butoxy pendant of IA-BVE, which is more hydrophobic than the ethoxy group of IA-EVE, is supposed to be an origin of the expanded phase. When compared with the isotherm on pure water, in case of $2C_{18}$DAE/IA-BVE, a more decreased molecular area at a given pressure was revealed on aqueous PAA. On the other
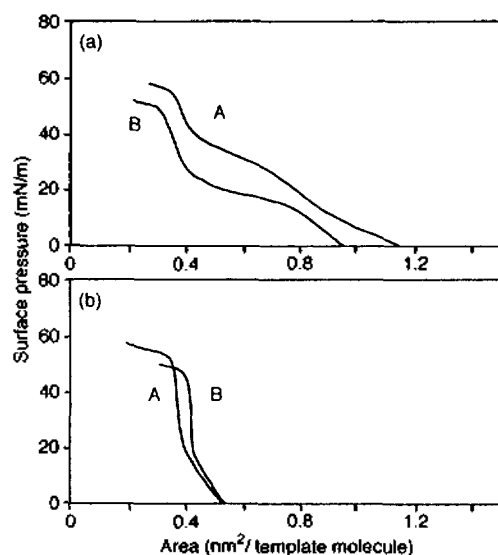
Figure 1. Surface pressure-area isotherms of $2C_{18}$DAE fixed electrostatically with itaconic acid copolymers; (a) IA-BVE, (b) IA-EVE; A, on pure water; B, on aqueous PAA at 30 °C. The concentration of the amino group of PAA is 2×10^{-4} M.

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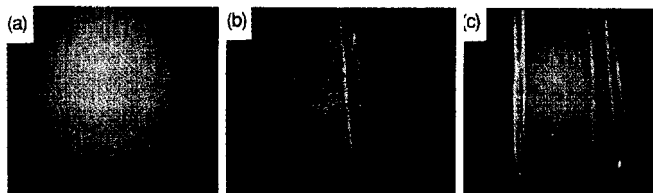
Figure 2. Fluorescence micrographs of monolayers $2C_{18}DAE/IA$-BVE on aqueous PAA: (a) at area of *ca.* 0.50 nm²/template molecule. (b) at area of *ca.* 0.25 nm²/template molecule; (c) at area of *ca.* 0.20 nm²/template molecule. Fluorescein-PE was used as fluorescence probe (0.5 mol% of the itaconic acid unit).
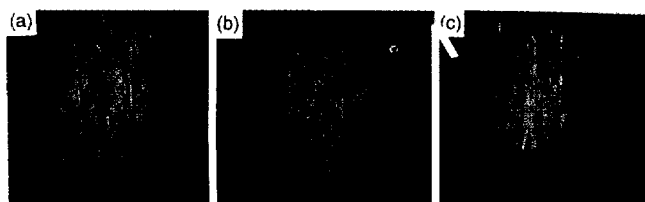
**Figure 3.** Fluorescence micrographs of monolayers $2C_{18}DAE/IA$-EVE on aqueous PAA: (a) at area of *ca.* 0.50 nm²/template molecule. (b) at area of *ca.* 0.45 nm²/template molecule. (c) at area of *ca.* 0.30 nm²/template molecule. Fluorescein-PE was used as fluorescence probe (0.5 mol% of the itaconic acid unit).

Figure 4. Transmission FT-IR spectra of LB films (1×2 layers) of $2C_{18}DAE/IA$-BVE/PAA on CaF_2 plates: (a) as-deposited film. (b) heat-treated film at 180 °C for 6 h under vacuum.

hand, the $2C_{18}DAE/IA$-EVE monolayer which was already condensed on pure water showed a more increased molecular area on aqueous PAA. This change between the two isotherms is attributed to the formation of a polyion complex at the interface and the consequent change of the monolayer organization, as previously described for other systems.[1,9,11] The results also support that two-dimensional molecular arrangement on the air-water interface can be controlled by subphase polymers with different lengths of ionically interactable repeat units.[3]

