Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

FORMATION OF DNA SELF-ASSEMBLED MONOLAYER ON A GOLD SUBSTRATE

Yosuke Sakao [a] , Nobuo Ueno [a] , Fumio Nakamura [b c] , Eisuke Ito [c] , Junko Hayasi [c] & Masahiko Hara [c]

[a] Faculty of Engineering, Chiba University, Yayoicho 1-33, Inageku, Chiba 263-0022, Japan

[b] PRESTO, JST, Honmachi 4-1-8, Kawaguchi, Saitama 332-0012, Japan and Toray Industries, Inc., Sonoyama 3-2-1, Otsu, Siga 520-0842, Japan

[c] RIKEN, Hirosawa 2-1, Wako, Saitama 351-0198, Japan

Version of record first published: 15 Jul 2010

To cite this article: Yosuke Sakao, Nobuo Ueno, Fumio Nakamura, Eisuke Ito, Junko Hayasi & Masahiko Hara (2003): FORMATION OF DNA SELF-ASSEMBLED MONOLAYER ON A GOLD SUBSTRATE, Molecular Crystals and Liquid Crystals, 407:1, 141-146

To link to this article: http://dx.doi.org/10.1080/744819024

Mol. Cryst. Liq. Cryst., Vol. 407, pp. 141/[537]–146/[542], 2003

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421400390263965

Taylor & Francis
Taylor & Francis Group

FORMATION OF DNA SELF-ASSEMBLED MONOLAYER ON A GOLD SUBSTRATE

Yosuke Sakao and Nobuo Ueno
Faculty of Engineering, Chiba University, Yayoicho 1-33, Inageku, Chiba 263-0022, Japan

Fumio Nakamura
PRESTO, JST, Honmachi 4-1-8, Kawaguchi, Saitama 332-0012, Japan and Toray Industries, Inc. Sonoyama 3-2-1, Otsu, Siga 520-0842, Japan

Fumio Nakamura, Eisuke Ito, Junko Hayasi, and Masahiko Hara
RIKEN, Hirosawa 2-1, Wako, Saitama 351-0198, Japan

We succeeded in forming a highly dense and uniform self-assembled monolayer (SAM) composed of thiolated double-stranded oligonucleotide (HS-dsDNA). The HS-dsDNA SAMs were prepared on gold substrates, and were characterized by surface plasmon resonance (SPR), X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). Formation of highly dense and uniform HS-dsDNA SAM was confirmed by the SPR and AFM measurements. XPS measurements bore out existence of the S-Au bond.

Keywords: AFM; DNA; self-assembled monolayer; surface plasmon resonance; XPS

INTRODUCTION

DNA sensors are crucial importance in genome researches and diagnoses of disease. It has recently become more important to establish higher-performance DNA sensors. For such DNA sensors we need to prepare well-oriented uniform probe-DNA films. However, current DNA sensors have some problems about the reproducibility of immobilization of probe

The authors thank Dr. K. Nakajima (RIKEN) for his help in measuring AFM images. One of them (F. N.) gratefully acknowledges the Grant-in-Aid for Encouragement of Young Scientists (No. 13740406) from the Ministry of Education, Culture, Sports, Science and Technology of Japan and Special Postdoctoral Researchers Program of RIKEN.

DNA on a substrate. That is, it is difficult to control the surface coverage of probe DNA on a substrate [1]. If a highly dense and uniform DNA self-assembled monolayer (SAM) is prepared, efficient and sensitive detection of hybridization can be realized. Although preparation methods of DNA SAMs have been actively studied so far, it was difficult to prepare a highly dense and uniform DNA SAM [1]. In this study, we attempted to prepare a highly dense and uniform thiolated double-stranded oligonucleotide (HS-dsDNA) SAM by selecting a suitable solvent. The characteristics of HS-dsDNA SAMs were studied by surface plasmon resonance (SPR), X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM).

EXPERIMENTS

As shown in Figure 1, the HS-dsDNA was prepared by annealing mixture of thiolated 20 bases single-stranded DNA (HS-ssDNA) and its complementary 20 bases single-stranded DNA (C-ssDNA) at 95°C. All DNAs were purchased from Sawady Technology Co. 20 mM aqueous $MgCl_2(H_2O)_6$ solution ($MgCl_2$aq) and 10 mM tri (hydroxymethyl) aminomethane buffer containing 1 mM EDTA (pH 7.0) called TE buffer were used as solvents. The concentration of HS-dsDNA was 3.0 μM in $MgCl_2$aq, and 3.0 μM in TE buffer.

