Single Molecular Anatomy of Host–Guest Chemistry Based on Atomic Force Microscopy Study of Cyclodextrin–Ferrocene Molecular Interaction

Satoshi YASUDA[1,5], Yoshitaka OKUTSU[1], Iwao SUZUKI[2], Ken-ichi SHINOHARA[3],
Makoto KOMIYAMA[4], Osamu TAKEUCHI[4], and Hidemi SHIGEKAWA[1*]

[1]*Institute of Applied Physics, 21st Century COE, CREST–JST, University of Tsukuba, Tsukuba, Ibaraki 305-8573, Japan*
[2]*Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan*
[3]*School of Materials Science, Japan Advanced Institute of Science and Technology, Nomi, Ishikawa 923-1292, Japan*
[4]*Research Center for Advanced Science and Technology, University of Tokyo, Tokyo 153-8904, Japan*
[5]*Research Center for Advanced Carbon Materials, AIST, Tsukuba, Ibaraki 305-8565, Japan*

The formation process of a typical host–guest compound, β-cyclodextrin–ferrocene system, in various solutions was analyzed on the basis of the results of unbinding-force measurement using atomic force microscopy (AFM). The ratio of the formation numbers determined by AFM is consistent with the complexation constant obtained from the macroscopic analysis. We also analyzed the unbinding force on the basis of the mechanism of surface tension, and solvophobic factors, the intrinsic van der Waals interaction and the effective length of surface tension for ferrocene, were evaluated at the single-molecule level.   [DOI: 10.1143/JJAP.46.5614]

1. Introduction

The understanding and control of the intermolecular dynamics, particularly the molecular recognition processes, have been fundamental subjects of biology in recent decades, and are also the primary goals of the development of future nanoscale molecular science and technology. Since most important systems are effective in solvents, interactions arising from solvent-related factors, such as the solvophobic effect, play important roles in intermolecular dynamics in addition to intrinsic forces such as the van der Waals (vdW) interaction and hydrogen bonding.[1]

The development of atomic force microscopy (AFM) has enabled the direct measurement of intermolecular interactions between individual molecules at the piconewton level.[2] Since the binding force can be measured at the single-molecule level, this technique has been used to investigate the molecular interaction on the basis of the analysis of the force amplitude.

For example, by applying this technique to a combination of β-cyclodextrin (β-CyD) and adamantane molecules, the contributions of solvent-related factors have been distinguished from the intrinsic vdW force at the single-molecule level.[3] With respect to dynamic force spectroscopy (DFS), which analyzes thermal activation processes of bond formation, the landscape of the interaction potential and the lifetime of the bonding structures are evaluated.[4]

From another point of view, since the bonds formed by several molecules are involved in the unbinding process, one can analyze the number of bonds with respect to the indication of a binding probability of the host–guest system at an equilibrium condition. In this study, we examined the validity of this approach using a β-CyD [Fig. 1(a)] and ferrocene complex system, the thermodynamic property of which has been well studied macroscopically.[5,6] The ratio of the formation numbers determined by AFM was consistent with the complexation constant obtained from the macroscopic analysis. Furthermore, the obtained unbinding force
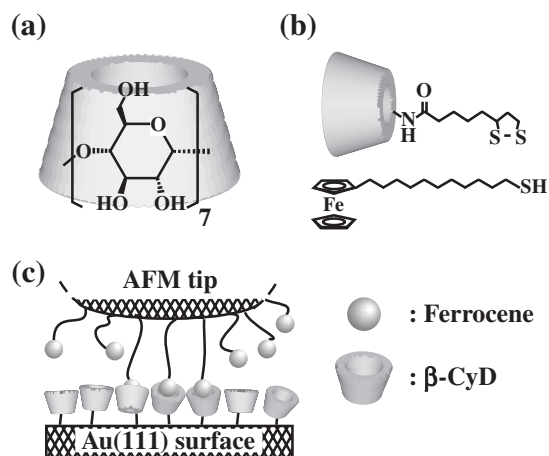
*E-mail address: hidemi@ims.tsukuba.ac.jp

Fig. 1. Structures of (a) β-cyclodextrin (CyD), (b) LP-β-CyD and 11-ferrocenyl-1-undecanthiol, and (c) experimental setup.

was quantitatively analyzed, and the contributions of solvophobic factors, as well as the intrinsic vdW interaction, were evaluated at the single-molecule level. After a comparison with the data, which were obtained for the adamantane molecule in previous work,[3] it was demonstrated that the analysis based on surface tension can be used to characterize the solvophobic effect at the single-molecule level.

