Molecular Patterning of a Guanidinium/Orotate Mixed Monolayer through Molecular Recognition with Flavin Adenine Dinucleotide

ARTICLE *in* LANGMUIR · FEBRUARY 1997

Impact Factor: 4.46 · DOI: 10.1021/la960112x

Molecular Patterning of a Guanidinium/Orotate Mixed Monolayer through Molecular Recognition with Flavin Adenine Dinucleotide

Yushi Oishi, Yuzuru Torii, Takayuki Kato, Miyuki Kuramori, Kazuaki Suehiro, Katsuhiko Ariga,† Kazuhiro Taguchi,† Ayumi Kamino,† Hiroshi Koyano,† and Toyoki Kunitake*,†

Department of Applied Chemistry, Faculty of Science and Engineering, Saga University, 1 Honjo-machi, Saga 840, Japan, and Supermolecules Project, JST (former JRDC), Kurume Research Park, 2432 Aikawa-cho, Kurume, Fukuoka 839, Japan

Received February 6, 1996. In Final Form: September 12, 1996[⊗]

Flavin adenine dinucleotide (FAD) undergoes multisite molecular recognition at the air-water interface with mixed monolayers that are capable of complementary hydrogen bonding. Molecular images were obtained with an atomic force microscope (AFM) for mixed monolayers of guanidinium (**G**) and orotate (**O**) amphiphiles transferred from pure water and from aqueous FAD. The AFM image of the **G/O** mixed monolayer on FAD showed a periodic oblique pattern composed of two kinds of methyl peaks with different heights, whereas that on pure water showed a periodic hexagonal pattern of only one kind of methyl peak. The two-dimensional molecular patterning as height difference is conceivably caused by rearrangement of monolayer components based on specific recognition by the FAD template molecule.

Introduction

Designed formation of nanometer-scale molecular patterns should provide exciting possibilities in fundamental supramolecular chemistry and in molecular scale electronic devices.[1] Several techniques that have been recently developed for two-dimensional patterning such as photolithography,[2] laser manipulation,[3] and cantilever techniques[4,5] do not supply a molecular resolution. This is because the patterning size is limited by the wavelength of light and the radius of curvature of the tip on a cantilever. In contrast, self assembly of molecules may be utilized for pattern formation with molecular precision, if the assembly process is properly controlled.

It has been demonstrated in previous studies that molecular monolayers can bind polar guest molecules dissolved in the aqueous subphase through complementary hydrogen bonding.[6–8] These results were extended to multisite molecular recognition, in which a guest molecule was specifically bound at two functional sites to two monolayer components.[9] The subsequent study implied that flavin adenine dinucleotide (FAD) was bound simultaneously to three kinds of monolayer components at three recognition sites.[10] This result suggests that we can produce molecular patterns from monolayer components by using multisite guest molecules.

An atomic force microscope (AFM) allows us to directly characterize the surface structure of organic materials in molecular precision. It has been shown that an AFM is capable of observing the molecular arrangement and structural defects in organic monolayers.[11–19] Hence, verification of the molecular patterning in the monolayer may be attained by using an AFM.

In this paper, as a first step for molecular patterning through molecular recognition, we employed two-component monolayers of a guanidinium amphiphile and an orotate amphiphile against complementarily functionalized nucleotide derivatives. The required molecular resolution was achieved by AFM observation of transferred films.

Experimental Section

1. Materials. Melting points were recorded on a Yanaco micro melting point apparatus and uncorrected. Chemical shifts of ^{1}H NMR spectra were recorded on a Bruker ARX-300 (300 MHz) spectrometer and reported relative to chloroform (δ 7.26) or tetramethylsilane (δ 0.00). Elemental analyses (C, H, and N) were performed at Faculty of Science, Kyushu University.

1,3-O-Dioctadecylglycerol. In a 500-mL three-necked round-bottomed flask equipped with a mechanical stirrer and a dropping funnel, octadecyl alcohol (200 g, 0.74 mol) and NaH (50% oil dispersion, 2.0 g, 0.083 mol) were placed and kept at 343 K. Epichlorohydrin (7.0 g, 0.075 mol) was added to this mixture from a dropping funnel over 30 min. The resulting mixture was stirred at the same temperature for 5 h. After the mixture was cooled to room temperature, volatile material was removed under vacuum. Hot ethanol (500 mL) was added to the residue, and the insoluble material was removed by filtration. The filtrate

* Author to whom correspondence should be addressed.
† Supermolecules Project, JST, Kurume Research Park.
⊗ Abstract published in *Advance ACS Abstracts,* January 15, 1997.