A SPR measurement is based on the utilization of the surface plasmon electromagnetic field, at a metal/dielectric interface, which is generated by a laser beam. The absorption of dielectric species on a metal surface can be directly monitored in real time by measuring the reflected light intensity as a function of time at an appropriate angle of the incidence (θ). The change

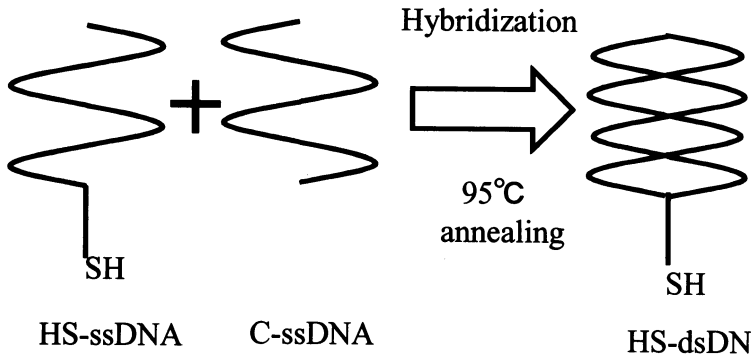

FIGURE 1 Schematic illustrations of hybridization of HS-ssDNA and its complementary ssDNA. The sequence of HS-ssDNA is HS-5′-ATGCATGCATTAG-CATGCTA-3′. The sequence of C-ssDNA is 3′-TACGTACGTAATCGTACGAT-5′′.

in the reflected intensity (ΔR) reflects sensitively the absorption of dielectrics on the metal surface. In our SPR measurements, the experimental setup of the Kretschmann configuration [2,3] was used with He–Ne laser ($\lambda = 632.8$ nm, 5 mW). The SPR measurements were carried out using gold-deposited glass substrates (LaSFN9, n = 1.85), which were contacted optically to the prism (LaSFN9, n = 1.85) bases. The gold surface was immersed in the HS-dsDNA solution for the SPR detection of the adsorption of HS-dsDNA onto the gold surface. The deposition of gold on the glass substrates was carried out by vacuum deposition under 10^{-5} Torr. The thickness of the gold film was ~ 50 nm. The temperature of the SPR cuvette was controlled to be 20°C.

XPS measurements were carried out by using monochromatized Al K_α line ($h\nu = 1486.6$ eV) with ESCALAB 250 system (Thermo VG Scientific). For XPS measurements, the SAMs were prepared on 150-nm-thick gold films evaporated on glass slides that had been covered with 2 nm chromium films to increase the stability of the gold films.

For AFM measurements, Au(111) films as the substrates of the HS-dsDNA SAMs were prepared by vacuum deposition on mica as reported in previous paper [4]. AFM images of the surface morphology of the HS-dsDNA SAMs were obtained in air at room temperature with a NanoScope IIIa (Digital Instrument, Santa Barbara, CA) in the tapping mode.

RESULTS AND DISCUSSION

Figure 2 shows the time dependence of ΔR in the SPR measurement, which corresponds to the adsorption behavior of HS-dsDNA onto the gold surface, for using different two solvents ($MgCl_2$aq and TE buffer). There is a marked difference between the adsorption behaviors obtained by using (a) $MgCl_2$aq and (b) TE buffer. In the case of $MgCl_2$aq the mean film thickness was evaluated to be 3.8 nm, assuming that refractive index of HS-dsDNA solid was 1.45 [5]. The evaluated film thickness suggests that the highly dense HS-dsDNA SAM was formed as shown in Figure 3 (a). In the case of using TE buffer as the solvent, on the other hand, the mean thickness was found to be $\sim$0.7 nm. This is much smaller than that by using $MgCl_2$aq. Furthermore, it is also smaller than the diameter of HS-dsDNA molecule ($\sim$2 nm). It can be speculated that HS-dsDNA was lying on the substrate, as shown in Figure 3(b). These results demonstrate that a highly dense HS-dsDNA SAM can be formed on a gold substrate when $MgCl_2$aq is used as the solvent.

