

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/231672558>

Chiral Discrimination in Langmuir Monolayers of N-Acyl Glutamic Acids Inferred from π -A Measurements and Atomic Force Microscopy

ARTICLE *in* LANGMUIR · JANUARY 2001

Impact Factor: 4.46 · DOI: 10.1021/la0013852

CITATIONS

19

READS

27

6 AUTHORS, INCLUDING:



Yanjie Zhang

James Madison University

36 PUBLICATIONS 2,773 CITATIONS

SEE PROFILE



Yanlin Song

Chinese Academy of Sciences

306 PUBLICATIONS 8,330 CITATIONS

SEE PROFILE

Letters

Chiral Discrimination in Langmuir Monolayers of *N*-Acyl Glutamic Acids Inferred from π -*A* Measurements and Atomic Force Microscopy

Yan Jie Zhang,[†] Yanlin Song,[‡] Yingying Zhao,[†] Tie Jin Li,[†] Lei Jiang,^{*,‡} and Daoben Zhu[‡]

Department of Chemistry, Jilin University, Changchun, China, 130023, and Research Center for Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing, China, 100080

Received October 2, 2000. In Final Form: December 15, 2000

A kind of amino acid amphiphile (*N*-stearoyl-glutamic acid) was synthesized and used to investigate chiral discrimination at the interface by surface pressure–area (π -*A*) isotherm measurements and atomic force microscopy (AFM). On the acidified water and aqueous CdCl₂ solution subphase at pH = 2, monolayers were built up from the L enantiomer and the racemate of *N*-stearoyl-glutamic acid. The π -*A* isotherms indicated that chiral discrimination could be observed on the aqueous CdCl₂ solution more obviously than on acidified water. The monolayers of the racemate were transferred onto newly cleaved mica surfaces for AFM investigation. Molecular images obtained on the monolayer prepared from the acidified water subphase gave a two-molecule rectangular unit cell that accommodates only racemates, whereas the mirror images could be obtained on the monolayer prepared from aqueous CdCl₂ solution, which indicates chiral discrimination in two dimensions.

Introduction

Chiral discrimination in monolayers at the air/water interface has aroused considerable interest because of the influence of chiral molecules on many enzymatic processes at membrane surfaces.^{1,2} *N*-acyl amino acid amphiphiles with a chiral center are good candidates to investigate the chirality effect at the air–water interface.^{3–9} π -*A*

isotherms have been used to demonstrate chiral discrimination effects by comparing monolayers of pure enantiomers and their racemic mixtures.^{2,3,10,11} Depending on the interaction between the enantiomeric forms, two types of enantiomeric mixtures can be distinguished at the interface.¹² Hühnerfuss et al.^{4,9,13} and Simon-Kutscher et al.¹⁴ showed that the presence of bivalent cations in the

* To whom correspondence should be addressed. E-mail: jianglei@infoc3.icas.ac.cn

[†] Jilin University.

[‡] Chinese Academy of Sciences.

(1) Vollbardt, D.; Emrich, G.; Gutberlet, T.; Fuhrhop, J.-H. *Langmuir* **1996**, *12*, 5659.

(2) Harvey, N. G.; Mirajovsky, D.; Rode, P. L.; Verbiar, R.; Arnett, E. M. *J. Am. Chem. Soc.* **1989**, *111*, 1115.

(3) Parazak, D. P.; Uang, J. Y.-J.; Turner, B.; Stine, K. J. *Langmuir* **1994**, *10*, 3787.

(4) Hoffmann, F.; Hühnerfuss, H.; Stine, K. L. *Langmuir* **1998**, *14*, 4525.

(5) Du, X.; Shi, B.; Liang, Y. *Langmuir* **1998**, *14*, 3631.

(6) Du, X.; Liang, Y. *Langmuir* **2000**, *16*, 3422.

(7) Du, X.; Liang, Y. *J. Phys. Chem. B* **2000**, *104*, 10047.

(8) Heath, J. G.; Arnett, E. M. *J. Am. Chem. Soc.* **1992**, *114*, 4500.

(9) Hühnerfuss, H.; Neumann, V.; Stine, K. J. *Langmuir* **1996**, *12*, 2561.

