Monolayer Formation on Silicon and Mica Surfaces Rearranged from *N*-Hexadecanoyl-L-alanine Supramolecular Structures

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The rearrangements of *N*-hexadecanoyl-L-alanine supramolecular structures in water to form monolayers on silicon and mica surfaces have been investigated using atomic force microscopy. It is the first time that such a monolayer with the polar groups on the solid surface and the alkyl chains up is obtained through a rational molecular design. The monolayer formation results from the strong interaction between the molecular headgroups and the surface, the intermolecular hydrogen bonding interaction via a six-membered ring structure in the case of silicon surfaces, and the additional attractive force in the case of mica surfaces. The anisotropic and dendritic growth structures, clearly observed for the monolayers rearranged on mica surfaces, are indicative of a homochiral effect. The differences in height and morphology of the monolayers on the two types of surfaces are considered to be relevant to the surface roughness and to the interactions between the molecules and the surface.

Introduction

Amphiphilic molecules tend to self-organize into various supramolecular structures in water.[1] In the presence of solid substrates, adsorbed supramolecular structures remain stable on surfaces if the intermolecular forces are stronger than the forces between the molecules and the surfaces.[2] It is known that phospholipid vesicles produce supported bilayers on solid surfaces through vesicle fusion.[3] Although monolayers with their polar groups on the solid surface and their alkyl chains up can be obtained by a simple method of organic solution spreading, i.e., octadecylphosphonic acid monolayers on mica and silicon surfaces,[4−6] such a monolayer rearranged from supramolecular structures in aqueous media has not to date been detected.

Over the past decade, considerable attention has been drawn toward the studies of *N*-acyl amino acid lipids since chirality is a very important phenomenon in nature.[7−16] The amino acid derivatives can assemble into supramolecular structures not only in water[17,18] but also in organic solvents (organogels).[19,20] The monolayer structure and recognition effects in *N*-acyl amino acid monolayers at the air−water interface[7−12,15] and in Langmuir−Blodgett (LB) films[12−16] have been investigated. It was shown that the intermolecular hydrogen-bonding interaction between secondary amide groups and chiral recognition between chiral centers significantly increased the interaction between the hydrophobic alkyl chains.[12] In this paper, *N*-hexadecanoyl-L-alanine is synthesized; on one hand, its hydrophilic headgroup is the simplest chiral amino acid, which is representative of the properties of this family of derivatives; on the other hand, its hydrophobic tail with a C_{16} chain length is suitable for the formation of both stable vesicles and monolayers.

Atomic force microscopy (AFM) has become an important tool to image materials with a high spatial resolution, and one of its significant advantages is the ability to image adsorbed biological molecules on nonconductive substrates. The AFM observation of vesicular morphologies on a mica surface was recently reported.[21] The observed vesicle diameters were consistent with the sizes obtained from transmission electron microscopy (TEM) and dynamic light scattering.[21] In this paper, AFM is used to study both vesicle formation and monolayer rearrangement in order to reduce system errors from different tools used. We not only obtain a deep insight into the interaction between the chiral molecules and the surface and the difference in monolayer morphology but also analyze the probable reason for the formation of such monolayers.

Experimental Section

1. Materials and Aqueous Dispersion Preparations. The synthesis of *N*-hexadecanoyl-L-alanine sample was the same as that of *N*-octadecanoyl-L-alanine reported previously.[12] The water used was double-distilled after a deionized Milli-Q exchange, and the corresponding pH value was about 5.5. The preparation of aqueous *N*-hexadecanoyl-L-alanine dispersion in water was described in the following: 0.2% (w/w) sample in water was heated to its boiling point and then cooled to room temperature. The heating/cooling cycles were repeated in order to wet the sample, after that, the sample sank to the bottom of container. The sonication of the mixture gave a uniform aqueous dispersion. The oily streaks were observed by shaking the dispersion, indicative of the formation of a concentrated lamellar phase. The pH value of the aqueous dispersion was around 6.5.

