consequently or simultaneously. In the different physiological processes or even in the different phases of the same physiological process, the major functioning signal might be different. Based on the fact that ACh is a neurontransmitter in mammals and it plays an important role in mammalian signal transduction, its role in root-shoot signal transduction and its relationship with other signals in plants need further studies.

Acknowledgements This work was supported by the National Key Basic Research Special Funds of China (Grant No. G1999011700).

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(Received December 9, 2002)

Chinese Science Bulletin 2003 Vol. 48 No. 6 573—576

Imaging surface structure of purple membrane partly dissolved by CHAPS with atomic force microscope

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Abstract **Atomic force microscope was employed to study the surface structure of purple membrane (PM) using contact mode in liquid. The surface structures of native PM and PM partly dissolved by detergent CHAPS were compared. Results show that 6 mmol/L CHAPS could induce some cracks and holes in PM. While Bateriorhodopsin (BR) was still presented as trimers in the two-dimensional hexagonal structure. We assume that it was caused by the interaction between CHAPS micellar and PM. Both absorption spectra and flash photolysis kinetic spectra indicated that BR's biochemical functions have been changed.**

Keywords: purple membrane, bacteriorhodopsin, detergent, CHAPS, atomic force microscope.

Purple membrane (PM) is part of the cell membrane of *Halobacterium halobium* and it contains one kind of protein, bacteriorhodopsin (BR). BR has the function of a light-driven electrogenic proton pump, so it has a high light-energy transforming ability. Many methods have been used to study the structures of PM and BR, such as X-ray diffraction, transmission electron microscope and atomic force microscope (AFM). As well known, BR naturally possesses two-dimensional (2D) hexagonal lattice as trimers in native PM[1,2].

In addition, PM has about 25% archeol lipid derivatives[3], and the lipid-protein interaction plays a crucial role in the physiological activities of BR[4]. Detergent is indispensable as a solubilizing agent to study lipid-protein interaction. It can be used to isolate, purify and dissolve membrane proteins without losing their biological functions[5]. The study of solvent process of membrane protein in detergent may benefit our understanding of the interaction between protein and lipids. A "three-stage" model has been proved to be a simple and useful explanation[5]. But detailed studies of solvent process of membranes by various kinds of detergent are still necessary to detecting the interactions between protein, lipid and detergent.

Kinetic study has shown that lipids in PM can be

removed by detergent to a different extent and detergent can change the kinetic process of BR[6]. Study of ^{13}C NMR has proved that detergent can cause the local conformation change of BR[4]. But direct imaging structure change of PM and BR during solvent process in nearly physiological conditions has not been reported.

According to the type of the headgroup, detergent can be divided into three categories: ionic, non-ionic and zwitterionic[15]. The behaviour of specific detergent depends on the character and stereochemistry of its headgroup and tail[15]. In our study we choose zwitterionic detergent CHAPS ((3-[(3-cholamidopropyl) dimethy-lammonio]-1-propanesulfonate). CHAPS are widely used to isolate, purify and pack purified membrane protein, and induce little loss of their biological function. Its critical micellar concentration (CMC) is 3—10 mmol/L[5]. As a preliminary experiment, we used 6 mmol/L CHAPS to treat native PM. On this concentration, CHAPS monomer and micellar exist in dynamic equilibrium, and PM has begun to be dissolved, but not dissolved completely. Therefore, its surface structure can be suitably characterized.

AFM is an important tool in the structural biology. Compared with other microscopes, AFM can be used to image and manipulate biology specimen in buffer solution. And its high signal-to-noise ratio provides fine spatial resolution. High resolution AFM images of native PM have been captured with 0.5 nm spatial resolution[7—9], and its structure change in photo-bleaching process has also been studied with AFM[10].

In this report we employed AFM to study the surface structure change of PM partly dissolved by CHAPS using contact mode in liquid. Absorption spectra and flash photolysis kinetic spectra were used to characterize its proton pump function.