The shapes of the n-A curves are closely related with those of the $2C_{18}DAE/a$ maleic acid copolymer system.[11] The n-A curve of $2C_{18}DAE/IA$-BVE is similar with that of $2C_{18}DAE/copoly(maleic acid-styrene)$ system. The structural similarity between the two copolymers is that the comonomers, n-butyl vinyl ether and styrene, have hydrophobic side groups. On the other hand, the monolayer of the $2C_{18}DAE/IA$-EVE system shows similar n-A curve with that of a maleic acid-methyl vinyl ether copolymer, which shows condensed phase.

The structure change of the monolayer on aqueous PAA was monitored with fluorescence microscopy during compression of the monolayer. Figure 2 and 3 show fluorescence micrographs of monolayers of IA-BVE and IA-EVE systems, respectively. In case of IA-BVE system, no noticeable structures were observed until the white lines appeared at the area of *ca.* 0.25 nm²/template amine beyond the collapse point. It seems that the monolayer is homogeneously spread and the monolayer collapse by compression occurs as a line shape. On the other hand, island structures appeared upon spreading and during the compression in case of IA-EVE system. The white lines owing to monolayer collapse appeared together with island structures. From the fluorescence
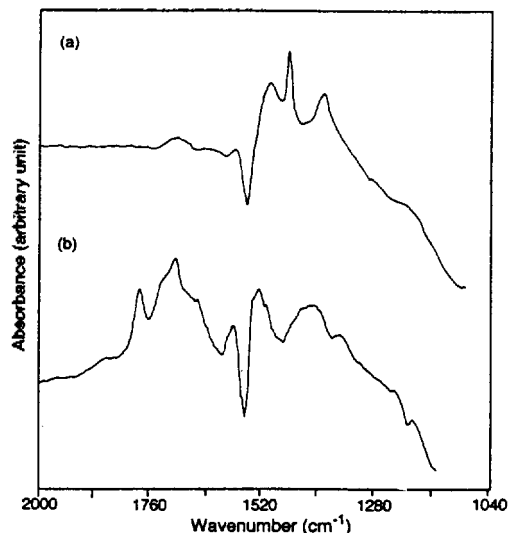
micrographs, we can deduce that the monolayer of IA-BVE system is macroscopically homogeneous, while the one of IA-EVE system consists of island structures even in a compressed state. The results agree with the patterns of n-A isotherms of IA-BVE and IA-EVE systems, which show expanded and condensed phases, respectively.

The monolayer of $2C_{18}DAE/IA$-BVE/PAA could be transferred on a fluorocarbon membrane filer (FP-010). The Y type deposition was obtained at the first stroke, but changed to Z type from the second run. When a CaF_2 plate was used as substrate, the transferred layer at upward stroke was peeled off during downward stroke. Thus, multilayer films on CaF_2 plates could not be made. The reason of the deposition phenomena is not clear at this point. The transfer ratio was 1.2 to 0.8 during 12 layers deposition on FP-010. The monolayer was so stable that any spontaneous decrease of monolayer area was not observed during the deposition at the surface pressure of 30 mN/m for 3 h. In case of the $2C_{18}DAE/IA$-EVE/PAA monolayer, Y type depositions were obtained on solid substrates such as FP-010 and a CaF_2 plate, although the transfer started from upward stroke in case of the CaF_2 plate. The transfer ratio was very constant from 1.1 to 1.0 for 14 layers deposition on FP-010. The monolayer was also so stable that any spontaneous decrease was not seen during the monolayer transfer at the surface pressure of 30 mN/m and at 30 °C for 4 h.

Polyion complex film. The salt formation in as-deposited LB film could be confirmed by FT-IR and XPS measurements. Figure 4 shows the IR spectra of the $2C_{18}DAE/IA$-BVE/PAA LB film deposited on a CaF_2 plate, which was taken from the monolayer LB film because multilayer deposition could not be carried out as described above. The strong carbonyl peaks of carboxylate salt (1508 cm⁻¹) and the small peak of carboxylic acid (1717 cm⁻¹) show that the itaconic acid units have the form of salts (Fig. 4a).