2. Substrate Cleaning and Sample Preparation. Silicon wafers were cut into the dimension 1 cm × 1 cm, and the cleaning of the silicon wafers was the similar to that reported previously.[22] First, the silicon wafers were ultrasonicated in $CHCl_3$ solvent to remove adsorbed organic contaminants. Second, the silicon wafers were cleaned in an O_2 plasma chamber for 2 min. Third, the silicon wafers were immersed into piranha solution (30% H_2O_2/98% H_2SO_4, 1:3 v/v) under ambient conditions for 30 min followed by copious washing with double-distilled water. Finally, the cleaned silicon wafers were dried in a vacuum for use. Mica plates were freshly cleaved
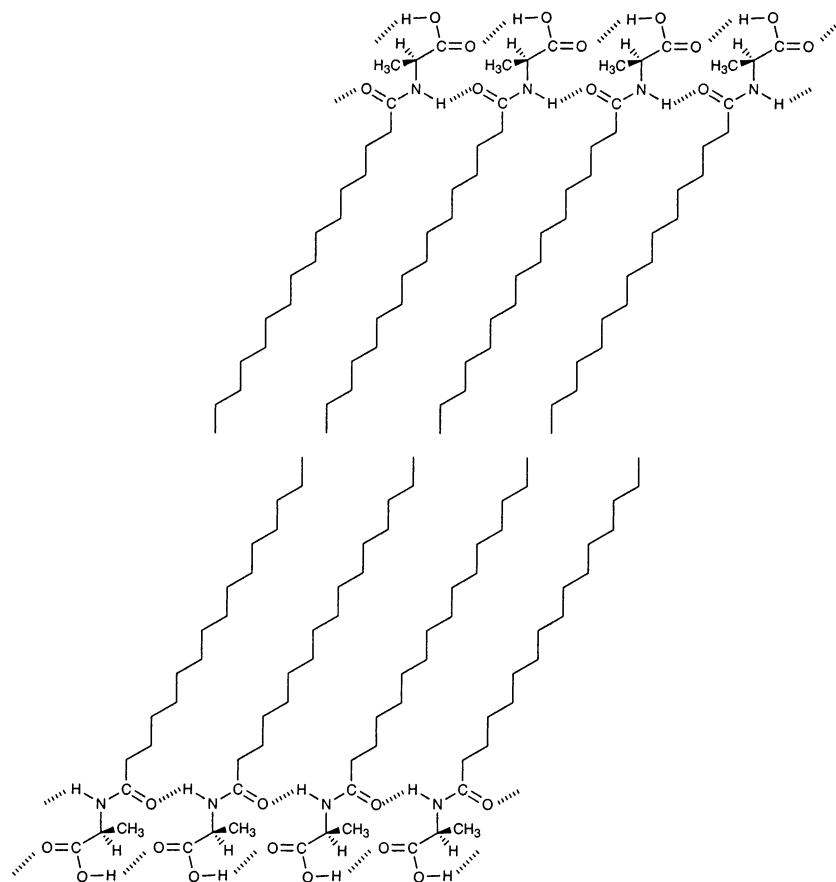
Figure 1. Schematic representation of *N*-hexadecanoyl-ʟ-alanine bilayer membrane in water: an almost parallel arrangement of hydrocarbon chains induced by two-layer arrays of intermolecular hydrogen-bonding interactions through adjacent carboxylic acid and amide groups as well as one-layer array of homochiral effect between chiral carbons.

just prior to use. The same procedure of sample preparation was applied on the silicon and mica surfaces. The desired volumes of the aqueous dispersion were sucked below the liquid surface with a microsyringe. A drop of the dispersion was placed on the substrate surface, and the liquid was rapidly spread. The excess liquid was blotted off with filter paper. The samples were left in ambient conditions (without any washing) for drying.

3. AFM Measurement. AFM images were acquired with contact mode in air on a Nanoscope II SPM (Digital Instruments, Inc.). The force constant of the cantilevers used was 0.06 N/m. The probes were irradiated under a high-intensity UV lamp for about 15 min just prior to imaging to remove organic contaminants from the tip surfaces.

