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AFM Observation of the Surface of Hydroxyapatite Single Crystal with and without L-B Monolayers

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The surfaces of a hydroxyapatite (HAp) single crystal were etched with a HCl solution. The surfaces were observed by an atomic force microscope (AFM). The (100) surface of HAp after the etching had steps corresponding to a height of 1/3 of the *a*-axis of the HAp unit lattice. Then, arachidic acid monolayers were formed on the etched HAp surface by the Langmuir-Blodgett (LB) method. A distance estimated by AFM between the HAp surface and carboxyl groups of the monolayers was suggested to be enough for an electrostatic interaction between them.

Key words : Hydroxyapatite, Single Crystal, L-B Monolayer, Surface Structure, AFM

1. INTRODUCTION

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, hereafter HAp) is the main constituent of bone and teeth which are a typical inorganic/organic nanocomposite of apatite and collagen.[1] Any information on the nature of the inorganic/organic heterointerface is important for the understanding of bioactive properties of natural bone and artificial bone substitutes.

In the present study, single crystal surfaces of HAp were etched by a dilute acid solution and then arachidic acid with the same functional group as collagen, i.e., carboxyl groups,[2] was accumulated on the etched HAp surfaces using the Langmuir-Blodgett (LB) method.[3] The interaction between the HAp surface and the carboxyl group was investigated by using an atomic force microscope (AFM) with an atomic resolution.

2. EXPERIMENTAL

2.1 Preparation of HAp Single Crystals and Surface Etching

Single crystals of HAp were grown by a flux method using $Ca(OH)_2$ under a hot isostatic pressure (HIP) of 50MPa in the following way.[4] Starting materials were the mixture of βTCP [$Ca_3(PO_4)_2$] and $Ca(OH)_2$ whose ratio was 2:3 by weight, and were molded into cylindrical pellets of 8mm in diameter. The pellets were then packed into a platinum crucible (10mm$^{\phi}$ × 100mm) as densely as possible. After weld sealing, the crucible was placed perpendicularly in a HIP furnace. The temperature was raised up to 1400°C at a rate of 466℃/h and kept for 1 hour. Then, the crystal growth of HAp was performed by gradually lowering the temperature to 900°C at 5°C/h.

HAp crystals grown were removed by dissolving the flux with an aqueous solution of 10% ammonium chloride at 80°C for one day. Thus obtained HAp crystals were hexagonal rods. The largest one was about 10mm in length. Crystals used in the present study are mainly 1～2mm in diameter and 3～5mm in length. All observations were carried out on the (100) faces, i.e., *a*-face. HAp single crystal surfaces were etched at room temperature by soaking in 0.05N-HCl aqueous solution without stirring for 3～30 minutes, washed by pure water, and dried in a desiccator for several hours. Thus prepared surfaces of HAp crystals were observed by an AFM, NanoScope Ⅲ (Digital Instruments Inc.). All AFM measurements were conducted with the tapping mode using a resonant frequency of 200 kHz.

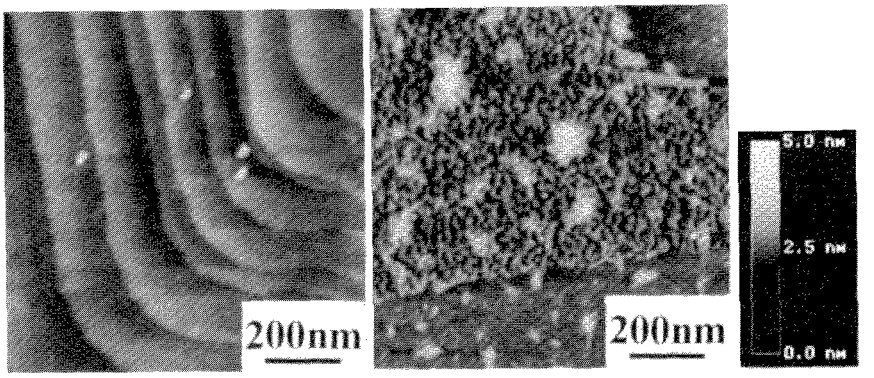
2.2 Accumulation of LB Thin Film

The LB method was applied to accumulate arachidic acid on the HAp crystal surface. First, a chloroform solution of 1mM arachidic acid, $C_{19}H_{39}COOH$, was prepared and spread on a pure water surface. A fixed surface pressure, typically 25mN/m, was applied along the water surface to form the monolayer film of arachidic acid. Then, a HAp single crystal soaked in advance in the water was perpendicularly pulled up with controlling the surface pressure by observing a π-A (surface pressure - surface area) curve. The carboxyl head groups of arachidic acid molecules adhered to the HAp crystal surface with the pulling up.

