Molecular Recognition and Photopolymerization of Nucleobase Monolayer Containing Diacetylene Group at the Air–Water Interface

Jin Matsumoto,[†] Kuniharu Ijiro,[†,††] and Masatsugu Shimomura*[†,††]
[†]*Research Institute for Electronic Science, Hokkaido University, N12W6, Kita-ku, Sapporo 060-0812*
[††]*CREST, Japan Science and Technology Corporation (JST), N12W6, Kita-ku, Sapporo 060-0812*

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An amphiphilic diacetylene with thymine (DA–Thy) was newly synthesized. The amphiphile can form solid-state monolayer and photopolymerize with UV light irradiation at the air–water interface to form blue and red forms of polydiacetylene. Shapes of the crystalline domain of the polydiacetylene monolayer were changed by base pair formation between DA–Thy and adenine nucleobase dissolved in the water subphase.

Nucleic acid is a functional polymer which stores and replicates genetic information playing the most important role of the life activity. Those functions are served by the precise molecular recognition between base pairs by complementary hydrogen bonding in the double helical DNA. Template polymerization of nucleoside analogs based on hydrogen bonding with nucleic acids has been proposed since 1970's.[1,2] Most study used homogeneous solutions for the polymerization environments. Nonpolar solvents were preferred for the hydrogen bonding formation between base analog monomers and nucleic acids.

In our previous report an octadecylcytosine monolayer at the air–water interface can form the complementary base pair with guanosine dissolving in the subphase.[3,4] Due to the high hydrophobicity of the air–water interface, hydrogen bond formation is enhanced in the monolayer.[5,6] To realize the template polymerization of the nucleobase monolayer directed by oligonucleotides dissolving in the subphase, a novel nucleobase amphiphile, DA–Thy, which has a diacetylene group in its long alkyl chain, was synthesized (Figure 1). It is known that the topochemical polymerization of diacetylene group by UV-light irradiation can be proceeded in crystalline state of the monolayer.[7,8] Moreover, it is expected that the monolayer of DA–Thy is organized by adenine nucleobases dissolving in the subphase through the complementary hydrogen bonding at the air-water interface. Molecular recognition and photopolymerization of DA–Thy monolayer at the air–water interface were investigated by using a fully computer controlled film balance (FSD-50, USI system), a UV–VIS reflection absorption spectrometer (LB-100, JASCO), and a fluorescence microscope (BH2-UMA, Olympus).

Active ester of a nucleobase derivative, 4-nitrophenyl-3-(1-thymidyl) propionate (PNP-Thy), was synthesized according to the previously reported procedure.[9] A mixture of 10,12-pentacosadiyne-1-ol and imidazole in dichloromethane was added to a DMF solution of PNP-Thy. The solution was stirred for a week and concentrated under reduced pressure. The crude product was purified by silica-gel column chromatography using toluene/ethyl acetate/chloroform = 4/2/1 (v/v) as an eluent and recrystallized from ethanol. The final product was characterized by ^{1}H NMR spectroscopy and elemental analysis.[10]

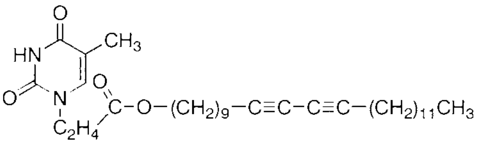

Figure 1. Chemical structure of DA-Thy.

The monolayers of DAThy were spread on the surface of 10 mM Tris-HCl buffer solution (pH 7.8) at 10 and 20 °C from the mixed solution of chloroform and ethanol. Figures 2a and 2b show the pressure-area isotherms of DAThy at 10 and 20 °C, respectively. At 20 °C the DAThy monolayer gives an expanded isotherm which is characteristic for a liquid state monolayer. Lowering the surface temperature to 10 °C, the isotherm shows a plateau region at ca. 10 mN/m and then steep pressure-up to 35 mN/m. Two-dimensional crystal formation in the monolayers can be observed by using fluorescence microscopy when a small amount of an amphiphilic fluorescence probe, octadecylrhodamine B, is added to the monolayers.[11] No crystal domain was formed in the DAThy monolayer at 20 ºC even at 30 mN/m. On the other hand, at 10 ºC the crystal domain was formed at the plateau region, ca. 10 mN/m, which was the phase transition pressure from liquid to crystalline state.

Figure 2c is the pressure-area isotherm of DAThy on a buffer solution containing 25 nM of deoxyadenylic acid 30 mer (dA$_{30}$). The phase transition pressure was decreased to ca. 5 mN/m. Remarkable change of the pressure-area isotherm was not observed when the non-complementary nucleic acids were dissolved in the subphase. This suggests that the DAThy monolayer can form complementary hydrogen bonds with the adenine bases at the airwater interface.

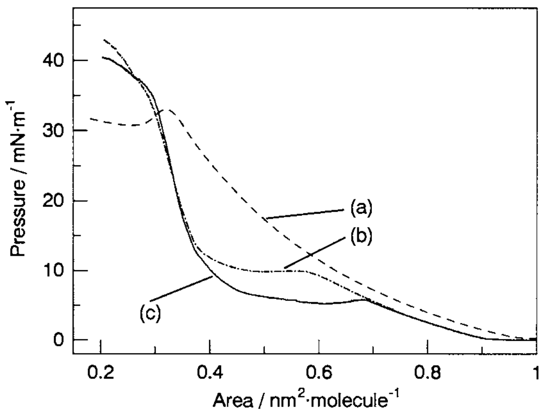

Figure 2. Pressure-area isotherms of DA-Thy on a 10 mM Tris-HCl (pH 7.8) buffer solution at 20 (a) and 10 °C (b) and on 25 nM dA$_{30}$ solution at 10 °C (c).

