Ionic channels in Langmuir–Blodgett films imaged by a scanning tunneling microscope

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ABSTRACT The molecular structure of channels formed by gramicidin A in a lipid membrane was imaged by a scanning tunneling microscope operating in air. The mono- and bimolecular films of lipid with gramicidin A were deposited onto a highly oriented pyrolitic graphite substrate by the Langmuir–Blodgett technique. It has been shown that under high concentration gramicidin A molecules can form in lipid films a quasi-regular, densely packed structure. Single gramicidin A molecules were imaged for the first time as well. The cavity of 0.4 ± 0.05 nm in halfwidth was found on the scanning tunneling microscopy image of the gramicidin A molecule. The results of direct observation obtained by means of scanning tunneling microscope are in good agreement with the known molecular model of gramicidin A. It was shown that gramicidin A molecules can exist in a lipid monolayer as individual molecules or combined into clusters. The results demonstrate that scanning tunneling microscope can be used for high spatial resolution study of ionic channel structure.

INTRODUCTION

Ionic channels of cell membranes are essential for the functioning of living organisms. For example, the ionic channels of brain membranes play a critical role in the process of signal transmission. Recently great progress has been made in studying the electrical properties of ionic channels due to the development of the patch clamp electrophysiological techniques (1) and new methods for forming model membrane bilayers (2). However, ionic channel molecular structure and functioning have not yet been investigated sufficiently because adequate experimental methods were not available.

In this study we show the possibility of using a scanning tunneling microscope to investigate the structure of ionic channels at the molecular level. We studied the ionic channels (half-pores) formed by gramicidin A in lipid films. Electrical properties of gramicidin A channels are described in reference 3.

MATERIALS AND METHODS

Gramicidin A and glycerylmonooleate were obtained from Sigma Chemical Company (St. Louis, MO). Dipalmitoylphosphatidylcholine was synthesized and testified biochemically by the method described in reference 4 by Dr. E. Ya. Kostetsky. The surface of highly oriented pyrolitic graphite was made hydrophilic by means of electrochemical oxidation as described in reference 5.

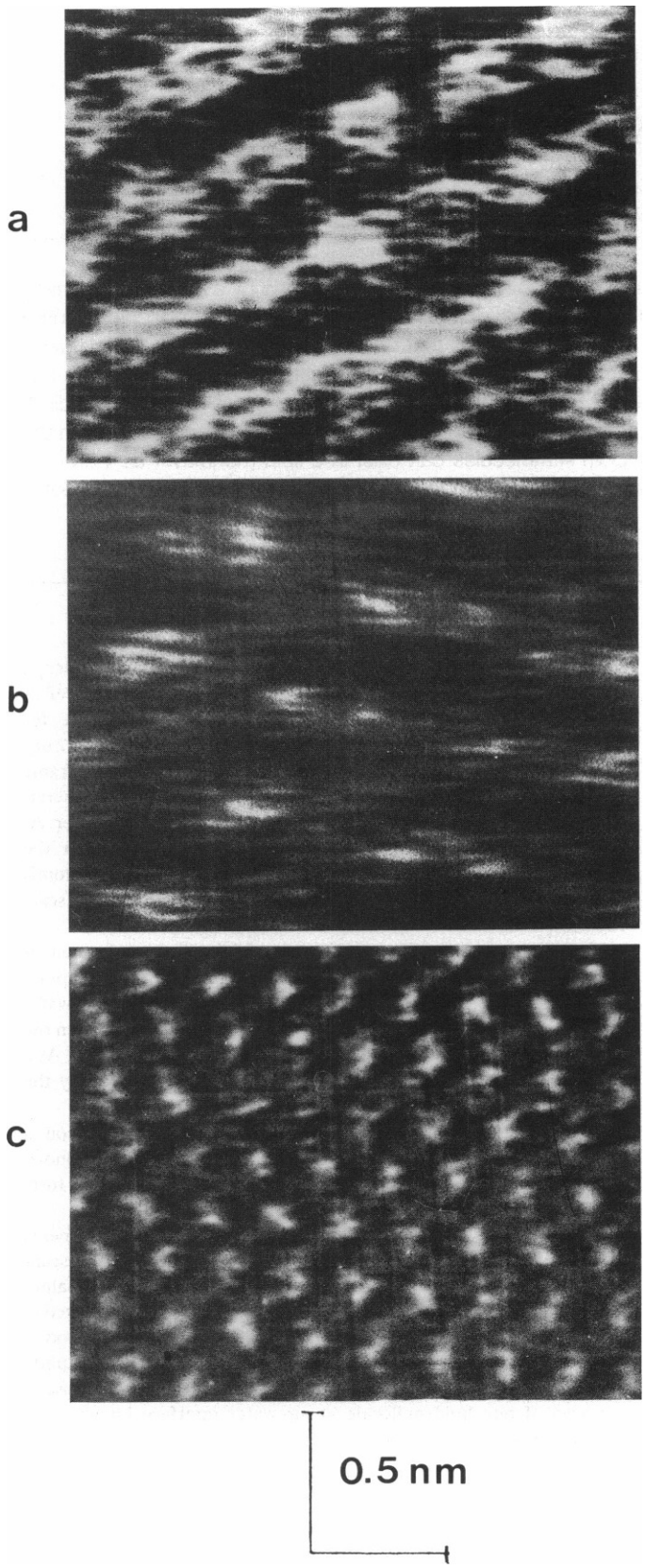
The scanning tunneling microscope operating in air was used. It has been described at the Institute for analytical instrumentation of the Academy of Sciences (Leningrad, USSR) (6). Tungsten tips were formed by both electrochemical polishing and mechanical cutting methods. The rough approach of a sample to the tip was made by means of an inertial piezowalker. The piezoelectric tube scanner was 30 mm long and of 12 mm external and 10 mm internal diameter. The maximum frame size was 5 × 5 μm^2. Scanning tunneling microscope measurements were performed at constant tunneling current as well as at the constant tunneling distant mode. The minimum time for measuring in one point at constant tunneling current mode was 0.2 ms. The number of points in one frame was 150 × 150. Maximum frame frequency under constant average distance mode was 20 Hz. Operating, measuring, and data analysis were made by means of computer. At constant average current mode the image was displayed on the oscilloscope screen. Images of a pyrolitic graphite surface with atomic resolution were used to calibrate the X and Y scales, Z scale calibration was made by optical interferometer (6).

