Polymer Journal, Vol. 31, No. 11-2, pp 1115—1120 (1999)

Preparation of DNA-Based Molecular Assemblies by Self-Organization. From Nanometer Scale to Mesoscopic Scale

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(Received February 12, 1999)

ABSTRACT: Toward the functional application of DNA as novel molecular devices, we have immobilized DNA as two-dimensional molecular assemblies by means of specific intermolecular interaction at the air-water interface. Preparation of DNA-mimetics at the air-water interface is briefly shown in the second part of this paper. The final part describes micropattern formation of DNA assemblies by using a novel lithography-free technique based on a general physical phenomenon. Mesoscopic two-dimensional patterns of DNA assemblies are prepared by freezing of dissipative structures formed in casting processes of DNA solution.

KEY WORDS DNA / DNA Mimetics / Polyion Complex / Surface Monolayer / Langmuir-Blodgett Films / Dissipative Structure / Micro Patterning /

Double-helical DNA is a supramolecular architecture composed of complementary base-pairs of adenine-thymine and cytosine-guanine based on specific hydrogen bonding, and carries genetic information. Stacking interaction of π-electrons at the ground states of the base-pairs is observed as a spectral hypochromic effect, reduction of the molecular extinction coefficient, in UV-Vis absorption spectrum. The hypochromism is ascribed to so-called "weak" intermolecular interaction classified by Förster, since the averaged distance between two base-pairs is about 0.34 nm in B-form DNA. Recently some articles have claimed that DNA can act as a π-electron medium for the photoinduced electron transfer because of its close stacking of base-pairs.[1,2] Mechanism of the DNA-mediated long range electron transfer is, however, just under hot discussions.[3,4]

In order to utilize DNA as novel functional materials, we have immobilized DNA as a monomolecular film by means of the electrostatic interaction with a cationic monolayer at the air-water interface. The first part of this manuscript describes the effect of DNA on photoinduced electron transfer in Langmuir monolayer deposited on an ITO electrode. To simplify mechanism discussions of the electron transfer we have attempted cutting stacked base-pairs out of DNA helices. The DNA-mimetics at the air-water interface are prepared from simple amphiphilic derivatives of the DNA bases. By using a novel lithography-free technique based on a general physical phenomenon, so-called dissipative structures, we have succeeded in preparing micropatterned DNA assemblies.

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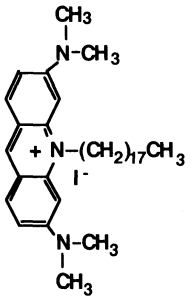
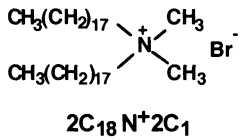
EXPERIMENTAL

Materials

Dioctadecyldimethylammonium bromide, $2C_{18}N^+2C_1$ was supplied by Sogo Pharmaceutical Company, Japan. Acridine orange hydrochloride was purchased from Aldrich, USA. Double-stranded DNA, (sodium salts from Calf thymus, Sigma, USA), double-stranded RNA, (polyA•polyU, sodium salts, Yamasa, Japan, and polyG•polyC, sodium salts, Sigma, USA), single-stranded RNA, (polyU sodium salt, Yamasa, Japan) and carboxymethylcellulose (CMC, sodium salts, Daicel Chemicals, Japan) were used without further purification. Chloroform, used as a spreading solvent, was of spectroscopic grade (Merck Uvasole). Dilute solutions of polyelectrolytes (10 mg / L Milli-Q water) were used as aqueous subphases.



