Projected structure of uroplakins in urothelium of rabbit urinary bladder at 20 Å resolution*

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Abstract Asymmetric unit membrane (AUM) was isolated and purified from the urothelium of rabbit urinary bladder. The electron micrographs were taken from negatively stained AUM patches. The Fourier filtering and lattice distortion correction were carried out by a computer. The projected structure of uroplakins in asymmetric unit membrane of rabbit urothelium at 20Å resolution was obtained.

Keywords: uroplakins, asymmetric unit membrane, two-dimensional crystal, image processing, projected structure.

Most functional processes in organism are related to biological membranes. The essential components of biological membrane are lipids and proteins. The membrane proteins play a dominant role in membrane functioning whereas the lipids constitute appropriate structure for the membrane protein functioning.

The surfaces of membrane proteins adjacent to lipids are mostly hydrophobic for which the well-ordered three-dimensional crystals are hard to be formed. This makes X-ray crystallography powerless in structure determination for membrane protein. Nuclear magnetic resonance (NMR) technique is also useless in this task because most of the membrane proteins have larger molecular weight. However, if the purified membrane protein is reconstituted into a lipid bilayer to form the two-dimensional (2D) protein crystal, it is possible to determine the spatial structure of such protein by an electron crystallographic method. Naturally formed 2D array sheets of membrane proteins have been found in some specialized cell membranes such as purple membrane[1], gap junctions[2] and asymmetric unit membrane (AUM). These sheets can be used directly for the structural analysis of related membrane proteins by electron crystallography.

Mammalian urothelia elaborate a highly specialized plasma membrane during an advanced stage of cellular differentiation. A large portion of the luminal surface of this urothelium is covered with scallop-shaped plaques, which are also present in the cytoplasm in the form of fusiform vesicles. When viewed in transverse section, plaque membranes exhibit a thickened luminal leaflet (8 nm) and a regular looking cytoplasmic leaflet (4 nm), hence no symmetry exists. Hicks observed such plaque

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membrane for the first time and named it "asymmetric unit membrane (AUM)" in 1965[3]. It has been shown[4] that the plaque in AUM is formed by protrudings of uroplakins particles that penetrate from the lipid bilayer into the luminal space. Each stellate-shaped particle of protein complex is composed of six inner and six outer domains, and the particles gather to form a plane array with symmetry p6. Such naturally formed 2D protein crystals are suitable for structural analysis with electron crystallography. So far most structural data on AUM with a resolution of about 22Å for sheets purified from bovine[4,5] or pig[6] bladders and negatively stained were obtained by electron crystallographic method. In this paper, the projected structure, at 20Å resolution, of uroplakins of AUM isolated and purified from rabbit urothelium is reported.

1 Experimental and image processing

1.1 Isolation and purification of AUM

The isolation and purification procedures described by Wu et al., Vergara et al. and Caruthers and Bonneville[7~9] were followed with slight modification. Briefly, rabbit's bladders were taken out rapidly after execution of the animals and washed with ice-cold distilled water, then inverted and rinsed with ice-cold phosphate-buffered saline (PBS) for three times. The urothelium was scraped from the luminal surface with a blunt scalpel, and suspended in PBS. After centrifuging at 4600 r/min at 4℃ for 10 min, the pellet was washed several times with PBS under the same conditions and then resuspended in 10 mmol/L HEPES buffer containing 1 mmol/L phenylmethyl-sulfonyl fluoride (PMSF), 1 mmol/L EDTA and 1 mmol/L EGTA (buffer A), pH 7.5. After homogenizing with glass homogenizer, the homogenate was centrifuged at 4℃, the pellet was washed again and resuspended in buffer A, then loaded onto 1.6 mmol/L sucrose. The centrifugation was performed in a JA 17 (Beckman) rotor at 17000 r/min at 4℃ for 20 min. The crude membranes located at the interface were collected and washed with buffer A for two times. The pellet was treated with 2% sodium sarcosine (N-lauroylsarcosine, sodium salt, Sigma) at room temperature for 10 min and the mixture was centrifuged at 17000 r/min at 4℃ for 30 min. After discarding the supernatant, the pellet was washed for three times with buffer A under the same conditions, and then treated with 25 mmol/L NaOH at room temperature for 10 min, the mixture was centrifuged again at 17000 r/min at 4℃ for 30 min, the pellet was washed three times with buffer A under the same conditions. Finally the pure AUMs were collected, suspended in buffer A, and stored at −70℃ before used.

1.2 Electron microscopy

The purified AUM samples were diluted to about 1.0 μg/mL with buffer A and mixed thoroughly. The 5 μL of the sample was dropped onto the carbon-coated grid and negatively stained with 10 μL of 1% uranyl acetate. For a good sample many AUM plaques could be observed under the electron microscope, while the overlap or stack of plaques were scarce. Micrographs of AUM were taken with a JEM 200CX electron microscope operated at 200 kV.

