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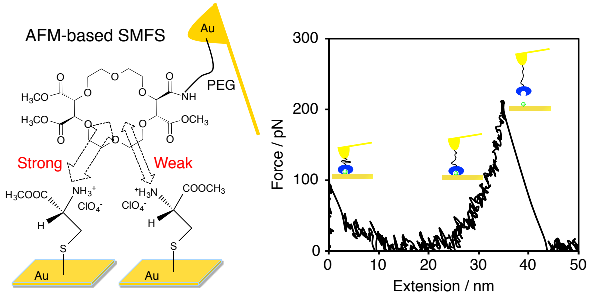
Graphical abstract

The chiral recognition of cysteine derivatives on the substrate was investigated using AFM tips modified with optically active crown ethers.



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Single-molecule force-spectroscopic study on chiral recognition of cysteine derivatives immobilized on gold substrate by using AFM tips modified chemically with optically active crown ethers

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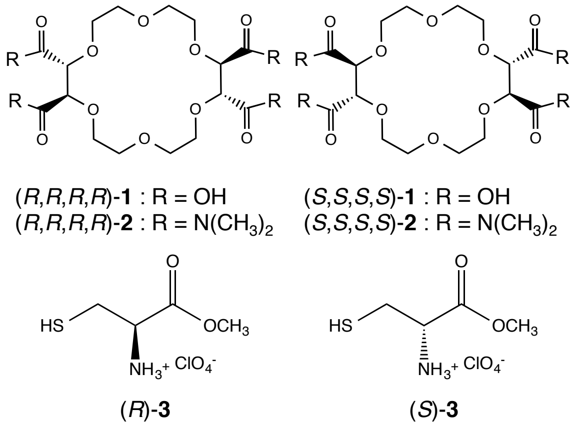
The chiral recognition of cysteine derivatives immobilized on the gold substrate by using atomic force microscopy (AFM) tips modified chemically with optically active crown ethers was quantitatively investigated with single molecule force spectroscopy (SMFS). Very interestingly, the chiral recognition ability of the optically-active-crown-ether-modified tip was entirely opposite to that of its-optical-isomer-modified tip. The difference of rupture forces for the chiral recognition was determined to be about 40 pN under our experimental conditions.

Introduction

Solid surfaces modified chemically with chiral host molecules have attracted considerable attention because they provide important applications such as enantiospecific sensors,[1] separation of chiral compounds[2,3] and asymmetric catalysts.[4-6] Despite this importance, the mechanism of the chiral recognition on the solid surface is hardly understood.[7] In order to elucidate the properties of chiral solid surfaces, a few analytical methods such as scanning tunnelling microscopy,[8-10] atomic force microscopy (AFM)[11] and quartz crystal microbalance method[12] have been developed but there still remains a lot of research subjects.[13,14]

Until now, host-guest interactions on the solid surface have been often investigated using AFM-based single molecule force spectroscopy (SMFS).[15-21] In these studies, host and guest molecules were immobilized on a tip and a substrate individually, and their host-guest binding events were revealed at the single-molecule level by analysing the force-extension curves reflecting the interaction between the tip and the substrate. Since SMFS can provide the insights into kinetics and thermodynamics of intermolecular association, this analytical method is highly expected as a useful tool to clarify the detailed mechanism of host-guest interactions on the solid surface.

Since optically active crown ethers were firstly reported by Cram et al. in the 1970s,[22,23] various kinds of chiral host molecules have been synthesized.[24,25] Among them, optically active (18-crown-6)-2,3,11,12-tetracarboxylic acid **1**[26] and its derivatives have been used most frequently for enantiomeric recognition.[27-30] In this study, the chiral recognition of cysteine derivatives on the solid surface by using AFM tips modified chemically with **1** was quantitatively investigated using AFM-based SMFS. In addition, the results of the chiral recognition on the solid surface obtained by the AFM study were compared with those in the solution evaluated by the NMR study. Fig. 1 shows chemical structures of chiral compounds used in this study. In the AFM study, **1** and cysteine methyl ester perchlorate salt **3** were immobilized on an AFM tip and a gold substrate, respectively. In the NMR study, optically active (18-crown-6)-2,3,11,12-tetrakis(*N*,*N*-dimethylcarboxamide) **2** was used instead of **1** because four carboxyl groups of **1** were capped with nonpolar functional groups in the AFM study. Any report about AFM-based SMFS for the chiral recognition of optically active crown ethers has not been published yet, as far as we know.