C18AO

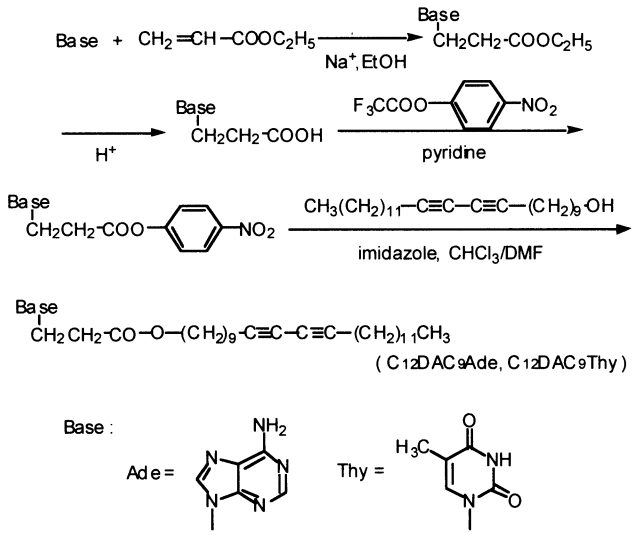


$2C_{18}N^+2C_1$

An amphiphilic intercalator, 10-octadecyl acridine orange iodide (C_{18}AO), was prepared by a quaternarization reaction of acridine orange with alkyl iodide.[5] Octadecyladenine (C_{18}-Ade) and octadecylthymine (C_{18}-Thy) were prepared by alkylation of adenine and thymine with octadecyl iodide in the presence of sodium hydride in dry DMF.

C_{12}DAC9Ade and C_{12}DAC9Thy, were newly synthesized.

Nucleobase propionic acids were sythesized from nucleobases and ethyl acrylate, followed by hydrolyses. Those acid compounds were activated by esterification with *p*-nitrophenol. A mixture of 10,12-pentacosadiyn-1-ol and imidazole in dichloromethane was added to dry DMF solution of *p*-nitrophenyl nucleobase propionate. The solution was stirred for one week at room temperature and concentrated by evaporation. The product was purified by column chromatography (sillica gel, toluene / ethyl acetate / chloroform = 4/2/1 as eluent) and recrystallized from methanol. *C12DAC9Ade* ; yield, 46% ; ^{1}H-NMR(400MHz, $CDCl_3$), d=0.88 (t, J = 6.8 Hz), 1.26 (m), 1.30 (m), 1.50 (m), 2.24 (t, J = 7.08 Hz), 2.93 (t, J = 6.35 Hz), 4.06 (t, J = 6.8 Hz), 4.51 (t, J = 6.1 Hz), 5.56 (s), 7.90 (s), 8.36 (s)
C12DAC9Thy ; yield, 47% ; ^{1}H-NMR(400MHz, $CDCl_3$), d=0.88 (t, J = 7.0Hz), 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)



RESULTS AND DISCUSSION

DNA Monolayers Complexed with Cationic Monolayers at the Air-Water Interface

The polyion complex technique is a useful method to assemble water-soluble polyelectrolytes on oppositely charged surfaces of monolayers at the air-water interface.[6] DNA, having phosphate groups in its backbone, was assembled with counter-charged amphiphiles and fixed as a cast oriented film on solid substrates.[5,7] DNA molecules are expected to be arranged orderly with keeping the double helical structure as counterparts of cationic monolayers at the air-water interface. Some cationic dyes, so called intercalators, can be incorporated into base-pairs of the double stranded DNA with high affinities. A new type of amphiphilic intercalator (C18AO) which can form a monolayer at the air-water interface is used to confirm the double helical structure of DNA complexed with the monolayer at the air-water interface.

Interaction between cationic amphiphiles and anionic polymers at the air-water interface is reflected in the pressure-area (π-A) isotherms of the monolayer. The π-A isotherm of C18AO on a DNA subphase is more expanded and has a higher collapse pressure than that on a pure water subphase.[5] Due to the hydrophobic microenvironment effect of the stacked base-pairs in the double helical DNA, fluorescence intensity of the intercalator is remarkably enhanced. Green fluorescence(535nm) attributed to monomeric acridine orange fluorophore is strongly enhanced when the C18AO monolayer is spread on the DNA subphase. Thus the DNA molecule complexed with the cationic monolayer keeps the double helical structure with the intercalation ability.

DNA Enhanced Electron Transfer from C18AO Monolayer to ITO Electrode

One-dimensionally stacked π-electron array of base-pairs in double-stranded DNA is expected to act as an electron- or a hole-transfer medium. We have investigated the effect of DNA on the fluorescence lifetime of a binary component monolayer of C18AO and $2C_{18}N^+2C_1$, which forms a matrix monolayer preventing the concentration fluorescence quenching of C18AO due to the chromophore aggregation. Fluorescence emission spectrum and life time was measured by a fiber optics spectrometer and a pico-second pulse laser system with a streak camera through a fluorescence microscope equipped with a Langmuir trough. Emission spectra of polyion complex monolayers are shown in Figure 1. Relative fluorescence intensity and life time of the polyion complex monolayers were summarized in Table I. Due to the intercalation of C18AO fluorescence intensity and life-time measured on the DNA subphase are larger and longer than those on the CMC (sodium carboxymethylcellulose) subphase, which is used as a control polymer without nucleobase, respectively. Strong fluorescence quenching was, however, found when polyG•polyC was dissolved in a water subphase. Static quenching of acridine fluorescence, probably due to the electron transfer from guanine base, is occurred because the fluorescence life-time is slightly shorter than that of the CMC subphase.