(1) Carter, F. L. *Molecular Electronic Devices*; Marcel Dekker Inc.: New York, 1982.
(2) López, G. P.; Biebuyck, H. A.; Härter, R.; Kumar, A.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 10774.
(3) Sasaki, K.; Koshioka, M.; Misawa, H.; Kitamura, N.; Masuhara, H. *Jpn. J. Appl. Phys.* **1991**, *30*, L907.
(4) Day, H. C.; Allee, D. R.; George, R.; Burrows, V. A. *Appl. Phys. Lett.* **1993**, *62*, 1629.
(5) Garnaes, J.; Bjørnholm, T.; Zasadzinski, J. A. N. *J. Vac. Sci. Technol.* **1994**, *B12*, 1839.
(6) Kurihara, K.; Ohto, K.; Honda, Y.; Kunitake, T. *J. Am. Chem. Soc.* **1991**, *113*, 5078.
(7) Kawahara, T.; Kurihara, K.; Kunitake, T. *Chem. Lett.* **1992**, 1839.
(8) Sasaki, D. Y.; Kurihara, K.; Kunitake, T. *J. Am. Chem. Soc.* **1991**, *113*, 9685.
(9) Sasaki, D. Y.; Kurihara, K.; Kunitake, T. *J. Am. Chem. Soc.* **1992**, *114*, 10994.
(10) Taguchi, K.; Ariga, K.; Kunitake, T. *Chem. Lett.* **1995**, 701.
(11) Marti, O.; Ribi, H. O.; Drake, B.; Albrecht, T. R.; Quate, C. F.; Hansma, P. K. *Science* **1988**, *239*, 50.
(12) Hansma, H. G.; Gould, S. A. C.; Hansma, P. K.; Gaub, H. E.; Longo, M. L.; Zasadzinski, J. A. N. *Langmuir* **1991**, *7*, 1051.
(13) Radmacher, M.; Tillmann, R. W.; Fritz, M.; Gaub, H. *Science* **1992**, *257*, 1900.
(14) Josefowicz, J. Y.; Maliszewakyj, N. C.; Idziak, S. H. J.; Heiney, P. A.; McCakey, J. P., Jr.; Smith, A. B., III. *Science* **1993**, *260*, 323.
(15) Viswanathan, R.; Zasadzinski, J. A.; Schwartz, D. K. *Science* **1993**, *261*, 449.
(16) Schwartz, D. K.; Viswanathan, R.; Garnaes, J.; Zasadzinski, J. A. N. *J. Am. Chem. Soc.* **1993**, *115*, 7374.
(17) Oishi, Y.; Hirose, F.; Kuri, T.; Kajiyama, T. *J. Vac. Sci. Technol.* **1994**, *A12*, 2971.
(18) Kajiyama, T.; Oishi, Y.; Hirose, F.; Shuto, K.; Kuri, T. *Langmuir* **1994**, *10*, 1297.
(19) Kajiyama, T.; Oishi, Y.; Suehiro, K.; Hirose, F.; Kuri, T. *Chem. Lett.* **1995**, 241.

S0743-7463(96)00112-6 CCC: $14.00

was treated with charcoal and allowed to stand at room temperature to give a colorless solid. This was collected and recrystallized from acetone (500 mL) to give 1,3-*O*-dioctadecylglycerol (30.0 g, 67%) as a colorless solid. Mp, 339.8–341.2 K; TLC R_f 0.90 (ether); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 6, J = 6.7 Hz, 2 CH_3), 1.2–1.4 (m, 60, 30 CH_2), 1.5–1.7 (m, 4, 2 OCH_2CH_2), 2.47 (d, 1, J = 4.1 Hz, OH), 3.4–3.6 (m, 8, 4 OCH_2), 3.9–4.0 (m, 1, OCH). Anal. Calcd for $C_{39}H_{80}O_3$: C, 78.52; H, 13.42. Found: C, 78.48; H, 13.56.

1′,3′-Bis(octadecyloxy)isopropyl orotate (O). Orotic acid monohydrate (16.6 g, 95.3 mmol) was suspended in benzene (350 mL) and refluxed for 16 h with a Dean–Stark water separator. After the mixture was cooled to room temperature, DMF (0.2 mL) was added. To this suspension thionyl chloride (25 mL, 343 mmol) was added slowly from a dropping funnel over 30 min. The resulting yellow suspension was refluxed for 16 h, filtered, and washed with benzene and dried in vacuo to give a yellow powder (16.1 g). A portion of this powder (200 mg) was suspended in THF (30 mL), and 1,3-*O*-dioctadecylglycerol (680 mg, 1.14 mmol) and NaH (55–60% oil dispersion, 60 mg) were added, and the whole mixture was refluxed for 16 h. After the mixture was cooled to room temperature, the insoluble material was removed by filtration and concentrated. The residue was chromatographed on SiO_2 (20 g) using CH_2Cl_2 and then 9:1 CH_2Cl_2/CH_3OH as eluents to give a solid, which was recrystallized twice from benzene to give **O** (360 mg, 41% based on orotic acid monohydrate) as a colorless powder. Mp, 375.4–376.4 K; TLC R_f 0.36 (20:1 CH_2Cl_2/CH_3OH); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 6, J = 5.0 Hz, 2 CH_3), 1.24 (m, 60, 30 CH_2), 1.53–1.59 (m, 4, 2 CH_2CH_2O), 3.43 (m, 4, 2 OCH_2CH_2), 3.62 (d, 4, J = 4.0 Hz, 2 OCH_2CH), 5.33 (tt, 1, J = 3.7 Hz, OCH), 6.41 (s, 1, vinyl), 8.43 (br s, 1, 1-NH), 8.56 (br s, 1, 3-NH). Anal. Calcd for $C_{44}H_{82}O_6$: C, 71.89; H, 11.24; N, 3.81. Found: C, 71.89; H, 11.26; N, 3.67.

