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Surface Imaging of Barley Aleurone Cell by Atomic Force Microscopy

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ABSTRACT: To observe and analysis ultra-microscopically barley aleurone cell surface, atomic force microscope (AFM) was used. Seed coat of early maturing germplasm, eam9, was dehulled and scanned by non-contact mode. We have obtained the high resolution topographic 3-dimensional image of barley aleurone layer with high resolution. These images showed the membrane proteins in barley aleurone cell. One channel protein and numerous peripheral or integral proteins were detected in a area of 100 μm^2. Furthermore, we found that their widths were ranged from 50 to 750 nm and lengths from 0 to 66 μm. The thickness of aleurone layer was measured by scanning electron microscope. The thickness at early developmental stage was about 16 and then the aleurone cell enlarged upto 57 μm at least until 42 days after anthesis. In this study, we firstly reported on the ultrastructural AFM analysis of living aleurone cell as a biological specimen. It was clearly suggested that AFM will become an powerful tool for probing both the structural properties of biological samples.

Keywords: Atomic force microscopy, Scanning probe microscopy, barley, aleurone layer, membrane protein

The development of the scanning tunnelling microscope by Nobel Prize winners G. Binnig and H. Rohrer in 1983 introduced an entirely new approach to visualization (Binnig and Rohrer, 1983). This revolutionary technique provided information with atomic resolution on many structures that are electrically conducting. Since then, a large family of scanning probe microscopes with all capable of generating 3D topographic profiles of surfaces at nanometer level were developed. Their common feature is a sharp probe that interrogates the local surface properties as its raster scans over the sample. Depending on the physico-chemical interactions responsible for the image formation, a whole range of material surface properties is accessible.

One of the major features and advantages that resulted in the great advance of the scanning probe microscope (SPM), in structural biology, the understanding of biological processes and the characterization of conventional and advanced materials, is the data acquiring ability both in ambient conditions as well as in liquid environment. This offers sharp contrast with other conventional imaging techniques such as scanning electron microscopy which operates under high vacuum. Also, the invention of atomic force microscope (AFM) has brought a new era of topographic research on biological samples (David *et al.*, 2001; Hansma *et al.*, 1999; Ridout *et al.*, 2003; Rinia *et al.*, 2001; Wang *et al.*, 2003). AFM can generate ultra high resolution and three dimentional nano-scale imaging with length measurement (Liu *et al.*, 2003). The significant advantage of the AFM is simple sample preparation. Imaging by AFM does not require complex or potentially damaging sample preparation because it is possible to image unmodified surface details without any additional coating treatment (Tojima *et al.*, 1996).

But the AFM tip (cantilever) can interact directly with structures on the membrane, which is, at the same time, more rigid due to the firm attachment (Gibson *et al.*, 2003).

Currently, the highest resolution with AFM are obtained on regular protein and lipid complexes. Moreover, cell surface can be observed in near real time, offering excellent opportunities for investigating dynamic biochemical and physiological processes simultaneously and functional information (Vansteenkiste *et al.*, 1998). These impressive achievements have recently resulted in the SPM techniques making a considerable impact upon various application in the field of biomaterials research (Yoshino *et al.*, 2003).

Since biological membranes consist of many different lipids, proteins, and other molecules and thus form heterogeneous mixtures, it has been speculated that lateral domains can form in biomembranes (Welti and Glaser, 1994). To function appropriately, membrane requires the correct assembly of various molecules in and around the cell membrane including receptors, transport proteins and specialized membrane domains such as caveolae, clathrin coated pits, or cell-cell and cell-substrate adhesion structures (Kusumi and Sako, 1996).

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Here we demonstrate that non-contact mode AFM leads high resolution topographical images of membrane proteins and channel proteins in barley aleurone cells. Also, we examined the aleurone thickness during the seed development stages.

MATERIALS AND METHODS

Barley (*Hordeum vulgare* L.) seeds (eam9, GSHO 1732 GS96) were sawn at November, 4th, 2002 and cultivated until Spring 2003. The developing seeds after anthesis were obtained and directly used for microscopic observation.

Seeds were divided for two experiments, scanning electron microscope and atomic force microscope.

Scanning electron microscopy

Isolated seed samples were washed 3 times with 0.05 M cacodylate buffer for 10 min after fixed in Karnovsky's solution at room temperature.