The photoinduced electron transfer to an ITO electrode was measured by an electrochemical method. The binary

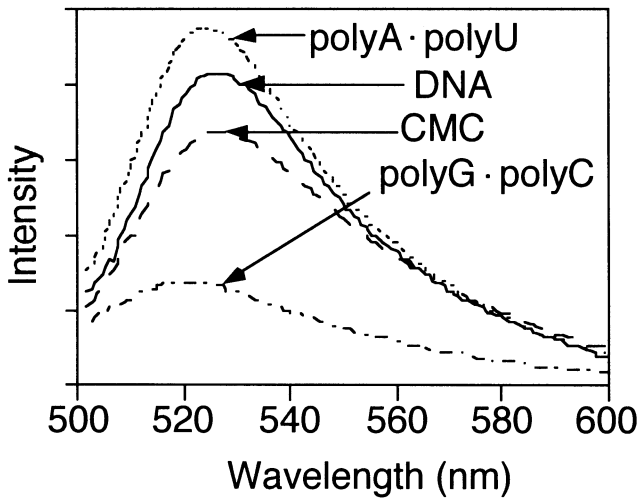


Figure 1. Fluorescence emission spectra of the binary component monolayer of C18AO and $2C_{18}N^+2C_1$ complexed with anionic polymers. excitation at 480 nm.

component monolayers complexed with anionic polymers were deposited on ITO electrodes, and the electrode was soaked in an aqueous 0.1M KCl solution containing EDTA as a sacrificial electron donor. As shown in Figure 2, the

Table I. Relative fluorescence intensity (If) and life time of the polyion complex monolayer deposited on a glass plate

Subphase	If	Life time / ns
PolyA•polyU	1.5	5.2
DNA	1.2	4.4
CMC	1.0	3.4
PolyG•polyC	0.4	2.8

polyion complex monolayer deposited on the ITO electrode generates photocurrent with visible light irradiation (> 450 nm). Anodic photosensitized current of C18AO is enhanced by the complex formation with nucleic acids. Long lived excited state of C18AO intercalated in the stacked base-pairs can transfer its photoexcited electron more effectively than the C18AO complexed with CMC. PolyG•polyC, whose guanine base is assumed to be oxidized by the excited state of C18AO, is the most effective nucleic acid for photocurrent generation (Figure 3). If the one-dimensional stacking of the guanine bases along the polymer backbone can transport a hole (the cation radical of guanine which is generated after the oxidative electron transfer to the excited state of C18AO), the guanine polynucleotide could act as a π-electron medium.

Formation of DNA-Mimetics at the Air-Water Interface Based on Hydrogen Bonding Interaction

Many efforts have been made to prepare artificial base-pairs at the air water interface.[8-10] We have already succeeded in preparing two-dimensional DNA mimetics by using molecular-recognition-directed self-assembly of C18-Cyt and guanine nucleosides based on specific hydrogen bonding at the air-water interface.[11,12] From a fluorescence imaging experiment, we have concluded that our DNA mimetics were composed of stacked cytosine-guanine base-pairs because the amphiphilic intercalator, C18-AO, was able to be incorporated into the crystalline domains of the nucleobase monolayer.

We report herein another type of artificial DNA-mimetic based on the mixed monolayer of octadecyladenine and octadecylthymine prepared at the air-water interface. Figure 4 shows the π-A isotherms of C18-Ade and C18-Thy on pure water subphase. Two amphiphiles were premixed at various molar ratio in $CHCl_3$ solution. In the case of single component monolayers of C18-Ade and C18-Thy, the π-A isotherms indicate formation of expanded monolayers, respectively. On the other hand, the isotherms indicate formation of stable and condensed monolayer by mixing of the two. A phase diagram of molecular area and surface pressure shows that the 1:1 mixture gives the minimum area. From the isotherm the molecular area of the 1:1 mixture can be estimated to be 0.35 nm^2/molecule. This value is consistent with the averaged edge area of purine and pyrimidine rings, which is calculated from CPK molecular modeling. Thickness of the mixed monolayer estimated by X-ray diffraction experiment (2.73 nm) is consistent with the schematic model of the base-pairing in Figure 4.