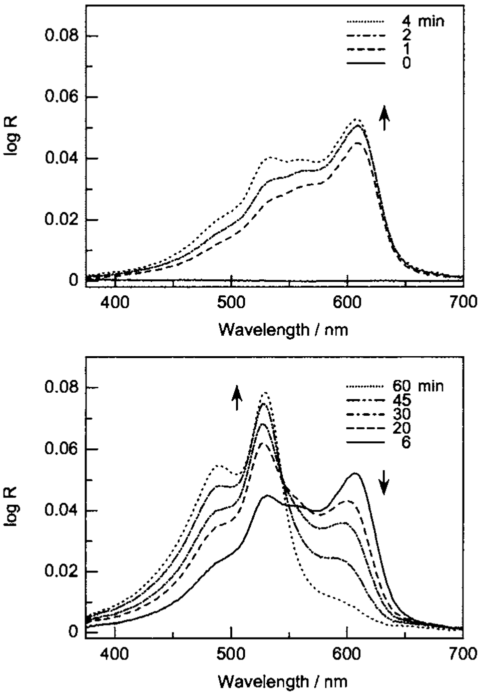

Figure 3. Irradiation time dependence of reflection spectrum of the DA-Thy monolayer at 15 mN/m on the buffer solution at 10 °C.

The diacetylene group is known to be polymerized by UV-irradiation in the crystalline state and polymerized diacetylene shows blue or red color. The crystalline monolayers of DA–Thy were expected to polymerized by irradiation of 254 nm light at the air–water interface. The reflection absorption spectra of the polymerized monolayers were measured by the fiber-optics spectrophotometer (Figure 3). No visible spectral changes were observed in the DA–Thy monolayer of the liquid state, below the phase transition pressure at 10 °C and at any pressure at 20 °C, respectively. While in the crystalline state monolayer at 10 °C, polymerization is accompanied by color change from blue to red as a function of UV-irradiation time. The color change was similar to that of the crystalline monolayer of 10,12-pentacosadiynoic acid.[12]

The "red form" monolayer shows fluorescence emission (λ_{em} = 635 nm). Fluorescence microscope images of the polymer monolayers deposited on a glass plate from the buffer solution, the dA_{30} subphase, and an adenosine subphase are shown in Figure 4. On the buffer solution lotus shaped polymer domains were formed (a). A polarized fluorescence image (b) indicates that the fibrous crystallites radially grow from the domain center to the edge. Addition of the adenine nucleobase, dA_{30} or adenosine, changed the domain morphology of the polymerized DA–Thy monolayer. Multilayered irregular shape domains were formed when the polymerization was carried out on the dA_{30} solution. The shape of the crystalline domain was changed to circle on the adenosine subphase. The polymerization behavior of DA–Thy on adenosine solution was similar to that on the buffer subphase. Morphological change of the polymerized DA–Thy monolayer is ascribable to the base pair formation with the adenine base at the air water interface.

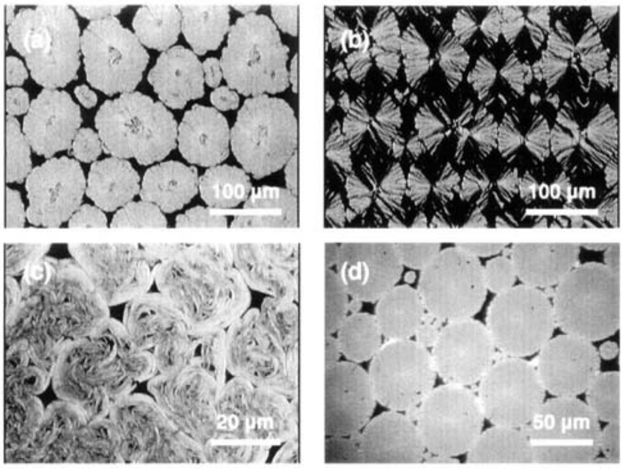

Figure 4. Fluorescence images of DA-Thy monolayers deposited from the buffer solution (a, b), the dA_{30} solution (c), and the adenosine solution (d) after UV irradiation.

In conclusion, the thymine monolayer with the diacetylene group can recognize the adenine base in the water subphase. The diacetylene group can photopolymerize in the crystalline monolayer. Shapes of the crystalline domains of DA–Thy were drastically changed by complementary base paring with adenine nucleobase. Template polymerization of the diacetylene monolayer based on molecular recognition at the air–water interface is now under investigation.

References and Notes

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10 DA–Thy ; yield, 47% ; ^1H NMR(400 MHz, $CDCl_3$), δ = 0.88 (t, J = 7.0 Hz), 1.26 (m), 1.37 (m), 1.54 (m), 1.91 (s), 2.75 (t, J = 6.8 Hz), 3.63 (t, J = 5.6 Hz), 3.96 (t, J=6.2 Hz), 4.08 (t, J=7.0 Hz), 7.19 (s), 8.20 (s). Anal. Calcd for $C_{33}H_{52}N_2O_4$: C, 73.29; H, 9.69; N, 5.18%. Found: C, 73.18; H, 9.79; N, 5.23%.

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