(10) Arnett, E. M.; Harvey, N. G.; Rose, P. L. *Acc. Chem. Res.* **1989**, *22*, 131.

(11) Stine, K. J.; Uang, J. Y.-J.; Dingnam, S. D. *Langmuir* **1993**, *9*, 2112.

(12) Andelman, D. *J. Am. Chem. Soc.* **1992**, *111*, 6536.

(13) Neumann, V.; Gericke, A.; Hühnerfuss, H. *Langmuir* **1995**, *11*, 2206.

(14) Simon-Kutscher, J.; Gericke, A.; Hühnerfuss, H. *Langmuir* **1996**, *12*, 1027.

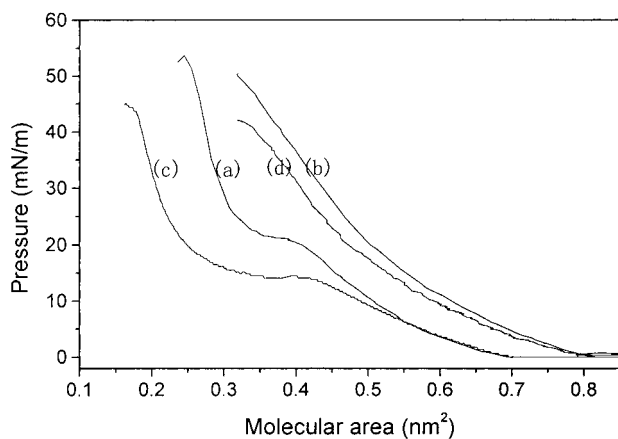
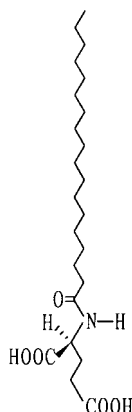


Figure 1. The π - A isotherms of *N*-stearoyl-glutamic acid on acidified water subphase and 2×10^{-4} M CdCl_2 aqueous solution at pH = 2 and 20 °C: (a) L enantiomer on acidified water subphase, (b) DL racemate on acidified water subphase, (c) L enantiomer on CdCl_2 aqueous solution, and (d) DL racemate on CdCl_2 aqueous solution.

Chart 1. Chemical Structure of *N*-Stearoyl-L-glutamic Acid



aqueous subphase may give rise to considerable compression or expansion of the film-forming molecules as well as to an increase or decrease in chiral discrimination in the respective monolayer depending on the kind of cation.

With the development of scanning probe microscopy (SPM), the absolute configuration in chiral molecules can be determined by scanning tunneling microscopy (STM).¹⁵⁻¹⁸ By using atomic force microscopy (AFM), a few chiral symmetry breaking phenomena in Langmuir-Blodgett films have been observed.^{19,20} In this letter, we investigated chiral discrimination at the interface by π - A measurement as well as AFM studies on monolayers transferred onto solid substrates. It is the first time that the chiral discrimination of chiral amino acid derivatives with one chiral center has been directly observed by AFM.

Experimental Section

L and DL *N*-stearoyl-glutamic acid (Chart 1) were synthesized from stearol chloride and L- and DL-glutamic acid by the method

(15) Walba, D. M.; Stevens, F.; Clark, N. A.; Parks, D. C. *Acc. Chem. Res.* **1996**, *29*, 591.

(16) Lopinski, G. P.; Moffatt, D. J.; Wayner, D. D. M.; Wolkow, R. A. *Nature* **1998**, *392*, 909.

(17) Fang, H.; Giancarlo, L. C.; Flynn, G. W. *J. Phys. Chem. B* **1998**, *102*, 7311.

(18) Feyter, S. D.; Gesquière, A.; Grim, P. C. M.; Schryver, F. C. D. *Langmuir* **1999**, *15*, 2817.

(19) Eckhardt, C. J.; Peachey, N. M.; Swanson, D. R.; Takacs, J. M.; Khan, M. A.; Gong, X.; Kim, J.-H.; Wang, J.; Uphaus, R. A. *Nature* **1993**, *362*, 614.

