Effect of Substrate Morphology on the Crystallization of Leucine on Gold Surface Modified with a Self-assembled Monolayer

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The effect of the surface morphology of gold-deposited quartz substrate on the enantioselective crystallization of leucine was investigated by using quartz crystal microbalance (QCM) and X-ray diffraction methods. The gold substrate was modified with a self-assembled monolayer with covalently attached D-leucine molecules. For a rough surface with root-mean-square (RMS) roughness of 17 nm, the QCM measurement showed a continuous decrease in frequency, while for a smooth surface with RMS roughness of 2 nm, essentially no change in frequency was observed. Nevertheless, leucine crystals with high crystallinity were clearly formed on the smooth surface.

Development of chiral sensors is important because even a small amount of enantiomeric impurities in the body may cause severe diseases. We previously investigated, using quartz crystal microbalance (QCM)[1,2] and X-ray diffraction (XRD) measurements,[3] enantioselective phenomena with the aim of constructing chiral sensors. Eun and Umezawa, using a QCM system as a chiral sensor, suggested that an enantioselective crystal growth of leucine (Leu) proceeds on the gold substrate modified with an enantiomer-attached, self-assembled monolayer (SAM).[4] The crystal growth on solid surfaces modified with an organic monolayer has been actively studied also in the field of crystal engineering, because the terminal functional groups of the monolayer control preferred orientation, polymorphism, and morphology of the grown crystals.[5–11] We also focused our attention on a QCM system and confirmed additionally by XRD that enantioselective crystal growth occurred depending on the chirality of the attached Leu enantiomer.[3] The present paper reports our finding that the substrate morphology significantly influences the QCM response at the initial stages of crystallization of Leu.

Two kinds of substrate with different morphologies were prepared by vapor deposition of gold on quartz crystals (AT-cut, 9 MHz) at different gold deposition rates and different polishing conditions of the quartz. A "rough" gold surface was prepared at the gold deposition rate of 2.0 Å s^{-1} on a quartz crystal lapped with #4000 emery paper, while a "smooth" gold surface was prepared at the deposition rate lower than 0.2 Å s^{-1} on a mirror finished quartz surface. The roughness factor (f_r: real surface area/geometrical area) of each gold surface was calculated from a cyclic voltammogram recorded in 1 M H_2SO_4 based on the charge associated with the electrochemical stripping of the oxide layer on gold surface. In addition, root-mean-square (RMS) roughness was measured by atomic force microscopy. The SAM of 11-mercaptoundecanoic acid (MUA) with covalently attached D-Leu molecules was formed on the gold surface by employing the procedure described in the literature.[3] The crystal growth on the SAM-modified surfaces was monitored by QCM using the following experimental procedure: 3 mL of D- or L-Leu was added carefully with stirring into 70 mL of a background solution (1.25×10^{-4} M of DL-Leu in 0.1 M phosphate buffer: pH 7.5). The concentration of added D-Leu solution was varied from 3 to 180 mM. Crystalline phases on the SAM-modified surfaces were analyzed by XRD (50-kV, 200-mA, Cu Kα radiation: $\lambda = 1.542$ Å). The diffraction angles of the crystallites grown on the SAM were calibrated against those of the gold substrate.

Figure 1 compares "rough" and "smooth" surfaces for the time dependence of the change in QCM frequency observed with the D-Leu attached SAM/gold/quartz after the addition of D- or L-Leu solution. After the frequency of QCM became stable within ±1 Hz for at least 5 min in the background solution, 3 mL of D- or L-Leu was added carefully to initiate the crystallization. Here, f_r and RMS roughness of the "rough" surface were 2.5 and 17 nm, respectively, while those of the "smooth" surface were 1.3 and 2 nm. No clear enantioselectivity was observed in the frequency change on the "smooth" surface (Figure 1b), whereas for the "rough" surface, enantioselectivity was reproducibly observed (Figure 1a) in agreement with the finding of Umezawa's group.[4] Namely, for the QCM with a "rough" surface, a continuous decrease in frequency was observed for more than 1000 s after the addition of D-Leu solution. The decrease in frequency amounting to ca. 20 Hz observed after 1000 s corresponds to a mass increase of ca. 0.12 μg cm^{-2}, which is approximately equivalent to one fifth of that area which is covered with an assumed bi-layer unit of Leu molecules.[11] On the other hand, after the addition of L-Leu, only spikes and unstable response were observed before the recovery of frequency to the initial values. In contrast, for the QCM with "smooth" surface, the fre-

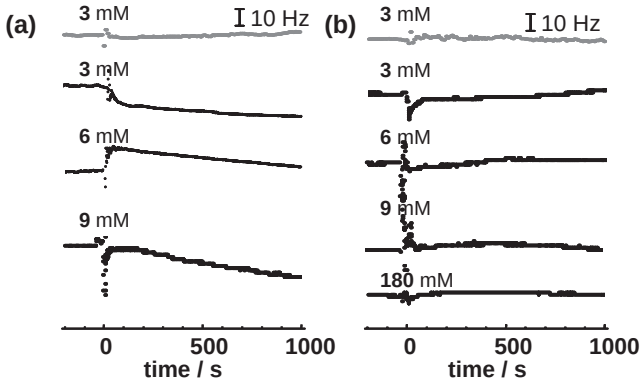

Figure 1. Change in frequency of D-Leu-attached QCMs with (a) "rough" and (b) "smooth" surfaces. At the time of 0 s, 3 mL portions of L- (gray curve) and D-Leu (black curve) of different concentrations were added into the background solution (1.25×10^{-4} M DL-Leu in 0.1 M phosphate buffer).