Results and Discussion

1. Supramolecular Structures and Monolayers on Silicon Surfaces. The aqueous *N*-hexadecanoyl-ʟ-alanine dispersion exhibits oily streaks by shaking, characteristic of a concentrated lamellar phase, which suggests the formation of vesicles and other ordered bilayer membranes in pure water. The FTIR spectrum of aqueous *N*-dodecanoyl-ʟ-serine gel, prepared with the procedure similar to that in this paper, indicated that most of the carboxylic acid groups were protonated,[23] while in aqueous 0.01 M KOH solutions, *N*-hexadecanoyl and *N*-octadecanoyl-ʟ-serine molecules formed micellar aggregates.[17] Our previous studies indicated that no significant carboxylate anion was detected from the FTIR spectra of *N*-octadecanoyl-ʟ-alanine LB films transferred from pure water subphase (pH ∼6.0) without the addition of acid.[12] Thus, the two-layer arrays of the intermolecular hydrogen bonding interactions through

adjacent carboxylic acid and amide groups as well as one-layer array of the homochiral effect between the neighboring headgroups induce an almost parallel arrangement of the corresponding hydrocarbon chains to achieve the balance of hydrophilic and hydrophobic interactions, so that the *N*-hexadecanoyl-ʟ-alanine molecules can self-organize into bilayer membranes in water as in the case of monoalkylethylenediamine.[24] The AFM images of the bilayer formation will be shown in the following sections, and the corresponding supramolecular structure is schematically illustrated in Figure 1.

The AFM images of a variety of aggregation morphologies of the dispersions on silicon surfaces are shown in Figure 2. Figure 2a show a large vesicle with the diameter about 500 nm. The vesicle size is very comparable to that of monoalkylethylenediamine in acidic aqueous solution obtained from TEM,[24] the vesicle is thus mostly composed of multilamellar structures. This provides AFM evidence for the formation of bilayer membranes of *N*-hexadecanoyl-ʟ-alanine in aqueous solution. Figure 2b shows the aggregation structure of a multilamellar cylindrical tubule. The shape and size of the tubule is very similar to those of the tubules formed from *N*-dodecanoyl-ʟ-serine observed by TEM[23] and ʟ-glutamic acid-docosadiynedioic acid imaged by AFM.[18] Some chiral amphiphilic molecules with a carbohydrate headgroup are known to self-assemble into nanostructures showing a large variety of morphologies, for example, cylindrical tubules and/or helical ribbons,[25,26] however, no helical ribbon is observed in the AFM images of the aqueous *N*-hexadecanoyl-ʟ-alanine dispersions on the silicon and mica surfaces. The formation of cylindrical tubules results from the contract of helical ribbons.[27] These

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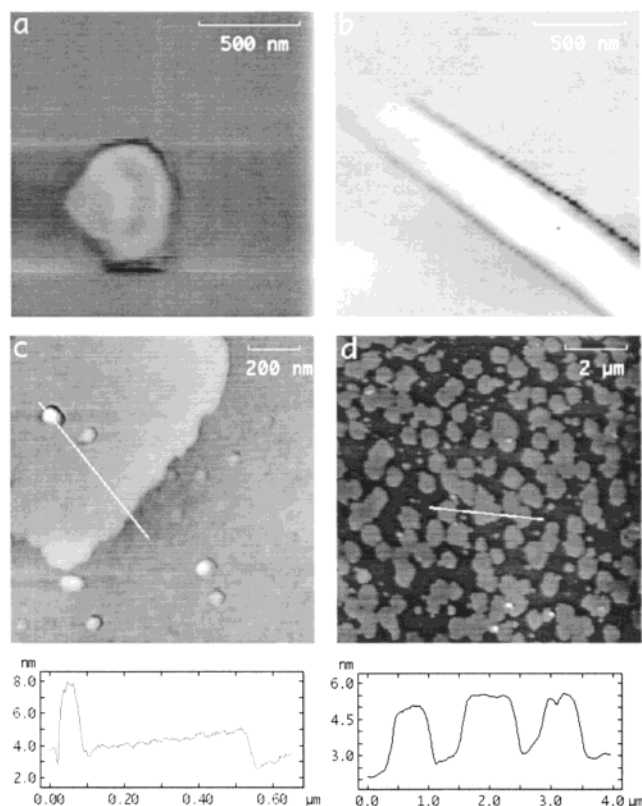

