J|A|C|S
COMMUNICATIONS

Published on Web 12/18/2003

Enantioselective Crystal Growth of Leucine on a Self-Assembled Monolayer with Covalently Attached Leucine Molecules

Naoki Banno, Takuya Nakanishi,* Mariko Matsunaga, Toru Asahi, and Tetsuya Osaka*

Department of Applied Chemistry, School of Science and Engineering, Waseda University, Shinjuku, Tokyo 169-8555, Japan

Received August 15, 2003; E-mail: osakatet@waseda.jp

Monolayer films with an ordered molecular arrangement, such as Langmuir monolayers at the air/solution interface, provide sites for crystal nucleation and affect the growth of crystals beneath the monolayer.[1] Self-assembled monolayers (SAMs) are also reported to influence the nucleation and growth of molecular crystals formed on the SAMs.[2,3] The monolayer-induced crystallization is important from the viewpoint of molecular recognition at the interface between the monolayer and solution. On the other hand, the attachment of amine molecules on the SAM with amide linkage formation[4] enables one to immobilize either one of the two enantiomeric forms of chiral amino acids, and the enantiomer-attached SAM thus prepared is expected to play a significant role in elucidating crystallization phenomena on the SAM. Indeed, the possibility of preferential crystallization of enantiomeric molecules has been suggested from the enantioselective mass change observed in a study using a quartz crystal microbalance (QCM) modified with enantiomeric seeds,[5] although no experimental evidence of crystallization has been shown. In the crystal of a hydrophobic amino acid such as leucine, the monolayers are packed to form a bilayer assembly, in which the linkage between side chains connects the hydrophobic edges of the planes through van der Waals forces (Figure 1).[6] In considering the fact that molecules in the solution first self-assemble into a prenucleation aggregate with a supramolecular structure resembling the crystal structure of a mature crystalline phase,[1−3] the interfacial interaction between the immobilized layer and the prenucleation aggregate is essential. We report here on the enantioselective crystal growth of leucine on a solid surface modified with the SAM, to which leucine molecules are covalently attached, as evidenced by the X-ray diffraction (XRD) method. It is suggested that the enantiomers attached to the SAM play an important role in inducing enantioselectivity through the interaction with prenucleation aggregates formed in solution.

A gold substrate with predominantly (111)-oriented surfaces was prepared by vapor deposition on a quartz crystal (AT-cut, 9 MHz). After being flame annealed, the substrate was immersed into a 2 mM ethanolic solution of 11-mercaptoundecanoic acid (MUA) for 24 h to prepare the SAM. The covalent attachment of leucine onto the SAM of MUA was performed by employing essentially the same procedure as described in the literature,[5] except for the use of a phosphate buffer (pH 7.5) instead of tris-HCl to avoid amide formation with tris(hydroxymethyl)aminomethane. The crystals on the SAM were grown by immersing the substrates in a saturated solution of D-, L-, or DL-leucine at 30 °C. To remove "excess" crystallites, which are simply adsorbed and not crystallized on the covalently attached leucine, the modified substrates were first carefully rinsed with ethanol, in which the grown crystal of leucine was hardly dissolved, and then exposed to a stream of nitrogen. XRD patterns were obtained with a Rigaku RINT-TTR diffractometer with 50 kV and 200 mA Cu Kα radiation (λ = 1.542 Å).

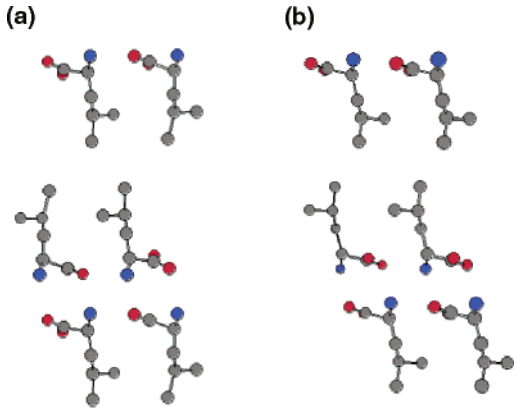

Figure 1. Schematic representation of the double layer of crystals of (a) L-leucine and (b) DL-leucine. Carbon, oxygen, and nitrogen atoms are represented by gray, red, and blue, respectively. Hydrogen atoms are not shown.