A typical AFM image of the HS-dsDNA SAM formed by using $MgCl_2$aq is shown in Figure 4. It is clearly seen that the terrace structure due to Au (111) was observed, indicating that the uniform SAM was formed. Although

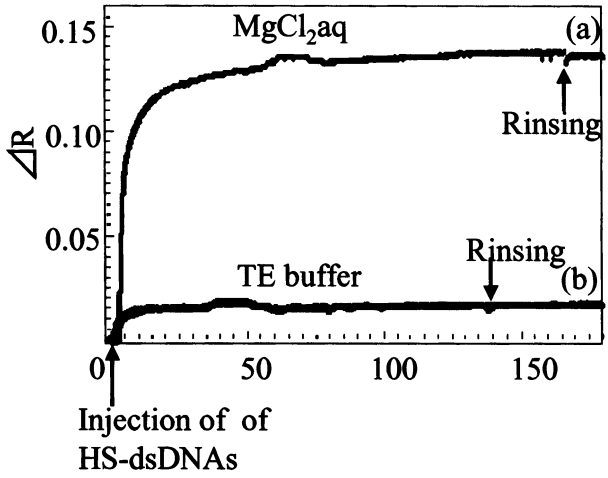

FIGURE 2 Adsorption behaviors of 20 base-pairs oligonucleotides onto gold substrates using an aqueous $MgCl_2$ solution (a) and TE buffer (b).

there were some small holes (dark spots in Figure 5) whose diameter and depth were ~20 nm and ~2 nm, respectively, the mechanism of the formation of holes is under consideration. The present AFM observation shows that surface morphology of the HS-dsDNA SAM is almost homogeneous on a gold substrate.

As a result of the SPR and AFM studies, we may conclude that highly dense and uniform HS-dsDNA SAM can be formed on a gold substrate using the $MgCl_2$ solution. Kelley *et al.* reported that thiolated ds oligonucleotide formed a monolayer on a gold substrate using a $MgCl_2$ solution [6]. The present result is consistent with their report.

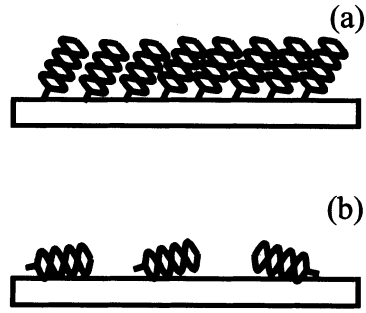

FIGURE 3 Schematic illustrations of formation of ds DNA SAM on gold substrates using an aqueous $MgCl_2$ solution (a) and TE buffer (b).

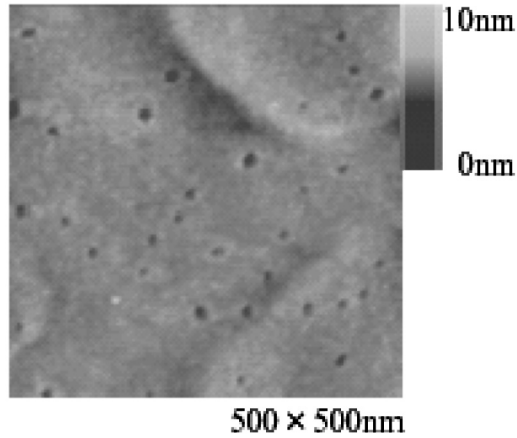

FIGURE 4 AFM image of ds DNA SAM on Au (111) substrate. This was taken by tapping mode in air at room temperature.

XPS measurements on the HS-dsDNA SAM prepared with $MgCl_2$aq showed that Mg existed in the SAM even after careful rinsing with pure water, since Mg1s peak was observed at the binding energy of 1305 eV (see Fig. 5(a)). As shown in Figure 5(b), on the other hand, a small but clear S2p peak was observed at approximately 162 eV, which can be assigned to the S-Au bond [7,8]. This means that HS-dsDNA molecules are anchored to the gold surface through chemical bonding between S and Au atoms. In passing we confirmed that Cl was not detected by the XPS measurements. These