Figure 2. AFM topographical images of self-organized morphologies of aqueous *N*-hexadecanoyl-L-alanine dispersions on silicon surfaces with contact mode in air: (a) large multilamellar vesicle, (b) cylindrical tubule, (c) monolayer and small unilamellar vesicles, and (d) monolayers. The cross-sectional profiles next to (c) and (d) correspond to the lines marked on the respective images.

Figure 3. AFM topographical images of *N*-hexadecanoyl-L-alanine monolayers after arrangement in small scales with contact mode in air.

Figure 4. AFM topographical images of self-organized morphologies of aqueous *N*-hexadecanoyl-L-alanine dispersions on mica surfaces with contact mode in air: (a) large-size monolayer, (b) small-size monolayers, (c) magnified monolayer, and (d) monolayer and vesicles. The cross-sectional profiles next to (c) and (d) correspond to the lines marked on the respective images.

aggregation morphologies indicate that the enantiomeric molecules self-organize into supramolecular structures in aqueous solution. It has been demonstrated that a chiral membrane in a tilted phase forms a cylinder because of an intrinsic bending force due to chirality.[28] This bending force arises because the chiral surfactants do not pack parallel but rather pack at a nonzero twist angle with respect to their neighbors, leading the whole membrane to twist into a helix and to further develop a cylindrical tubule. The vesicle in Figure 2a and the cylindrical tubule in Figure 2b are the aggregation structures of the *N*-hexadecanoyl-L-alanine molecules in aqueous dispersion and remained on the silicon surfaces after the spreading of the dispersion. TEM observations of the cationic surfactants with a sugar headgroup and amino coumarinic acid in water showed a large variety of the morphologies of assemblies occurring in a single sample: vesicles, helices, and tubules.[27] However, the presence of a chiral headgroup was not always sufficient to lead to the formation of helices or tubules.[27] The aggregation morphologies of the *N*-hexadecanoyl-L-alanine in aqueous dispersion are very similar to those previous observations.

Figure 2c shows a large patch on the silicon surface and a few aggregation particles with various sizes. From the corresponding cross-sectional profile of the line marked in Figure 2c the size of the particle lying on the patch can be obtained. It is well-known that the width of separate particles is larger than their actual sizes due to the geometrical tip/sample convolution effect. This does not, however, affect the total vertical displacement of the cantilever. To reduce the influence caused by the tip convolution effect, the full width of half-maximum height of the particle is measured as its size, which may be closer to the actual width than the full width at the baseline level for the
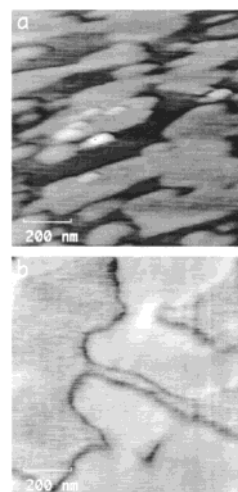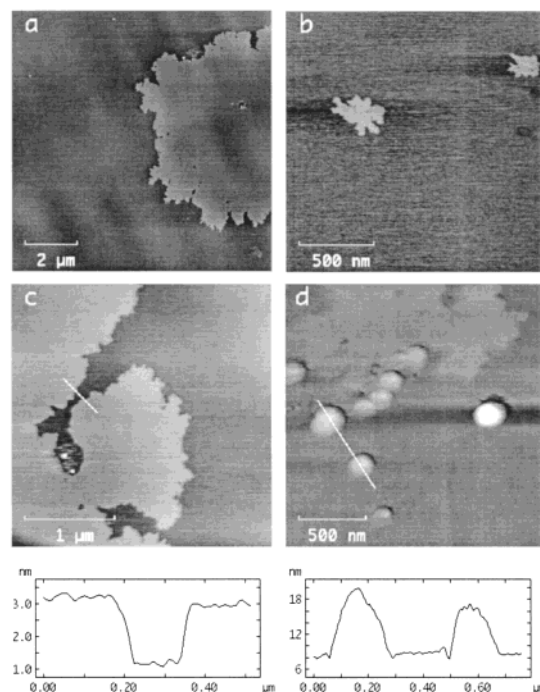
almost spherical particles. The full width of half-maximum height of the particle is shown to be about 53 nm, which is apparently larger than the sizes of spherical micellar aggregates in the range of 2−4 nm and coincides with typical range for small unilamellar vesicles 20−30 nm.[29]

