JOURNAL OF PHYSICAL ORGANIC CHEMISTRY
J. Phys. Org. Chem. 2005; **18:** 957–961
DOI: 10.1002/poc.934

Rational model for chiral recognition in a silica-based chiral column: chiral recognition of *N*-(3,5-dinitrobenzoyl)phenylglycine-terminated alkylsilane monolayer by 2,2,2-trifluoro-1-(9-anthryl)ethanol derivatives by chemical force microscopy

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Received 16 June 2004; revised 22 January 2005; accepted 18 February 2005

ABSTRACT: Direct measurement of the chemical force between chiral molecules was investigated by chemical force microscopy (CFM). 2,2,2-Trifluoro-1-(9-anthryl)ethanol (TFAE) and *N*-(3,5-dinitrobenzoyl)phenylglycine (DNBP), a well-known pair of enantiomers, were strongly immobilized on the surface of the cantilever tip and the substrate surface, respectively, by the use of aminosilane, to propose a chiral stationary phase model system of a silica-based chiral column. The modification of TFAE on a cantilever tip and DNBP on the substrate surface was confirmed by x-ray photoelectron spectroscopic and time-of-flight secondary ion mass spectrometric measurements. The force curve between (*R* or *S*)-TFAE and (*R* or *S*)-DNBP enantiomers were measured using the force measurement mode scanning force microscopy to determine the magnitude of the interaction. The histograms of the adhesion force for a different chirality pair [(*R*)-ATEA vs (*S*)-DNBP and (*S*)-ATEA vs (*R*)-DNBP] showed a broader distribution than those for the identical chirality pair [(*R*)-ATEA vs (*R*)-DNBP and (*S*)-ATEA vs (*S*)-DNBP]. Since CFM measurement cans recognize the difference of in nanonewton forces, the results can be regarded as a rational design for chiral recognition in a silica-based chiral column. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: chiral recognition; chemical force microscopy; surface modification; aminosilane monolayer

INTRODUCTION

Many kinds of enantiomers have been synthesized for application to agricultural chemicals, pharmaceuticals, etc. Chiral separation of enantiomers is very important in order to improve safety and drug efficiency. Recently, various methods for chiral separation such as recrystallization, a diastereomer method and chromatography have been developed. Chiral recognition by chromatography originates from the difference in the stability of diastereomer complexes formed between enantiomers and a chiral stationary phase (CSP). Since the successful development of high-performance liquid chromatography (HPLC) using CSPs by Pirkle and co-workers,[1–3] considerable research on chiral separation using CSPs has been carried out and various CSPs have been developed.

Since a CSP can distinguish between enantiomers, it is suggested that over three-point interaction is necessary for a specific interaction, including ionic interaction, dipole–dipole interaction, hydrogen bonding, π–π interaction, charge-transfer interaction and hydrophobic interaction, between the stationary phase and enantiomers. However there are few examples of the direct systematic evaluation of the interaction between them.[4]

To measure directly the interaction between a CSP and enantiomers, measurements of the adhesion force and friction force between chiral functional groups by scanning force microscopy (SFM) have been employed. Recently, SFM has attracted considerable attention owing to its capability of sensing the interaction force between its cantilever tip and substrate surfaces and acquiring topographical images of sample surfaces. Chemical modification on the surface of the probe tip and substrates allows the investigation of various chemical interactions

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Contract/grant sponsor: Ministry of Education, Culture, Sports, Science and Technology, Japan; *Contract/grant numbers:* 1200875189; 08CE2005.
Contract/grant sponsors: Japan Society for the Promotion of Science; Foundation Advanced Technology Institute.

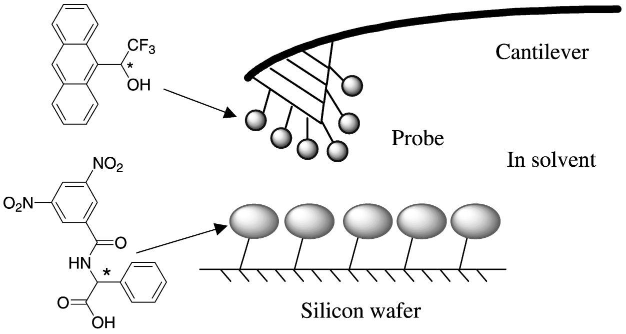


Figure 1. Schematic representation of the interaction between chiral molecules on the probe tip and substrate

on a molecular scale. SFM can measure, e.g., intermolecular forces,[5–9] host–guest interaction force,[10,11] intermolecular force between base pairs of DNA[12] and antigen–antibody interaction force.[13,14] The main potential advantage of chemical force microscopy (CFM) over other techniques which measure intermolecular forces, such as the surface force apparatus, is its speed, combined with the ability to map the spatial arrangement of ligands on the surface. McKendry *et al*. reported the chiral recognition of mandelic acid attached by means of an alkanethiol to a gold surface by using a gold-coated probe tip modified with acylated phenylglycine with a thiol group.[15]