The lipid monolayers containing gramicidin A were formed at air–water interface and deposited on an oxidized pyrolitic graphite substrate by the Langmuir–Blodgett technique (5, 7). At air-water interface a monolayer of lipid and gramicidin A was formed. Then the oxidized graphite substrate was slowly removed from water to air. As a result, the surface of the oxidized graphite became covered by the monolayer of the lipid and gramicidin A.

The lipid bilayers containing gramicidin A were formed on a nonoxidized graphite by the similar technique. In this case a nonoxidized graphite was immersed under water and then removed from water to air.

Pure lipid films on graphite were studied in the control experiments as well. Spatial modulation of conductivity was observed when scanning was performed above the glycerylmonooleate and dipalmitoylphos phatidylcholine monolayers (Fig. 1). The images presented in Fig. 1 are reproducible. It is seen from Fig. 1 that the spatial period of lipid monolayer structures is several times larger than the graphite one. The area per one white spot in Fig. 1 *a* (0.175 nm^2) is very close to the area of one lipid molecule at air–water interface known from surface pressure measurements (8). We conclude therefore that the molecular structure of the lipid monolayer appeared in the scanning tunneling microscope image. Molecular resolution was obtained also when scanning was performed above the dipalmitoylphosphatidylcholine bilayer.

The possibility of tunneling current passing through mono- and bimolecular films of arachidonic acid and dipalmitoylphosphatidylcho-



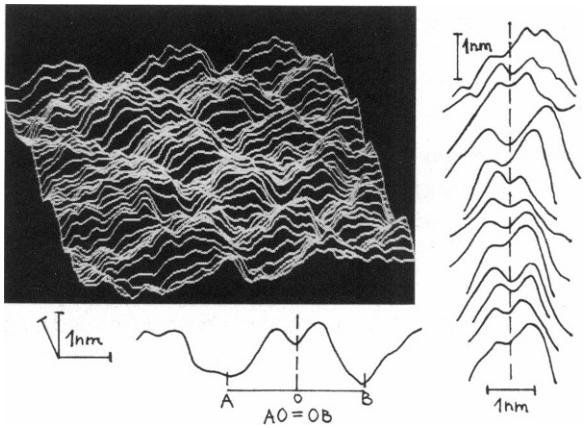


FIGURE 2 Side-view of the structure formed at relatively high concentration of gramicidin A in the glycerylmonooleate monolayer (gramicidin A/glycerylmonooleate is 1:1 in weight). The scanned area is 7.5 × 10 nm^2. The constant tunneling current mode was used. One image was obtained in 10 s. To the right cross-sections of individual objects are presented.

line has been previously shown (5, 7). Our scanning tunneling microscope images are similar to the lipid films images obtained earlier.