Figure 2d shows a number of irregular patches with different sizes. The average height of the patches in Figure 2c,d is in the range of 2.5−2.6 nm obtained from the corresponding height profiles. The height is very close to the molecular length (2.64 nm) of *N*-hexadecanoyl-L-alanine estimated from the CPK model, which indicates that *N*-hexadecanoyl-L-alanine monolayers are formed on the silicon surfaces due to the rearrangements of the supramolecular structures. The AFM images are similar to those of the transferred monolayers of *N*-octadecanoyl-
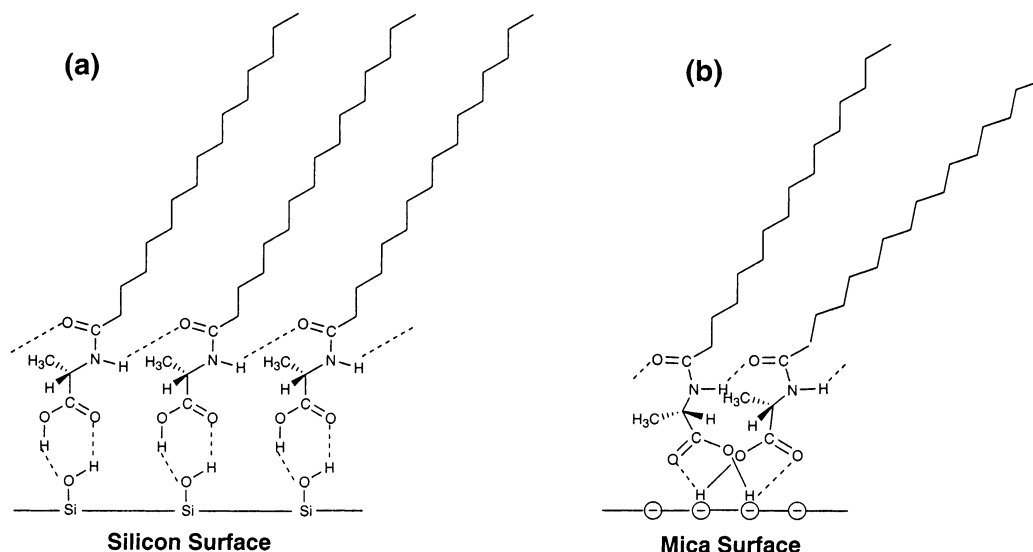
Figure 5. Schematic illustrations of *N*-hexadecanoyl-L-alanine monolayers on two surfaces: (a) intermolecular hydrogen-bonding interaction via six-membered ring structures between carboxylic acid groups and surface hydroxyl groups on silicon surface; (b) additional attractive interaction between hydrogen atoms in carboxylic acid groups and mica surface, together with an out-of-plane dimmer between adjacent carboxylic acid groups and a nonzero twist angle between adjacent hydrocarbon chains.