1-Octadecylguanidinium p-Toluenesulfonate (G). Methylisothiourea sulfate (1.63 g, 11.8 mmol) was dispersed in methanol (30 mL), and sodium (0.311 g, 10.5 mmol) dissolved in methanol (30 mL) was added slowly at 273 K, and the mixture was stirred for 1 h at 273 K with a $CaCl_2$ tube. Octadecylamine (2.65 g, 9.83 mmol) in methanol (20 mL) was added slowly to the dispersion at room temperature. The reaction mixture was stirred for 2 h at room temperature and for 46 h at 313 K. *p*-Toluenesulfonic acid monohydrate (3.96 g, 20.8 mmol) was added to the solution. The solution was heated to 333–343 K, and the precipitate was filtered. Colorless crystals appeared when the filtrate was kept in a freezer. Recrystallization of the crystals from acetonitrile (100 mL) gave **G** as fine colorless crystals (0.904 g, 19%). Mp, 391–392 K; ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3, J = 6.6 Hz, CH_3), 1.24 (m, 30, 15 CH_2), 1.44 (m, 2, CH_2CH_2N), 2.29 (s, 3, $ArCH_3$), 3.07 (m, 2, NCH_2), 7.11 (d, 2, J = 8.0 Hz, Ar), 7.47 (d, 2, J = 8.0 Hz, Ar), 6.80 and 7.37 (br and m, respectively, 5, guanidinium). Anal. Calcd for $C_{26}H_{49}N_3O_3S$: C, 64.55; H, 10.21; N, 8.69. Found: C, 64.53; H, 10.19; N, 8.52.

2. Surface Pressure–Area (π–A) Isotherms. Water used as the subphase was deionized and doubly distilled by a Nanopore II-4P and Glass Still D44 System (Barnstead). Spectroanalytical grade benzene (Wako Pure Chemicals) was used as spreading solvent. When lipids were not satisfactorily soluble in benzene, small amounts of spectroanalytical grade ethanol (Wako Pure Chemicals) were added. π–A isotherms were measured with a computer-controlled film balance system FSD-50 (USI System, Fukuoka). The sample solution was spread on water with a surface area of 350.8 × 100.0 mm^2. The molecular area before compression was ca. 1.2–1.5 nm^2 molecule^{-1}. The mean molecular area was estimated by dividing the total monolayer area by the number of all the lipid molecules. The compression rate was 0.2 mm s^{-1} (or 20 mm^2 s^{-1} based on surface area), and the subphase temperature was maintained at 293.2 ± 0.2 K. Surface pressures were measured by a Wilhelmy plate which was calibrated with the transition pressure of an octadecanoic acid monolayer.

3. π–A Isotherm Analysis of Mixed Monolayer. In order to make a quantitative analysis, all the data of π–A isotherms were normalized as follows. When component a and component b were mixed, the theoretical molecular area (A_{ideal}) is given by eq 1,

$$A_{ideal} = A_a x + A_b(1 - x) \quad (1)$$

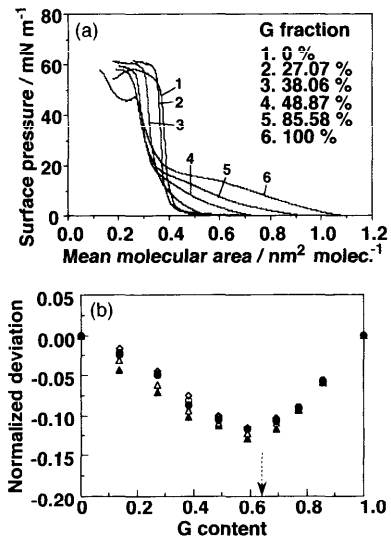


Figure 1. (a) π–A isotherms of the **G/O** mixed monolayers at 293 K on 0.01 mM aqueous FAD. **G** fraction: a, 0%; b, 27.07%; c, 38.06%; d, 48.87%; e, 85.58%; f, 100%. (b) Plot of normalized deviation vs **G** content (see eq 2): ▲, 20 mN m^{-1}; △, 25 mN m^{-1}; ●, 30 mN m^{-1}; ○, 35 mN m^{-1}; ◇, 40 mN m^{-1}. The statistical error for the normalized deviation was less than 0.01.

where A_a, A_b, and x are the molecular area of component a, the molecular area of component b, and the mole fraction of component a, respectively. A_{ideal} represents the mean molecular area of the mixed monolayer without any intercomponent interaction. Deviation of the observed molecular area (A_{obsd}) from the theoretical value (A_{ideal}) can be normalized as follows.