To reveal the interaction between C18-Ade and C18-Thy, we measured IR-RAS (reflection absorption spectroscopy) spectrum of 11 layers of C18-Ade and C18-Thy transferred onto a gold substrate by Langmuir-Blodgett technique under the constant surface pressure, 40 mN/m. Due to interaction in the LB film, the frequency of C=O stretching vibrations of thymine shows lower shift (1698 cm^{-1} in KBr and 1685cm^{-1} in the mixed LB film, respectively), and streching bands of three

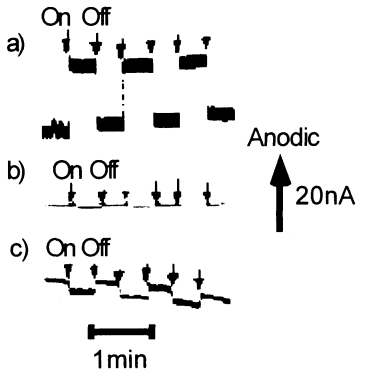


Figure 2. Photocurrent generation of polyion complex of the binary component monolayer and DNA. at (a) 1.5 V (b) 0 V (c) -0.3 V vs. SCE.

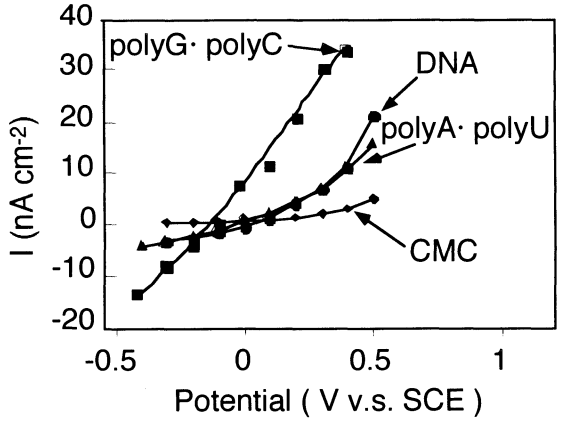
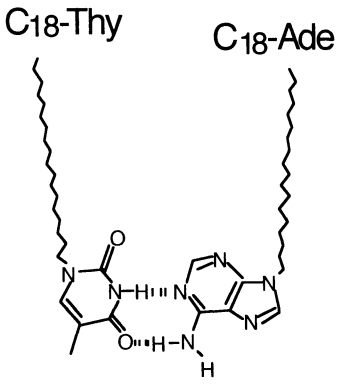


Figure 3. I-V curve of the photocurrent generation of the polyion complex LB monolayer deposited on an ITO electrode.



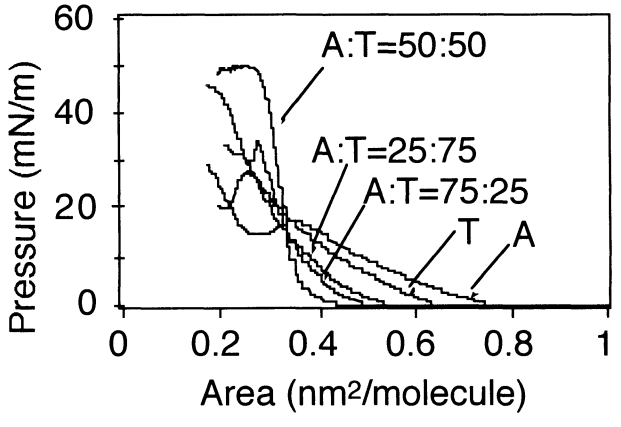


Figure 4. Pressure-area isotherms of mixed monolayers of C18-Thy and C18-Ade on pure water subphase.

amino groups, $\nu_{N\text{-}H}$, split to four bands (3387, 3333, 3257 and 3221cm^{-1}). These spectral changes indicate that the interaction between C18-Ade and C18-Thy is attributed to the Watson-Crick type hydrogen bond formation.