RESULTS AND DISCUSSION

The main purpose of the present work was visualization of ionic channels formed by gramicidin A in lipid membranes. We have obtained scanning tunneling microscope images of monolayers formed of lipid with gramicidin A. Quasi-regular, densely packed structure was obtained under high concentration of gramicidin A. Typical image of such region is given in Fig. 2.

It is important to point out that we never found such objects when we were studying pure lipid layers. This fact and also the size of the objects (2 ± 0.5 nm) lead to the conclusion that these objects are scanning tunneling microscope images of gramicidine A molecules built into lipid monolayer.

The cross-sections of individual molecules are given in Fig. 2, to the right. One can see that the characteristic size of a gramicidine A molecule is 2 ± 0.5 nm. Moreover, in the central part of the scanning tunneling

FIGURE 1 Scanning tunneling microscope images of glycerylmonooleate (*a*) and dipalmitoylphosphatidylcholine (*b*) monolayers on a graphite substrate. The image of the pyrolitic graphite substrate (*c*) is shown for comparison. Fast scanning at the constant tunneling distant mode was used. One image was obtained in 1 s, 300 mV were applied to the tip. White areas in pictures mean higher tunneling current in comparison with black areas.

microscope molecule image the cavity is present of 0.4 ± 0.05 nm in halfwidth (diameter at 50% depth).

We also tried to obtain the images of individual gramicidin A molecules in lipid monolayer with low concentration of antibiotic. In these experiments we saw as a rule the structures of pure glycerylmonooleate monolayer. Total area of 4,500 nm^2 was visualized. But six times we met regions with significant local rise of tunneling current. They looked like hills with an average diameter of 2 ± 0.5 nm (Fig. 3) on the images obtained at constant tunneling current mode. The size and the shape of these hills were similar to the same characteristics of the objects obtained under high concentration of gramicidin A (Fig. 2). Therefore we are inclined to think that the object shown in Fig. 3 is an individual molecule of gramicidin A in the lipid monolayer.

It should be noted that parameters of tunneling current and voltage necessary for obtaining good quality images of gramicidin A and lipid molecules differed. Therefore it is difficult to obtain good images of gramicidin A and lipid molecules at the same picture. But the control experiment with different scanning parameters showed that tightly packed lipid molecules were presented in space between gramicidin A molecules. Analysis of the pictures showed that the total area of spots imaged in Fig. 2 was ~50% of the area of a monolayer. The films were made of a mixture of gramicidin A and lipid in weight ratio 1:1. Therefore the obtained result

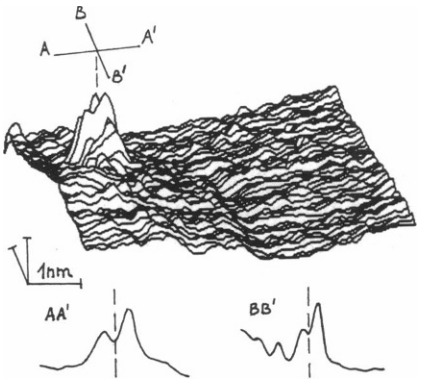


FIGURE 3 Side-view of the structure formed at low concentration of gramicidin A in the glycerylmonooleate monolayer (0.1 nM of gramicidine A was in solution when the monolayer was formed on the water-air interface). The scanned area is 7.5 × 10 nm^2. Scan parameters are the same as the ones in Fig. 2. Cross-sections of the individual object are presented below.

supported the conclusion that gramicidin molecules were imaged in Figs. 2 and 3.

To confirm our conclusions the experiments with another type of lipid layer, dipalmitoylphosphatidylcholine bilayer, have been carried out.

Typical result is given in Fig. 4, where molecules combined into clusters of chain type with average