$$(A_{obsd} - A_{ideal})/A_{ideal} \quad (2)$$

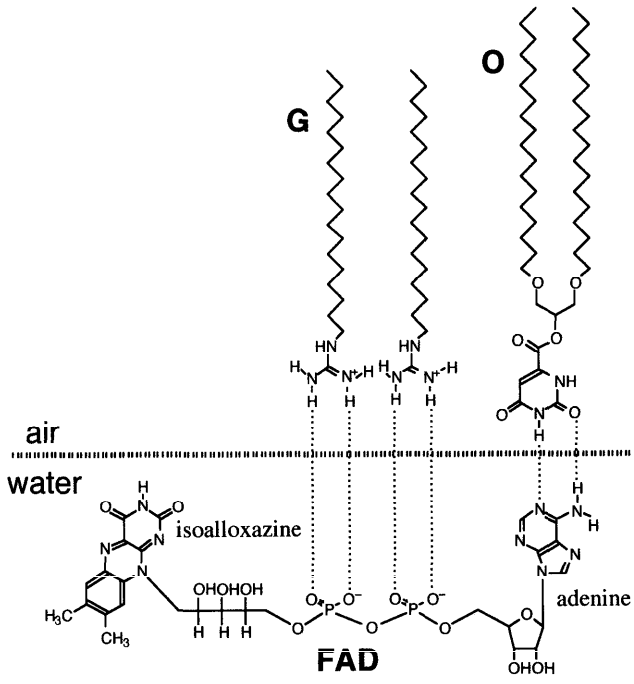
This calculation was carried out at constant surface pressures in the condensed phase.

4. Monolayer Preparation. The guanidinium unit is known to interact with phosphates through hydrogen bonding and electrostatic interaction. The orotate unit would form complementary hydrogen bonds with the adenine unit.[9] The π–A isotherm measurements of the **G/O** mixed monolayer revealed that the 2:1 molar ratio was suitable for the complexation on aqueous FAD, as is demonstrated by the maximum deviation in Figure 1. However, the mixed monolayer became mechanically unstable when the **G** content was increased. Therefore, we spread an equimolar **G/O** mixture in benzene/ethanol (4:1 in volume) with the combined concentration of 1 × 10^{-3} M on pure water and on 0.01 mM aqueous FAD at a subphase temperature of 293 K. The monolayer was compressed up to a surface pressure of 25 mN m^{-1} and was maintained as such for 1 h, in order to prepare mechanically stable monolayers sufficient for obtaining a molecular-resolution AFM image.[17,18] Each monolayer was transferred by the horizontal drawing-up method[20] onto a freshly cleaved mica (Okabe Mica, Fukuoka) and on a SiO substrate that was prepared by vapor-deposition of SiO onto a Formvar thin film, for AFM and TEM observations, respectively. The transfer ratio for each monolayer was unity, indicating that mica and SiO substrates were completely covered with each monolayer.

5. Atomic Force Microscopy. AFM images of the monolayers were obtained with a SFA300 (Seiko Instruments) in air at 293 K. A 0.8 mm × 0.8 mm scan head and a silicon nitride tip on a cantilever with a spring constant of 0.022 N m^{-1} were used. Images were recorded in the "constant-height" mode, that is, feedback electronics and the corresponding software were used to keep the sample height and to measure the cantilever deflection. The AFM stage with the monolayer sample was allowed to equilibrate for a few hours to eliminate any thermal gradients that cause thermal drift in the image. The influence of mechanical drift was minimized by using a fast scan rate of 10 μm s^{-1}. Mica was used for nanometer calibration of the

(20) Oishi, Y.; Kuri, T.; Takashima, Y.; Kajiyama, T. *Chem. Lett.* **1994**, 1445.

Chart 1. Scheme of Multisite Molecular Recognition between FAD and a G/O Mixed Monolayer at the Air–Water Interface (G, 1-Octadecylguanidinium *p*-Toluenesulfonate; O, 1′,3′-Bis(octadecyloxy)isopropyl Orotate; FAD, Flavin Adenine Dinucleotide).



piezoscanner. The applied force during the scanning was about 10^{-10} N in an attractive force region. In order to reduce the noise component in raw AFM images, only low-pass filter treatment was carried out.

6. Transmission Electron Microscopy. Electron diffraction (ED) patterns were taken with a Hitachi H-7000 electron microscope at 293 K, which was operated at an acceleration voltage of 75 kV and a beam current of 0.5 μA. The electron beam was 10 μm in diameter.

Results and Discussion

Figure 1a shows π–A isotherms of mixed monolayers of **G** and **O** with various mixing ratios on 0.01 mM aqueous FAD. The FAD molecule tends to expand the **G/O** mixed monolayer compared to those on pure water, indicating binding of FAD onto the mixed monolayer.[10]

In order to elucidate the binding stoichiometry of **G** and **O** in the presence of FAD, normalized deviation of the averaged molecular area from the ideal mixture (eq 2) was plotted as a function of the **G** content (Figure 1b). Similar analyses have been reported on complexation between phospholipids and cholesterol monolayers.[21–23] The mixing ratio at the maximum deviation might reflect the stoichiometry of the complex. Figure 1b clearly shows the maximum deviation at about 60% **G**. This suggests that **G** and **O** form a 2:1 complex in the presence of FAD. In order to confirm the complex stoichiometry, the ratio of N over P in the transferred film was evaluated by X-ray photoelectron spectroscopy (XPS) measurements. The XPS measurements of the **G/O** (2:1) film transferred from 0.01 mM aqueous FAD onto Au-coated glass indicated that one FAD molecule is formed to one unit of **G/O** (2:1).[24] Therefore, the XPS result supports the proposed binding stoichiometry between **G**, **O**, and FAD.