DL-glutamic acid.[30] The *N*-hexadecanoyl-L-alanine molecules self-organize into bilayer membranes in water with a variety of aggregates such as vesicles (small unilamellar and large multi-lamellar structures) and tubules, and some of the supramolecular structures can rearrange to the molecular monolayers on the silicon surfaces. The clear AFM images of the molecular monolayers in different regions of Figure 2d are given in Figure 3. These monolayer patches have irregular shapes with comparable height. It has been shown that adsorbed supramolecular structures remain stable on surfaces if the intermolecular forces are stronger than the forces between the molecules and the surface.[2] That means that the rearrangements of the supramolecular structures will take place if the intermolecular forces are weaker than the forces between the molecules and the surface. After being treated with O_2 plasma cleaning and H_2O_2/H_2SO_4 immersion, the silicon surfaces are coated with a layer of surface hydroxyl groups. The carboxylic acid groups of the *N*-hexadecanoyl-L-alanine molecules can form six-membered ring structures with the hydroxyl groups on the silicon surfaces through hydrogen bonding interactions (see Figure 5a), which is the driving force for the rearrangements of the supramolecular structures to form monolayers on the silicon surfaces.

2. Monolayers on Mica Surfaces. Figure 4 shows the aggregation morphologies of the aqueous *N*-hexadecanoyl-L-alanine dispersion on mica surfaces. In Figure 4d, several vesicles on the mica surfaces are observed, as well as a large patch of aggregates in the upper part of the image. The cross-sectional profile of the line marked shows that the vesicles in Figure 4d are 154 and 131 nm in diameter, respectively, based on the full width of half-maximum height. Figures 4a, b, and c show the aggregation patches with the sizes ranging from about 200 nm to several micrometers. The average height of the patches in Figure 4c is approximately 1.96 nm, smaller than the extended length of the enantiomeric molecule 2.64 nm. This confirms the formation of enantiomeric monolayers on the mica surfaces as well as on the silicon surfaces. Mica is an atomically flat substrate, while silicon surface has a higher roughness. It is reported that the average roughness of silicon surfaces is 0.35 nm after cleaning with organic solvents, and 0.2−0.42 nm after etching by 53% HF solution with time.[31] The surface roughness of the silicon surfaces is a probable reason for the difference in

monolayer height on the silicon and mica surfaces. In Figure 4, the small-size monolayers exhibit the feature of dendritic growth, and the large-size monolayers display the characteristics of anisotropic growth. Whereas in Figures 2 and 3, neither anisotropic growth nor dendritic growth is observed for the monolayers but the irregular shapes. This discrepancy is considered to result partly from the higher roughness of the silicon surfaces. The fingerlike structures for the rearrangement of metastable micelles to bilayers were observed on the mica surface, and not on the silicon surface.[32] It is well-known that mica is a natural aluminosilicate material with a hydrophilic negatively charged surface. An additional attractive force (possible hydrogen-bonding interaction[5]) between the hydrogen atoms in the carboxylic acid groups of the enantiomeric molecules and the negatively charged surface may be a probable reason for the formation of the stable monolayers.

The anisotropic and dendritic growth of the *N*-hexadecanoyl-L-alanine monolayers on the mica surfaces observed by AFM is very similar to that of the same molecular monolayers on water surface observed by fluorescence microscopy,[8] which is a sensitive means for studying the chiral discrimination of enantiomeric and racemic monolayers.[8] This provides new evidence for the occurrence of the homochiral effect between the enantiomeric molecules. On the basis of Nandi's effective pair potential approach,[33] which may predict the senses of helical amphiphilic assemblies including chiral monolayers and bilayers, the pairwise intermolecular interaction between the groups attached to the chiral carbons of the neighboring molecules depends on the size of attached groups, their separation distance, and their relative orientation. The sizes of the four groups attached to the chiral carbons of the molecules decrease as follows: $CH_3(CH_2)_{14}CONH > COOH > CH_3 > H$. In the plane of CH_3 and H groups, the adjacent CH_3 groups are farther away from each other compared to the two H groups. Similarly, in the plane of the $CH_3(CH_2)_{14}CONH$ and COOH groups, the adjacent $CH_3(CH_2)_{14}CONH$ groups are farther away, and the neighboring COOH groups are closer to each other, which would force the two COOH groups to rotate around their respective C^*-C bonds to deviate from the $C-C-C$ planes of alkyl chains and to form an out-of-plane dimer between the neighboring molecules. The formation of the out-of-plane carboxylic acid