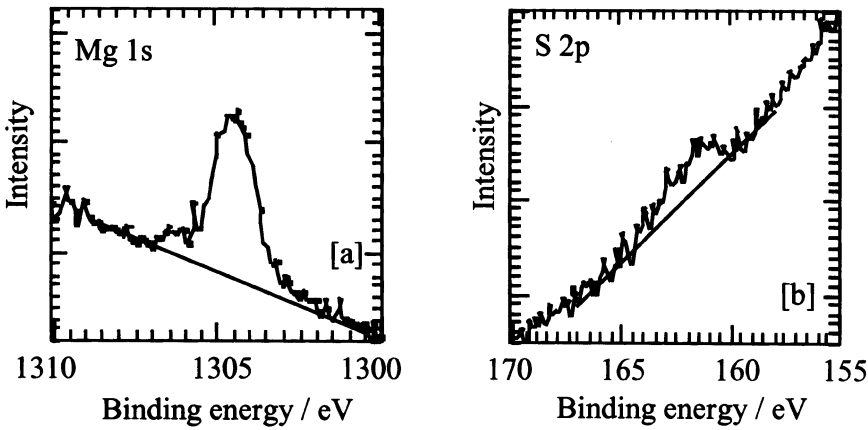

FIGURE 5 XP spectra of the HS-dsDNA SAM.

results indicate that the S-Au bond is important to the formation of the highly dense and uniform HS-dsDNA SAM. It is considered that (i) the lateral interaction between HS-dsDNA molecules is necessary for the formation of the highly dense SAM and (ii) incorporation of Mg atoms in the SAM plays an important role as mediators of the intermolecular interaction of HS-dsDNAs on the substrate.

CONCLUSION

We succeeded to prepare a highly dense and uniform HS-dsDNA SAM by using aqueous $MgCl_2$ solution on a gold substrate. Such highly dense and uniform SAM is very useful in improving the immobilization of probe DNA on a substrate. We believe that a probe-DNA SAM can be prepared by introducing single-stranded part into the present DNA SAM. The present method is of epoch-making for the development of sensitive and highly efficient DNA sensors.

REFERENCES

[1] Levicky, R., Herne, T., Tarlov, M. J., & Satija, S. K. (1998). Using self-assembly to control the DNA monolayers on gold: A neutron reflectivity study. *J. Am. Chem. Soc.*, *120*, 9787–9792.

[2] Kambhampati, D. K., Jakob, T. A. M., Robertson, J. W., Cai, M., Pemberton, J. E., & Knoll, W. (2001). Novel silicon dioxide sol-gel films for potential sensor applications: A surface plasmon resonance study. *Langmuir*, *17*, 1169–1175.

[3] Weisser, M., Nelles, G., Wohlfart, P., Wenz, G., & Neher, S. M. (1996). Immobilization kinetics of cyclodextrins at gold surfaces. *J. Phys. Chem.*, *100*, 17893–17900.

[4] Noh, J., Murase, T., Nakajima, K., Lee, H., & Hara, M. (2000). Nanoscopic investigation of the self-assembly processes of dialkyl disulfides and dialkyl sulfides on Au(111). *J. Phys. Chem. B*, *104*, 7411–7416.

[5] Wink, T., Beer, J. D., Hennink, W. E., Bult, A., & Bennekom, P. V. W. (1999). Interaction between plasmid DNA and cationic polymers studied by surface plasmon resonance spectrometry. *Anal. Chem.*, *71*, 801–805.

[6] Kelley, S. O., Barton, J. K., Jackson, N. M., McPherson, L. D., Potter, A. B., Spain, E. M., Allen, M. J., & Hill, M. G. (1998). Orienting DNA helices on gold using applied electric fields. *Langmuir*, *14*, 6781–6784.

[7] Ishida, T., Choi, N., Mizutani, W., Tokumoto, H., Kojima, I., Azehara, H., Hokari, H., Akiba, U., & Fujihira, M. (1999). High-resolution X-ray photoelectron spectra of organosulfur monolayers on Au(111): S(2p) spectral dependence on molecular spesies. *Langmuir*, *15*, 6799–6806.

[8] Ishida, T., Hara, M., Kojima, I., Tsuneda, S., Nishida, N., Sasabe, H., & Knoll, W. (1998). High resolution X-ray photoelectron spectroscopy measurements of octadecanethiol self-assembled monolayers on Au(111). *Langmuir*, *14*, 2092–2096.