Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

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Version of record first published: 24 Sep 2006

To cite this article: Fumio Nakamura, Keita Mitsui, Tomohide Murase, Kazutoshi Kobayashi, Masahiko Hara, Wolfgang Knoll & Hiroyuki Sasabe (2000): Immobilization of DNA on Self-Assembled Monolayer, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 349:1, 219-222

To link to this article: http://dx.doi.org/10.1080/10587250008024904

Mol. Cryst. Liq. Cryst., 2000, Vol. 349, pp. 219-222
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Immobilization of DNA on Self-Assembled Monolayer

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We attempted the immobilization of DNA onto self-assembled monolayer (SAM) which contains the intercalator for DNA. Anthryl moieties were attached to the terminal OH groups in SAM of 1-Mercapt-11-undecanol (MU-SAM) by esterification. In the case of the SAM containing anthryl moieties (Anth-SAM), surface plasmon resonance measurement indicates that the adsorption of DNA onto the SAM was observed in an aqueous DNA solution, and the DNA remained on the surface even after rinsing. Although the adsorption of DNA onto MU-SAM was also observed, little DNA remained on the SAM after rinsing. These results indicate that the anthracene which was attached to the SAM can interact with DNA because the binding ability was increased by introduction of anthracene moieties to the SAM.

Keywords: DNA; intercalation; self-assembled monolayer; surface plasmon resonance; IR-RA spectroscopy; atomic force microscope

INTRODUCTION

Currently, considerable interest has been centered on photo-function[1,2] and/or sequencing[3,4] of DNA by introducing two-dimensional immobilization of DNA on inorganic substrates. Here we paid attention to an intercalation which is incorporation phenomenon of chromophore in stacked base-pairs of DNA and attempted the immobilization of DNA on self-assembled monolayer (SAM) which contains the intercalator

(FIGURE 1).

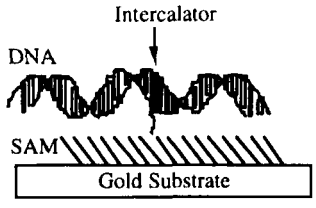

FIGURE 1. Interaction between intercalator and DNA on SAM.

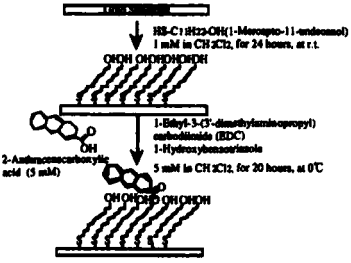

FIGURE 2. Schematic procedure for attachment of anthryl groups to SAM.

EXPERIMENTS

Schematic procedure of the introduction of anthracene to SAM is shown in FIGURE 2. Anthryl moieties were attached to the terminal OH groups in SAM of 1-mercapto-11-undecanol (MU-SAM) by esterification in CH_2Cl_2 solution at 0 ℃ for 24 hours. DNAs used in this study were calf thymus DNA and plasmid DNA (pBR322) which were diluted in buffer solution. Esterification of anthryl moieties and adsorption of DNA onto the SAM on gold substrate was confirmed by IR –RAS (Mattson, Infinity 60AR). Surface plasmon resonance was measured at room temperature. Topology of the SAM was observed by tapping mode of atomic force microscope (AFM) (Dedital Instrument, NanoScope Ⅲ).

RESULTS AND DISCUSSION

1. Characterization of SAM

The esterification was confirmed by FT-IR RAS. The IR spectrum of Anth-SAM shows that the peak which attributed to C=O stretching of ester group was appeared in around 1720 cm^{-1}. This indicates that anthryl moieties were attached to UM-SAM through ester bindings.

2. Adsorption of DNA onto SAM

It is shown in FIGURE 3 that the kinetic curves for adsorption-desorption of DNA to the Anth-SAM or MU-SAM by surface plasmon resonance(SPR). In the case of Anth-SAM, the kinetic curve shows that the increase of reflectivity was observed in addition of DNA solution, and the DNA was remained on the surface even after rinsing. The increasing thickness of the SAM is estimated to be 1.0 nm by the SPR spectrum. Although the increment of reflectivity could be also observed in the UM-SAM, little DNA remained on the SAM after rinsing.

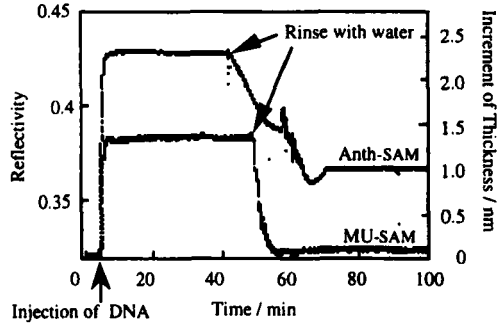

FIGURE 3. Binding behavior of DNA onto SAM: DNA (Calf thymus, 300-600 base pairs), 50 mg/l.

3. AFM observation of DNA on SAM

Figure 4b shows an AFM image of Anth-SAM in air which was immersed in DNA aqueous solution, taken in air after rinsing with water. Network structure was observed in the image although such a structure was not observed on Anth-SAM (FIGURE 4a). The observed height of the network is 0.7-1.3 nm, well below 2.0 nm of DNA diameter in solution but consistent with the reported height of DNA on substrates between 0.1 - 1.5 nm measured by AFM.[5,6] The image implies DNA is immobilized onto the Anth-SAM two-dimensionally. These results indicate that the anthracene attached to the SAM can interact with DNA at the solid-liquid interface because the binding ability of SAM for DNA was increased by introduction of anthracene moieties to the SAM. AFM image of Anth-SAM which was immersed in plasmid DNA buffer solution is shown in FIGURE 4c. Circular structure of plasmid DNA can be observed in the

image. The observed height of the DNA is 1.1-1.3 nm. These AFM images indicate that DNAs could be immobilized on the Au substrate two-dimensionally in our method.

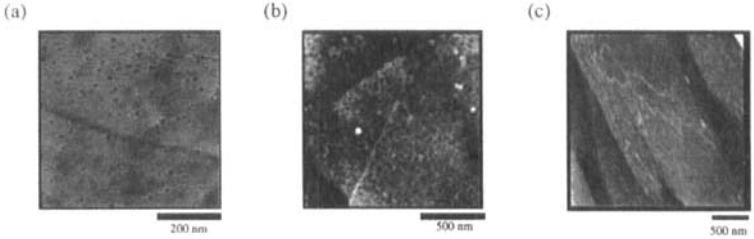

FIGURE 4. AFM image of DNA on (a)Anth-SAM , (b) calf thymus DNA/ Anth-SAM and (c) plasmid DNA/ Anth-SAM (Height image of tapping mode).

CONCLUSION

We succeeded an efficient immobilization of DNA onto two dimensional surface by means of introduction of intercalator to the surface. The advantage of our method is little influence for structure of DNA which is immobilized onto SAM because the interaction between intercalator and DNA is due to van der Waals force. Then, the DNA may interact with other molecules which have a specific binding for DNA. It is also expected to elucidate the electrochemical and photochemical characteristics of DNA in the 2-D immobilization method.

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