Probing the microelastic properties of nanobiological particles with tapping mode atomic force microscopy

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Abstract

We have studied untreated photosystem II (PSII) membrane using tapping mode atomic force microscopy (AFM). The individual PSII particles distribute randomly in the membrane. Near the center of each particle, our AFM reveals an intramolecular cavity which confirms the previous electron microscopy of stained samples. The cavity can be reversibly enlarged from a few nm to as many as 40 nm in diameter by increasing the force on the AFM tip. A study of the particle's apparent height and cavity size under various forces provides unique information about the microelastic properties of single PSII particles. © 1997 Published by Elsevier Science B.V.

1. Introduction

Photosystem II (PS II) is a pigment–protein complex integrated into the thylakoid membrane of higher plants, cyanobacteria, red and green algae. Its major biological function is to split water to form molecular oxygen, proton and electron by capturing solar energy which is vital for maintaining the present level of biomass on earth and for sustaining an oxygenic atmosphere. Despite many years of intensive studies [1–13], the exact structure of PS II is still not well understood. The techniques that have been predominantly used to probe PS II structure are X-ray crystallography [3,4] and electron microscopy (EM) [5–9]. While these techniques have made important contributions to our understanding of PS II structure, the samples require crystallization, staining or metal coating. Scanning tunneling microscopy (STM) and atomic force microscopy (AFM) have the capability of imaging biological molecules under conditions close to their native states [14]. However, since electrons cannot directly tunnel through large insulating molecules such as PSII, platinum replicates or metal coated PS II membranes were used in recent STM studies [10,11]. AFM works both for conductive and non-conductive samples therefore requires no metal coating of the samples, but the AFM tip can seriously distort and even damage soft biological materials when operated in the contact mode. Furthermore, the large lateral tip force requires the samples to be strongly attached to a substrate, which results in a large sample–substrate interaction that may distort the natural structure of the sample. AFM has been recently used to image titanium coated PS II crystals [12] and Langmuir–Blodgett (LB) films of PS II membranes isolated from bacteria Rhodopseudomonas viridis deposited on glass substrates [13].
In the present study, we report a tapping mode AFM [15,16] study of untreated PSII membranes from spinach deposited on an atomically flat mica substrate using the Langmuir–Blodgett method. Since the oscillating tip in the tapping mode AFM only briefly touches the sample during each cycle of oscillation, it drastically reduces tip-induced distortion to soft biological materials. The use of atomically flat mica as substrate removes the complication in the image interpretation due to rough features on substrates such as glass. We have found that the PSII particles distribute in the membrane in a disordered fashion with a density of \( \approx 10^{-3} / \text{nm}^2 \) in all the areas surveyed by AFM. Our AFM has revealed a cavity near the center of each PSII particle. In addition to structural studies, we have investigated the microelastic properties of the PSII by utilizing the unique advantage that the AFM tip can apply a local force onto each individual PSII particle.

2. Experiments

The PSII membranes in this study were extracted from spinach using a procedure described in Ref. [17]. The sample was characterized by performing low-temperature LDS–PAGE and by measuring oxygen evolution using the Clark-type electrode. The oxygen evolution measurement demonstrated that the PSII membranes used in our experiments were active. The PSII membranes were spread at the interface of air and aqueous solution (2 mM CdCl₂, 2 mM sodium ascorbate, and 2 mM MES (pH 6.5)), compressed to a desired pressure and then deposited onto a freshly cleaved mica using the vertical method at a speed of 10 mm/min. The coverage of the PSII membranes on each substrate was examined with fluorescence spectroscopy (Fig. 1). The peak position and shape of the fluorescence spectrum of the membranes deposited on mica are nearly identical to those obtained in buffer solution. The peak height, as expected, increases as the pressure increases.

The tapping mode AFM study was carried out on a MultiMode Nanoscope system in air at room temperature. Etched Si tips with a resonant frequency of \( \approx 319 \) kHz, force constant of \( \approx 50 \) nN/nm and nominal radius of curvature of 5–10 nm were used. The quality factor, \( Q \), of the tip at the resonant frequency was determined to be \( \approx 500 \). The tip was driven to oscillate at a slightly lower frequency than the resonant frequency with an amplitude of \( \approx 30 \) nm. The images were obtained with various setting points which allows us to obtain information about the elastic properties of the sample.

3. Results and discussion

Fig. 2A is a tapping mode AFM image of the PSII membrane prepared at 10 mN/m. The image reveals the PSII particles as blob-like features that appear to be randomly distributed on the surface. The height of the particles above the membrane surface measured from the AFM image varies from 0.5 nm to 2.5 nm, comparing to 3 nm estimated from the transmission electron microscopy [7]. The dimension of the particles measured from the image is about 25 ± 5 nm, which is somewhat greater than 17–19 nm determined by the electron microscopy [5]. This discrepancy is at least partially due to the finite radius of the AFM tip. If we approximate the PSII particle with a sphere embedded in the membrane with a slight protrusion out of the membrane surface, then the broadening due to the finite tip radius is about \( 2Rh/W_0 \), where \( R \) is the radius of curvature of the tip, \( h \) is the amount protrusion and \( W_0 \) is the actual width of the particle. Using \( R = 10 \) nm, \( h = 2.5 \) nm and \( W_0 = 17 \) nm, the broadened amount is estimated to be 3 nm.
Fig. 2. AFM images of PSII membrane LB film obtained with a tip force of $\approx 0.3$ nN (A), $\approx 5$ nN (B), $\approx 0.3$ nN (C) and $\approx 5$ nN (D). The film was deposited at 10 mN/m.