(20) Viswanathan, R.; Zasadzinski, J. A.; Schwartz, D. K. *Nature* **1994**, *368*, 440.

described by M. Takehara et al.²¹ A solution of disodium glutamate was formed by adding sodium hydroxide (2 mol) to a suspension of glutamic acid (1 mol) in the mixture of acetone and water. To this solution, octadecanoyl chloride (1 mol) and sodium hydroxide (1 mol) in water were added dropwise with stirring at 30 °C and pH 12. The reaction mixture was stirred for two additional hours, cooled, and acidified to pH 1 with hydrochloric acid. The precipitated crude crystals were washed in petroleum ether to obtain pure crystals. The melting points of L and DL *N*-stearoyl-glutamic acid are 113–115 and 121–123 °C, respectively. The structures of the compounds were checked by FT-IR and ¹H NMR. IR (cm^{-1}): 3339 (N-H), 2918 and 2850 ($-\text{CH}_3$, $-\text{CH}_2-$), 1731 (carboxyl group), 1644 (amide I), 1542 (amide II). The ¹H NMR measurements were carried out on a Bruker DMX-400 instrument operating at 400 MHz in CD_3SOCD_3 . ¹H NMR (CD_3SOCD_3 , δ , ppm): 0.97 (t, 3H, CH_3), 1.35 (m, 28H, $-\text{CH}_2-$), 1.58 (t, 2H, $-\text{CH}_2-$, attached to the chiral center), 1.86 and 2.05 (m, 2H, $-\text{CH}_2-$, linked to carboxyl group), 4.3 (m, 1H, $-\text{CH}-$, chiral center).

The LB trough used in this study was an RMC-2T multicompartmental round trough from Mayer-Feintech (Germany). Deionized water (Mili-Q system, 18.2 m Ω cm) and CdCl_2 aqueous solution (2×10^{-4}) were acidified by HCl to pH = 2 at 20 ± 0.5 °C. L and DL *N*-stearoyl-glutamic acid were dissolved in chloroform with the concentration of 5×10^{-4} M. The monolayer was compressed at a constant barrier speed of $(3.8-4.0) \times 10^{-4}$ nm²/ (molecule s). The transfers of the monolayers were accomplished by the vertical method onto a newly cleaved mica surface at 25 mN/m. The transfer speed is 10 cm/min. AFM investigations were conducted using a commercial system (Seiko Instruments Inc., SPA300HV, Japan) with a 20 μm scanner. A triangular-shaped Si_3N_4 cantilever with a spring constant of 0.02 Nm⁻¹ was used to acquire images in contact mode. All images were recorded under ambient conditions at 22 °C.

Results and Discussion

For surface-active compounds with a chiral center, the recognition between each other can be sorted into two categories: a preferential D/D or L/L interaction is denoted as "homochiral" behavior, and a preferential D/L interaction is called "heterochiral" behavior.^{4,13} Homochiral interactions are of particular interest because they raise the possibility of phase separation into regions of the L and D enantiomers.²² This is so-called "chiral discrimination" or "chiral symmetry breaking".²³ Because the chiral discrimination at the air-water interface is very sensitive to temperature, we selected 20 °C for the experiments.⁴ Surface pressure/area (π - A) isotherms of the enantiomeric and racemic *N*-stearoyl-glutamic acid spread on an acidified water subphase (pH = 2) are shown in curves a and b of Figure 1, respectively. The isotherm of the L enantiomer exhibits a characteristic of a more condensed monolayer than the isotherm of the racemate, which implies a slightly homochiral interaction. For the enantiomeric monolayer, there is a platform at the pressure of 20 mN/m, which corresponds to the two-phase coexistence. Curves c and d of Figure 1 show the π - A isotherms determined on an aqueous CdCl_2 solution (2×10^{-4} M) at pH = 2 adjusted by HCl. The presence of cadmium ions in the subphase strongly affected the π - A characteristics. These π - A curves are indicative of comparatively compressed monolayers. The platform in the monolayer for the enantiomer on aqueous CdCl_2 solution emerged at 15 mN/m and was more pronounced than that on the acidified

(21) Takehara, M.; Yoshimura, I.; Takizawa, K.; Yoshida, R. *J. Am. Oil Chem. Soc.* **1972**, *49*, 157.

(22) Andelman, D.; Orland, H. *J. Am. Chem. Soc.* **1993**, *115*, 12322.