1.3 Image processing

The negative films with sharp diffraction spots, good symmetry and high resolution were selected by optical diffraction method[10,11] and then digitized with the LeafScan 45[12], a line-illuminating

micro-densitometer, at a step size of 10 μm.

Spectra, a special software programmed by Schmid et al.[13], was used to process the digitized images on the SGI work station.

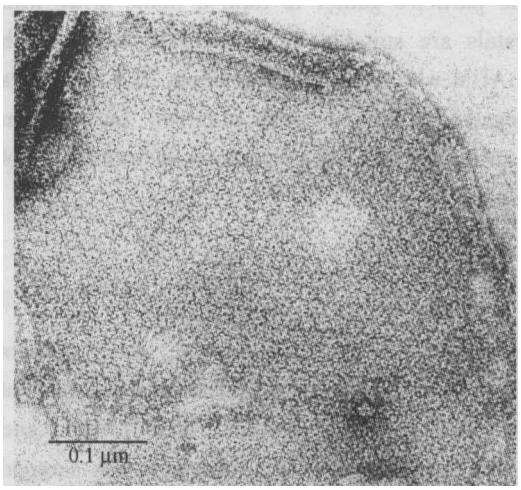

Fig. 1. A micrograph of AUM from rabbit urothelium.

2 Results and discussion

Figure 1 is a micrograph of rabbit bladder's AUM, where the well-ordered uroplakins particles can be seen clearly. The Fourier transform was carried out for the digitized image. Some strong reflections were indexed, based on which the least-square refinement on reciprocal lattice parameters was performed. According to the refined reciprocal lattice parameters expressed in pixel, the micrograph magnification and the scan step in digitizing the negative film, the lattice parameters of 2D crystal of uroplakins from rabbit bladder were calculated: $a = b = 16.5$ nm, $\gamma = 120°$.

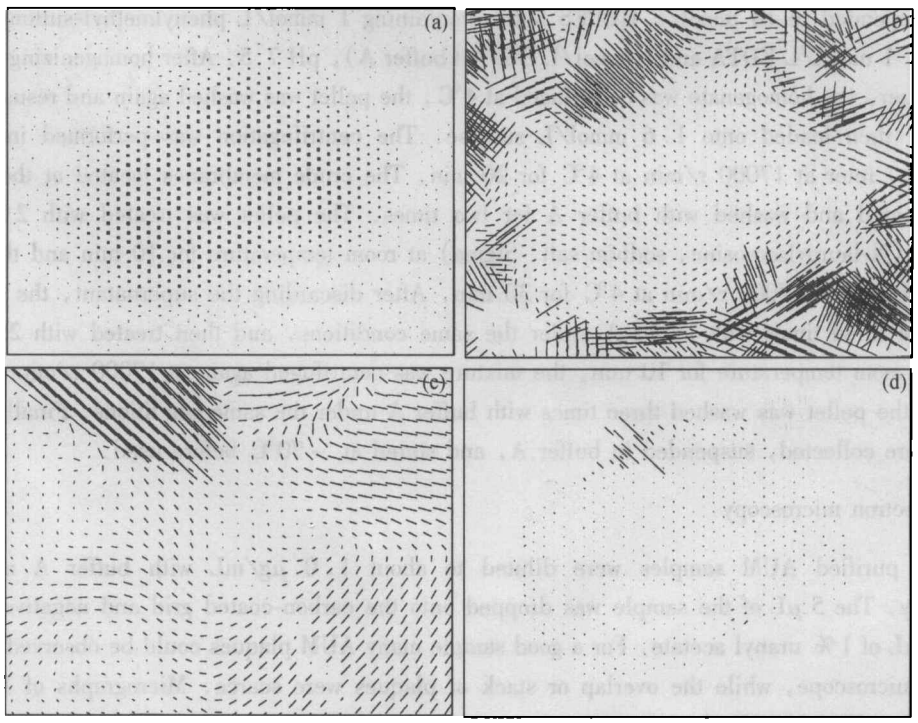

Fig. 2. Correction of lattice distortion in 2D AUM crystal. (a) Peaks of the cross-correlation function, bigger dots indicate better correlation; (b) vector displacements showing the deviation of correlation peaks from positions predicted based on a perfect lattice, and indicating the distortion of all individual lattice cells; (c) actual distortion correction for reinterpolating the new image; (d) vector displacements after correcting the lattice distortion.