In the course of our studies on surface chemistry using organosilane monolayers,[16–20] the organosilane monolayers were recognized as good models for the stationary phases of silica-based columns. In this work, we used 2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE) and *N*-(3,5-dinitrobenzoyl)phenylglycine (DNBP) as a well-known pair of enantiomers.[21] As shown in Fig. 1, TFAE and DNBP were strongly immobilized on the surface of the cantilever tip and the substrate surface, respectively, by the use of aminosilane, enabling us to propose a CSP model system for a silica-based chiral column. Adhesion force measurement was carried out between TFAE and DNBP using the force measurement mode of SFM. The mechanism of chiral recognition is discussed based on the magnitude of the interaction between chiral functional groups.

EXPERIMENTAL

Materials

1-(9-Anthryl)-2,2,2-trifluoroethoxyacetic acid (ATEA) was synthesized by the method reported previously[1] via Williamson-type alkylation of 2,2,2-trifluoro-1-(9-anthryl)ethanol with ethyl bromoacetate followed by hydrolysis. Either enantiomer of ATEA can be obtained from the corresponding enantiomer of 2,2,2-trifluoro-1-(9-anthryl)ethanol. Silicon wafers, polished n-type Si(111) wafers with 0.5 mm thickness, were purchased from Sumitomo-Mitsubishi Silicon. They were immersed in H_2SO_4–30% H_2O_2 (70:30, v/v) at 373 K for 1 h to obtain an Si—OH terminated surface before use. Aminopropylmethyldiethoxysilane (APDMS) was used as received (Chisso). Solvents were distilled before use. All other reagents were purchased from Kanto Chemicals or Wako Pure Chemical Industries and were used as received.

Measurements

A Physical Electronics TRIFT-II TOF instrument was used for time-of-flight secondary ion mass spectrometric (TOF-SIMS) imaging. Negative ion images were obtained with a 15 kV primary pulsed Ga^+ ion beam (pulse width 13 ns) with a 2 nA beam current. The scan area was $100 \times 100\,\mu m$. A Nano Scope IIIa instrument (Digital Instruments) was used for adhesion force measurements. The measurement was carried out in 2-propanol at room temperature. The adhesion force data were collected from force curves as histograms for 200 measurements for each sample.

Preparation of ATEA-modified cantilever tip

The cantilever tip, made of silicon nitride (Si_3N_4), was treated with vacuum ultraviolet (VUV) irridiation ($\lambda = 172$ nm) for 5 min to decompose organic contaminants. The cantilever tip was placed together with 1.0 ml of APDMS in the container under a dry nitrogen atmosphere. To confirm the degree of modification, the silicon wafer was also enclosed in the reaction vessel. The container was heated in an oven at 383 K under 15 mmHg pressure for 2 h. The cantilever tip and silicon wafer were washed with ethanol and heated at 383 K for 5 min. The APMDS-modified cantilever tip and silicon wafer were mixed with ATEA (8.2 mg, 0.025 mmol), *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (7.3 mg, 0.029 mmol), and THF (5 ml) and stirred at 303 K for 2 h. The cantilever tip and silicon wafer were washed with THF and ethanol.

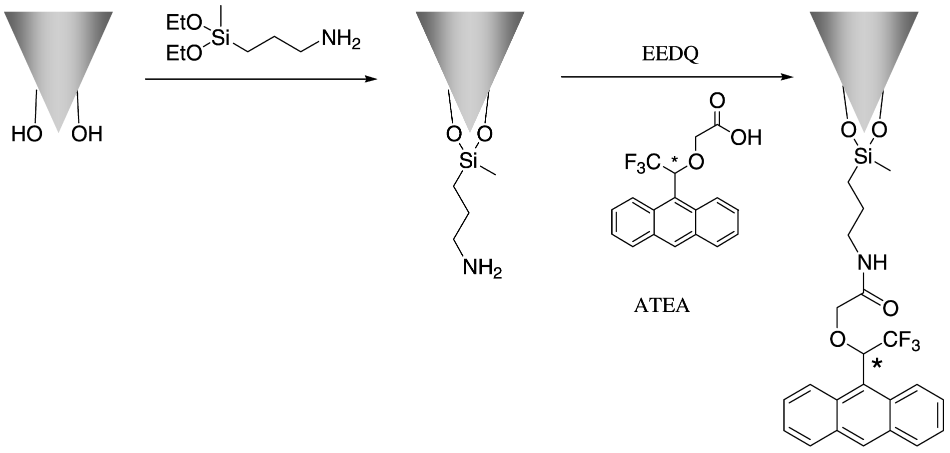
Preparation of DNBP-modified silicon wafer