(*R*,*R*,*R*,*R*)-**1** : R = OH
(*R*,*R*,*R*,*R*)-**2** : R = $N(CH_3)_2$

(*S*,*S*,*S*,*S*)-**1** : R = OH
(*S*,*S*,*S*,*S*)-**2** : R = $N(CH_3)_2$

(*R*)-**3**

(*S*)-**3**

Fig. 1 Chemical structures of optically active crown ethers and cysteine derivatives used in this study.

Experimental

Materials

All chemicals for the preparation of optically-active-crown-ether-modified tips except for **2**[31] and 1-{[5-(1,2-dithiolan-3-yl)pentanoyl]oxy}-2,5-pyrrolidinedione,[32] and are of commercially available purity, and used without further purification. Also, **3** was obtained from cysteine methyl ester chloride salt by anion exchange. Organic solvents for the synthesis were purified by conventional methods, such as distillation. Deionized water (resistivity: 18 MΩ cm) was prepared using a Milli-Q system. Ethanol for AFM measurements was obtained from Sigma-Aldrich (Japan) and used without further purification.

Chemical modification

The tips and the gold-coated substrates used for AFM force measurements were modified chemically with thiol and disulfide derivatives.[33] The V-shaped AFM cantilevers were commercially available: Si_3N_4 type coated with Au/Cr on both sides (k = 0.02 N m^{-1}, OMCL-TR400PB-1, Olympus, Japan). The cantilevers were pretreated by immersion into a piranha solution (concentrated H_2SO_4/28% H_2O_2, 7/3, v/v) at room temperature for 30 min for cleaning their surface (**CAUTION**: Piranha solution is extremely dangerous and should be handled with great care). The cantilevers were then thoroughly rinsed with deionized water.

The AFM tips modified chemically with optically active crown ethers were prepared according to Fig. 2. The cleaned tips were treated by immersion into an ethanol solution containing 1.0 mM 1-{[5-(1,2-dithiolan-3-yl)pentanoyl]oxy}-2,5-pyrrolidinedione at room temperature for 24 h. After being rinsed with ethanol, the tips were fully dried in the atmosphere. The tips were treated by immersion into a DMF solution containing 0.20 mM α-aminopropyl-ω-aminopropoxy, polyoxyethylene (M_w = 3500, M_w/M_n = 1.4) and 5.0 mM *N*,*N*-dimethylaminopyridine (DMAP) at room temperature for 2 h. After being rinsed with DMF, the tips were fully dried in the atmosphere. Next, the tips were treated by immersion into an 1.0 wt % $NaHCO_3$ aqueous solution containing 1.0 mM **1** and 10 mM 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (WSC) at room temperature for 24 h. After being rinsed with ion-exchange water, the tips were fully dried in the atmosphere. Furthermore, the tips were immersed into acetic anhydride at room temperature for 2 h for the remaining amino groups to be nonpolarized. After being rinsed with ethanol, the tips were fully dried in the atmosphere. Then, the tips were immersed into HCl aqueous solution (pH 3) at room temperature for 2 h. After being rinsed with ion-exchange water, the tips were fully dried in the atmosphere. Finally, the tips were immersed into a diethylether solution containing 2.0 M trimethylsilyldiazomethane at room temperature for 30 min. After being rinsed with ethanol, the crown-ether-modified tips were fully dried in the atmosphere. Also, the crown-ether-unmodified tips for the control experiment were prepared as shown in Fig. S1.

Gold-coated mica substrates were prepared by sputtering of gold (99.999%, Nilaco, Japan) using a JFC-1600 Auto Fine Coater (JEOL, Japan) onto a mica substrate prepared by fresh cleavage of a sheet of natural mica (Nilaco, Japan). The gold-coated substrate was immersed into a 2.5 mM **3** methanol solution at room temperature for 24 h. After being rinsed with ethanol, they were dried with nitrogen gas before use.