The most important step in image processing is the correction of lattice distortion. The Fourier filtering of the digitized image was carried out using a mask of moderate hole-radius. A comparatively perfect part of the filtered image, normally the central area, was taken as the reference area. The cross-correlation map (CCM) (fig. 2(a))[14] was obtained by convoluting the reference area and the filtered image. The higher the peaks on CCM, the better correlation it has. The locations of the peaks on CCM were searched[15] and the accurate positions of centre of gravity for all correlation peaks were determined by the least-square method[16]. And a plot of vector displacement was used to describe the deviation from perfect lattice in both direction and distance for each unit cell[17] (fig. 2(b)). The bicubic spline fitting was employed to determine the coefficients which describe the image distortion

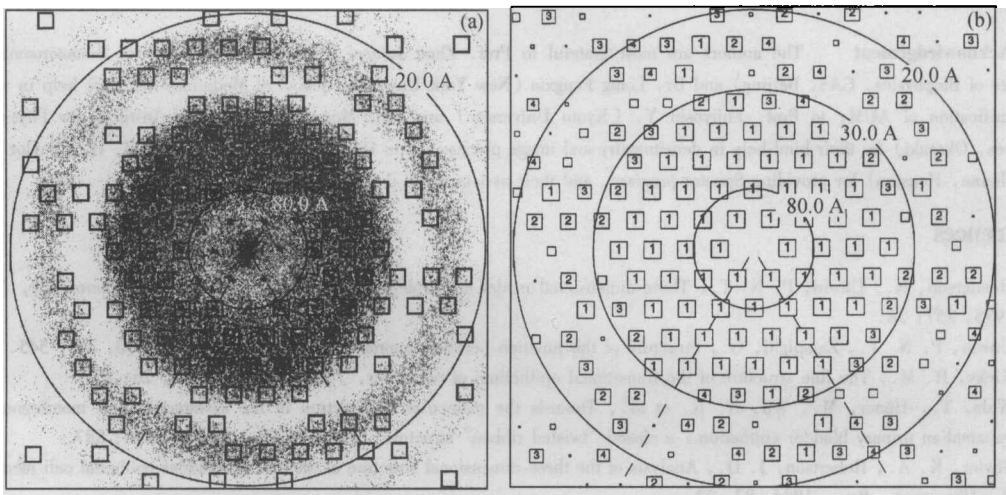

Fig. 3. Fourier transform of corrected image (a) and the IQ values for all the reflections within 20Å (b).

with a relatively few parameters, and hence the correction needed for each point in the image can be calculated (fig. 2(c)). Fig. 2(d) shows the vector displacements after the distortion correction. Obviously fig. 2(d) is much improved. After two cycles of the correction, the distortion in the crystal lattice becomes negligible. Generally, the next step of image processing is the correction of contrast transfer function (CTF). However, CTF refinement was not performed in this work because it would not affect the final results at the present resolution. Fig. 3(a) shows the Fourier transform of the corrected image.

Figure 3(b) presents the signal-to-noise ratios (IQ values)[17] for all reflections within 20Å. It can be seen that the resolution is at least 20Å if the IQ values equal to or less than 3 are taken into consideration. The inverse Fourier transform of fig. 3(a) yields the projected map of uroplakins in

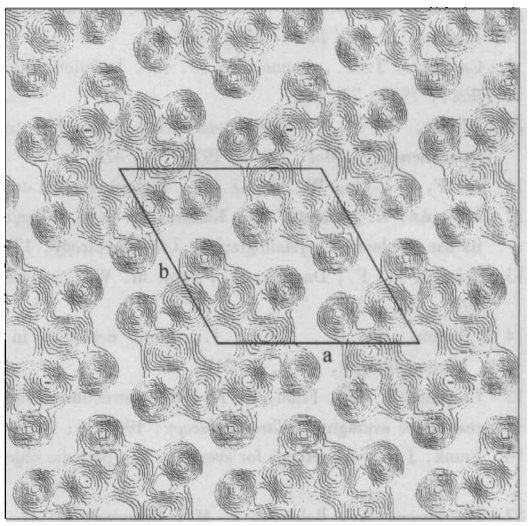

Fig. 4. Projected map of uroplakins in AUM of rabbit urothelium at 20Å resolution.

AUM of rabbit urothelium (figure 4).

It is shown clearly that the complex of AUM proteins (uroplakins) is composed of six domains, each of which can be resolved into the inner and outer rings. This result is consistent with the previous results obtained from bovine, pig or mouse bladders[3~6] and verifies further the high conservation of the specialized AUM structure in mammalian. However, it is still impossible to elucidate the biological functions of AUM clearly with this projected structure of insufficient resolution. A detailed analysis and investigation on biological function and functioning mechanism of this specialized AUM can only be carried out on the basis of the high resolution (atomic or near atomic resolution) three-dimensional structure of AUM.

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