(23) Selinger, J. V.; Wang, Z. G.; Brunisma, R. F.; Knobler, C. M. *Phys. Rev. Lett.* **1993**, *70*, 1139.

(24) McConnell, H. M. *Annu. Rev. Phys. Chem.* **1991**, *42*, 171.

(25) Green, J.-B. D.; McDermott, M. T.; Porter, M. D.; Siperko, L. M. *J. Phys. Chem.* **1995**, *99*, 10960.

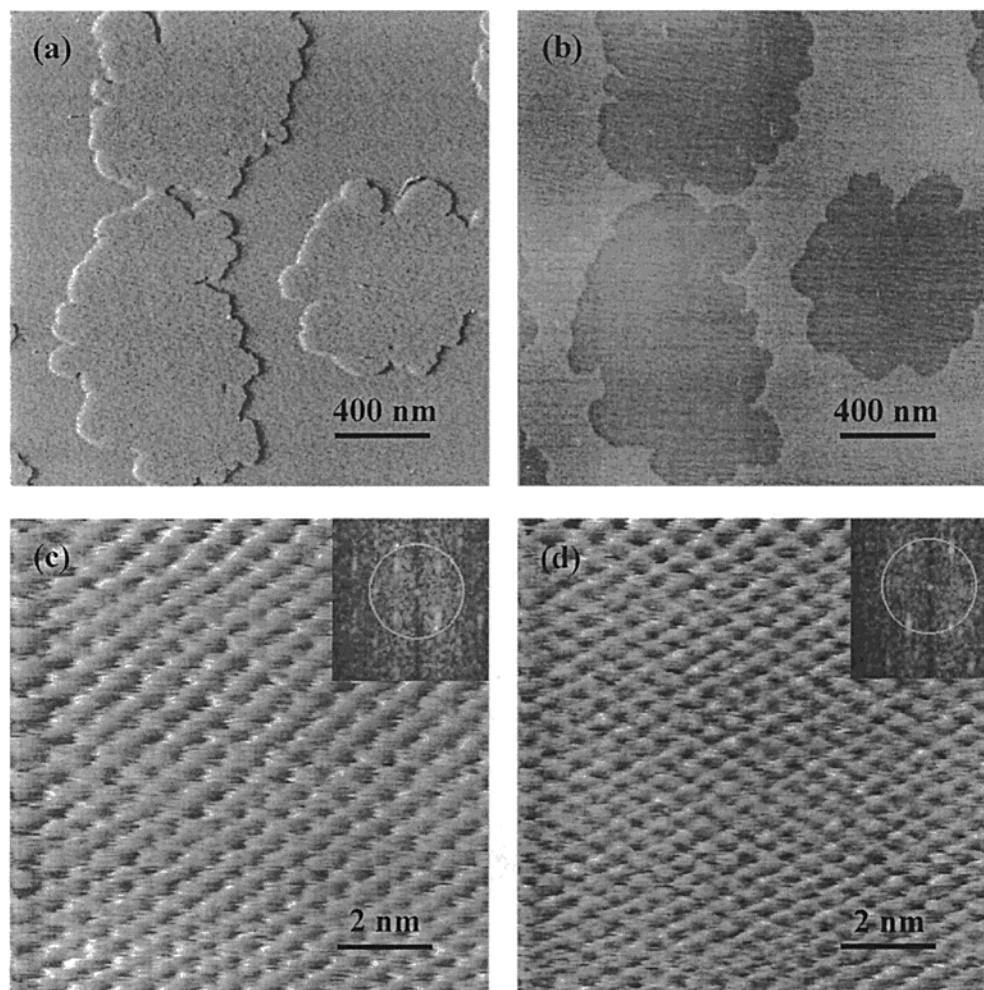


Figure 2. AFM image of a DL *N*-stearoyl-glutamic monolayer on a mica surface prepared from acidified water at pH = 2. (a) Large scale image ($2\ \mu\text{m} \times 2\ \mu\text{m}$). (b) The friction force map of (a). (c) The molecular image of the mica substrate with a corresponding Fourier pattern inset that shows a hexagonal crystal lattice. (d) The molecular image of the DL *N*-stearoyl-glutamic acid monolayer with a corresponding Fourier pattern inset that shows a two-molecule rectangular unit cell.

water subphase. Clear homochiral discrimination was found in the region between $0.4\ \text{nm}^2/\text{molecules}$ and the collapse point. One can expect that the chiral discrimination on the CdCl_2 aqueous solution is more obvious than that on the acidified water. The monolayers were transferred onto a newly cleaved mica surface at the solid region ($25\ \text{mN/m}$) to investigate the morphology by AFM.