AFM force measurements

AFM force measurements were carried out in an ethanol solution at room temperature (ca. 298 K) using a Nanoscope 3D MultiMode AFM with PicoForce (Veeco, USA). The probe tip and the substrate were mounted on the apparatus using a liquid cell. The spring constants (typically 0.02 N m^{-1}) of the cantilever were always calibrated using the thermal tune method before each measurement.

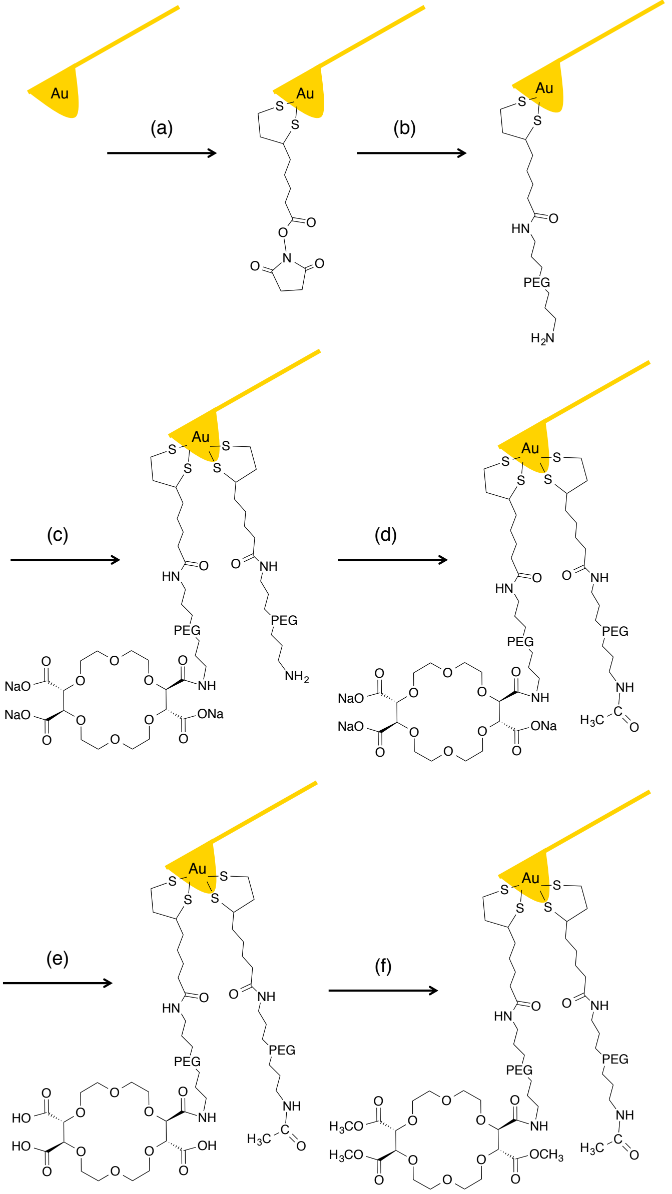


Fig. 2 Preparation of AFM tips modified chemically with optically active crown ethers. Reagents and conditions: (a) 1-{[5-(1,2-dithiolan-3-yl)pentanoyl]oxy}-2,5-pyrrolidinedione, ethanol, rt, 24 h; (b) α-aminopropyl-ω-aminopropoxy, polyoxyethylene, DMAP, DMF, rt, 2 h; (c) **1**, WSC, $NaHCO_3$ aq., rt, 24 h; (d) acetic anhydride, rt, 2 h; (e) HCl aq., rt, 2 h; (f) trimethylsilyldiazomethane, diethylether, rt, 30 min.

All force-distance curves were obtained in a contact mode using an AFM software of the manufacturers at a scan rate of 310 nm s^{-1}. The surface delay, defined as the resting time after the tip touches the substrate surface, was set at 1.0 s. The scan rate and surface delay were determined to maximize the observable probability for the stretching of a single PEG chain. Typical measurements were made 400-1,200 times at different positions of the substrate for each tip/substrate combination. The experimentally obtained force-distance curves were converted to the force-extension curves.[17] Then, a theoretical model for polymer extension, worm-like chain (WLC) model, was used to perform the curve fitting of the force-extension curves in order to obtain the characteristic fitting parameters. The average observable probability for the stretching of a single PEG chain was about 0.84% in total. The representative force-extension curve except for the stretching of a single chain is shown in Fig. S2.