These samples postfixed in 1% osmic acid for 2 hr at 4 and then followed by a wash for 10 min in 0.05 M cacodylate buffer. The samples were dehydrated in a series of ethanol solution (50-100%) and critical point dried (HCP-2, Hitachi) had used with liquid CO_2. Isoamyl acetate was used as the intermediate fluid. After the dried samples were longitudinally sectioned with blade, it were directly mounted on circular aluminum stubs with double-sided sticky tape, coated for 2 min with Au-Pd ion coater (E-1010, Hitachi), then examined and photographed in a Hitachi scanning electron microscope (S-3500N, Hitachi) at an accelerating voltage of 15 kv.

Atomic force microscopy

For AFM observation, the barley grain outer and inner glume were removed with razor blade and forceps (Fig. 1).

Dehulled seed was placed on a G scanner (XE 150 mode PSIA, Korea) (Fig. 2), and then scanned in the non-contact mode and recorded as the topographical images. The scanning area was limited to 1×1 μm^2 to 10×10 μm^2 and scan speed was on 0.5 Hz. Images were composed of 256×256 μm^2 pixels. The operating voltage was set as low as possible to avoid damaging the samples.

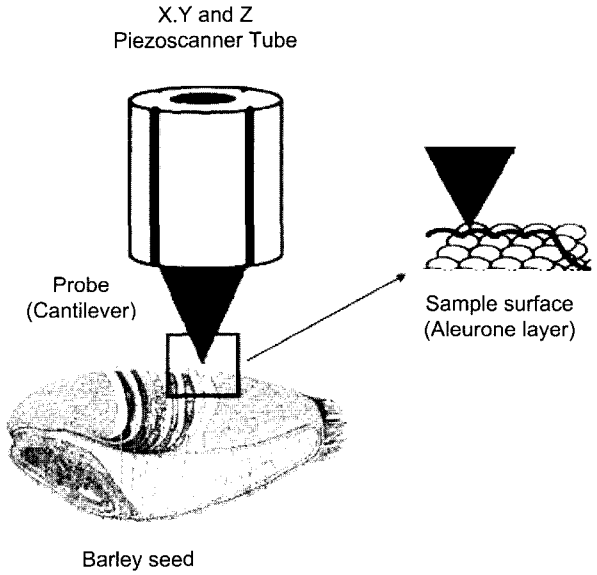

Fig. 1. Schematic description of the non-contact mode atomic force microscopy (AFM) and the sample measuring process.

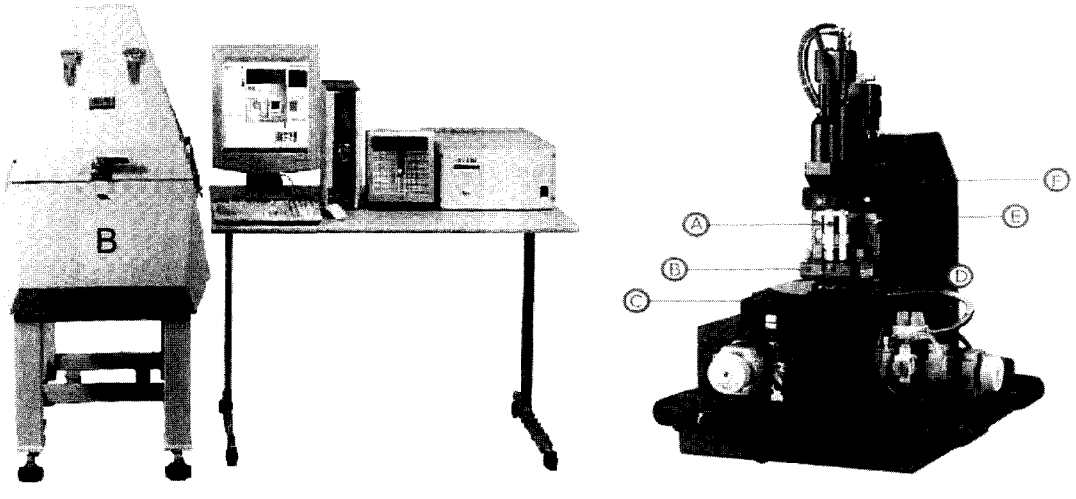

Fig. 2. Used scanning probe microscope(XE150, PSIA) (ⓐ : High quality optical microscope, ⓑ : Advanced scan system, ⓒ : Guided fexure scanner, ⓓ : EZ snap probe tip exchange, ⓔ : Dovetail lock head mount ⓕ : Ball screw driven z-stage)

RESULTS AND DISCUSSION

Scanning electron micrography

It was clearly observed that the differentiation of aleurone cell layers and subaleurone layers were rapidly progressed between 18 and 21 days after anthesis (DAA, Fig. 3). During this period, the enlargement of starchy endosperm cells were profoundly developed. Thereafter, starch granules were filled fast. It seemed that barley aleurone layers consisted of two or three unicellular cells. The formation of aleurone cell layer was not commenced until 21 DAA.