3. RESULTS AND DISCUSSION

3.1 Surface Morphology of Etched Single Crystal

Figure 1 shows an AFM image of *a*-face before the etching. Step/terrace structures observed on the surface of as-grown HAp crystals suggested that the HAp crystal grew by a layer-by-layer mechanism. However, the step/terrace



(a) (b)

Fig. 1, AFM image of as-grown HAp single crystal at the *a*-face. (a) right after synthesis and (b) 8days after.

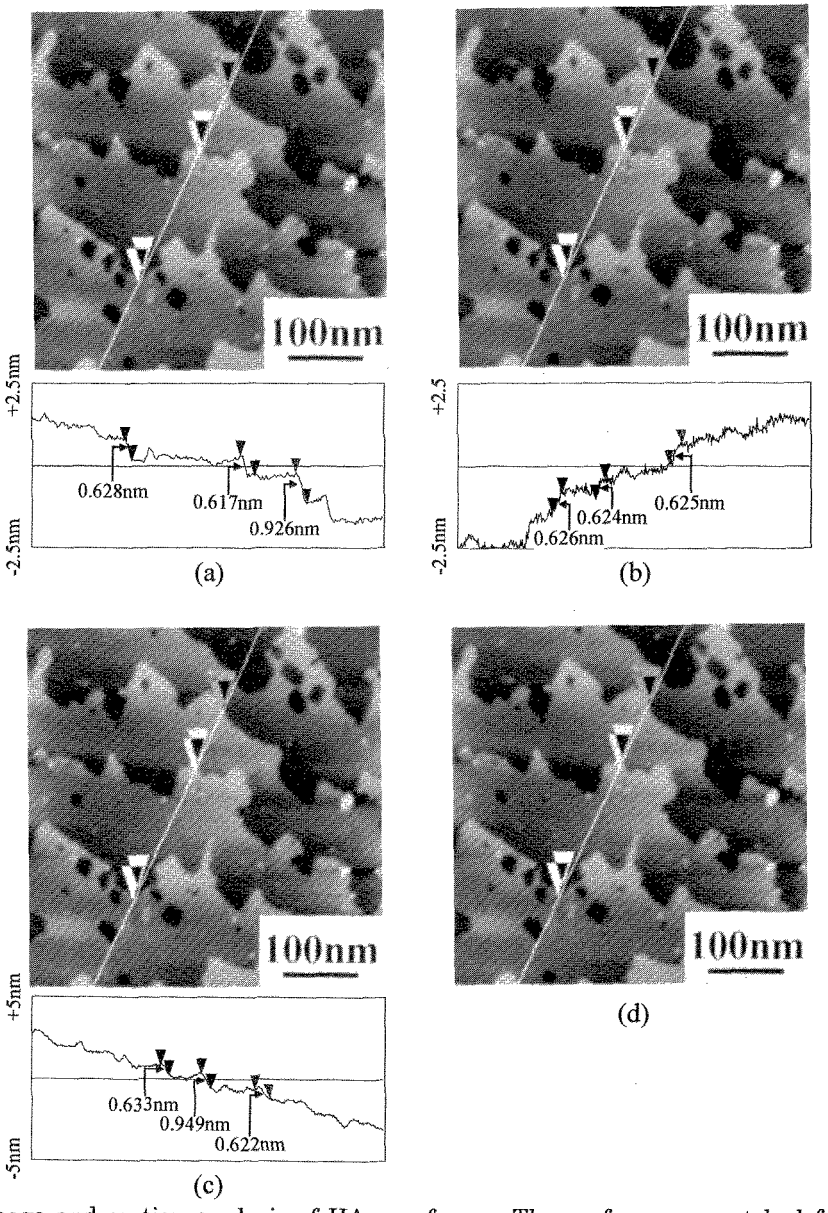


Fig. 2, AFM image and section analysis of HAp surfaces. The surfaces were etched for (a) 3min., (b) 10min., (c) 20min. and (d) 30min. by 0.05N-HCl aqueous solution.