The incorporation of PAA into the LB film and the formation of ammonium salt were also confirmed by XPS measu-
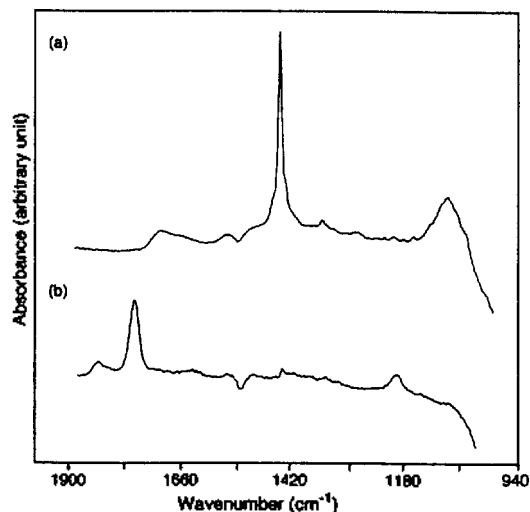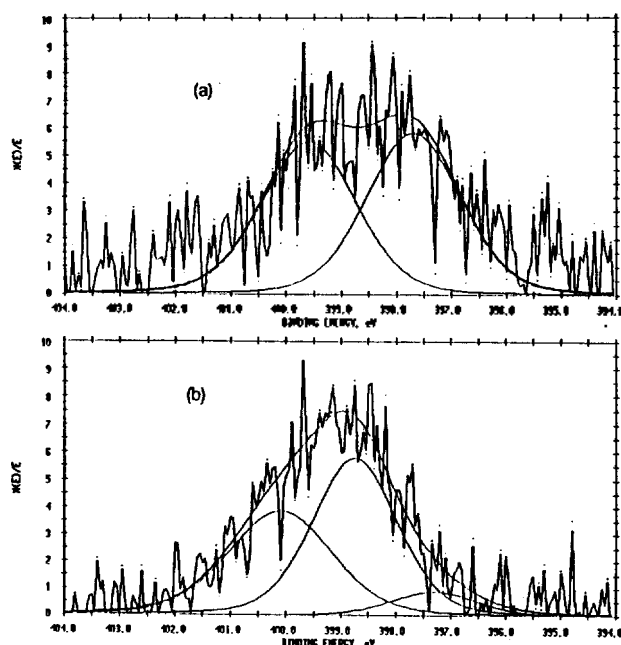
Figure 5. Transmission FT-IR spectra of LB films (15×2 layers) of 2C₁₈DAE/IA-EVE on CaF₂ plates: (a) as-deposited. (b) heat-treated at 180 ℃ for 6 h under vacuum.
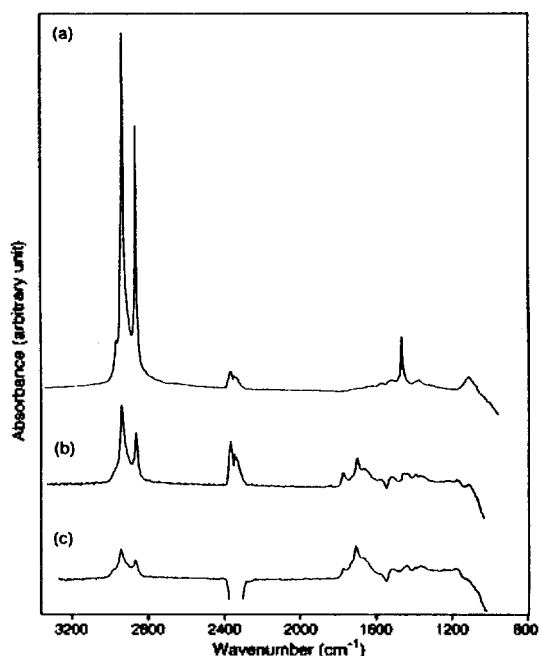
Figure 6. Transmission FT-IR spectra of LB films (15×2 layers) of 2C₁₈DAE/IA-EVE/PAA on CaF₂ plates: (a) as-deposited. (b) heat-treated at 180 ℃ for 6 h under vacuum. (c) extracted by chloroform after immersion in aq. NaCl followed heat treatment.