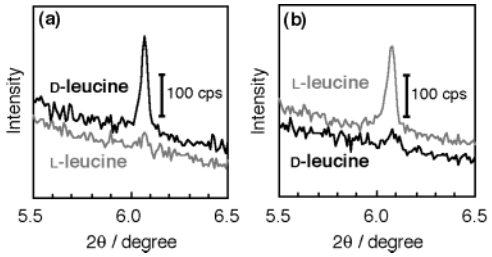

Figure 2. XRD patterns of (a) D- and (b) L-leucine-attached SAMs after immersion in pure enantiomeric leucine solutions.

The diffraction angles of the crystallites grown on the SAM were calibrated against those of the gold substrate.

Figure 2 shows XRD patterns of the specimens with an enantiomeric leucine-attached SAM after immersion in a 175 mM D- or L-leucine solution for 3 h.[7] For the D-leucine-attached SAM, the diffraction peak was observed only after it was immersed in the D-leucine solution, whereas no peak appeared when it was immersed in the L-leucine solution (Figure 2a). With the L-leucine-attached SAM (Figure 2b), exactly the opposite results were obtained. The diffraction angles of the two observed peaks were identical to each other and equal to 6.07°. As both L-leucine and D-leucine crystallize in the monoclinic space group $P2_1$ (a = 14.666 Å, b = 5.324 Å, c = 9.606 Å, β = 94.06°),[8] the observed peaks are attributed to (100) reflection of the leucine crystal. These results demonstrate that one enantiomer with the same chirality as that immobilized on the SAM crystallizes preferentially as compared with the other enantiomer. In other words, the above-mentioned cross inversion between D- and L-leucine strongly suggests that the crystallization on the leucine-attached SAM is highly enantioselective. This enantioselectivity was also confirmed by the increase in surface mass resulting from the grown leucine crystals on the

10.1021/ja037942z CCC: $27.50 © 2004 American Chemical Society

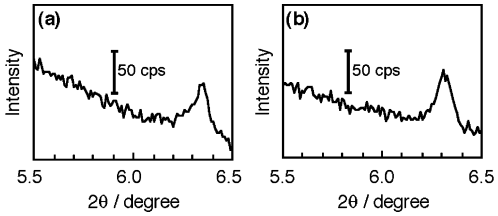

Figure 3. XRD patterns of (a) D- and (b) L-leucine-attached SAMs after immersion in racemic leucine solution.

modified SAM detected by QCM, which allows one to estimate the change in mass from the change in oscillating frequency of the quartz crystal. A decrease in frequency by ~1100 Hz, corresponding to a mass increase of ~6 μg cm^{-2}, was observed after the specimens were subjected to the same treatment as that for the XRD samples,[9] whereas no significant frequency shift was observed for the specimens of D/L pairs. The sharpness of the observed diffraction peaks suggests a high crystallinity of the phase formed on the SAM, resembling that of bulk single crystals. These results indicate that SAM with one immobilized enantiomer serves as a suitable substrate for crystallization of the same enantiomer but not for the other enantiomer because of the geometric match between the enantiomers attached on the SAM and the prenucleation aggregates. The reason the nucleation on the SAM precedes the bulk crystallization is likely to be that heterogeneous nucleation on the substrate surface is more favorable than homogeneous nucleation, as the former generally causes interfacial interaction with prenucleation aggregates to lower their surface energy.[2]

As shown in Figure 3, a diffraction peak was observed at 6.34° for both D- and L-leucine-attached SAMs after they were soaked for 21 h in a 76 mM DL-leucine solution.[10] The formation of a crystalline phase corresponding to the mass increase of ~2 μg cm^{-2} was suggested by the result of a QCM experiment performed by using specimens treated under the same conditions.[9] Because the DL-leucine crystal belongs to the triclinic space group $P\bar{1}$ (a = 14.12 Å, b = 5.39 Å, c = 5.19 Å, α = 111.1°, β = 97.0°, γ = 86.4°),[11] the observed peak can be attributed to the (100) plane of the DL-leucine crystal. In the presence of equimolar amounts of D- and L-enantiomers of leucine, the racemic crystal tends to form instead of either one of the two pure enantiomeric crystals. Because of the lower solubility of DL-leucine than L-leucine, it is assumed that the growth of prenucleation aggregates or clusters into racemic crystals predominates over the formation of pure enantiomeric crystals. As shown in Figure 1, leucine molecules with two different conformations appear in each enantiomorphic crystal, while leucine molecules of racemate form only one conformation. A single layer of enantiomeric or racemic crystals always contains only the L- or D-configured molecules; the relative orientation of the layers is also different in the L and the DL crystals. Thus, only the side chains determine the mode of stacking of the layers.[6] Molecules of leucine are packed together with their side chains located in an asymmetric manner, in which chiral effects are prominent as is reflected on the solubility.

