Control of Arrangement for DNA Molecules Chemisorbed to the Organized Molecular Films of Comb Copolymers Containing s-Triazine

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Adsorption behavior of DNA molecules to comb copolymers containing 2-vinyl-4, 6-diamino-1, 3, 5-triazine (VDAT) at air/water interface and their molecular arrangement have been investigated by surface pressure-area (π-A) isotherm, IR spectroscopy, polarized UV-vis spectroscopy, and atomic force microscopy. Newly hydrogenated and fluorinated binary comb copolymers containing VDAT as adsorption template were synthesized by radical copolymerization. From the result of π-A isotherm of monolayer on DNA aqueous solution, an increase of value for molecular area per VDAT unit corresponds to 20 Å$^2$. The IR spectrum of transferred LB multilayer indicates adsorption of DNA molecules by hydrogen bonding to the copolymer templates. AFM images show the circular domain at 50-60 nm diameters. Adsorbed DNA molecules showed the absorption band at 260 nm and emitted fluorescence at 400 nm. From the results of polarized UV-vis spectroscopy, these absorption bands indicate faint polarized dependency. In addition, emission bands in fluorescence spectrum of adsorbed DNA to copolymer film showed red-shift and became sharper than the one of their solution.

Key words: 2-vinyl-4, 6-diamino-s-triazine, Comb Copolymer, DNA Molecule, Hydrogen Bonding, Molecular Recognition, Organized Molecular Films

1. INTRODUCTION

In recent times, many researchers have attempted to utilize poly(2-vinyl-4, 6-diamino-s-triazine) (PVDAT) such as agricultural chemicals, a weed-killer agent and a candidate for drag delivery system because PVDAT exhibits selective ion capturing properties and self-aggregated properties. Further, PVDAT is expected to be used as an absorbing material to DNA molecules.

On the other hand, it is essential to control the molecular orientation and the arrangement at the monomolecular level in order to drive these organic molecular recognizing sensors in an efficient manner. However, in many cases, it is difficult to control the molecular arrangement and the homopolymer packing because PVDAT itself readily forms an amorphous polymer. In the previous study, we have investigated to control solid-state structures and the formation of organized films of newly synthesized comb copolymers containing hydrogenated and fluorinated side-chains. These comb polymers form a side-chain crystal in the bulk state due to the van der Waals interaction between the side-chains and extremely stable condensed monolayers at the air/water interface. The 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, regardless of the low crystallinity of the polymers.

Recently, fine structures in the solid-state of synthesized comb polymers containing 2-vinyl-4, 6-diamino-s-triazine (VDAT) in the main chains obtained by co-polymerization with hydrogenated or fluorinated long-chain compounds were investigated using wide-angle X-ray diffraction. In this study, adsorption behavior of DNA molecules to comb copolymers containing VDAT at air/water interface and their molecular arrangement have been investigated by surface pressure-area (π-A) isotherm, IR spectroscopy, polarized UV-vis spectroscopy, and atomic force microscopy.

2. EXPERIMENTAL

2.1 Materials

Binary random comb copolymers used in this study were obtained by the copolymerization of VDAT with octadecyl acrylate (OA) or 2-(perfluorodecyl)ethyl acrylate (FF$_{16}$EA) at various monomer ratios (Fig.1). Copolymerizations were carried out in an acetone solution at 50 °C for 48 h using 1.0 mol% azobisisobutyronitrile (AIBN) as an initiator. These monomers and the initiator were purchased from Tokyo Kasei Co. Ltd. and Daikin Fine Chemicals Co. Ltd., and were used without further purification. The precipitated polymers were washed with acetone until they were free of monomers. The comb copolymer compositions were determined by $^1$H NMR (Nihon Densi Co. Ltd. EX270 NMR) spectroscopy. An average molecular weight of copolymer was estimated to be approximately $M_w = 2.02 \times 10^6$ on the basis of GPC (JASCO-860-CO) measurements.

According to the theory of the $Q$-$e$ scheme proposed by T. Alfrey and C.C. Price, these comb polymers form random copolymers. In this case, the $e$ values of VDAT and OA are 0.59 and 1.12, respectively.
DNA molecules in this study come from a salmon spermary.

2. Estimation of formation and structure of organized molecular films.

The comb copolymers used in this study were spread from chloroform solution (approximately $10^{-4}$ M) onto DNA aqueous solution composed by distilled water (approximately 18 MΩ·cm) containing DNA molecules (20 μg ml$^{-1}$). The π–A isotherms were measured by a USI-3-22 film balance (USI Co. Ltd.) at 20 °C. These monolayers were transferred onto CaF$_2$ (IR sample), quartz (UV-vis and fluorescence spectroscopy), and mica (AFM) substrates using the LB method. IR spectra were measured by system 2000 spectrometer (Parkin-Elmer Co. Ltd). Uv-vis spectra were measured by JASCO V-650 spectrometer. The surface morphologies of the transferred monolayers were observed using a scanning probe microscope (Seiko Instrument, SPA300 with SPI-3800 probe station) at tapping mode utilizing microfabricated rectangular Si single crystal cantilevers with integrated pyramidal tips with a constant force of 1.7 Nm$^{-1}$. In order to estimate optical property of DNA adsorbed film, fluorescence spectra were measured by JASCO FP-6500.