NMR titrations

^{1}H-NMR spectra were collected on a JNM-ECA 400 FT NMR (JEOL, Japan). As an example, the titration experiment for the complexation of the crown ether (*R*,*R*,*R*,*R*)-**2** with the cysteine derivative (*R*)-**3** is described. A 3.0 mM solution of (*R*,*R*,*R*,*R*)-**2** in 600 μL of CD_3CN was prepared, and an initial NMR measurement of this solution was recorded. A 91 mM solution of (*R*)-**3** in 400 μL of CD_3CN was separately prepared. Samples were then made by adding various amounts of the guest solution into the host solution. The spectra of these eight different solutions were recorded. The stability constants were calculated from the chemical shift changes using an iterative nonlinear least squares curve-fitting program.[34]

Results and discussion

SMFS measurements

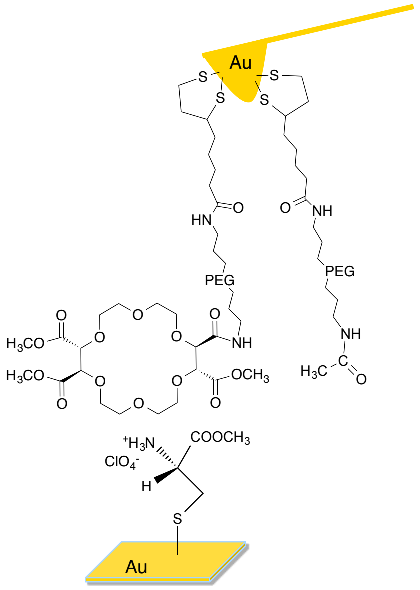


Fig. 3 Schematic diagram of our AFM-based SMFS study.

Fig. 3 outlines the strategy of our AFM-based SMFS study, wherein **1** was covalently anchored to an AFM tip through a polyethyleneglycol (PEG) spacer and **3** was immobilized on a gold-coated substrate based on a well-known thiol-Au interaction. After the chemical modification, the tips were immersed into acetic anhydride for the capping of the remaining amino groups by nonpolarized functional groups. Furthermore, the remaining carboxylic groups on the crown ether moiety were esterified by trimethylsilyldiazomethane diethylether to remove the effect of electrostatic interactions between $–COO^-$ and $–NH_3^+$. The tips and substrates were thoroughly dried with nitrogen gas before the measurements.

Fig. 4 shows a typical force-extension curve observed using the tip and the substrate modified chemically with (*R*,*R*,*R*,*R*)-**1** and (*R*)-**3**, respectively, in ethanol. The representative force-extension curves in the other combinations of the tips and substrates are shown in Fig. S3. The force-extension curves obtained in all the combinations of tips and substrates exhibited similar deformational characteristics: a nonspecific adhesion based on the contact between the tip and the substrate, a rising force with the increase of the extension, followed by a force dropping (rupture force) upon the rupture of a polymer bridge between the tip and the substrate. Here, the gentle slope observed at the rupture does not mean the slow dissociation of the host-guest complex. Interestingly, more than 100 pN of rupture forces were not observed in the presence of 100 mM KSCN (Fig. S4). This result suggests that the added K^+ in the solution interferes with the complexation of the 18-crown-6 moiety on the tip with $–NH_3^+$ site on the substrate.[35] Furthermore, such rupture forces were not also observed in the control experiment using the crown-ether-unmodified AFM tip and the cysteine–modified substrate (Fig. S5). Therefore, it is assumed that the rupture forces observed here mainly reflect the specific interaction between (*R*,*R*,*R*,*R*)-**1** and (*R*)-**3**.

