Morphological Control for Aggregates of DNA Molecules Chemisorbed on the Organized Films of Ternary Comb Copolymers Containing s-Triazine Rings

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We investigated adsorption behavior of DNA molecules to ternary comb copolymers containing 2-vinyl-4, 6-diamino-1, 3, 5-triazine (VDAT) at air/water interface and their molecular arrangement. A newly ternary comb copolymers as adsorption template was synthesized by radical copolymerization of VDAT, octadecyl acrylate (OA), 2-(perfluorodecyl)ethyl methacrylate (FF\textsubscript{10} EMA). The IR spectrum and UV-vis spectrum of transferred LB multilayers indicates adsorption of DNA molecules by ternary copolymer templates. There were hydrogenated domains at 40–50 nm diameter scales in phase separated surface structure of Z-type monolayers. Furthermore, adsorbed DNA molecules showed segment domain at 100–200 nm scales. Fluorescence emission bands of adsorbed DNA to copolymer film showed red-shift and became sharper than the one of their solution. It was caused by formation of aggregate of π-conjugated system in the DNA with uniform aggregation number. In the case of copolymer film containing N-vinyl carbazole (NVCz), small domain at 30-40 nm scales and DNA aggregate with various aggregation number were confirmed.

Key words: 2-Vinyl-4,6-diamino-s-triazine, Ternary Comb copolymer, DNA molecule, Hydrogen bonding, Molecular recognition

1. INTRODUCTION

Recently, many researchers have attempted to utilize poly(2-vinyl-4,6-diamino-s-triazine) (PVDAT) in various applications such as agricultural chemicals, weed-kill ing agents, and as a candidate for drug-delivery systems.\textsuperscript{1} These applications are made possible by the selective-ion-capturing properties and self-aggregation properties of PVDAT.\textsuperscript{2} Furthermore, PVDAT may find use as an absorbing material for DNA molecules.\textsuperscript{3–6}

It is essential to control molecular orientation and arrangement at the monomolecular level in order to efficiently use these organic molecular recognition sensors.\textsuperscript{7, 8} However, it is difficult to control the molecular arrangement and homopolymer packing in many cases because PVDAT readily forms an amorphous polymer.\textsuperscript{9}

In a previous study, we investigated the control of solid-state structures and the formation of organized films of newly synthesized comb copolymers containing hydrogenated and fluorinated side chains.\textsuperscript{10–12} These comb polymers form a side-chain crystal in the bulk state through van der Waals interactions between the side chains and the extremely stable and condensed monolayers at the air/water interface. Incorporation of bulky and flat functional groups in the main chains of side-chain crystalline polymers may enable us to control the arrangement of bulky groups at the monomolecular level, despite the low crystallinity of the polymers.

Recently, fine structures in the solid state of the synthesized comb polymers containing 2-vinyl-4,6-diamino-s-triazine(VDAT) in the main chains have been investigated using wide-angle X-ray diffraction.\textsuperscript{13} These comb polymers were obtained by copolymerization with hydrogenated or fluorinated long-chain compounds.

In the present study, the adsorption behavior of DNA molecules on comb copolymers containing VDAT at the air/water interface\textsuperscript{14–18} and their molecular arrangement were investigated using surface pressure–area (π–A) isotherms, infrared (IR) spectroscopy, UV–vis spectroscopy, and atomic force microscopy (AFM).

2. EXPERIMENTAL

2.1 Materials

Comb copolymers used in this study were obtained by the copolymerization of VDAT with octadecyl acrylate (OA), 2-(perfluorodecyl)ethyl methacrylate (FF\textsubscript{10} EMA), and N-vinyl carbazole (NVCz) at various monomer ratios (Fig.1).
2.2. Investigation of formation and structure of organized molecular films

The comb copolymers used in this study were prepared in a solution of chloroform (10⁻⁴ M) into a solution composed of distilled water (18 MΩ cm) and DNA molecules (20 μg mL⁻¹). The π–A isotherms were obtained using a USI-3-22 film balance (USI Co. Ltd.) at 20°C. The monolayers formed were transferred onto CaF₂ (IR sample), quartz (UV–vis and fluorescence spectroscopy), and mica (AFM) substrates using the LB method. IR spectra were acquired on a System 2000 spectrometer (Perkin Elmer Co. Ltd.). UV–vis spectra were recorded on a JASCO V-650 spectrometer. The surface morphologies of the transferred monolayers were observed under a scanning probe microscope (Seiko Instrument, SPA300 with SPI-3800 probe station) at tapping mode; microfabricated rectangular Si single-crystal cantilevers with integrated pyramidal tips were utilized with a constant force of 1.7 Nm⁻¹. In order to estimate the optical property of the DNA-adsorbed film, fluorescence spectra were recorded on a JASCOFP-6500.