Template Polymerization of Nucleobase Monolayers Based on Molecular Recognition

As DNA is regarded to be a molecular weight- and sequence-regulated polymer, it should be possible to devise a method for selective polymerization of nucleobase monolayers using DNA as a template. For example, polymerizable nucleo base monomers could be spread out into a monolayer film on the water subphase, arranged on a specific sequence by forming Watson-Crick type base-pairs with a DNA template, and finally polymerized. However, this idea has a fundamental flaw. That is, as soon as four types of monomer are dissolved together in a solvent, they form base-pairs with each other, and do not recognize the oligonucleotide template. In order to solve this problem, we focused our attention on the A-T-T and G-C-C base-trimer in triple helix DNA. These trimers are formed by Hoogsteen type hydrogen bonding as well as the Watson-Crick type.

Figure 5 shows π-A isotherms of the Watson-Crick type DNA mimetic monolayer of C18-Ade and C18-Thy on various polynucleotide subphases. It clearly indicates that only single stranded polyU, which is known to form A-T-U(T) base-triplex by Hoogsteen type hydrogen bond, can change the isotherm.

Amphiphilic nucleobase derivatives having a diacetylene moiety, C12DAC9Ade and C12DAC9Thy are newly synthesized to achieve template polymerization based on molecular recognition of single-stranded nucleic acids. Diacetylenes can be photopolymerized topotactically in monolayers, thus the ordered structure of the two-dimensional assembly can be preserved. Pressure-area isotherm of a 1:1 mixture of the two amphiphiles indicates that Watson-Crick type complementary base-pair is formed at the air-water interface. UV light irradiation was carried out on water surface to polymerize the diacetylene moiety in the monolayer. UV-Vis absorption and fluorescence spectra clearly showed that the diacetylene moiety was converted in a blue formed polymer in the initial stage of polymerization and then transformed to a red formed polymer (Figure 6). Fluorescence imaging by polarized fluorescence microscopy clearly shows that the polymerized monolayer of C12DAC9Thy forms a dendritic single crystalline monolayer.

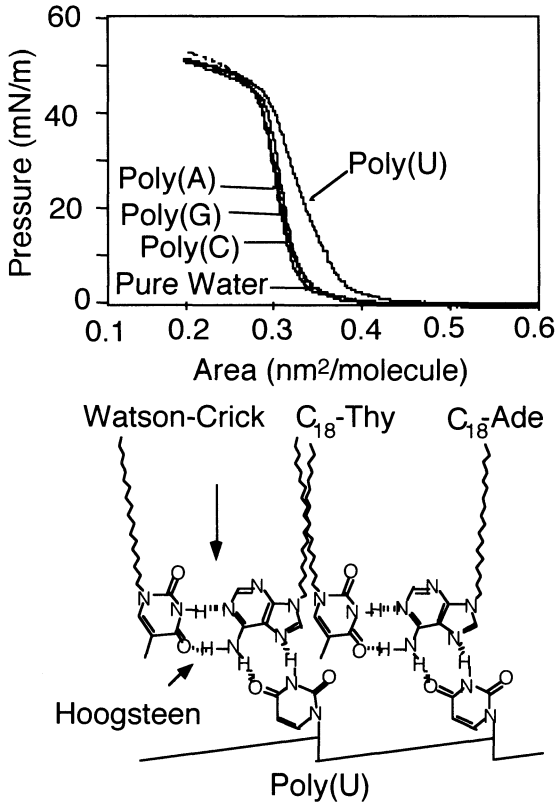


Figure 5. Pressure-area isotherms of equimolar complex of C18-Ade and C18-Thy on polynucleotide subphases and schematic illustration of triplex formation in the DNA mimetics at the air-water interface.