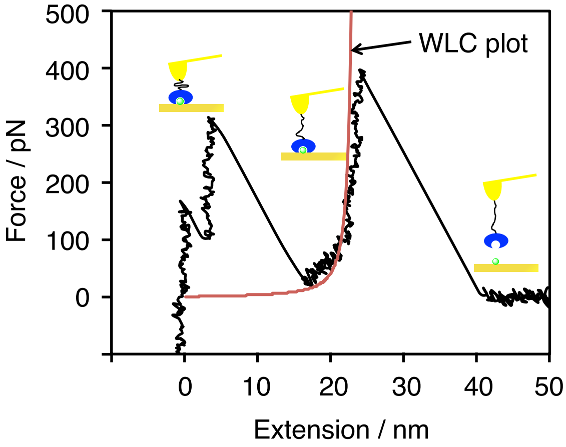


Fig. 4 Typical force-extension curve observed using (*R*,*R*,*R*,*R*)-**1**-modified tip and (*R*)-**3**-modified substrate in ethanol.

As has been reported by some researchers,[36] single polymer chain stretching can be described by several theoretical models. In order to confirm that the rupture force was observed at the single-molecule level, we analysed the force-extension curves for the stretching of the PEG linker based on a theoretical model, WLC model.[16] The WLC model describes a single polymer chain as a string of constant bending elasticity with a worm-like conformation. The following expression has been widely used to describe the force (F) as a function of chain extension (x)

$$F(x)=\frac{k_BT}{l_p}\left[\frac{1}{4}\left(1-\frac{x}{L}\right)^{-2}+\frac{x}{L}-\frac{1}{4}\right]$$

where L represents the contour length of the polymer chain, k_B is the Boltzmann constant, T is the temperature, and l_p is the persistence length, indicating the rigidity/flexibility of the polymer chain. Based on the above-mentioned equation, the curve fitting was conducted in order to gain the characteristic fitting parameter L. Fig. 4 also shows the fitting curve based on the WLC model. As some force-extension curves, which seem to be originated from the stretching of plural polymer chains, were not obviously fitted with the WLC model curve, they were excluded from the analysis. The typical force-extension curve badly fitting the WLC model plot is shown in Fig. S6. Furthermore, we regarded the curves with 10-50 nm of L as the full stretching of the single PEG chain among all the fitting curves because the length of the PEG linker used in this study was calculated to be about 30 nm according to the reference.[37] The range of contour length was enlarged to some extent because it also depends on the molecular weight distribution and the introduction position of the PEG linker.[38,39] After all, we regarded 176 fitting curves as the stretching of the single PEG chain among all of 21,000 force measurements. On behalf on the above-mentioned SMFS analysis, we can quantitatively discuss the chiral recognition ability of optically active crown ethers at single-molecule level.

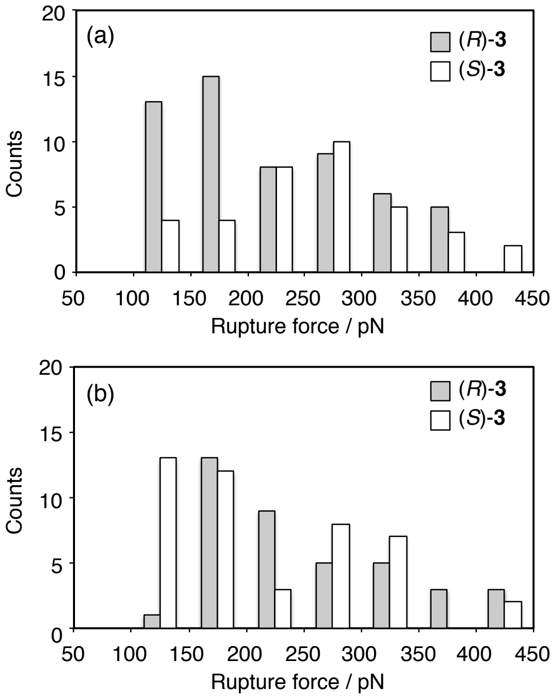


Fig. 5 Histograms of rupture forces observed using tips modified chemically with (a) (*R*,*R*,*R*,*R*)-**1** and (b) (*S*,*S*,*S*,*S*)-**1**.