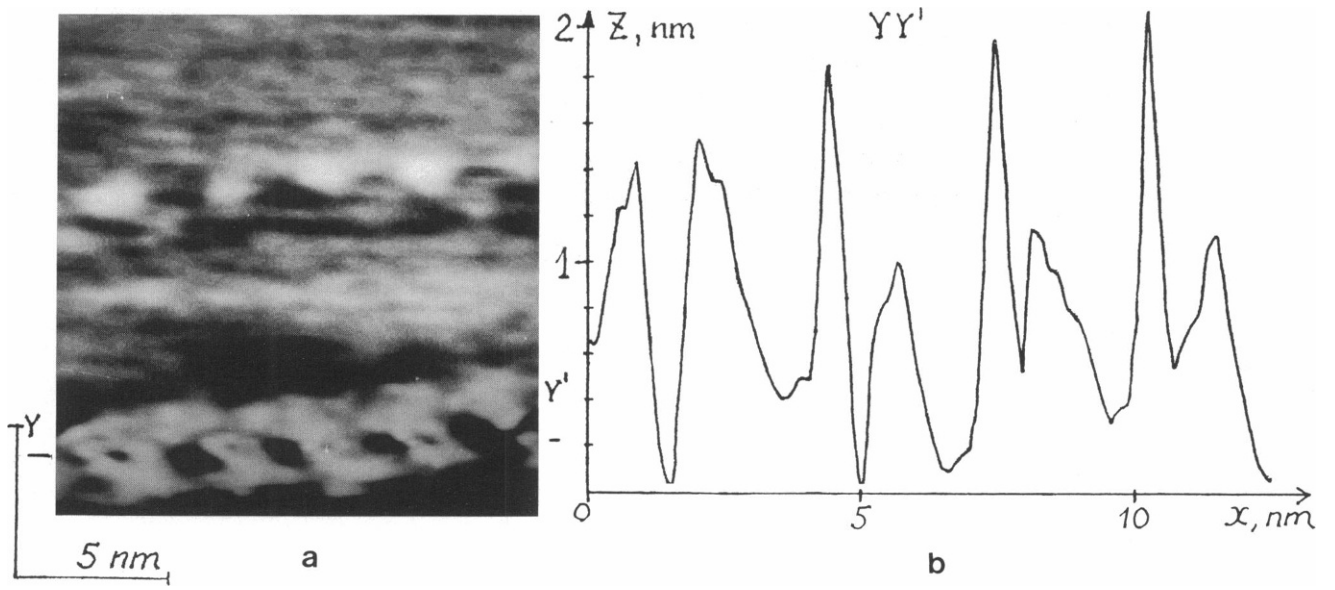


FIGURE 4 Scanning tunneling microscope image of "chain-type" clusters of gramicidin A molecules in lipid bilayer formed from dipalmitoylphosphatidylcholine on graphite substrate. Scans were performed with a frame size 15 × 15 nm^2 in 0.15-nm steps at a bias voltage of 30 mV. Constant tunneling current of 0.3 nA was employed. One image was obtained in 10 s. The ratio of lipid/gramicidin A in the bilayer was 20:1 in weight. (*a*) Top view. (*b*) Cross-section in line Y-Y'.

intermolecular distance of 3.0 ± 0.1 nm are visualized. It could be seen from cross-sections given to the right part of Fig. 4 that the cavity exists in the center of the scanning tunneling microscope image of gramicidin A molecules.

The image obtained in repeated scanning in the smaller frame which corresponds to the left edge molecule from this chain cluster is given in Fig. 5 *a*. One can see that the cavity in the centre of this molecule has the halfwidth of ~0.4 nm.

Let us compare the obtained images with a molecular model of gramicidin A channel. Urry proposed two $\beta^6_{3,3}$-helixes dimerized by head-to-head hydrogen bonding as the dominant channel structure in a bimolecular lipid membrane (9). Each $\beta^6_{3,3}$-helix is located in one lipid monolayer. In Fig. 5 *b* the molecular model of a gramicidin A channel is shown. It is seen that hydrophobic butt-end of the gramicidin "half-pore" in a channel has triangular form because of tryptophan residues. A pore 0.4 nm in diameter is located in the center of $\beta^6_{3,3}$-helix. We can see that the scanning tunneling microscope image of a gramicidin molecule shown in Fig. 5 *a* has similar form and size as hydrophobic butt-end of the gramicidin $\beta^6_{3,3}$-helix. Location and dimension of black area in Fig. 5 *a* and the channel pore in Fig. 5 *b* are quite similar to each other. Therefore it is possible to con-