3. RESULTS AND DISCUSSION

Figure 2 shows π–A isotherms of monolayer on the water surface and aqueous DNA solution at 20 °C. (a) VDAT:OA=1:1, (b) VDAT:FF$_{10}$EA=1:1 copolymers.

Figure 3 shows IR spectra of DNA solution and LB multilayers of VDAT:OA=1:1 copolymer and their adsorbed DNA. In the spectrum of DNA powder, the C=O stretching vibration around 1620-1750 cm$^{-1}$ comes from an existence of various hydrogen bonded carbonyl groups in base pairs. From the result of IR for multilayers of VDAT:OA=1:1 copolymers, the C=O stretching vibration appeared at 1750 cm$^{-1}$. Further, in the case of LB multilayers transferred by monolayer on aqueous DNA solution, the C=O stretching vibration...
band became broad around 1620-1750 cm\(^{-1}\). This result supports that DNA molecules adsorb to VDAT units of copolymer monolayer by hydrogen bonding. Figure 4 shows AFM images of X-type monolayers on solid substrates of VDAT:OA=1:1 comb copolymer and their adsorbed DNA. In the AFM images of X-type monolayers of VDAT:OA=1:1 comb copolymer, remarkable flat surfaces in mesoscopic scales were observed in several transferred surface pressures. On the other hand, hydrogenated copolymer monolayers which adsorbed DNA molecules show irregular surfaces based on the adsorption from subphase. In the images of 1 × 1 μm\(^2\) probing areas, many circular domains were confirmed. Confirmable minimum size of these domains are about 50-60 nm diameters. It seems that other large domains are coagulated by any micro domains. Further, the increased value of molecular area brought about DNA adsorption in π-A isotherms correspond to the 20 Å\(^2\). That
is to say, it is speculated that the minimum nano-domains are also coagulated by DNA molecules.

Figure 5 shows Uv-vis spectra of LB multilayers of DNA absorbed copolymers and DNA aqueous solution. Almost all samples indicated to the absorption band at around 260 nm. LB multilayers of VDAT:OA=1:1 and VDAT:FF₁₀EA=1:1 copolymers themselves did not show any absorption bands. The maximum absorption band at 260 nm comes from any nucleotide bases in DNA (Table 1). Hence, polarized Uv-vis spectroscopies are performed in order to estimate molecular arrangement of adsorbed DNA molecule. Figure 6 shows the measurement of polarized UV-vis spectra for LB multilayers of VDAT:OA=1:1 and VDAT:FF₁₀EA=1:1 which adsorbed DNA molecules. Although polarized dependency is relatively faint, calculated orientation angles of transition dipole moments correspond to 37.7° and 46.9° in hydrogenated and fluorinated polymer templates, respectively. Since this band comes from convolution of any kinds of bases, the resultant faint polarized dependency may be confirmed.

Figure 7 shows fluorescence spectra of LB multilayers for the VDAT : OA = 1:1 and VDAT : FF₁₀EA = 1:1 which adsorbed DNA molecules, and DNA aqueous solution. All excitation wavelengths are 260 nm. DNA molecules in solution show broad emission at 387 nm. On the other hand, adsorbed DNA molecules of VDAT:OA copolymer multilayers emitted fluorescence at 401 nm. This emission band became sharper than that of solution. Further, emission band of adsorbed DNA molecules for the VDAT:FF₁₀EA copolymer multilayers shifted at 441 nm. The tendency of red-shift in adsorbed films was commonly confirmed. It is speculated that the origin of appearance for sharper band bases on the aggregation of π-electron system of nucleotide base in adsorption DNA to the arranged VDAT units of copolymer monolayers. In addition, this aggregation may bring about changes in retention process of excited π-electron.

As mentioned previously, we could control the orientation of the adsorbed DNA molecules by using several copolymer templates containing VDAT units. Also, optical properties of these adsorbed DNA were remarkable influenced on aggregation of π-electron system.

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, polarized 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 was expanded 20 Å² per units. Further, the IR spectra of corresponding LB multilayers indicate adsorption of DNA molecules by hydrogen bonding. The circular DNA domains at 50-60 nm diameters confirmed by AFM observation. Adsorbed DNA molecules showed the absorption band at 260 nm. This material also shows fluorescence emission at around 400 nm. From the result of polarized UV-vis spectroscopy, this absorption band indicates faint polarized dependency. It is found that conjugated π-electron system in adsorbed DNA is oriented. Emitted fluorescence band of adsorbed DNA indicates red-shift and becomes sharper to the one of their solution.

REFERENCES


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