Figure 7. XPS spectra of the N_{1s} region of a 12 layer LB film of 2C₁₈DAE/IA-BVE/PAA on a fluorocarbon membrane filter (FP-010): (a) as-deposited. (b) after heat treatment at 180 ℃ for 6 h under vacuum. The charging shift was not corrected.

rement of 12 layers LB film deposited on FP-010. The spectra of N_{1s} region were shown in Figure 7. The formation of carboxylate-ammonium salt can be confirmed from the appearance of the ammonium N peak in N_{1s} region. Figure 7a shows that the N_{1s} peak is composed of two components of ammonium N (399.0 eV) and amino N (397.0 eV). From the quantitative analysis of elements (C_{1s}, N_{1s}, O_{1s}) and components of N_{1s} peak, it is estimated that PAA is incorporated into the film as 1.8 times of allylamine units with respect to itaconic acid units.

The FT-IR and XPS spectra of the 2C₁₈DAE/IA-EVE/PAA LB film were similar with those of the 2C₁₈DAE/IA-BVE/PAA LB film. Figure 5 shows the FT-IR spectra of the 2C₁₈ DAE/IA-EVE LB film deposited as Y type on a CaF₂ plate from pure water subphase. In the spectrum of as-deposited LB film (Fig. 5a), we can clearly see two carbonyl peaks derived from carboxylic acid (1716 cm⁻¹) and carboxylate salt (1576 cm⁻¹). It means that the polymer IA-EVE in the LB film has the structures of carboxylate salt form with template amine and free acid form. The FT-IR spectrum (Fig. 6a) of the LB film deposited from the aq. PAA subphase shows much more reduced carbonyl peak (1716 cm⁻¹) of carboxylic acid than that obtained from pure water subphase, while the carbonyl peak of carboxylate salt (1576 cm⁻¹) is increased. Thus, the free acid groups are supposed to form the carboxylate-ammonium salts by subsequent electrostatic interaction with amino groups of PAA when the 2C₁₈DAE/IA-EVE monolayer is spread on the aq. PAA subphase. The extent of PAA incorporation into the LB film was estimated from XPS analysis by the same method as performed in the 2C₁₈DAE/IA-BVE/PAA LB film. As a result, it is revealed that PAA is incorporated as 1.5 times of allylamine units with respect to itaconic acid units.

When the polyion-complexed monolayers were transferred on FP-010 membrane filters, the covering of the pores by the LB films could be observed by SEM. Figure 8 shows the SEM micrographs of 2C₁₈DAE/IA-BVE/PAA LB films on FP-010. The whole covering of the pores was achieved with 6 layer LB films (Fig. 8b), while some hole defects were observed as dark holes in Fig. 8c (indicated with arrows) even in 6 layers when the LB film was prepared from the pure water subphase. It means that the polymer-polymer