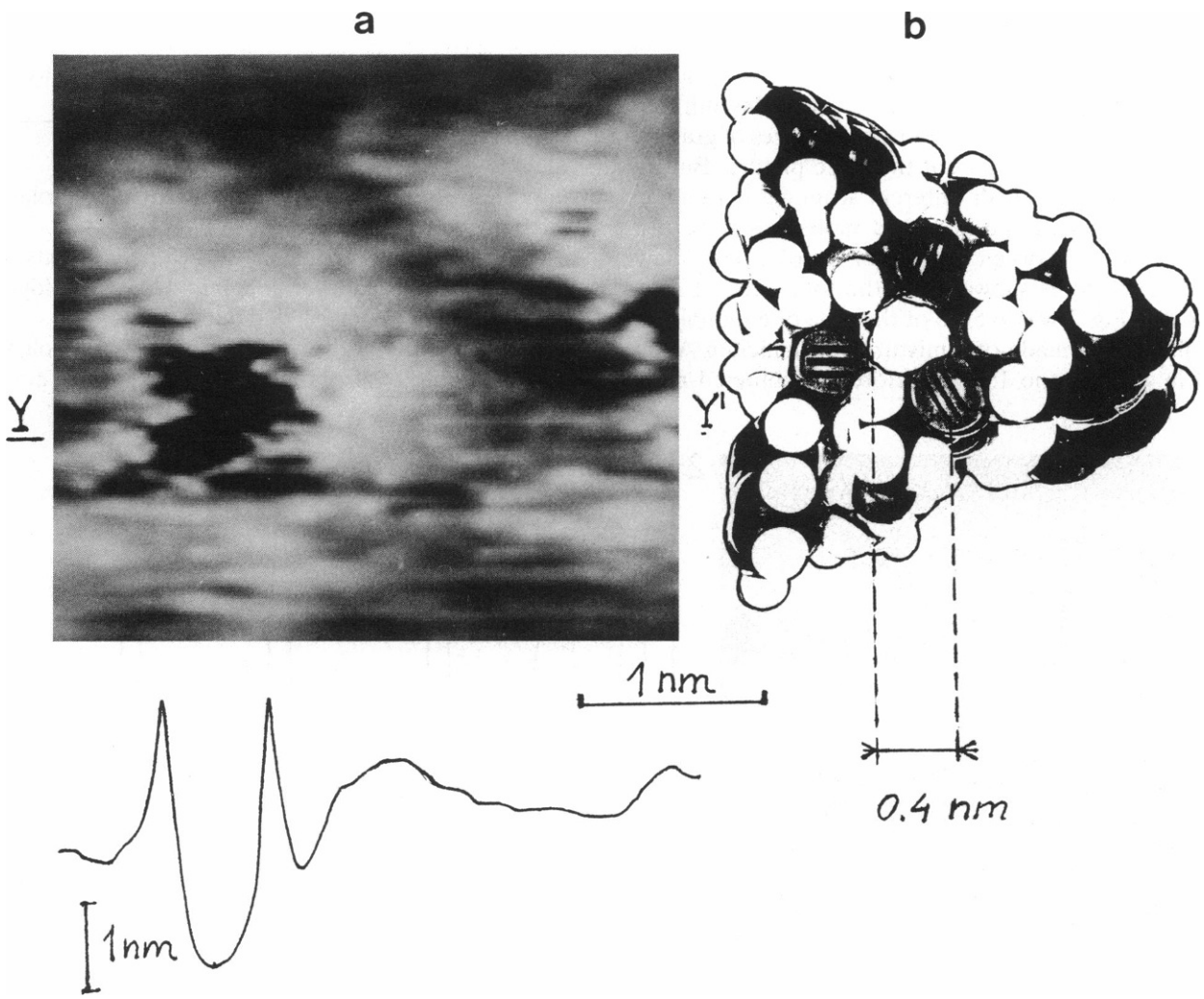


FIGURE 5 Scanning tunneling microscope images and molecular model of gramicidin A molecule. (*a*) Scanning tunneling microscope top-view image of gramicidin A molecule in the dipalmitoylphosphatidylcholine bilayer. The scanned area is 4 × 4 nm^2. Cross-section in Y-Y^1 plane is shown below. (*b*) Channel view (helix axis perspective) of Corey-Pauling-Koltun molecular model of gramicidin A in the $\beta^6_{3,3}$-helical conformation is taken from reference 9.

clude that the image of the gramicidin $\beta^6_{3,3}$-helix was obtained.

There is no answer to the question now why tunneling current can flow across such thick area as lipid layers with incorporated peptides. Generally speaking one can consider that the spatial distribution of electron density is displayed on the scanning tunneling microscope image. But it required additional experimental and theoretical study to understand the nature of contrast of the scanning tunneling microscope images of such complex objects as peptides. At present we can compare the peculiarities and characteristic sizes of molecule models with scanning tunneling microscope images.

The obtained results demonstrate that the scanning tunneling microscope can be used for high spatial resolution study of the structure of the molecules forming ionic channels in a lipid membrane.

It is interesting that gramicidin A molecules can be combined into clusters (Fig. 4). It is possible that existence of such clusters should be taken into account when time parameters of channel gate functioning in a lipid bilayer are estimated (10).

It is known that a scanning tunneling microscope is able to operate in water (11). Therefore one could expect that the direct experiments of both ionic channel visualization and conductivity measurements may be carried out by means of the scanning tunneling microscope.

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