structure disappeared upon leaving the crystal in air for 8 days. According to an Auger electron spectroscopic experiment by Suetsugu et al.,[5] calcium carbonate formed on the HAp surface by reacting with carbon dioxide in the atmosphere, and calcium ions could move near the surface even at room temperature. So, the disappearance of the step/terrace structure indicates that some ions moved from the inside to the surface and then a secondary chemical phase formed. Figure 2 shows etched surfaces of HAp as a function of soaking time. Figure 2(a) shows the surface after 3 minutes etching. Terraces with about 100nm in width were observed and steps ran approximately parallel to the *c*-axis. According to the etching and dissolution experiments of fluoroapatite single crystals by Jongebloed et al.,[6] triangularly shaped etchpits was observed at the *a*-faces. And the one side of the triangle etch pits was parallel to the *c*-axis. Therefore, it is conjectured that the step/terrace structures at the *a*-faces of HAp crystals were originated from the triangle etchpits. The variation of the surface heights observed by the AFM section analysis was also shown in Figure 2; the step heights were typically 0.63nm or 0.92nm.

Figure 2(b) shows the surface after 10 minutes etching. In this case, the terrace width became narrower than 100nm, which indicates that the dissolution had been progressing. Since the step height did not change in comparison to Figure 2(a), it is considered that the dissolution occurred more selectively along the horizontal direction of the terrace than along the perpendicular direction. Figure 2(c) shows the surface etched for 20 minutes. The dissolution of the terraces progressed further and the step became rectilinear. The step height of Figure 2(c) almost did not change in comparison with those of Figures 2(a) and 2(b). With respect to Figure 2(d) etched for 30min, although narrowness of the terrace width prevented the precise section analysis, the height was estimated to be the same as that for Figure 2(a)~(c).

As a result of a detailed section analysis, the step height was 2/3 or 1/3 of the lattice constant of the *a*-axis (0.9418nm) or the lattice constant of the *a*-axis. These step heights must correspond to the HAp crystal structure in which ionic density changes along the *a*-axis with a periodicity of 1/3 of the unit lattice.[7] Accordingly, the composition and cycle of the terraces at the *a*-faces might be assumed as shown in Figure 3.

The terraces have charges of -4, +2 or +2 per unit area. Even if any lattice relaxations take place in the terraces to reduce the surface charges, the total surface charges probably differ from terrace to terrace. We supposed that unique adsorption characteristics of HAp are related to such a difference in charge between terraces.

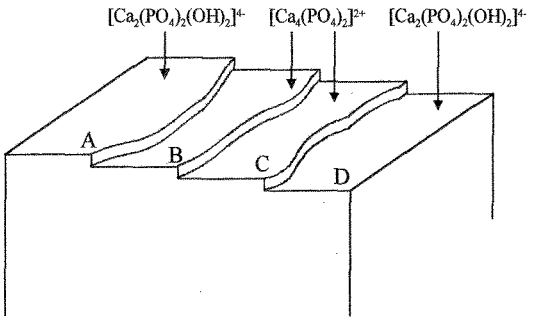


Fig. 3, Surface compositions the *a*-face terraces of HAp single crystal.

3.2 Adsorption State of Arachidic Acid

Arachidic acid molecules were accumulated on the etched HAp *a*-face by the LB method. Figure 4 shows the AFM image of the resulting accumulated surface.