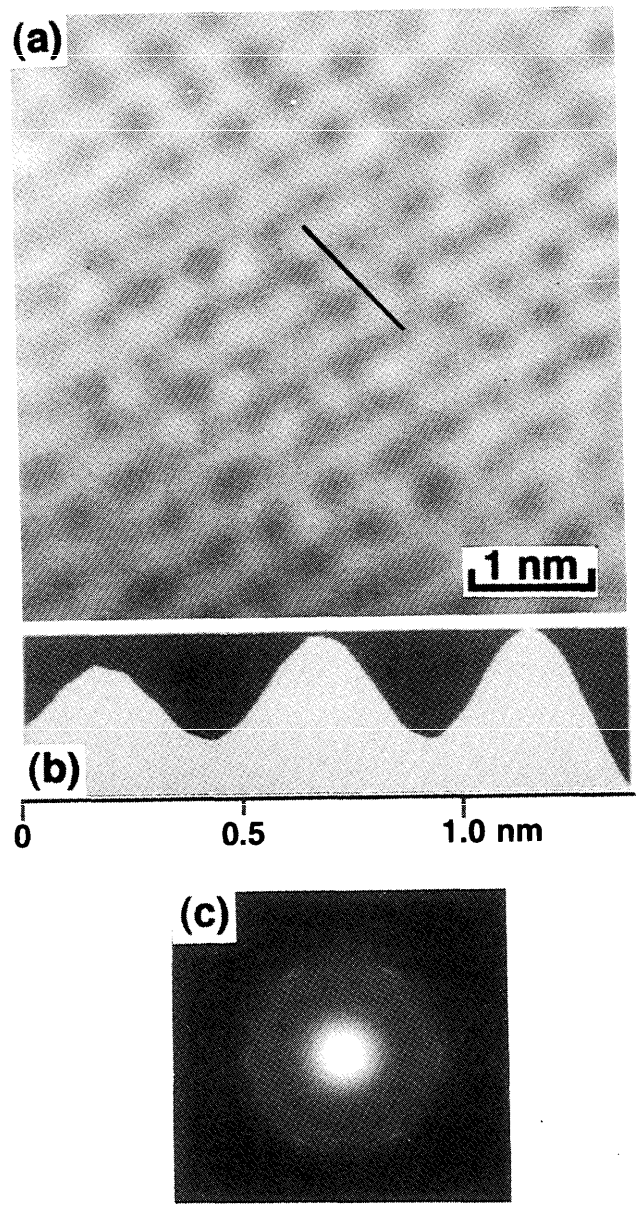


Figure 2. AFM image (5 × 5 nm^2) of the monolayer prepared from an equimolar mixture of **G** and **O** on the pure water subphase (a), the contour (b), and the ED pattern (c).

A plausible interaction model between FAD and the mixed monolayer that is consistent with the experimental data is illustrated in Chart 1. The guanidinium unit interacts with the phosphate group in FAD through hydrogen bonding and electrostatic interaction. The orotate unit forms complementary hydrogen bonds with the adenine group in FAD. Unfortunately, the **G/O** mixed monolayer with high **G** contents was mechanically unstable, and the decrease in surface area became greater with increasing **G** contents on pure water, because of monolayer collapse and/or dissolution into the subphase at a constant surface pressure. Thus, we prepared mechanically stable monolayers containing equimolar **G** and **O** for AFM observation.[25]

Figure 2 shows a filtered AFM image of an equimolar **G/O** monolayer on pure water with a scan area of 5 × 5

(21) (a) Demel, R. A.; De Kruyff, B. *Biochim. Biophys. Acta* **1976**, *457*, 109. (b) Joos, P.; Demel, R. A. *Biochim. Biophys. Acta* **1969**, *183*, 447.
(22) Pethica, B. A. *Trans Faraday Soc.* **1955**, *51*, 1402.
(23) Kamino, A.; Ariga, K.; Kunitake, T.; Birault, V.; Pozzi, G.; Nakatani, Y.; Ourisson, G. *Colloids Surf.* **1995**, *103*, 183.
(24) Taguchi, K.; Kamino, A.; Koyano, H.; Ariga, K.; Kunitake, T. Manuscript in preparation.
(25) XPS and FT-IR data suggest that **G** and **O** molecules form a stable 1:1 interlipid complex on pure water.