1 Materials and methods

(i) Sample preparation. The culture of the *Halobacterium halobium* and the isolation of purple membrane were operated according to the standard procedure[8]. Purified purple membrane in deionized water was stirred gently with KCl, Tris-HCl buffer (KCl 150 mmol/L; Tris-HCl 10 mmol/L; pH 7.2—7.4) and CHAPS (6 mmol/L) to achieve the final sample solution. Samples were kept in dark at 4℃ for over 48 h before measurement.

(ii) AFM experiment. AFM was performed with a commercial microscope (NanoScope IIIa, MM-AFM, DI) in the contact mode. Imaging was done with a 10 μm scanner (E-scanner) and a liquid cell without O-ring seal. We used NP-S (Si_3N_4) tips with length of 200 μm and spring constant of about 0.06 Nm^{-1}.

Thermal equilibrium of the system and force exerted by the tip to the sample are two key factors in the experiment. After mounting the sample, 2 h were generally needed to reach thermal equilibrium. The force exerted by the tip to sample can be calculated by force curves, i.e. the curve of tip deflection vs. tip-sample distance. The force was set as low as possible by adjusting the setpoint value. In our experiment, the force can be kept below 300 pN in order to get high resolution images of PM. During scanning the values of integral gain, proportional gain and scan rate need to be adjusted often to get perfect images.

(iii) Absorption spectra and flash photolysis kinetic spectra. Absorption spectra were recorded with a Hitachi U-3200 spectra-photometer. The flash photolysis kinetic spectra were recorded on our homemade instrument[12], with probing light at 412 nm and stimulating light at 570 nm. The final absorption curves were averaged from six results, and the data were analyzed by Govindjee's method[13].

2 Results

Fig. 1(a) is a typical topography of native PM sheets in a large scan size of 2 μm. The contour of the native PM is round or elliptical with clear edge. As contrast, Fig. 1(b) is a typical topography of the PM treated with 6 mmol/L CHAPS. Its contour appears irregular with rough edge. And there are some "white dirties" on the membrane, indicating that they are much higher than the membrane surface. It is assumed that these "dirties" were a collection of CHAPS micellar or dissolved membrane protein. In the experiment, they can stick on tips, resulting in worse images than the native PM. It should be pointed out that for the membrane sheets with quite different shape, their thickness was almost the same. In fact, the average thickness of the native PM is about 5.9 nm, and the PM treated by CHAPS is about 5.8 nm.

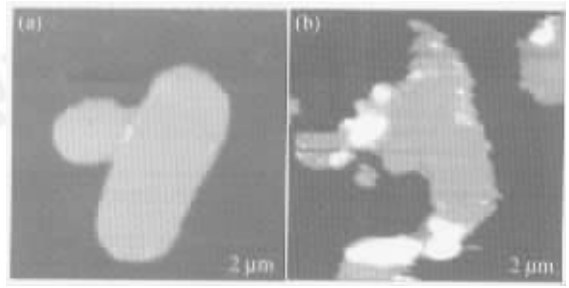

Fig. 1. Typical membrane sheets of the native PM (a) and the PM with 6 mmol/L CHAPS (b). The contour of the native PM is round or elliptical with clear edge, however, the PM treated by CHAPS appears irregular and the edge looks rough. The gray levels are 40 nm.

But the surface structure of the two kinds of membrane sheets are much different from each other, as shown in Fig. 2 in a small scan size of 300 nm. Fig. 2(a) is the typical surface topographic image of native PM, in which the 2D lattice structure is clearly shown. Fig. 2(b) is the typical surface topographic image of the PM treated by 6

mmol/L CHAPS, in which there obviously exist some cracks and holes, but 2D lattice can still be seen on the flat areas of the surface. Notice that the gray level of Fig. 2(b) is much larger than that of Fig. 2(a), thus the change of the topography of PM by adding CHAPS is impressive. We believe that the rupture of 2D lattice in the forms of cracks and holes indicates that PM has begun to be dissolved, resulting in the irregular shape of the membrane sheets (Fig. 1(b)). The average width of the cracks is about 12 nm, and the hole depth is 1.5—2.7 nm.