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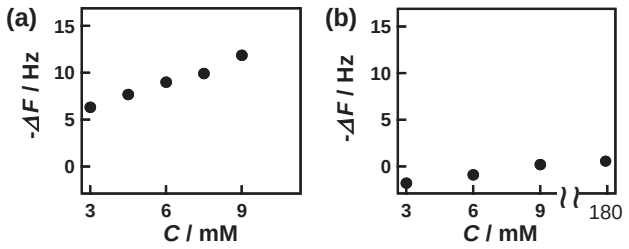

Figure 2. Concentration dependence of frequency change of QCM observed in 10 min with (a) "rough" and (b) "smooth" surfaces. *C* is the concentration of the added D-Leu solution.

quency became stable within ±2 Hz for 1000 s after the appearance of spikes and unstable response not only after the addition of L-Leu but also after the addition of D-Leu.

Figure 2 shows the concentration dependency of frequency change ($-\Delta F$) during the period of 10 min after the appearance of spikes and unstable response. This dependence is suggested to reflect the crystallization rate. For the "rough" surface, the increase in concentration of added D-Leu solution (C) increased $-\Delta F$ within the concentration range of 3 to 9 mM (Figure 2a). For the "smooth" surface, it appears that the increase in C slightly increased $-\Delta F$ in the concentration range of 3 to 180 mM although this dependence is not clearly visible in Figure 1b. These results suggest that the "smooth" surface as well as the "rough" surface initialize the crystallization, although the conditions are not optimal for chiral sensing. Because the difference in $-\Delta F$ between the "rough" and "smooth" substrates is too large to be attributable solely to the difference in real surface areas, it is considered that the crystallization behavior depends on the surface morphology of the substrate.

To investigate the difference in crystallization behavior of Leu, the crystalline phases grown on both the "rough" and "smooth" surfaces were examined by XRD. With the solutions of the same concentrations as were used for the QCM measurements, it was difficult to detect XRD peaks attributable to the crystalline phase of Leu even after the incubation period of 72 h. Therefore, the concentration of background solution was increased 60 times that used in the QCM measurements, i.e., 70 mL of 9.0×10^{-3} M of DL-Leu with 3 mL of 1.82×10^{-1} M D- or L-Leu. As shown in Figure 3, the XRD peaks at 2θ values of 6.07 and 6.31° attributable to (100) of D-Leu crystal[12] and (100) of DL-Leu crystal,[13] respectively, were observed with D-Leu, whereas no peak was observed with L-Leu for either substrate. It is important to note that the intensities of observed peaks were different between the "rough" and "smooth" substrates. The mass increases on the "rough" and "smooth" surfaces were roughly estimated to be 3.0 and 5.0 μg cm^{-2}, respectively. They were measured by QCM in air for the specimens before and after the same treatment as for the XRD samples, because precise measurements could not be performed in the highly concentrated background solution. In view of the fact that the difference in XRD peak intensity shown in Figure 3 was relatively large compared to that in mass increase, it is suggested that crystals with low crystallinity grew on the "rough" surface, whereas crystals with high crystallinity grew on the "smooth" surface.

In view of the general rule that the more frequently the nucleation takes place, the lower the crystallinity, it is considered that the continuous frequency change in Figure 1a reflects continuous nucleation taking place on the "rough" surface, while the very small frequency change in Figure 1b reflects essentially no continuous nucleation progressing on the "smooth" surface.

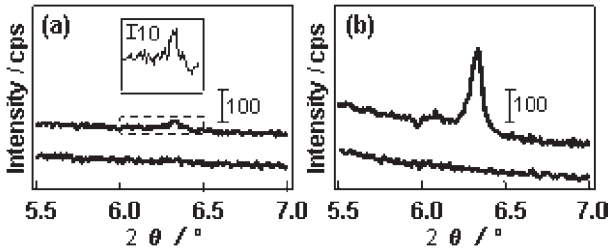

Figure 3. XRD patterns of specimens on (a) "rough" and (b) "smooth" substrates modified with D-Leu-attached MUA SAM. Each modified substrate was incubated in 9.0×10^{-3} M DL-Leu for 20 h after addition of 3 mL of 1.82×10^{-1} M D- (upper curves) and L-Leu (lower curves).

In summary, we confirmed that nucleation as well as crystal growth on the gold substrates modified with D-Leu attached SAM is influenced greatly by the surface morphology of substrate. Our finding is believed to be significant for optimizing substrate conditions in the fabrication of sensors utilizing initial stages of crystallization as well as for designing SAM-controlled crystals on solid surfaces.

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