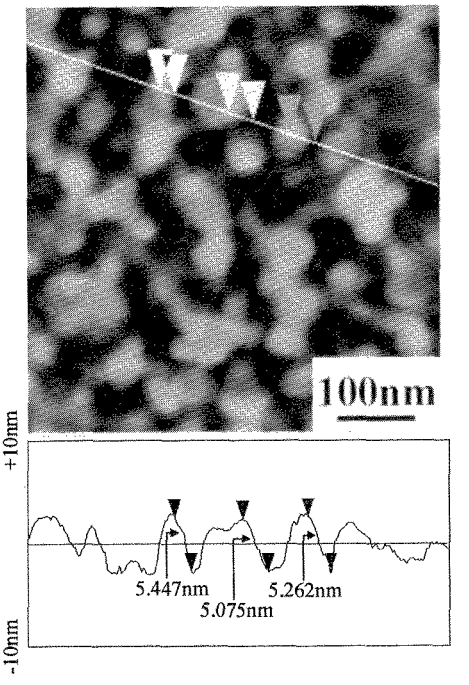


Fig. 4, The AFM image and section analysis of arachidic acid accumulated on a HAp single crystal etched for 30min.

Bright and dark areas correspond to arachidic acid and HAp surface, respectively. Though the monolayer of arachidic acid forms generally a planar triangular lattice on the water surface under the application of surface pressure, the AFM image of the accumulated surface did not hold such an arrangement, i.e., the LB film obtained was inhomogeneous as a whole. According to the section analyses, the thickness of the built-up film was 5.1~5.4nm which was approximately equal to the twice molecule length

of arachidic acid, i.e., 2x2.7nm.[3,8] This result suggested the formation of a bi-layer film of arachidic acid, in which the hydrophilic carboxyl groups of arachidic acid faced the HAp surface.

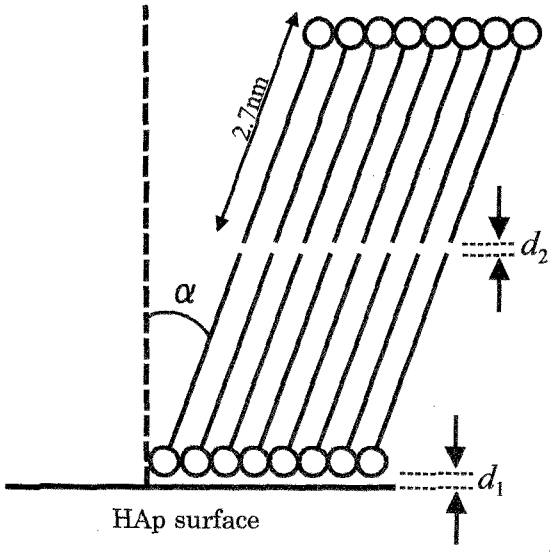


Fig. 5, The accumulation state of arachidic acid on a HAp surface.

In general, arachidic acid molecules are roughly perpendicularly accumulated on a substrate but slightly lean toward the HAp single crystal surface. For example, the leaning angle (α) is about 7° for cadmium stearate on a hydrophilic substrate and about 20° for arachidic acid on a hydrophobic substrate.[9] As shown in Figure 5, we defined here d_1 as a distance between the HAp surface and the carboxyl group, and d_2 as an accumulation distance between arachidic acid molecules. If α is less than 30°, the sum of d_1+d_2 is less than 0.7nm; thus d_1 should be 0.5nm or less on the basis of the thickness of the dyad film determined, i.e., 5.1~5.4nm, and the molecule length of arachidic acid, 2.7nm. The resulting 0.2nm for d_2 is reasonable compared to 0.3nm for the case of a clay mineral.[10] The length estimated for d_1 is sufficiently short for an electrostatic interaction to occur between the carboxyl head group of arachidic acid and the HAp surface.

4. CONCLUSIONS

HAp single crystals were grown by a flux method, and etched by 0.05N-HCl aqueous solution, a step height of 1/3 unit of the *a*-lattice constant was appeared, and the direction of the steps was almost parallel to the *c*-axis.

Arachidic acid molecules were accumulated on the HAp crystal surfaces by the Langmuir-Blodgett method. The hydrophilic carboxyl group of arachidic acid was sufficiently adjacent to the HAp surface, probably less than 0.5nm. It was considered that the adjacent distance was enough to an electrostatic interaction took place between the charged HAp surface and the hydrophilic carboxyl group of arachidic acid.

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