2. Experimental Methods

To form self-assembled monolayers (SAMs) of β-CyD molecules on a Au(111) surface, we used lipoamide (LP)-β-CyD molecules, which are β-CyD molecules modified with a lipoamide residue chemically bound to the primary hydroxyl side. Scanning tunneling microscopy (STM) measurements revealed that a LP-β-CyD monolayer was formed all over the Au(111) surface as shown in Fig. 2(a), and densely packed homogeneous LP-β-CyD molecules were confirmed [Fig. 2(b)]. The formation process and the structure were examined and confirmed in detail using STM and cyclic voltammogram measurements.[7,8]
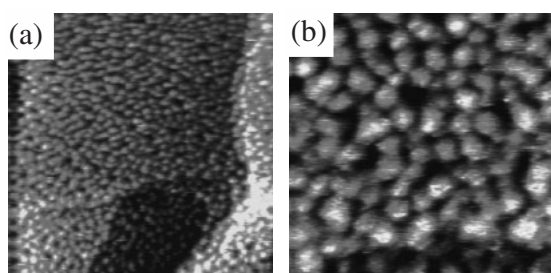
Fig. 2. STM images of LP-β-CyD molecules self-assembled on a Au(111) surface (a) $75 \times 75\,\text{nm}^2$, $V_t = +1.0\,\text{V}$, $I_t = 0.3\,\text{nA}$, (b) $50 \times 50\,\text{nm}^2$, $V_t = +1.0\,\text{V}$, $I_t = 0.15\,\text{nA}$.
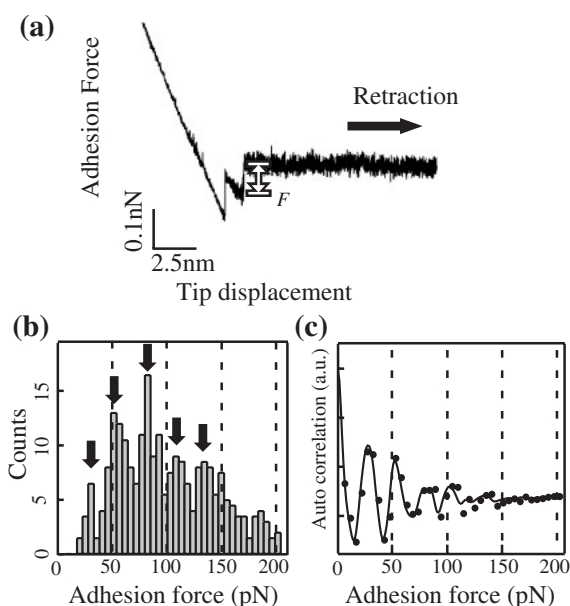

Fig. 3. (a) Typical force curve obtained for DMF. (b) Histogram derived from the force measurement in DMF. (c) Autocorrelation analysis of the histogram shown in (b).

Ferrocene molecules were chemically tethered on a gold-coated Si_3N_4 AFM cantilever, using 11-ferrocenyl-1-undecanthiol (11-FUT) molecules, that is, ferrocene modified with an undecanthiol chemically added to one cyclopentadienyl group. Schematic illustrations of the modified molecules and the experimental setup are shown in Figs. 1(b) and 1(c), respectively.

3. Results and Discussion

Figure 3(a) is a typical force curve obtained in dimethylformamide (DMF) solution. Bond-breaking events occurred step by step, because the tip–sample bonding consists of many individual host–guest bonds. For the analysis of the host–guest interaction, the unbinding force in the final step, indicated by "F" in Fig. 3(a), was recorded. More than 300 force measurements were performed for methanol, DMF, and water. A histogram of the unbinding forces measured for DMF is shown in Fig. 3(b). As indicated by arrows, there is a certain periodic feature in the histogram, which is more clearly confirmed from an autocorrelation analysis of the histogram, as shown in Fig. 3(c). This result indicates that the obtained unbinding forces are composed of integer multiples of an elementary force for breaking a single β-

CyD–ferrocene complex. Through a similar procedure, the unbinding forces in all solutions were determined. The unbinding forces between ferrocene and β-CyD molecules measured in methanol, DMF, and water are 21 ± 3, 26 ± 3, and $54 \pm 6\,\text{pN}$, respectively.[9] The measurement error (12%) mainly arises from that in the spring constant of the cantilever.[10]

The unbinding force in water is close to that obtained in previous work, $55 \pm 10\,\text{pN}$,[9] and is smaller than that for the case of the β-CyD–adamantane complex, $97 \pm 12\,\text{pN}$.[3] The magnitude relationship of the unbinding force between β-CyD–ferrocene and β-CyD–adamantane complexes is in good agreement with the relationship of the free energy macroscopically obtained for both cases, $\Delta G = -4.5$ and $-6.2\,\text{kcal/mol}$, respectively.[6,11] The analysis for the detailed treatment of the comparison will be our future work.