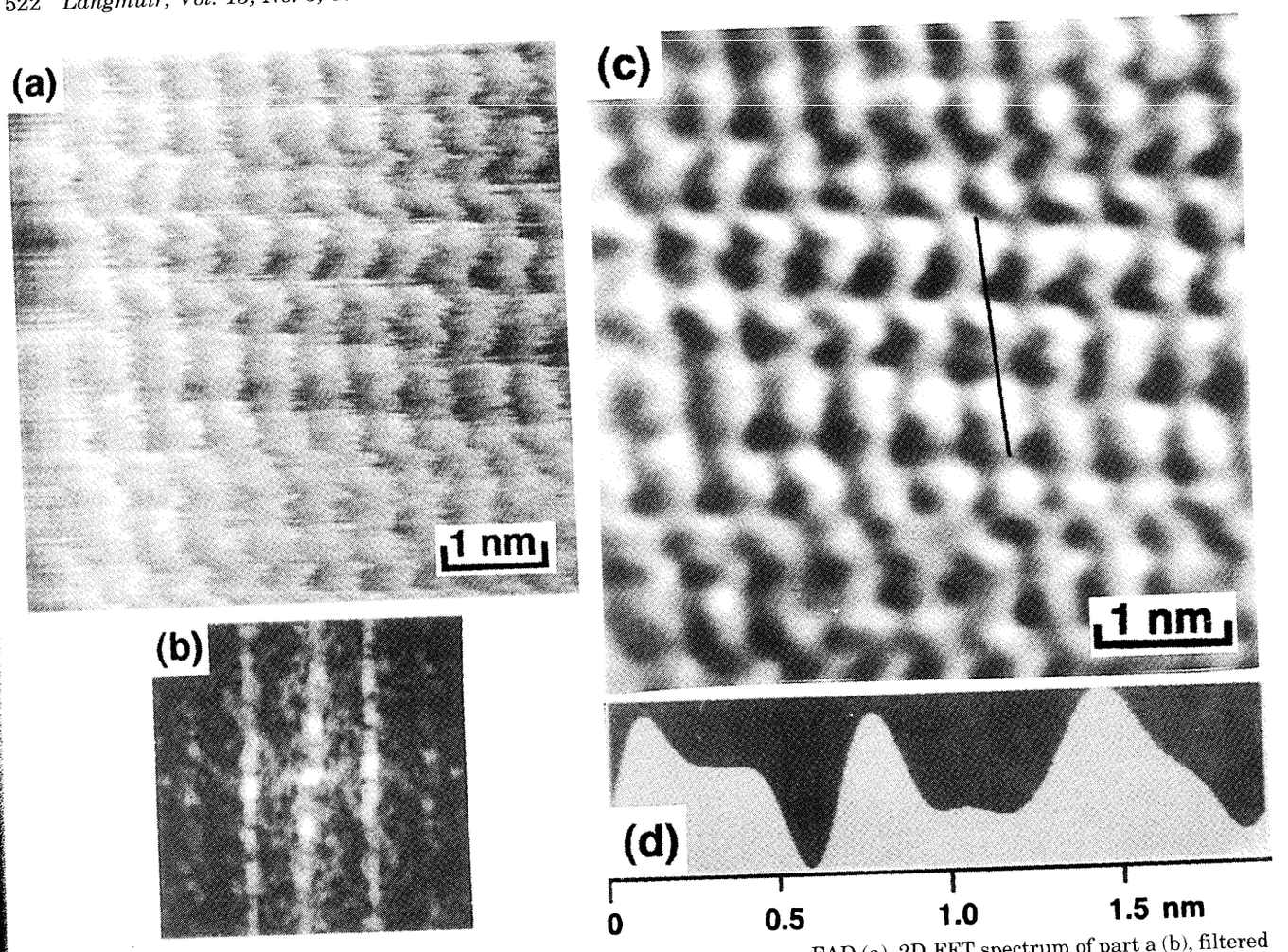


Figure 3. Raw AFM image (5 × 5 nm^2) of the **G/O** mixed monolayer on aqueous FAD (a), 2D-FFT spectrum of part a (b), filtered AFM image of part a (c), and the contour (d).

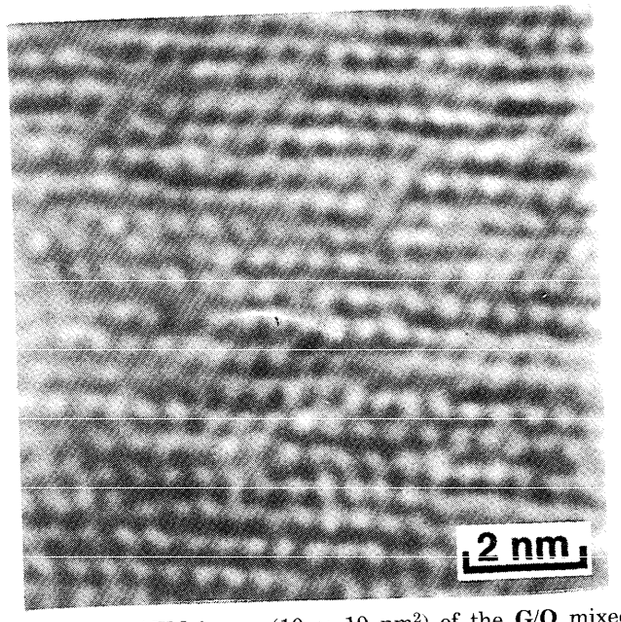


Figure 4. AFM image (10 × 10 nm^2) of the **G/O** mixed monolayers on aqueous FAD.

nm^2 (a), a topographical contour plot taken along the black line overlaying the AFM image (b), and an ED pattern of the monolayer (c). The AFM image is typical and is given in a top-view presentation in which the brighter and darker portions correspond to higher and lower regions of the monolayer surface, respectively. Though scanning was

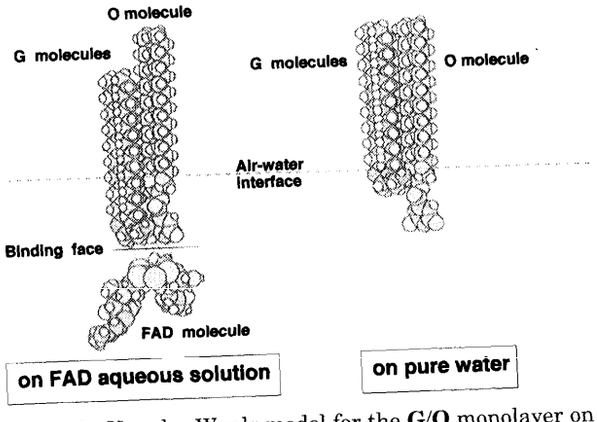


Figure 5. Van der Waals model for the **G/O** monolayer on a pure water subphase (a) and aqueous FAD (b).