It should be noted that the primary interaction between the enantiomers attached on the SAM and prenucleation aggregates formed in the bulk of the solution is not due to hydrogen bonding but to hydrophobic or van der Waals interaction. Thus, the origin of the enantioselectivity seen in Figure 2 is accounted for by the hydrophobic (or van der Waals) interaction. It is assumed that the crystal growth proceeds only when the chirality of the prenucleation aggregate of pure enantiomers formed in solution is the same as that of the attached enantiomer. In the racemic solution, on the contrary, most of the prenucleation aggregates are considered to be in DL-form, resulting in the growth of the racemic crystalline phase on both D- and L-leucine-attached SAMs as shown in Figure 3. Furthermore, we investigated the crystallization from an unsaturated leucine solution containing one enantiomeric form in excess over the other. It should be noted here that, for the D-leucine-attached SAM, the distinct peaks attributable to both enantiomeric and racemic crystals were observed at 2θ of 6.10° and 6.37°, respectively, after immersion in the solution containing excess D-form, while neither peaks were observed with the solution containing excess L-form. This phenomenon is peculiar to monolayer-induced crystallization. Details including the effect of the degree of enantiomeric excess are currently under investigation.

In summary, enantioselective crystal growth was found to occur on the SAM depending on the chirality of the enantiomer attached. Further investigations on other amino acids may lead to the elucidation of the origin of enantioselectivity in the crystallization of amino acids.

Acknowledgment. This work was supported in part by the Grant-in-Aid for COE Research "Molecular Nano-Engineering" from the Ministry of Education, Culture, Sports, Science, and Technology, Japan. This work was carried out under the 21st Century COE program "Center for Practical Nano-Chemistry". T.N. acknowledges the Research Fellowship of the Japan Society for the Promotion of Science for Young Scientists. Assistance in manuscript preparation provided by Dr. Yutaka Okinaka is gratefully acknowledged.

References

(1) Rapaport, H.; Kuzmenko, I.; Berfeld, M.; Kjaer, K.; Als-Nielsen, J.; Popovitz-Biro, R.; Weissbuch, I.; Lahav, M.; Leiserowitz, L. *J. Phys. Chem. B* **2000**, *104*, 1399−1428.

(2) Frostman, L. M.; Bader, M. M.; Ward, M. D. *Langmuir* **1994**, *10*, 576−582.

(3) (a) Kang, J. F.; Zaccaro, J.; Ulman, A.; Myerson, A. *Langmuir* **2000**, *16*, 3791−3796. (b) Lee, A. Y.; Ulman, A.; Myerson, A. S. *Langmuir* **2002**, *18*, 5886−5898.

(4) (a) Duevel, R. V.; Corn, R. M. *Anal. Chem.* **1992**, *64*, 337−342. (b) Frey, B. L.; Corn, R. M. *Anal. Chem.* **1996**, *68*, 3187−3193.

(5) Eun, H.; Umezawa, Y. *Anal. Chim. Acta* **2000**, *413*, 223−227.

(6) Schade, B.; Fuhrhop, J.-H. *New J. Chem.* **1998**, *22*, 97−104.

(7) Immersion for a longer period of time resulted in the formation of precipitates on the bottom of the beaker and at the edges of the substrate.

(8) Coll, M.; Solans, X.; Fony-Altaba, M.; Subirana, J. A. *Acta Crystallogr.* **1986**, *C42*, 599−601.

(9) QCM measurements were carried out in air before and after the specimens were treated by the same procedure as that used for the XRD samples.

(10) No precipitate was observed in the beaker.

(11) Di Blasio, B.; Pedone, C.; Sirigu, A. *Acta Crystallogr.* **1975**, *B31*, 601−602.

JA037942Z