Scanning tunneling microscopy of gramicidin A molecules embedded in Langmuir-Blodgett film

S. A. Vinogradova, A. O. Golubok, O. V. Kolomytkin, and S. Ya. Tipisev
Institute of Analytic Instrument Manufacture, Academy of Sciences of the USSR

(Submitted 12 April 1990)
Pis'ma Zh. Eksp. Teor. Fiz. **51**, No. 10, 513–515 (25 May 1990)

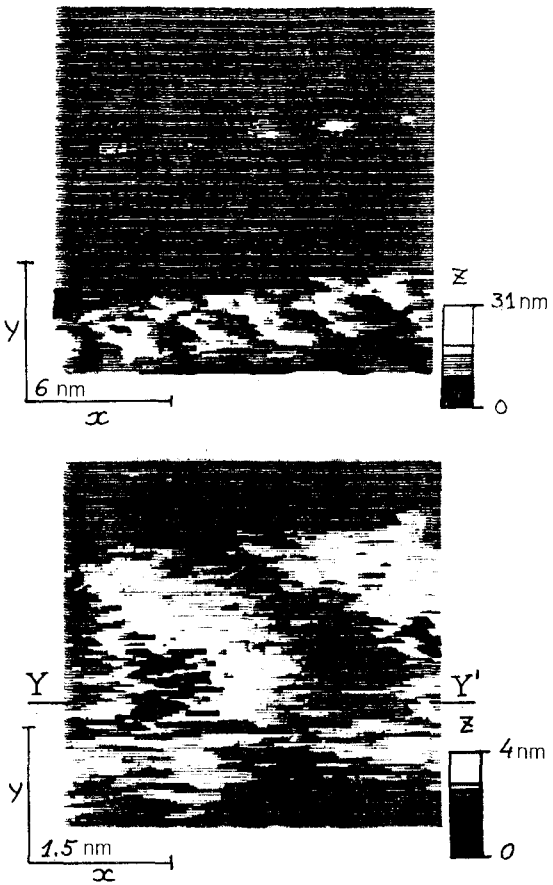
The ion channels formed by gramicidin A molecules embedded in a Langmuir-Blodgett film have been visualized by scanning tunneling microscopy at atmospheric pressure. The film was a bilayer of dipalmitoyllecithin molecules on a graphite surface. The results of the study agree with existing molecular models.

The structure and electronic properties of Langmuir-Blodgett films are of interest in the effort to solve a variety of fundamental and applied problems. For example, Langmuir-Blodgett films of lipid and monomolecular and bimolecular layers modified by molecules are used as models of cell membranes with ion channels and are also used to decipher the molecular mechanisms for the ionic conductivity of cell walls.[1] The experimental methods which have been used in previous studies detect only the functional properties of the channels. A new approach to studying the ion channels embedded in lipid membranes is to use a scanning tunneling microscope (STM),[2] which has already been used to study pure Langmuir-Blodgett films.[3–5] Tunneling microscopy[6,7] differs from the standard methods of electron microscopy and spectroscopy in that the energy applied is small, and the spatial resolution is high, not only in vacuum but also in gases and liquids.[8] This flexibility is particularly important for studying biological specimens.[9,5]

In this letter we are reporting a study of a Langmuir-Blodgett film of dipalmitoyllecithin (DPML) with embedded ion channels formed by gramicidin A molecules. The substrate was a clean cleaved surface of pyrolytic graphite, which has been studied thoroughly by scanning tunneling microscopy and which has a sufficient number of atomically smooth regions.[10] A bilayer of DPML molecules was formed on the substrate by the OLB method.[3] We then added an alcohol solution of the gramicidin (10^{-4} g/liter) to the surface of the substrate coated with the lipid bilayer. The gramicidin molecules became embedded in the lipid bilayer as a result. The experiments were carried out at atmospheric pressure in the STM described in Refs. 10 and 11.

For comparison we studied STM images of a clean graphite substrate, of a lipid bilayer, and of a lipid bilayer modified with gramicidin molecules. In the case of the clean graphite we observed a hexagonal structure with the known period;[6,7,10] this period was then used for spatial calibration of the subsequent images. After the deposition of the DPML bilayer, we observed a considerably "noisier" regular structure with a length scale $\sim$0.6–0.7 nm, which is much larger than the length scale of graphite. We accordingly associate the observed STM image with the outer monolayer of the membrane.

Figure 1 shows an STM image of a lipid bilayer modified with gramicidin A



FIGS. 1 and 2. Scanning tunneling microscopy image of ion channels (top view) in a Langmuir-Blodgett film of dipalmitoyllecithin. $V_T = 30$ mV, $I_T = 0.3$ nA. The positive potential is applied to the tip.

molecules. Figure 2 shows an STM image obtained during a repeated scan of a region of smaller area near the characteristic features in Fig. 1. Figure 3 shows a section of the STM image along the Y–Y' in Fig. 2.