done repeatedly, the monolayers were not damaged by the tip. However, a hole could be artificially pierced through the monolayer with an applied force stronger than 10^{-9} N. The hole was about 3 ± 0.03 nm deep and was comparable to the calculated molecular length (**G**, 2.8 nm; **O**, 3.0 nm) based on the extended CPK molecular model. Therefore, the hole depth represents the thickness of the mixed monolayer. It is reasonable to expect that the brighter portion in the AFM image on pure water represents individual methyl terminals of **G** and/or **O** molecules, because the hydrophobic part of the **G** or **O** molecule must be oriented toward air in the monolayer transfer by the horizontal drawing-up method. It is clear

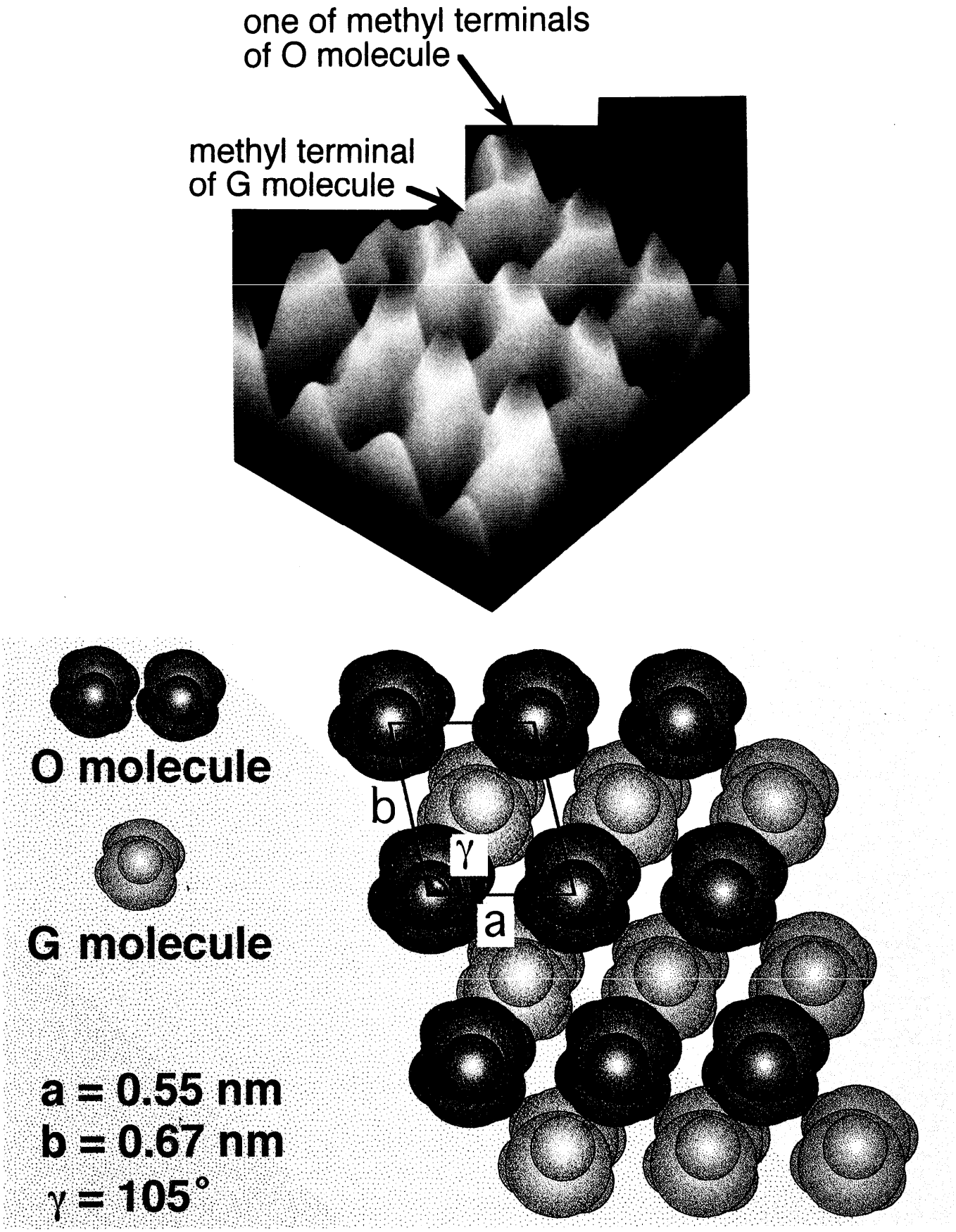


Figure 6. Three-dimensional AFM image of the **G/O** mixed monolayer on aqueous FAD and the corresponding molecular arrangement.

that **G** and/or **O** molecules are regularly arranged in a hexagonal array with the (1 0) spacing[26] of 0.43 ± 0.02 nm. This is consistent with the spacing of 0.43 ± 0.01 nm which was estimated from the ED pattern (Figure 2c) of the monolayer but is different from the 0.46 ± 0.02 nm spacing of the mica substrate. The area occupied by a single chain in the **G/O** mixed monolayer was evaluated from these data as 0.21 ± 0.02 nm^2. This magnitude is comparable to the well-known cross section (0.20−0.21 nm^2 in a hexagonal array) of the monoalkyl chain.[17,18] It is, therefore, reasonable to conclude that the brighter portion in the AFM image represents single methyl groups of the component molecules that are regularly arranged in hexagonal arrays. The contour plot on pure water (Figure 2b) showed a periodic wavelike pattern composed of only one kind of peak. Therefore, the heights of the terminal methyl group of the **G** and **O** components are identical. This result is derived from regular spatial alignment of the same C_{18} chains of the two components above the water surface.