With regard to possible methods that can be used for the investigation of chiral amphiphiles, fluorescence or Brewster angle microscopy is well suited for the studies of chiral discrimination in Langmuir monolayers, if chiral symmetry breaking is manifested in the shape of micrometer-sized domains of the ordered phase curving in either direction or showing a dendritic morphology.^{3,24} However, these methods are limited to structures larger than $1\ \mu\text{m}$. AFM is a powerful tool to image surface area for macroscopic and microscopic studies from molecular scale-up tenths of millimeters in size. Figure 2a,b shows the AFM image and the friction force mapping taken from the same area of the DL *N*-stearoyl-glutamic monolayer on a mica surface prepared from acidified water at pH = 2. Domains with an average size of about $1\ \mu\text{m} \times 1\ \mu\text{m}$ were observed over the surface. The friction force mapping is based on the frictional interactions between the chemical functional groups at the outermost few angstroms of microscopic contacting area.²⁵ High brightness in the

friction map corresponds to a high value of surface free energy.²⁶ The friction force mapping (Figure 2b) showed a clear correspondence with the domains in Figure 2a. This indicates that the low area is corresponding to the exposed mica surface. The molecular image of mica and its corresponding Fourier pattern inset are shown in Figure 2c that shows a hexagonal crystal lattice. While the monolayer of DL *N*-stearoyl-glutamic acid on the mica surface shows a two-molecule rectangular unit cell (Figure 2d and the inset), which can accommodate only racemates.²⁰ It suggests that there was no phase separation between the D and L enantiomers in the racemate. D and L enantiomers exist in the same phase although there is an obvious difference in the π -*A* isotherms under this condition.

AFM images of a DL *N*-stearoyl-glutamic monolayer on the mica surface prepared from $2 \times 10^{-4}\ \text{M}$ CdCl_2 aqueous solution at pH = 2 are shown in Figure 3. Small domains with considerably high coverage can be observed in Figure 3a and its zoomed-in image (Figure 3b). The height difference over the surface is less than $0.2\ \text{nm}$. The incorporation of metal ions dramatically changes the morphology of the monolayer. The sizes of the small domains are almost uniform with the average dimension

(26) Takano, H.; Kenseth, J. R.; Wong, S.-S.; O'Brien, J. C.; Porter, M. D. *Chem. Rev.* **1999**, *99*, 2845.