3. RESULTS AND DISCUSSION

Fig. 2 shows the π–A isotherms of the monolayer for VDAT:OA:FF₁₀EMA = 6:5:1 copolymer on the water surface and aqueous DNA solution at 20°C.
an influence on the band shift of C=O stretching vibration to low wavenumber side bringing about the formation of hydrogen bonding between triazine ring in copolymer and adenin-thymin (A-T) base pair of DNA. This result suggests that DNA molecules adsorb to VDAT units of the copolymer monolayer by hydrogen bonding. Fig. 4 shows UV–vis spectra of LB multilayers of DNA adsorbed on copolymers and DNA aqueous solution. LB multilayers of VDAT:OA:FF$_{10}$EMA = 6:5:1 copolymer adsorbed DNA produced an absorption band at 261.0 nm (Fig. 4(a)). The LB multilayers of VDAT:OA:FF$_{10}$EMA = 6:5:1 copolymer themselves did not show any absorption bands. In addition, aqueous DNA solution produced an absorption band at around 260 nm (Fig. 4(b)). From these results, it can be confirmed adsorption of DNA molecule to organized molecular film of copolymer, too.

Fig. 5-1 shows AFM images of Z-type, X-type monolayers for VDAT:OA:FF$_{10}$EMA = 6:5:1 copolymer, and X-type monolayer adsorbed DNA. LB depositions can be classified in different types. When the monolayer deposits only in the aqueous solution containing DNA, it produces a circular domain. In the case of Z-type monolayer, many circular domains were observed (Fig. 5-1(a)). It is well-known that characteristic phase separation occurs in the mixed materials containing fluorocarbons and hydrocarbons because of the lack of miscibility. Therefore, it is speculated that hydrogenated side chains formed these circular domains. In the case of X-type monolayer, irregular surface was confirmed (Fig. 5-1(b)). It is speculated that this irregular surface was formed by phase separation of side chains. Furthermore, X-type monolayer that adsorbed DNA molecules showed segment domain (Fig. 5-1(c)). Furthermore, VDAT:OA:FF$_{10}$EMA:NVCz = 6:5:1:0.6 copolymer was newly synthesized. Fig. 5-3 shows AFM images of Z-type, X-type monolayers for VDAT:OA:FF$_{10}$EMA:NVCz = 6:5:1:0.6 copolymer copolymers, and X-type monolayer adsorbed DNA. In the case of Z-type monolayer, many circular domains were observed (Fig. 5-3(a)). In the case of X-type monolayer, irregular surface was confirmed (Fig. 5-3(b)). In the case of X-type monolayer that adsorbed DNA molecules, small DNA domains were confirmed (Fig. 5-3(c)). From the result of AFM, it was confirmed that adsorbed DNA molecules formed domains of different sizes by several copolymer templates containing VDAT units.

Fig. 6 shows fluorescence spectra of LB multilayers for the VDAT:OA:FF$_{10}$EMA = 6:5:1 and VDAT:OA:FF$_{10}$EMA:NVCz = 6:5:1:0.6 copolymer that adsorbed DNA molecules and the DNA aqueous solution. All excitation wavelengths are 260 nm. DNA molecules in solution show broad emission at 387 nm. The emission bands of the adsorbed DNA molecules for VDAT:OA:FF$_{10}$EMA = 6:5:1 copolymer multilayers shifted at 401 nm. The tendency of red-shift in the adsorbed films was confirmed. It seems that red-shift of the emission band brings about formation of aggregate of π-conjugated system in the DNA molecules. These experimental findings are caused by the DNA adsorption on the arranged VDAT of the copolymer monolayer (Fig. 7(a)). Furthermore, the emission bands of the adsorbed DNA molecules of these copolymer multilayers were sharper than that of the solution.
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Previously, it has been reported that formation of uniform particle size brings about sharpening of band of the fluorescence spectra. Therefore, the attainment of formation of aggregates with uniform aggregation number (Fig. 7(b)) is speculated in the case of these copolymer systems. These experimental findings relate to well-ordered arrangement of functional groups caused by the π-π interaction between the s-triazine rings. On the other hand, the adsorbed DNA molecules of VDAT:OA:FF$_{10}$EMA:NVCz = 6:5:1:0.6 copolymer multilayers emitted fluorescence at 370 nm and 454 nm. The attainment of formation of aggregates with various aggregation number (Fig. 7(c)) is speculated in the case of VDAT:OA:FF$_{10}$EMA:NVCz = 6:5:1:0.6 copolymer systems.

As mentioned previously, we could control the arrangement of the adsorbed DNA molecules by using several copolymer templates containing VDAT units. Finally, optical properties of these adsorbed DNA were remarkably influenced by the aggregation of π-electron systems.

4. CONCLUSION

We investigated adsorption behavior of DNA molecules to comb copolymers containing VDAT at air/water interface and their molecular arrangement by π-A isotherm, IR spectroscopy, UV-vis spectroscopy, and AFM. Adsorption templates in this study are newly synthesized hydrogenated and fluorinated comb copolymers containing VDAT. Comb copolymers form stable condensed monolayers on distilled water. When DNA molecules were present in the subphase, the π-A isotherm indicated expand behavior. Furthermore, the IR spectra and UV-vis spectra of corresponding LB multilayers indicated adsorption of DNA molecules by hydrogen bonding. From the result of AFM, nanometer order phase separated structure was confirmed. Furthermore, adsorbed DNA molecules showed segment domain. Emitted fluorescence band of adsorbed DNA indicates red-shift and becomes sharper to the one of their solution. It was caused by formation of aggregate of π-conjugated system in the DNA with uniform aggregation number. In the case of copolymer film containing NVCz, small domain and DNA aggregate with various aggregation number were confirmed.

REFERENCES


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