The thickness of aleurone was about 16 μm at initial stage (18 DAA) and finally 57 μm at mature stage (42 DAA, Fig. 4). It was observed that the size of starch granules were about 10 μm at the fully matured kernels. This magnitude was slightly smaller than that in cultivar Amaki (Tang *et al.*, 2002).

Atomic force micrography of aleurone cell

We successfully performed topographic imaging of barley aleurone layer surface. AFM was able to image surface structure without additional sample preparation steps. It was found that the large channel proteins (Fig. 5, arrow) were composed of at least 4 different head groups. It appeared that these pinnacle shape on AFM was derived from hydrophobic properties.

The heights and width of the observed membrane proteins were ranged from 0 to 66 μm and 50 to 750 nm, respectively, the basis of lipid bilayer surface line. We had also tried to measure precisely the histogram of the 2-D topographic image in 10×10 μm^2 (Fig. 6).

In atomic force micrography, the lipid bilayer contained many integral proteins. Percec and Bera (2002) have also suggested that the lipid bilayer of cell membrane in its ordered state accommodates proteins with various tertiary structures. The fluid mosaic model of the cell membrane consists of a self-assembled lipid bilayer containing protein-based active elements that are co-assembled in the bilayer structure.

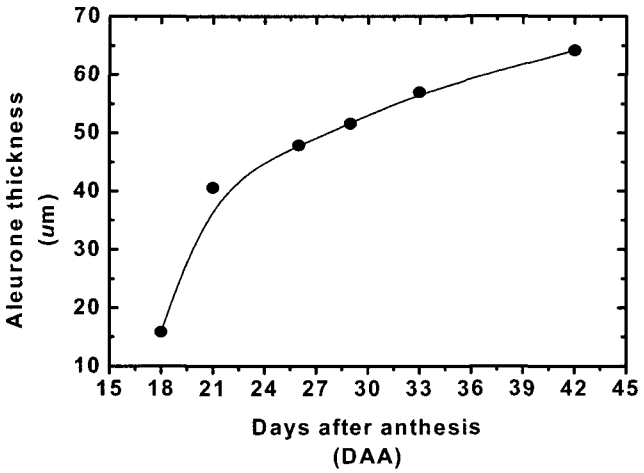

Fig. 4. Changes of aleurone layer thickness in developing barley kernels.

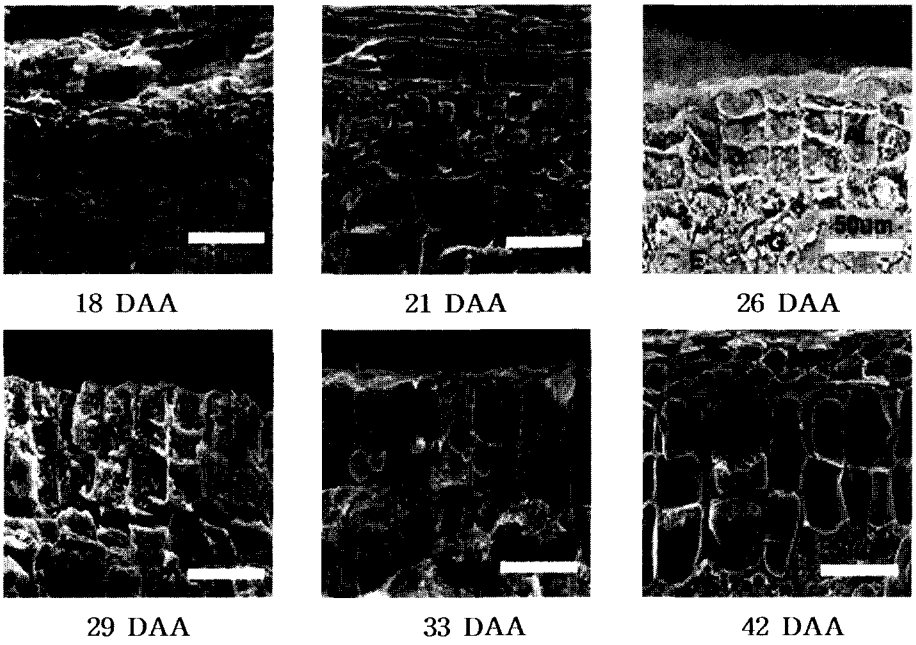

Fig. 3. SEM observation of cross section of barley kernel during the seed maturation. AL, aleurone layer; E, endosperm; Th, testa and hyaline layer; G, starch granule; Per, pericarp.