The APMDS-modified silicon wafer, DNBP (8.6 mg, 0.025 mmol), EEDQ (7.3 mg, 0.029 mmol) and THF (5 ml) were mixed and stirred at 303 K for 2 h. The silicon wafer was washed with THF and ethanol.

RESULTS AND DISCUSSION

Immobilization of chiral groups

2,2,2-Trifluroethanol has frequently been used as a chiral chemical shift reagent for NMR spectroscopic



Scheme 1

measurements. In order to immobilize 2,2,2-trifluoroethanol on the tip of the cantilever, the carboxylic acid derivative, 1-(9-anthryl)-2,2,2-trifluoroethoxyacetic acid (ATEA), was designed and synthesized. 2,2,2-Trifluoroethanol was treated with sodium hydride and reacted with ethyl bromoacetate to give the ethyl ester derivative. The ester derivative was hydrolyzed in 90% ethanol in the presence of potassium hydroxide to afford ATEA. The characterization of ATEA was carried out by IR and NMR measurements.

The ATEA-modified cantilever probe tip was prepared according to Scheme 1. Since the cantilever tip is made of Si_3N_4, the treatment with VUV radiation produced hydroxyl groups on the surface. The OH-terminated cantilever tip was modified with APDMS under a nitrogen atmosphere to prepare an NH_2-terminated surface. Chemical vapor adsorption (CVA) of APDMS was performed at 383 K for 2 h. To confirm the modification reaction, the OH-terminated silicon wafer was also added to the reaction vessel for X-ray photoelectron spectroscopic (XPS) analysis. Figure 2 shows the F_{1s} spectra of the APDMS monolayer on the Si wafer, (*R*)-ATEA immobilized APDMS monolayer and (*S*)-ATEA immobilized APDMS monolayer. The F_{1s} peak was not observed in the APDMS monolayer; in contrast, the F_{1s} peak located at 687.5 eV appeared for (*R*)-ATEA and (*S*)-ATEA immobilized monolayers. The immobilization of ATEA on APDMS monolayer by the condensation reaction was confirmed.

Scheme 2 illustrates the preparation of the DNBP-modified silicon wafer. The immobilization of DNBP was carried out by a similar method to the immobilization of ATEA. Figure 3 shows the N_{1s} spectra of the APDMS monolayer on the silicon wafer and DNBP immobilized APDMS monolayers. The APDMS monolayer exhibited N_{1s} peaks at 399.5 eV originating from amino groups. In the case of (*R*)-DNBP and (*S*)-DNBP immobilized APDMS monolayers, the N_{1s} peak was also observed at 405.9 eV, which can be assigned to the nitrogen of nitro groups. The N_{1s} peak at 399.5 eV can be ascribed to NH and NH_2 groups. These results indicate that DNBP was immobilized on the substrate surface.