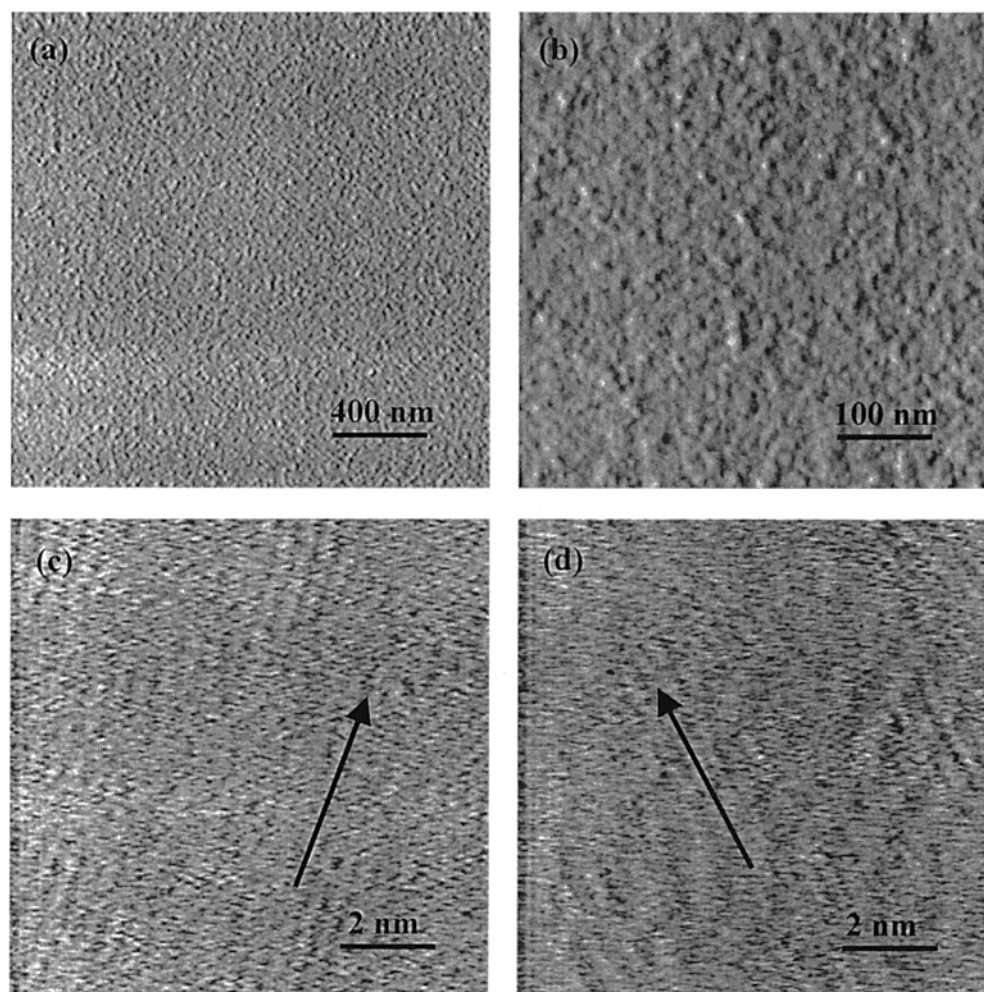


Figure 3. AFM images of a DL *N*-stearoyl-glutamic monolayer on a mica surface prepared from 2×10^{-4} M CdCl_2 aqueous solution at pH = 2. (a) Large scale image ($2 \mu\text{m} \times 2 \mu\text{m}$). (b) Zoomed-in image of (a). (c) A $10 \text{ nm} \times 10 \text{ nm}$ area of the monolayer. (d) A $10 \text{ nm} \times 10 \text{ nm}$ area of the same monolayer as in (c) but from a region where the packing is a mirror image of that observed in (c). The arrows indicate the directions of the molecular packing.

of about $(15 \pm 5) \times (15 \pm 5) \text{ nm}^2$. Figure 3c,d shows typical unenhanced molecular images collected from two different domains. These are the only two structures that can be identified on the surface. Figure 3d has a different sense of molecular packing than Figure 3c. The directions of the molecular packing are indicated by the arrows. The rolls of the molecular packing are a mirror image of those in the other area. This phenomenon had been observed in the monolayer of a chiral tetracyclic alcohol racemate on mica surface.¹⁹ The existence of two mirror images is the direct proof of separate chiral phases. This indicates that the addition of cadmium ions into the subphase enhanced the chiral discrimination effect between the L and D enantiomers at the air–water interface. This effect is induced by the chiral center that exists as a unique asymmetric element in the molecule. This separation of chiral phases on the nanoscale in two dimensions may be considered analogous to the spontaneous resolution of enantiomers in three-dimensional crystallization.

Summary

A kind of chiral amino acid amphiphile was synthesized and used to investigate chiral discrimination at the interface. The surface pressure–area (π – A) isotherms indicated that chiral discrimination could be enhanced on the aqueous CdCl_2 solution as compared to acidified water. Molecular images obtained on the monolayer prepared from the acidified water subphase showed that D and L enantiomers exist in the same phase, whereas the mirror images could be obtained on the monolayer prepared from aqueous CdCl_2 solution, which indicates chiral discrimination in two dimensions. These results suggest that the chiral discrimination effect of chiral amphiphiles that contain only one chiral center in two dimensions could be observed by AFM directly.

Acknowledgment. The authors acknowledge the National Natural Science Foundation of China (NNSFC) for financial support.

LA0013852