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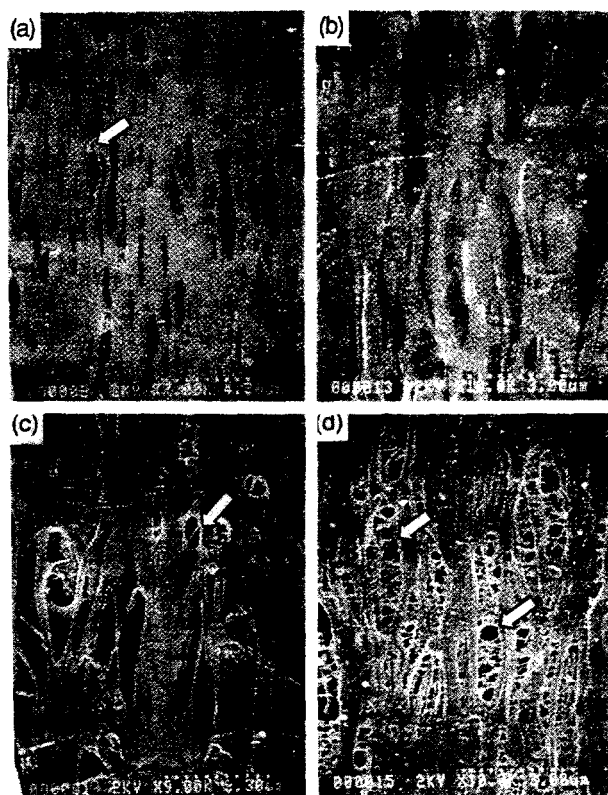
Figure 8. Scanning electron micrographs of LB films of 2C$_{18}$-DAE/IA-BVE/PAA deposited on fluorocarbon membrane filters (FP-010): (a) as-deposited film of 4 layers. (b) as-deposited film of 6 layers. (c) as-deposited film of 6 layers from pure water subphase. (d) heat-treated film of 6 layers at 180 ℃ for 6 h under vacuum. The samples were coated with Pt-Pd and observed at 2 kV. The defects are indicated with arrows.

Scheme 1. Network formation in the polyion complex LB film during heat treatment.

polyion complex at the air-water interface enhances the covering ability by electrostatic crosslinking between the two different polymers. The covering ability of 2C$_{18}$DAE/IA-BVE/PAA LB film was better than 2C$_{18}$DAE/IA-EVE/PAA LB film, *i.e.*, 8 layers were required in order for the latter film to cover the pores of the same membrane filter.

Covalently-crosslinked film. The polymer network in the LB films was achieved by covalent crosslinking through heat treatment of the polyion-complexed film. Heat treatment was carried out at 180 ℃ for 6 h under vacuum. The formation of covalent bonds was confirmed by FT-IR and XPS measurements. Figure 4b shows the FT-IR spectrum of the heat-treated 2C$_{18}$DAE/IA-BVE/PAA LB film. New peaks at 1701 cm^{-1} and 1778 cm^{-1} appeared after heat treatment. These peaks are attributed to symmetric and asymmetric modes of imide group. From the change of carbonyl peaks, the change of the salt structure to an imide structure can be deduced. However, the structural changes accompany with formations of itaconic anhydride and amide groups, which can be estimated from the small peak at 1841 cm^{-1} and the shoulder peak around 1690 cm^{-1} (see Scheme 1). The anhydride formation became clear when the 2C$_{18}$DAE/IA-EVE LB film deposited from pure water subphase was heat-treated. Figure 5b shows the characteristic peaks of anhyd-

ride at 1854 cm^{-1} and 1779 cm^{-1}. Figure 6b is the spectrum of the heat-treated 2C$_{18}$DAE/IA-EVE/PAA LB film. The interpretation of the spectrum can be similarly done as that of the 2C$_{18}$DAE/IA-BVE/PAA LB film. The strong peaks of imide (1701 cm^{-1} and 1778 cm^{-1}) are noticeable in this spectrum.

The XPS spectra support the formation of imides by appearance of a new third peak (at 398.4 eV in Figure 7b) corresponding to the region of imido N. Although the formation of a bit of amides could be assumed from the FT-IR spectra, the new peak of the XPS spectra was assigned as a component of imides N. The extent of imides (practically including amides) is estimated to be 56.5% and 51.3% of total nitrogens in the 2C$_{18}$DAE/IA-BVE/PAA and the 2C$_{18}$ DAE/IA-EVE/PAA films, respectively, which was calculated from the components areas of the N$_{1s}$ spectra.