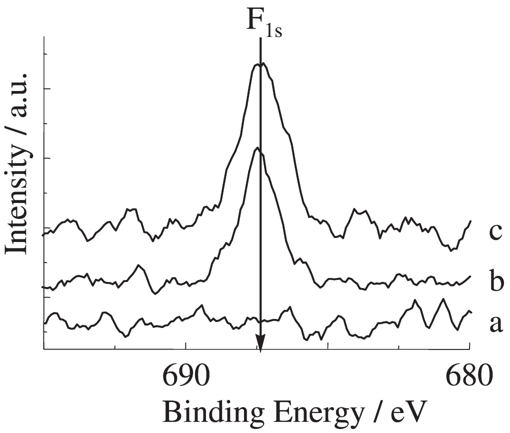
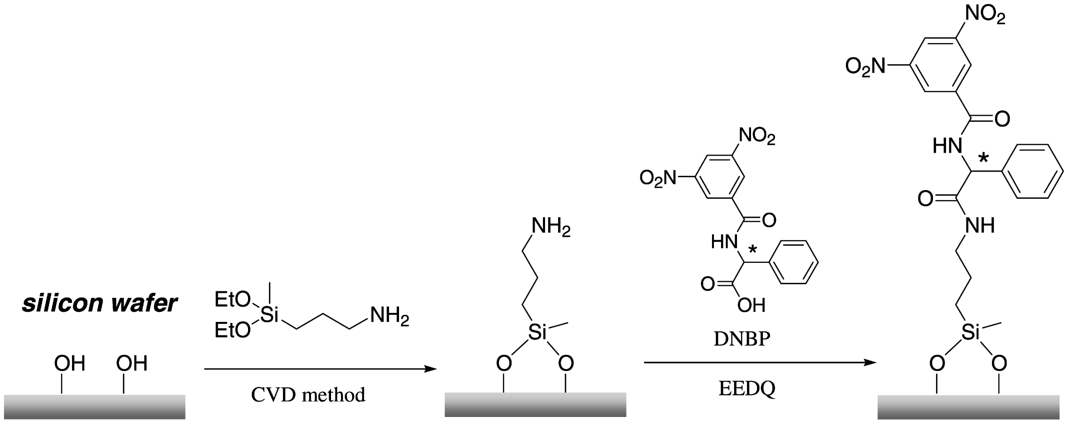


Figure 2. XPS F_{1s} spectra of the APDMS- and ATEA-modified silicon wafer: (a) APDMS, (b) (*R*)-ATEA, (c) (*S*)-ATEA

The modification was also characterized by TOF-SIMS of DNBP- and ATEA-modified APDMS monolayers. In the positive and negative SIM spectra of the ATEA immobilized surface, the fragments at *m/z* 69 (CF_3^- group of ATEA) and *m/z* 178 (anthracene group of ATEA) indicated the immobilization of ATEA on the APDMS monolayer. On the other hand, the fragments at *m/z* 167 and 195, which originated from a dinitrobenzyloxy group, suggested the presence of DNBP on the APDMS monolayer. From these results obtained by XPS and TOF-SIMS, it can be concluded that DNBP and ATEA were successfully immobilized on APDMS monolayers.

Adhesion force measurements

The adhesion force between chiral molecules was determined from force versus cantilever displacement curves. There are four possible combinations of chiral surfaces and tips. In these measurements, the deflection of the



Scheme 2

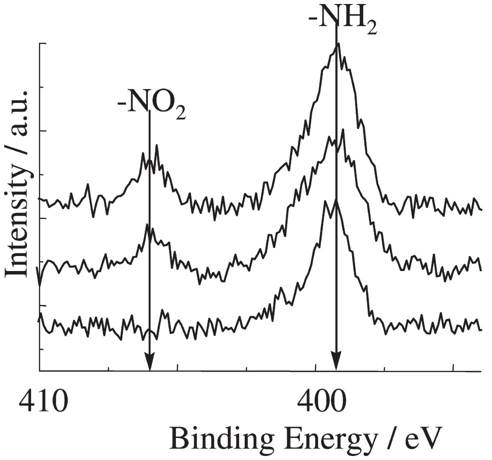


Figure 3. XPS N_{1s} spectra of the APDMS- and DNBP-modified silicon wafer: (a) APDMS, (b) (*R*)-DNBP, (c) (*S*)-DNBP

cantilever was recorded as the sample approaches, contacts and withdraws from the cantilever tip. Figure 4 shows typical force curves obtained by SFM measurements using the ATEA-modified probe tip and DNBP-modified silicon wafer in 2-propanol: (a) (*S*)-ATEA vs (*S*)-DNBP and (b) (*S*)-ATEA vs (*R*)-DNBP. The observed cantilever deflection was converted into a force using the cantilever spring contact. These measurements were carried out in 2-propanol rather than air to eliminate uncertainties arising from capillary forces.

Figure 5 shows the histograms of the adhesion force between chiral molecules obtained using the force curve measurement mode of SFM. The average values of the adhesion force for (*R*)-ATEA vs (*S*)-DNBP and (*S*)-ATEA vs (*R*)-DNBP were greater than those for (*R*)-ATEA vs (*R*)-DNBP and (*S*)-ATEA vs (*S*)-DNBP. In the case of the different chirality pair, an adhesion force >10 nN was observed. These results revealed that the adhesion force for a different chirality pair is stronger than that for the identical chirality pair. The histograms of the adhesion force for the different chirality pair [(*R*)-ATEA vs (*S*)-DNBP and (*S*)-ATEA vs (*R*)-DNBP] showed broader distribution than those for the identical chirality pair [(*R*)-ATEA vs (*R*)-DNBP and (*S*)-ATEA vs (*S*)-DNBP]. Importantly, there was a close resemblance between the histograms obtained in the case of the different chirarity pairs, and a resemblance was also observed in the case of the identical chirarity pairs. This means that the detected adhesion forces are based on the interaction between the enantiomers immobilized on the surface of the probe tip and silicon wafer. Although the chiral recognition of ATEA and DNBP is