The characteristic structures in Figs. 1 and 2 appeared only in the case of a lipid layer modified with gramicidin molecules. This fact, along with the size and shape of the observed structures, suggests that gramicidin A molecules embedded in the lipid bilayer are being visualized in Figs. 1 and 2. The typical size of the molecule according to the STM image is ~ 1.5 nm. At the center of the image there is a "dip" with a typical size ~ 0.5 nm (Fig. 3). These results agree with existing molecular models,[12] according to which the gramicidin A molecule is a cylinder 1.2 nm in diameter and 1.5 nm long with an internal channel 0.4 nm in diameter.

We determined the optimum values of the tunneling current I_T and of the voltage V_T—i.e., the values which result in high-quality STM images of the molecules. These values turned out to be slightly different for the gramicidin A and the DPML. We were thus unable to select conditions under which the periodic structure of the lipid

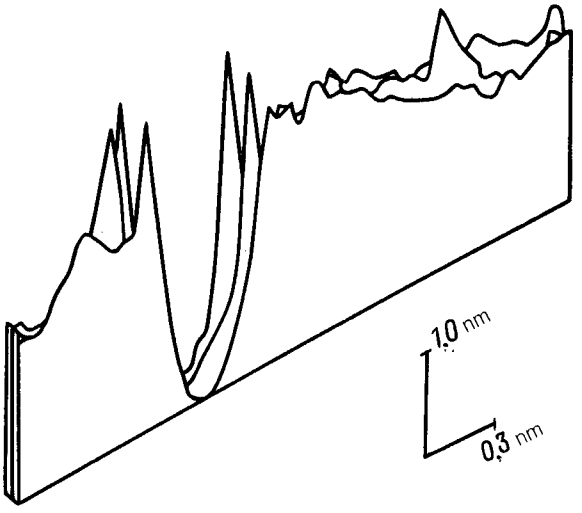


FIG. 3. Section of an STM image of an ion channel.

bilayer would be manifested along with the STM image of individual gramicidin molecules on a single frame. However, a control experiment in which we varied the scanning conditions revealed that the gramicidin molecules are separated by a layer of close-packed DPML molecules with an intermolecular distance of 0.6–0.7 nm.

In summary, this study has demonstrated a new capability: Individual ion channels in a lipid membrane can be visualized by STM. This capability opens up some important opportunities for learning about the molecular mechanisms by which these channels function. We wish to stress that the contrast of these STM images may be due to either geometric factors or local electronic properties of the specimens (the height of the tunnel barrier, the local density of states, etc.). To determine the nature of the contrast, we are carrying out some additional measurements of the local work function, i.e., of $I_T(V_T)$ and dI_T/dV_T.

We thank D. N. Davydov and V. A. Timofeev for participation in these experiments, and we thank N. A. Tarasov and M. L. Fel'shtyn for useful discussions.

[1]O. V. Kolomytkin and V. I. Kuznetsov, *Studia Biophisica* **115**, 157 (1986).
[2]O. V. Kolomytkin *et al.*, in *Fourth International Conference on STM/STS-89*, Japan, July 9–14, 1989, Vol. 19.
[3]D. P. E. Smith *et al.*, Proc. Natl. Acad. Sci. U.S.A. **84**, 969 (1987).
[4]T. R. Albrecht *et al.*, J. Appl. Phys. **64**, 1178 (1988).
[5]J. H. Coombs *et al.*, Thin Solid Films **159**, 293 (1988).
[6]P. K. Hansma and J. Tersoff, J. Appl. Phys. **61**, R-1 (1987).
[7]V. S. Édel'man, Prib. Tekh. Eksp. No. 5, 25 (1989).
[8]J. S. Khaĭkin and A. M. Troyanovskiĭ, Pis'ma Zh. Tekh. Fiz. **11**, 1236 (1985) [Sov. Tech. Phys. Lett. **11**, 511 (1985)].
[9]S. D. Alekperov *et al.*, Dokl. Akad. Nauk SSSR **303**, 341 (1988) [Sov. Phys. Dokl. **33**, 828 (1988)].
[10]A. O. Golubok *et al.*, Poverkhnost' **3**, 146 (1989).
[11]A. O. Golubok *et al.*, in *Scientific Instrument Manufacture. Electron-Ion Optics (Collected Scientific*

Works, Scientific and Engineering Department, Academy of Sciences of the USSR), Nauka, Leningrad, 1989, p. 72.
[12]L. N. Ermishkin and A. Ya. Zil'bershteĭn, *Scientific and Technological Progress. Membrane Biophysics, Vol. 2,* VINITI, Moscow, 1982.

Translated by Dave Parsons