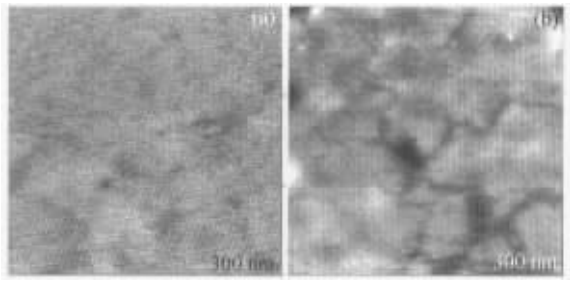

Fig. 2. Lattice structure of PM surface. (a) The native PM. The image clearly shows a 2D lattice structure. But it is not so clear at the top of the image as at the bottom because the force exerted by the tip was increased due to thermal drift in scanning. (b) The PM partly dissolved by 6 mmol/L CHAPS. Cracks and holes appear on the membrane surface. But the 2D lattice structure can be still seen on the flat areas. The gray levels are 2 and 5 nm for (a) and (b), respectively.

In order to make clear whether BR trimers have been affected or not, a high resolution image with 100 nm scan size was captured for the PM with 6 mmol/L CHAPS (Fig. 3(a)). The image of native PM with the same scan size is not presented here, please refer to our former paper[8] or ref. [6]. Fig. 3(a) shows the 2D lattice structure with some defects (arrows). Then 2D spectrum analysis was carried out to highlight the lattice. The result is shown in Fig. 3(b), in which the three protrusions, i.e. the BR trimers, can be clearly distinguished (in circle) the same as in native PM[6,8]. The holes and cracks disappear in Fig. 3(b) because only the periodical structural information can be preserved during 2D spectrum analysis. From Fig. 3(b) the lattice length of 6.1 nm and the distance of 3.1 nm between three protrusions were measured. The data are similar to that of native PM, as previously reported[11].

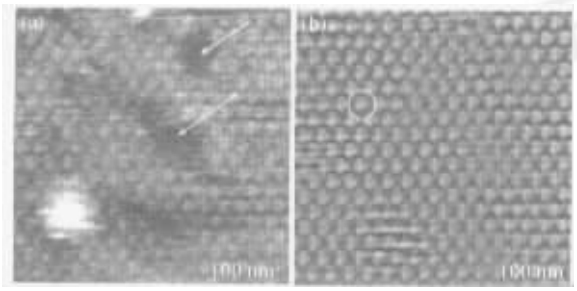

Fig. 3. (a) High resolution topography of PM partly dissolved by 6 mmol/L CHAPS. It shows both the lattice structure and some defects (arrows) of PM; (b) the image obtained after two-dimensional spectrum analysis of (a). The three protrusions can be clearly distinguished (in circle), indicating that BR trimmers still exist the same as in native PM. The defects in (a) do not appear in (b) because some structure information was lost during spectrum analysis. The gray levels of (a) and (b) are 1.5 and 0.8 nm, respectively.

In addition, absorption spectra and flash photolysis kinetic spectra were measured to characterize the function changes of BR. Fig. 4(a) is the absorption spectra of native PM and the PM with 6 mmol/L CHAPS. As shown in Fig. 4(a), the peak wavelength of native PM is 569.6 nm, but for the PM after being treated by 6 mmol/L CHAPS, its peak wavelength moved down to 564.8 nm. Fig. 4(b) is the flash photolysis kinetic spectra of BR, which represents the decay of one intermediate M_{412} of BR in photocycle. As shown in Fig. 4(b), the decay of M_{412} in native PM is much faster than that in the PM with 6 mmol/L CHAPS. By fitting the curves using two-order exponential decay function, the calculated half life ($t_{1/2s}$) of M_{412} is 2.6 and 11.6 ms for native PM and the PM with 6 mmol/L CHAPS, respectively. The increase of the decay rate of M_{412} indicates the changes of the light-driven electrogenic proton pump function of BR, which results from the lipid