These films showed poor stability against heat treatment. Figure 8d shows the SEM micrograph of heat-treated 6 layer LB film of 2C$_{18}$DAE/IA-BVE/PAA. We can see many hole defects which are seen as dark spots. On the other hand, much more instability against heat treatment was revealed in the case of LB film deposited from pure water. It is as expected because the itaconic acid copolymer interacts with only template amine and not with PAA. In the case of 2C$_{18}$ DAE/IA-EVE/PAA films, most of the films were destroyed even in 8 layers, and only strand-like structures were seen. When we consider the heat stability with relation to the monolayer structure observed by fluorescence microscopy, it is shown that the homogeneity of the monolayer is important to obtain stable LB films.

Almost template amines are removed during heat treatment, which are evidenced from the reduced C-H stretching peaks in Figure 5b and 6b. To complete the removal of residual template amines, the heat-treated films were dipped into aq. NaCl (1.0×10^{-3} M) for 1 day followed by immersion in chloroform for 1 day. The immersion in aq. NaCl is carried out in order to exchange the counter ion of the template amine from polymeric carboxylate ion to chloride ion, since this exchange makes the template amine extractable in chloroform. Figure 6c shows the IR spectrum of LB films after

immersion in chloroform following aq. NaCl treatment. The remarkably reduced C-H stretching peaks (2917 cm^{-1} and 2850 cm^{-1}) show the removal of residual template amines. Meanwhile, it is noticed that the peaks of carbonyl region remain intacted. From the relative density of C, N, and O determined from XPS measurement as described above, the extent of the amine removal was estimated to be completed.

Conclusion

A LB method for two-dimensionally crosslinked polymer network employing an tert-amine as template was applied to the system of an itaconic acid copolymer and poly(allylamine). The formations of a three-component polyion-complexed monolayer, a covalent crosslinking in the LB film, and subsequent removal of template amine, which are the basic steps for the method, could be established in an itaconic acid copolymer and PAA system. The template LB method is supposed to be applicable to other polymer systems.

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Adsorption Characteristics of Endo II and Exo II Purified from *Trichoderma viride* on Microcrystalline Celluloses with Different Surface Area

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The adsorption behaviors of two major components purified, endo II and exo II, from *Trichoderma viride* were investigated using microcrystalline cellulose with different specific surface area as substrates. Adsorption was found to apparently obey the Langmuir isotherm and the thermodynamic parameters, ΔH, ΔS, and ΔG, were calculated from adsorption equilibrium constant, K. The adsorption process was found to be endothermic and an adsorption entropy-controlled reaction. The amount of adsorption of cellulase components increased with specific surface area and decreased with temperature and varied with a change in composition of the cellulase components. The maximum synergistic degradation occurred at the specific weight ratio of the cellulase components at which the maximum affinity of cellulase components obtains. The adsorption entropy and enthalpy for respective enzyme system increased with specific surface area increase. The adsorption entropy was shown to have a larger value with enzyme mixture.

Introduction

One of the most difficult and undeveloped area in enzyme kinetics relates to enzymatic reactions involved with insoluble substrates. Since native cellulosic materials are water-insoluble solid substrates, the cellulose-cellulase system is heterogenous, and the hydrolysis reaction involves several steps. Among these, the adsorption of cellulase is very important because adsorption is a prerequistic step in enzymatic hydrolysis reaction of cellulose and detailed studies on adsorption may lead to a better understanding of mechanism of the enzymatic hydrolysis process.[1-3]

Recently, some important observation on cellulase adsorption have been reported.[4-9] These include the effects of various pretreatments of cellulose and sample preparation on the adsorption of cellulase components, the relation between the specific surface area of the cellulose particle and the adsorbed amount of soluble protein, and the competitive adsorption of cellulase components. Nevertheless, the adsorption characteristics of a mixture of various cellulase components and the mechanism of cellulase adsorption on cellulosic material are still not fully understood. A good understanding of adsorption phenomena concerning cellulase components may provide some clues to the true reaction mecha-