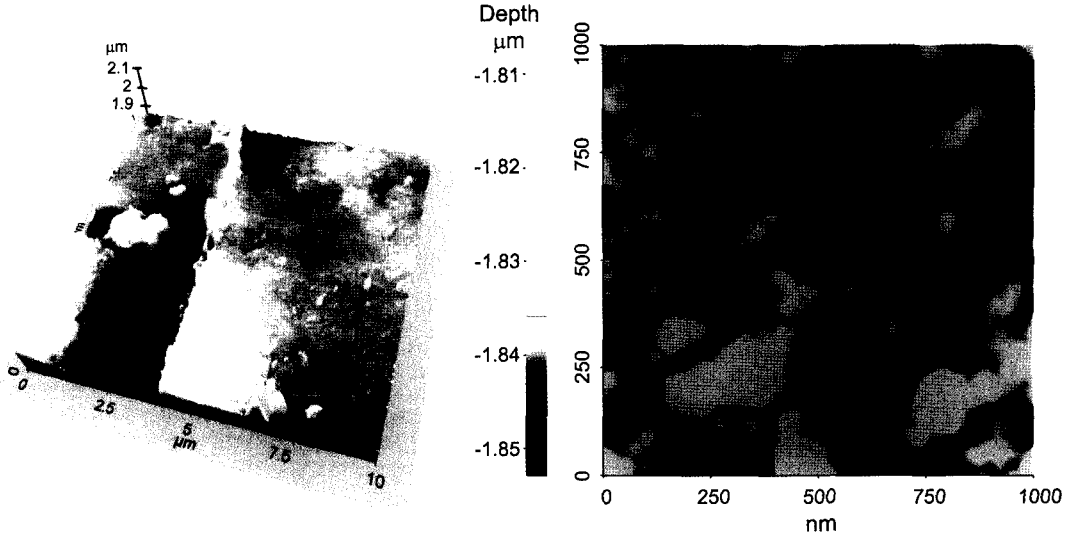

Fig. 5. Three-dimensional (A) and 2-dimensional (B) images of barley aleurone layer using non-contact mode AFM (A, 10×10 μm^2) and topographic image (B, 1×1 μm^2).

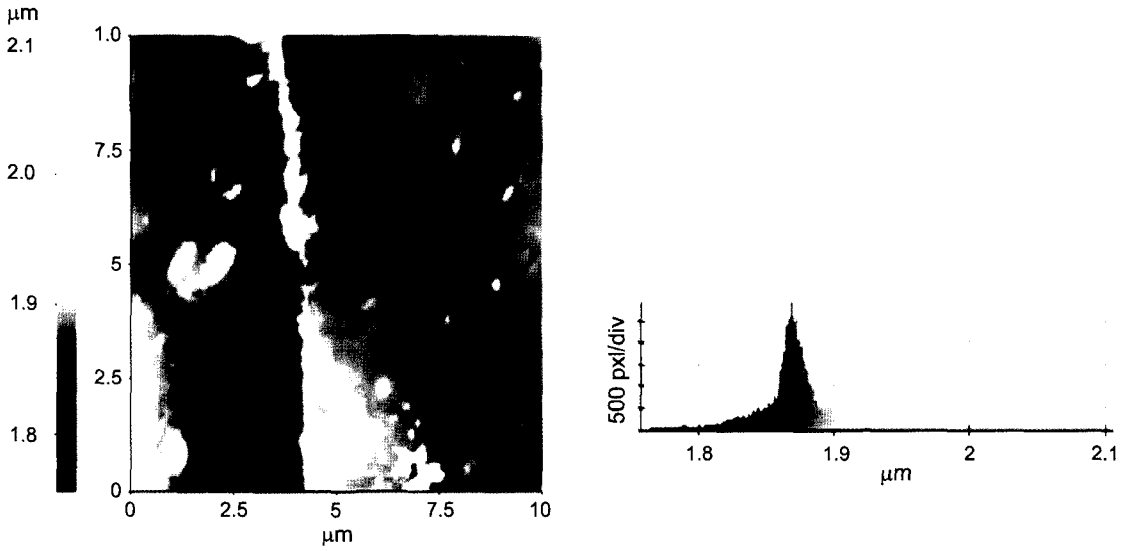

Fig. 6. The histogram of aleurone layer topographic image.

In this paper, we have found that non-contact mode AFM is a powerful imaging tool for soft and weakly attached biological sample like the internal structure of barley aleurone layer. Furthermore, we would favor that the high resolution images obtained support the fluid mosaic model of membrane.

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