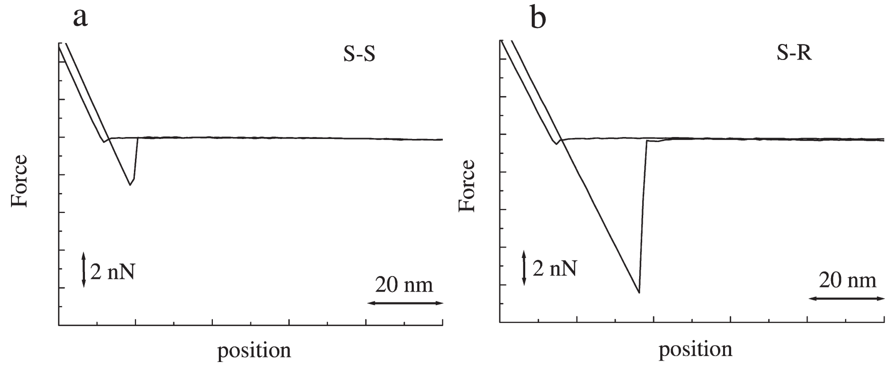


Figure 4. Typical force curves obtained by scanning force microscopy using the ATEA-modified probe tip and DNBP-modified silicon wafer in 2-propanol: (a) (*S*)-ATEA vs (*S*)-DNBP and (b) (*S*)-ATEA vs (*R*)-DNBP

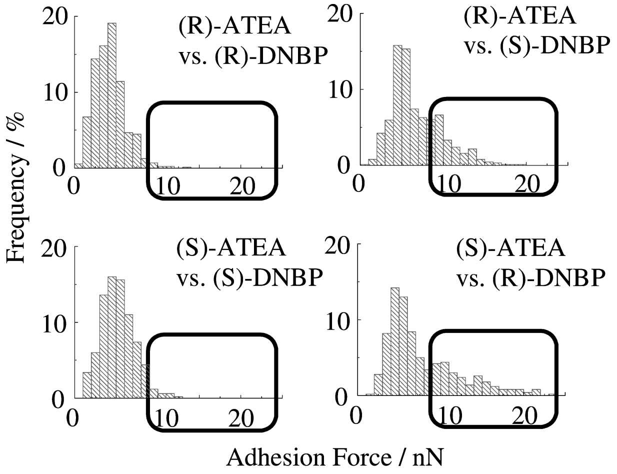


Figure 5. Histograms for adhesion forces between the ATEA-modified probe tip and DNBP-modified silicon wafer in 2-propanol

accomplished by weak interactions such as hydrogen bonding and charge transfer, which have directivity, the present CFM measurement was successfully applied to the model system for chiral recognition in a silica-based column. In the chiral separation of TFAE by HPLC with an (*R*)-DNBP-modified chiral column, it was reported that (*R*)-TFAE was the first to be eluted. The results presented above are in good agreement with the separation behavior in HPLC using silica-based chiral column.

CONCLUSION

We immobilized TFAE and DNBP, a well-known pair of enantiomers, on the cantilever tip and silicon wafer surface, respectively, by the use of aminosilane. The force curves between TFAE and DNBP enantiomers were measured using the force measurement mode of SFM to determine the magnitude of the interaction. Since the SFM measurement can recognize the difference of nanonewton forces, the results obtained can be regarded as a rational design for chiral recognition in a silica-based chiral column and may open the door to the design of novel silica-based chiral columns.

Acknowledgments

This research was partially supported by Grant-in-Aid for Scientific Research No. 1200875189 and COE Research ‘Design and Control of Advanced Molecular Assembly Systems’ (08CE2005) from the Ministry of Education, Culture, Sports, Science and Technology, Japan. T.K. acknowledges financial support from the Japan Society for the Promotion of Science. H.O. acknowledges the financial support from the Foundation Advanced Technology Institute (ATI). The authors thanks Ms M. Tozu and Dr T. Hoshi (Ulvac Phi., Inc) for TOF-SIMS measurements.

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