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dimer in a 1-monolayer LB film of *N*-octadecanoyl-L-alanine has been confirmed by FTIR spectroscopy.[12] The minimum-energy configuration of a pair of the same enantiomers favors a twisted angle between them, and the twist from neighbor to neighbor gives rise to chirality of the aggregate. The macroscopic anisotropic growth of the monolayers in Figure 4 originates from the microscopic twisted orientations of groups attached to the chiral carbons in the condensed phases. The molecular structures of the *N*-hexadecanoyl-L-alanine monolayers on the silicon and mica surfaces are schematically shown in Figure 5. Seen from this figure, the difference in the interaction between the carboxylic acid headgroups and the surface may have an influence on the interaction between the adjacent enantimeric molecules, which would result in differences in chain orientation and chiral recognition of the enantiomeric molecules on the silicon and mica surfaces. This may be another contribution to the differences in the height and morphology of the monolayers on the two surfaces.

3. Probable Reason for Monolayer Formation. As shown above, the stronger interaction between the chiral molecules and the surface is necessary for adsorbed supramolecular structures to rearrange. It was reported that the metastable micelles of Ru-coordinated bis(2,2′-bipyridyl) dihexadecyl-2-(2,2′-dipyridyl-methylene) malonate rearranged to different molecular bilayers on silicon and mica surfaces.[32] It is obvious that the metastable aggregates easily undergo rearrangements on the solid surface. Taking into account the shape-structure or packing parameter concept,[34] the molecules with large cross-sectional metal-coordinated headgroups cannot form stable monolayers but interdigitated bilayers on the surface. *N*-Octyl-D-gluconamide fibrous aggregates were only observed to rearrange to bilayers on gold and silicon surfaces.[35] It is known that the amphiphiles with a C_8 chain length are unfavorable to the formation of stable monolayers. The chiral molecules *N*-hexadecanoyl-L-alanine can self-organize into supramolecular structures (bilayer membranes) in water. It is possible for the chiral molecules to form stable monolayers on the solid surface due to suitable headgroup size and alkyl chain length. Stable *N*-hexadecanoyl-L-alanine monolayers at the air−water interface have been reported.[8,9] It is shown that the intermolecular hydrogen-bonding interaction and homochiral effect between the hydrophilic headgroups considerably enhance the interaction between the hydrophobic chains. The monolayers, with their polar headgroups on the solid surface and their alkyl chains up, are rearranged from the corresponding supramolecular structures in water through a rational molecular design.

Conclusions

The self-organized morphologies of aqueous *N*-hexadecanoyl-L-alanine dispersions on silicon and mica surfaces have been studied using atomic force microscopy. A variety of morphologies of organized molecular assemblies are observed on the two types of surfaces: vesicles (large multilamellar and small unilamellar structures), cylindrical tubules, and enantiomeric monolayers. The existence of the vesicles and tubules indicates that ordered bilayer membranes are formed from the enantiomeric molecules in aqueous solution. The formation of the monolayers results from the rearrangements of the bilayer membranes. The driving force for the monolayer formation is

considered to be the additional interaction between the carboxylic acid headgroups of the molecules and the surfaces of the substrates, such as intermolecular hydrogen bonding interaction in the case of silicon surfaces and strong attractive force in the case of mica surfaces. The anisotropic and dendritic growth structures of the monolayer aggregates are observed on the surface of atomically flat mica, indicative of homochiral discrimination between the enantiomeric molecules. The differences in height and morphology of the monolayers on the silicon and mica surfaces are due to the higher roughness of the silicon surfaces, as well as chain orientation and intermolecular interaction induced by the interaction between the carboxylic acid headgroups and the surface.

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