Mesoscopic Pattern Formation of Polymers

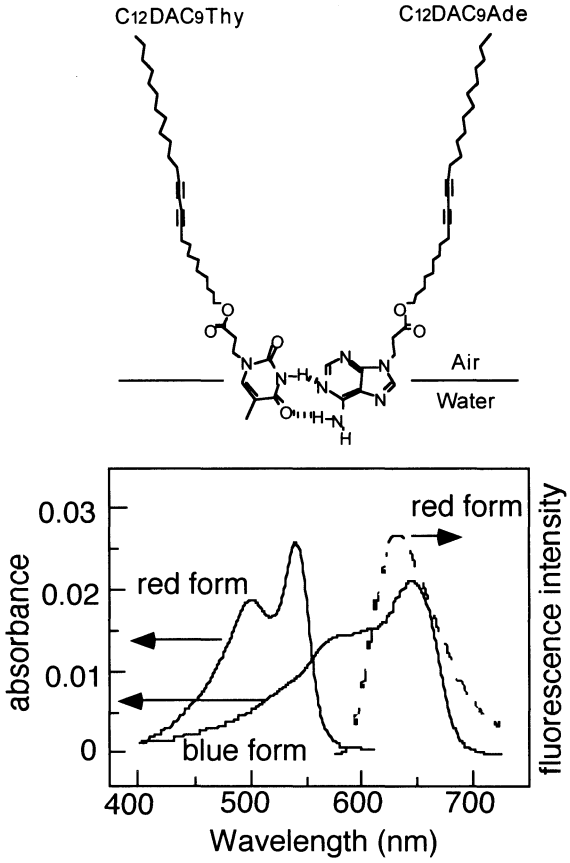


Figure 6. Schematic illustration of Watson-Crick base-pairing of the diacetylene amphiphiles at the air-water interface and spectral evidence of photopolymerization of C12DAC9Thy monolayer.

Patterning is an essential technique for the device fabrication. Great interests are growing in mesoscopic pattern formation based on self-assembling natures of block copolymers, organothiol derivatives and semiconductor materials for micro phase separation, mesoscopic surface-patterning (micro contact printing) and quantum-device fabrication, respectively, with or without lithographic procedures. On the other hand, spatio-temporal patterns are generated as dissipative structures under the chemical or physical conditions far from equilibrium. Several types of regular patterns; spirals in the Belousov-Zhabotinsky reaction systems, honeycomb and strips of Rayleigh-Bénard convection, are formed in dissipative processes[13] with various spatial scales from mesoscopic scale of submicrons to

macroscopic scale toward kilometer size. Here we report a new general method of the mesoscopic pattern formation of polymer assemblies based on freezing spatio-temporal structures generated in an evaporation process of polymer solutions.[14]

Due to the evaporation of the solvent, many physical properties, e.g. viscosity, temperature, surface tension, etc. of polymer solutions dynamically change during casting process on solid substrates. The casting process is complex enough to form dissipative structures. If the dissipative structures are formed in the casting solution, some regular patterns of polymer assemblies can be fixed on the solid surface after rapid solvent evaporation. Pattern formation process was traced by *in situ* microscopic observation of a chloroform solution of polymer. A small amount of a fluorescence probe, octadecylrhodamine B, was added. As shown in Figure 7, typical dissipative structures are found in the casting process of the polymer solution. The highly diluted chloroform solution of complex of dihexadecyldimethyl ammonium and polystyrene sulfonate was placed on hydrophilic substrates (mica, glass, silicon wafer, etc.) and allowed to air-dry under dry atmosphere. Several numbers of circular domains of Bénard-type convection cells are formed in the central part of the solution. Radial convectional flows from the center to the solution front are observed, too. Fingering instability is clearly found at the three-phase-line (the solution front) and the fingers seem to be connected to the central Bénard cells via the radial convectional streams. The fingering instability can be ascribable to Marangoni effect based on the surface tension gradients at the three-phase-line.

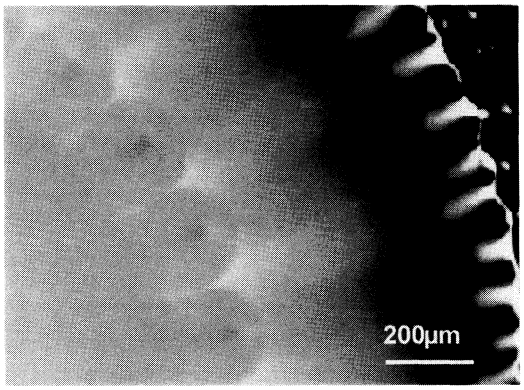


Figure 7. Bénard cells and fingering instability formed in casting polymer solution.

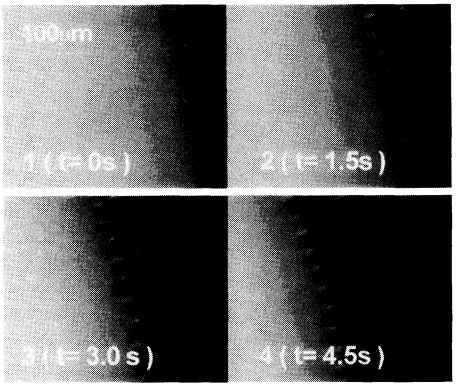


Figure 8. Stripe formation from the fingering instability generated at the solution front.