Figure 3 shows a raw AFM image of an equimolar **G/O** monolayer on aqueous FAD with a scan area of 5 × 5 nm^2 (a), the two-dimensional fast Fourier transform (2D-FFT) spectrum of Figure 3a (b), a filtered AFM image of Figure 3a (c), and a contour plot for Figure 3c (d). The brighter portions corresponding to individual methyl terminals of **G** or **O** molecules are regularly arranged in a two-dimensional oblique array with a nearest-neighbor spacing of 0.57 ± 0.02 nm (*a*-axis), a next-nearest-neighbor spacing of 0.66 ± 0.02 nm (*b*-axis), and an interaxis angle γ of 105 ± 3° as illustrated in Figure 6.[27] The contour plot for the **G/O** mixed monolayer on aqueous FAD (Figure 3d) displayed a periodic wavelike structure composed of two different peaks. The height difference between the two peaks is estimated to be several angstroms on the basis of the height relative to the above-mentioned monolayer thickness. However, the oblique arrays with a height difference cannot be found over the entire monolayer surface. An AFM image on aqueous FAD at a wider scan area of 10 × 10 nm^2 (Figure 4) exhibited a dislocation-like molecular arrangement[19] in the horizontal direction. The overall **G/O** composition of the mixed monolayer was set at 1:1 by considering monolayer stability, as described in the Experimental Section. This composition does not agree with the 2:1 stoichiometry that is deduced from structural complementarity and is confirmed by the π−*A* isotherm experiment (Figure 1b). Apparently, the dislocation-like molecular arrangement is derived from the concentration of the extra **O** component. The same dislocation-like molecular arrangement was observed even for mixed monolayers on FAD with lower **G** contents. This indicates that the 2:1 (**G/O**) complex is locally formed irrespective of the overall monolayer composition. Anyway, we cannot deny from the difference in the AFM images of Figures 2 and 3 that the molecular arrangement in the **G/O** mixed monolayer is altered by FAD.

Figure 5 shows packing models for **G** and **O** molecules in the monolayer on pure water and on aqueous FAD that are consistent with preceding results. The longer polar region of the **O** component would then be buried in the pure water subphase deeper than the shorter guanidinium unit. On the other hand, the binding of FAD with the mixed monolayer would dispose two functional units at the same level and produce a height difference between the two terminal methyl groups. The flavin unit in FAD may be buried under the phosphate group owing to a conformational flexibility of the FAD molecule. Hence, the higher and lower peaks in the contour plot (Figure 3d) may be assigned to one of the two methyl terminals of **O** and the methyl terminal of **G**, respectively, and the **O** molecule appears as a brighter portion (higher top).

Figure 6 shows a three-dimensional AFM image of a part of Figure 3c and the corresponding molecular arrangement. The molecular arrangement is composed by using van der Waals radii of 0.20 nm for C, 0.14 nm for O, and 0.12 nm for H, interatomic distances of 0.154 nm for C−C, and 0.109 nm for C−H, and the bond angle of 109.5° for H−C−H. The red methyl terminal is higher than the pink methyl terminals by one methylene length. The highest peaks colored by red and pink in the three-dimensional AFM image should correspond to one of the two methyl terminals of the **O** molecule and one methyl terminal of the **G** molecule, respectively, as discussed above. Thus, a regular arrangement of the former methyl terminals is seen as a molecular pattern in the mixed monolayer.

Conclusion

The AFM results are consistent with the interaction scheme depicted in Chart 1. Thus, two-dimensional molecular patterning has been achieved by rearrangement of monolayer components as directed by specific recognition by an aqueous template molecule. Regularly repeating patterns are readily prepared by this approach. The two-dimensional arrangement of interacting groups in a template molecule is transformed into height patterns of alkyl chains. Therefore, proper molecular design of templates and mixed monolayers would yield a number of novel molecular patterns and the consequent functionalities.

Acknowledgment. The authors thank Prof. T. Kajiyama (Kyushu University) for the use of a transmission electron microscope.

LA960112X

(26) (1 0) represents the two-dimensional lattice plane with Miller indices of $h = 1$ and $k = 0$.

(27) The occupied area of an alkyl chain in the monolayer of **G/O** (2:1) on FAD is calculated to be about 0.18 nm^2 on the basis of the packing model in Figure 6. The occupied area of two **G** molecules and one **O** molecule (corresponding to four alkyl chains) is estimated to be about 0.71 nm^2. Thus, the averaged molecular area in the monolayer is about 0.24 nm^2, which is comparable to that estimated by the π−*A* isotherm (0.28 nm^2).