Ferrocene is known to dissolve well in polar DMF, which results in a lower complexation. This higher dissolvability is due to the high polarizability of ferrocene. In fact, according to the macroscopic analysis, the complexation constants K of ferrocene and CyD in water, DMF, and methanol are 2400, 67, and $125\,\text{mol}^{-1}$ (100 : 2.8 : 5.2), respectively. This effect on solvation is predicted to occur in AFM measurement. By dividing the value of the adhesion force by the unbinding force of a single β-CyD and guest molecular complex, we can obtain the number of bonded β-CyD–ferrocene complexes in each measurement. Here, "adhesion force" is the averaged value of the forces obtained from the bond-breaking events, where events occur as a single-step unbinding process instead of the step by step shown in Fig. 3(a). The ratio of the formation numbers determined for the processes in water, DMF, and methanol is 100 : 3 : 17 [(7800/54) : (110/26) : (510/21)], and the order of the intensity ratio components is consistent with that of K, 100 : 2.8 : 5.2. Here, the difference in the value for methanol may be related to the difficulty in the macroscopic measurement of the complexation constant K in methanol due to the low solubility.

The intermolecular interaction and solvophobic effect were then analyzed. How can we distinguish the solvophobic effect from other effects? The solvophobic force is related to the force required to form a space in a liquid to accommodate a guest molecule, and its strength is proportional to the surface tension of the solvent.[1] Although only on the basis of the macroscopic physicochemical and thermodynamic measurements, the free energy for the formation of β-CyD and adamantane inclusion complex was found to exhibit a linear relationship with respect to surface tension; that is, $\Delta G_{\text{solvent–solvent}} \propto \gamma \Delta(v^{2/3})$, where γ and $\Delta(v^{2/3})$ are the solvent surface tension and surface area change in the reaction, respectively.[12] The surface tension contributes to the stability of the complex.

From this standpoint, the obtained unbinding forces for the ferrocene molecule were plotted as a function of surface tension of the solvents macroscopically determined[13] [Fig. 4-(I)]. Here, the results obtained for a combination of β-CyD and adamantane molecules in methanol-water solutions of various volume ratios with different surface tensions are presented together for comparison [Fig. 4-(II)].[3]

The unbinding forces obtained for the combination of β-CyD and adamantane molecules linearly increase for an
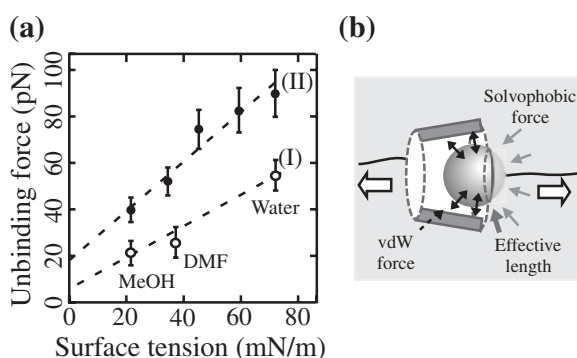
(a)

(b)

Fig. 4. (a) Unbinding forces obtained for (I) ferrocene and (II) adamantine molecules,[3] as a function of surface tension of solvents. (b) Intermolecular interactions.

increasing surface tension of the solvents. Since the observed linear relationship is based on the measurements of the intermolecular interaction at the single-molecule level, the result indicates that the solvophobic effect on a single host–guest interaction can be analyzed using the mechanism of surface tension at this level.[3] From the gradient of the linear relationship shown in Fig. 4(a)-(II), the length of this system related to surface tension ["effective length", Fig. 4(b)] was estimated to be 1.07 ± 0.13 nm, which is consistent with the perimeter of the adamantane molecule, $\sim$1.3 nm.