Owing to evaporation of the solvent, the three-phase-line is starting to recede toward the solution center. When the polymer solution is cast on a smooth surface, e.g. mica, the finger can grow as a line parallel to the receding direction of the three-phase-line. Figure 8 shows snap shots of stripe formation from the periodically generated fingers. The dimension of the line is several hundred micrometers in length, a few hundred nanometers in width, and several nanometers in height. When dewetting of polymers occurs in each line, the line ruptures to small polymer droplets. If the polymer dewetting synchronizes with the strip formation, orderly arranged droplets of polymer aggregates (polystyrene, dendrimer, etc.) are formed as the receding traces.[15,16]

Immobilization of Single DNA Molecule Based on Dissipative Structure

By using the simple casting method we have prepared mesoscopic stripe pattern of DNA-amphiphile complex on a mica substrate cast from chloroform solutions.[17] Complexation with amphiphilic counter ions is indispensable to solubilization of DNA in chloroform. Here we show other methods of DNA patterning from water solution without using amphiphilic counterions. DNA patterns were prepared from water solutions by following two methods. (1) An aqueous DNA (from calf thymus) solution mixed with an aqueous $CaCl_2$ solution was cast onto a freshly cleaved mica surface. The casting solutions were dried in air at room temperature.

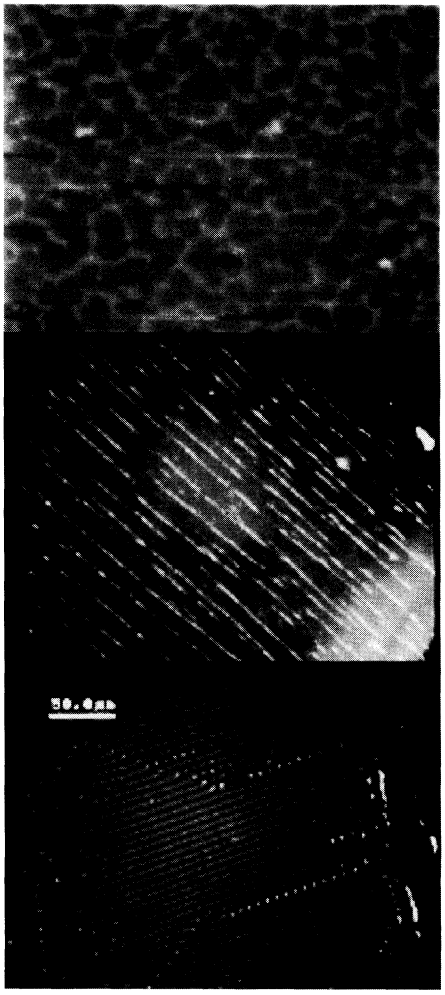


Figure 9. Mesoscopic pattern of DNA assemblies, a (upper); with Ca^{2+} ion prepared by method (1), b.(center); cast at high temperature, c.(lower); cast with alginic acid. (scale bar 50μm)

(2) An aqueous DNA solution (containing a fluorescence probe, acridine orange) mixed with/without an aqueous alginic acid solution was dropped onto a fleshly cleaved mica surface and dried by heating up to ca. 80°C. An AFM imaging of DNA-Ca^{2+} complex film prepared by the method (1) in propanol shows formation of network structures of DNA (Figure 9a). Height of the network is about 2-3 nm and almost comparable to the diameter of DNA. Figure 9b shows a fluorescence image of DNA film without alginic acid prepared by the method (2). The mesoscopic lines parallel to the receding direction of the solvent were observed in the cast film. When alginic acid was added to the aqueous DNA solution, more regularly aligned stripe patterns were obtained (Figure 9c). Height of those lines was below 10 nm. Orientation of the DNA molecules in the line was supported by fluorescence measurement with a scanning near-field optical microscope.

CONCLUDING REMARKS

In this article we show how we can organize DNA and DNA-mimetics as novel functional materials both in the nanoscopic and the mesoscopic scales. Conductivity measurements of cast oriented DNA film[18] and patterned mesoscopic structures, and single molecule detection of DNA molecule embedded in the mesoscopic polymer pattern are now in progress.

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