Fig. 5a and 5b show the histograms of rupture forces observed using the tips modified chemically with (*R*,*R*,*R*,*R*)-**1** and (*S*,*S*,*S*,*S*)-**1**, respectively. In Fig. 5a, the larger rupture forces (260 ± 82 pN) in the case using the (*S*)-**3**-modified substrate were observed than those (219 ± 83 pN) in the case using the (*R*)-**3**-modified substrate. In Fig. 5b, the larger rupture forces (250 ± 84 pN) in the case using the (*R*)-**3**-modified substrate were observed than those (212 ± 86 pN) in the case using the (*S*)-**3**-modified substrate. Summarizing the above results, we can say that the chiral recognition ability of the optically-active-crown-ether-modified tip was entirely opposite to that of its-optical-isomer-modified tip. Namely, this result demonstrates that the chiral recognition on the solid surface was successfully observed by our SMFS study. The difference of rupture forces between (*R*)-**3**- and (*S*)-**3**-modified substrates was determined to be about 40 pN under our experimental conditions. Also, the statistical test (Welch's *t*-test) revealed that p-value was less than 0.05 between two histograms in both the cases using the same tip. Accordingly, it was shown that the differences of rupture forces observed between the substrates modified chemically with (*R*)- and (*S*)-cysteine derivatives were considered statistically significant. The difference may be due to the chiral recognition of optically active crown ethers because both measurements were carried out under the identical experimental conditions except for the absolute configuration of cysteine derivatives.

NMR measurements

Next, the chiral recognition in the solution was investigated by the NMR measurements. Firstly, the stoichiometry of the **2**-**3** complex was determined to be 1:1 by a Job plot. Table 1 shows the stability constants, which were determined by the ^{1}H NMR titration method, between chiral crown ethers **2** and chiral ammonium salts **3** in CD_3CN. In the NMR study, the selectivity for chiral recognition of the optically active crown ether was opposite to that of its optical isomer. Namely, this result demonstrates that chiral crown ethers **2** have the chiral recognition ability towards chiral ammonium salts **3**. However, the selectivity for chiral recognition in the solution was opposite to that on the solid surface. The previous study about the chiral recognition between (*R*,*R*,*R*,*R*)-**1** and amino acids also reported that the selectivity for chiral recognition in the solution was different from that on the solid surface in some cases and that the hydrogen bond between the side chain on the crown ether ring and amino acids plays an important role in the determination of the chiral selectivity.[40] In this study, the structures of the side chain on the crown ether ring were different between the AFM and NMR studies. This fact may affect the selectivity of the chiral recognition. In addition, as the distance between the ammonium moiety and the substrate was very short in the AFM study, the substrate might affect the selectivity. Therefore, the introduction of the spacer between the guest compound and the substrate would be required in the next study.

Table 1 Stability constants of **2** for **3** in CD_3CN determined by NMR titration method

	log K (M^{-1})	
	(*R*)-**3**	(*S*)-**3**
(*R*,*R*,*R*,*R*)-**2**	3.58	3.34
(*S*,*S*,*S*,*S*)-**2**	3.25	3.50

Conclusions

In this paper, we firstly reported AFM-based SMFS for the chiral recognition of cysteine derivatives on the solid surface by using AFM tips modified chemically with optically active crown ethers. Remarkably, the chiral recognition ability of the optically-active-crown-ether-modified tip was entirely opposite to that of its-optical-isomer-modified tip. On the other hand, the chiral selectivity on the solid surface obtained by the AFM study was different from that in the solution evaluated by the NMR study. The reason for this interesting phenomenon is not fully understood at the present stage. Further observation and analysis of the chiral recognition using AFM-based SMFS is underway in our laboratory, aiming at understanding chiral surfaces in more detail.

Notes and references

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Electronic Supplementary Information (ESI) available: Preparation of crown-ether-unmodified tip, representative force-extension curve except for the stretching of a single chain, representative force-extension curves in the other combinations of the tips and substrates, force-extension curve using (*R*,*R*,*R*,*R*)-**1**-modified tip and (*S*)-**3**-modified substrate in the presence of KSCN, and force-extension curve in the case using crown-ether-unmodified tip and (*S*)-**3**-modified substrate, and typical force-extension curve badly fitting with WLC model plot. See DOI: 10.1039/b000000x/

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