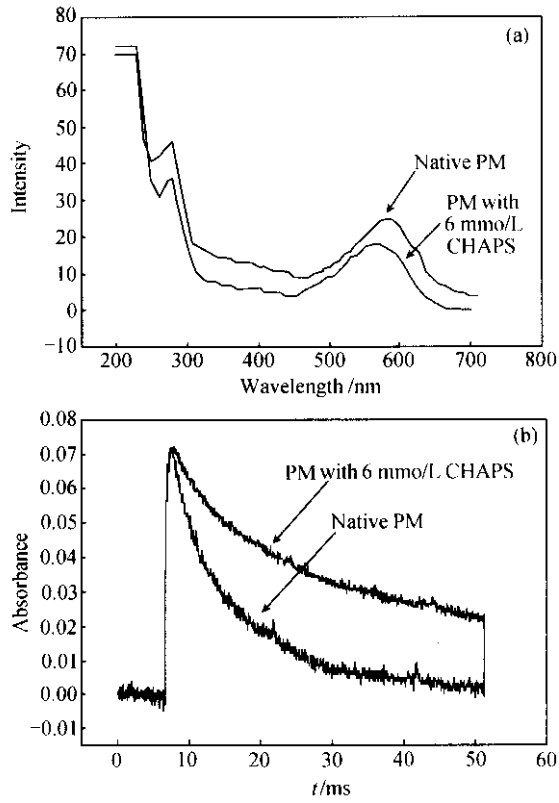

Fig. 4. (a) Absorption property of native PM and the PM treated by 6 mmol/L CHAPS. (b) Results of flash photolysis kinetic spectra.

environment changes around BR.

3 Discussion

The amphipathic character of detergent makes it able to be inserted into bio-membrane to substitute the lipids in the membrane. In our experiment, the concentration of CHAPS is 6 mmol/L, which is just in the range of its CMC. At this concentration, CHAPS monomers and micelles exist in dynamic equilibrium[6]. Former studies have proved that in the interaction between detergent monomer and membrane the dominant way is that the detergent is inserted into membrane to substitute the native lipid. However, the dominant way for the interaction between detergent micelles and membrane is that micelles extract the lipid or protein in membrane[5].

Our results show that the trimer structure of BR is not strongly affected by 6 mmol/L CHAPS, but the 2D lattice structure is partly ruptured in the form of cracks and holes. This manifests that BR trimer is more stable than 2D lattice structure in detergent environment. Considering the size of the cracks and holes, it can be assumed that the cracks and holes should be caused by the extraction of some lipid or protein of PM by CHAPS micelles, instead of the CHAPS monomer inserting into PM.

Although the trimer structure of BR was not changed after adding 6 mmol/L CHAPS, from absorption spectra and flash photolysis kinetic spectra it can be seen that its special absorption peak and light-driven electrogenic proton pump function have changed, indicating that the changes of secondary or tertiary structure of BR occurred. We attribute it mainly to the interaction between PM and detergent monomer. In fact, when monomers are inserted into the inner area of PM and substituted for its native lipid, the local lipid environment of BR will be changed, and the absorption and kinetic property of BR will be affected.

Our experiments have proved the surface structure changes of PM after adding 6 mmol/L CHAPS. According to the "three-stage" model, if the concentration of CHAPS is below its CMC (<3 mmol/L), only detergent monomers are inserted into membrane, therefore, the surface structure will not be largely changed. If the concentration of CHAPS is higher than its CMC, more cracks and holes will appear and PM will be eventually dissolved. If we dialyze CHAPS in the dissolved PM solution, the extracted protein or lipids might also be dialyzed out. As a result, the surface structure of PM could no longer be restored. However, the above speculation needs further experiments to confirm.

4 Conclusion

The contact mode AFM in liquid cell was used to investigate the surface structure of native PM and the PM with 6 mmol/L CHAPS, high resolution images were obained. Comparison of the images of native PM and the PM with 6 mmol/L CHAPS shows that the 2D lattice structure of PM was ruptured. It can be attributed to the interaction between PM and CHAPS micelles. But BR trimers still exist, indicating that BR trimer is more stable than 2D lattice structure in the detergent environment. Both the absorption spectra and flash photolysis kinetic spectra show that the absorption and kinetic property of BR have been changed. We attribute it mainly to the interaction between PM and detergent monomer.

Acknowledgements This work was supported by the State Key Basic Research and Development Plan Project (Grant No. 199801012).

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(Received December 6, 2002)