Since AFM probes tip–sample interactions, it may be used to study microelastic properties of biological material from the tip-induced deformation on the sample [18,19]. We have used this unique advantage to study elastic properties of the PSII particles by applying various forces on the individual particles via the AFM tip. Fig. 2A was obtained with the smallest possible force that allowed the tip to follow the sample surface topography. The force, estimated from the amplitude setting point and resonant response of the AFM cantilever, is about 0.3 nN. The image clearly reveals individual PSII particles as blobs. Upon increasing the tip force applied on the sample, a cavity shows up gradually near the center of each PSII particle (Fig. 2B). This confirms the electron microscopic studies of negatively stained two dimensional crystals of several PSII complexes [5–7]. These intramolecular cavities are believed to serve as special intramolecular micro-environment for oxygen evolution [20]. The cavities cannot be clearly resolved by the AFM under a small force. This is probably because they are filled up with small molecules such as solvent and salt. Under a large force, the tip penetrates into the cavity by enlarging the cavity. As expected, we found that the cavity size increases with the increases in force. Decreasing the force back to the lowest level ($\approx 0.3$ nN), the cavities in almost all the PSII particles disappear completely and the particles return to their original shape (Fig. 2C). Occasionally we have observed these cavities in some particles (pointed by an arrow) do not disappear, indicating a permanent damage to the particles. By controlling the tip force applied on the sample, we have reproducibly made the cavities to appear (Fig. 2D) and to disappear in 9 different samples prepared under various pressures. We note that cavities also appear on the ‘bright’ particles which are not clearly shown in the image because the image contrast was adjusted to show the cavities of the majority particles. However we have observed that $\approx 3\%$ particles never exhibit cavities even under the largest applied force ($\approx 10$ nN). This is possibly due to the placement of PSII membranes with luminal side away from the mica substrate (inverted position).

We have monitored the deformation of a single PSII particle under various forces (Fig. 3A–C) to understand their nature and avoid any erroneous

Fig. 3. AFM tip-induced deformation of a single PSII particle. A–C are images obtained with various tip forces and D–E are the height profile across the center of the particle.
Fig. 4. AFM images of PSII membrane LB films deposited at 10 (A), 12.5 (B) and 15 mN/m (C). The inset in each image is a higher magnification image of the corresponding film which shows more clearly the individual PSII particles.

We have studied PSII membrane LB films prepared at pressures, 10, 12.5 and 15 mN/m. The corresponding typical AFM images are shown in Fig. 4. At 10 mN/m, the particles are basically uniformly distributed on the surface indicating a complete coverage of a monolayer (Fig. 4A). The coverage of the particles determined from the AFM images is about 1 particle per 1000 nm². At 12.5 mN/m, aggregate-like features begin to appear, corresponding to the formation of a second layer due to the collapse of the film at the air–solution interface under the surface pressure (Fig. 4B). A higher magnification image shows more clearly the PSII particles in both the first layer and the second layer aggregates (Fig. 5). Further increasing the deposition pressure, the second layer expands and eventually covers the entire surface (Fig. 4C). Higher resolution images of the second layer clearly resolve individual PSII particles (insets in Fig. 4B–C). First glance at the images in Fig. 4A and B may give one an impression that the PSII particles are more densely packed in the film deposited at 12.5 mN/m than that at 10 mN/m. However, by carefully counting the particles per unit area, we found that the particle densities at the two pressures are about the same. The false impression is due to that the particle height variation in the first layer (10 mN/m) is much greater than that in the second layer (12.5 mN/m). The difference in the
particle height variations can be attributed to that the first layer is on the flat and relative rigid mica while the second layer is on the first layer which has many PS II particles protruding out of the surface and is also softer than mica. From the AFM images, the second layer is determined to contribute ≈ 80% and 100% times to the total particle coverage in the 12.5 and 15 mN/m films, respectively. This result agrees reasonably well with the fluorescence intensities which show the films at 12.5 and 15 mN/m are about 100% and 120% more intense than the film at 10 mN/m.

In summary, we have studied the structural and microelastic properties of PS II membrane prepared with the Langmuir–Blodgett technique by using the tapping mode AFM. The PS II particles are fairly uniformly distributed in the membrane with a density of $10^{-3}$ particles/nm$^2$, but they do not pack into an ordered structure in 9 different samples and in all the areas surveyed by AFM. The particle coverage in the films prepared at three different pressures determined from the AFM images are in good agreement with the fluorescence spectra. An intramolecular cavity located near the center of each particle is observed. Under a large AFM tip force, the particle can be compressed in the vertical direction which allows us to determined the elastic constant to be $\approx 5$ nN/nm in the direction. The large tip force can also enlarge the intramolecular cavity by penetrating into the cavity which provides unique information about the binding energy between the four domains that make up each PS II particle. Releasing the tip force, the particle fully recovers its original size and shape even after enlarging the cavity to a diameter twice as large as the original diameter of the entire particle.

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