A similar analysis was performed for the β-CyD–ferrocene system. From the gradient of the linear relationship in Fig. 4(a)-(I), the effective length of the ferrocene was estimated to be 0.66 ± 0.13 nm. The ferrocene and adamantane molecules are similar in size, and thus, the difference in the effective length may be related, for example, to the difference in the conformation and solvation effects on the unbinding process. In this study, only three data points were obtained because only three solutions are available to use in the process of measuring the unbinding force for ferrocene. Since it is difficult to find an adequate number of solutions, the use of surface tension in the analysis of the solvophobic effect would be advantageous at the single-molecule level.

The intrinsic vdW force, in this case obtained by extrapolating the linear relationship between the surface tension and the unbinding force, is 5.2 ± 5.9 pN (less than $\sim$10 pN). Clearly, the value is smaller than that obtained for the case of the adamantane molecule, 18 ± 7 pN. The ratio of the surface tensions macroscopically obtained for water, DMF and methanol is $72 : 37 : 22$ ($= 100 : 51 : 31$). In contrast, the ratio of the solvophobic forces (unbinding forces—vdW) for water, DMF and methanol obtained in this experiment is $49 : 21 : 16$ ($= 100 : 43 : 33$), when the vdW force is assumed to be 5 pN. The smaller ratio in DMF measured from the unbinding force, unlike the macroscopic value, can be related to the decrease in surface tension,

which is expected to occur in the measurement of the host–guest system because of the solvation effect.

4. Conclusions

The formation process of a typical host–guest compound, β-CyD–ferrocene system, was analyzed based on the unbinding-force measurement by AFM. The ratio of the formation numbers determined by AFM is consistent with the complexation constant obtained from the macroscopic analysis. The results demonstrate the potential of this method to provide more accurate understanding of the results of the macroscopic analysis. Regarding the solvophobic effect, the comprehensive results obtained for the ferrocene and adamantane molecules suggest the importance of using the effective length for the analysis of the host–guest interaction. Precise images of host–guest complexes drawn at the molecular level are expected to pave the way for a more straightforward molecular design.

1) O. Sinanoğlu: in *Molecular Associations in Biology*, ed. B. Pullman (Academic Press, New York, 1968) p. 427.
2) V. T. Moy, E.-L. Florin, and H. E. Gaub: Science **266** (1994) 257.
3) S. Yasuda, I. Suzuki, K. Shinohara, and H. Shigekawa: Phys. Rev. Lett. **96** (2006) 228303.
4) O. Takeuchi, T. Miyakoshi, A. Taninaka, K. Tanaka, D. Cho, M. Fujita, S. Yasuda, S. P. Jarvis, and H. Shigekawa: J. Appl. Phys. **100** (2006) 074315.
5) M. L. Bender and M. Komiyama: *Cyclodextrin Chemistry* (Springer, Berlin, 1978).
6) L. A. Godinez, L. Schwartz, C. M. Criss, and A. E. Kaifer: J. Phys. Chem. B **101** (1997) 3376.
7) S. Yasuda, I. Suzuki, T. Nakamura, M. Matsumoto, M. Komiyama, and H. Shigekawa: Appl. Phys. Lett. **76** (2000) 643.
8) S. Yasuda, I. Suzuki, J. Sumaoka, M. Komiyama, and H. Shigekawa: Trans. Mater. Res. Soc. Jpn. **31** (2000) 853.
9) As was pointed out in H. Schönherr, M. W. J. Beulen, J. Bügler, J. Huskens, F. C. J. M. Veggel, D. N. Reinhoudt, and G. J. Vancso: J. Am. Chem. Soc. **122** (2000) 4963, the unbinding force does not change in the measurement range of 2.0×10^4–3.0×10^5 pN/s in the case of the β-CyD–ferrocene system.
10) The spring constant of the cantilevers coated with a 50 nm Au film was calibrated by the thermal resonance method [J. L. Hutter and J. Bechhodefer: Rev. Sci. Instrum. **64** (1993) 1868]. The range of the measured spring constant was 0.11 to 0.25 N/m. Each value was obtained as an average of more than 20 measurements for each cantilever.
11) W. C. Cromwell, K. Bystrom, and M. R. Eftink: J. Phys. Chem. **89** (1985) 326.
12) J. Harrison and M. Eftink: Biopolymers **21** (1982) 1153.
13) *CRC Handbook of Chemistry and Physics* (CRC Press, Boca Raton, FL, 1991–1992) 72nd ed., p. 6-117.