3P-203 ハイドロゲル界面人工膜を用いたチャネル電流計測 Lipid Bilayers at the Gel Interface for single Ion Channel Recordings

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Single channel recording using artificial lipid bilayers is along with the patch-clamp technique a very powerful tool to physiologically and pharmacologically study ion-channels. However, the fragility of the bilayers and the difficulty to incorporate ion-channels into them significantly compromises measurement efficiency. We have developed a novel method for forming artificial lipid bilayers at a hydrogel-water interface that significantly improves the measurement efficiency. Bilayers formed almost instantly and were able to incorporate various types of ion-channel proteins within a short time enabling multichannel measurements. We have also developed a practical method to produce artificial lipid bilayers using a hydrogel-hydrogel interface for ion channel measurements. Bilayers were formed by forcing a hydrogel-bead into contact with the hydrogel plate in a lipid solution. The immediate formation of a bilayer was observed. This allows channel recordings to be repeated more easily and quickly as compared to conventional methods. Currents of various types of channel such as gramicidin, hemolysin, BK-channel, and channels from skeletal muscle SR and Torpedo electric organ have been recorded. These results indicate that these methods can potentially be applied to developing high-throughput screening devices for drug design.

3P-204 流動電位測定による KcsA カリウムチャネルのイオン透過機構の検討

Water-ion coupling in ion permeation through KcsA potassium channel revealed by streaming potential measurements

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Potassium channels permeate K^+ ions at rates approaching 10^8 ions per second with strict selectivity. This function is crucial for generating rapid changes in membrane potentials. For KcsA potassium channel, high resolution crystal structures in the closed state have revealed the single-file distribution of ions and water molecules in the pore. Many simulation studies have been performed based on these static pictures, providing microscopic processes in ion permeation. On the other hand, single-channel analyses gave throughput rates of ion conduction. To fill the gaps between microscopic and macroscopic processes of ion permeation, experimental data for the ratios of ion and water flux through the channel are crucial. Here we examined the streaming potential (V_{stream}) of ion permeation in the KcsA channel using liposome patch-clamp technique. V_{stream} provides a quantitative index of the number of water molecules accompanying a conducting ion (the water-ion coupling ratio), the knowledge of which is a prerequisite for elucidating the coordination of water and ion during permeation. Current-voltage relationship of KcsA currents was recorded under varying osmotic pressure and the V_{stream} was evaluated as a shift of the reversal potentials. Effects of concentration and species of permeating ions on the V_{stream} were investigated. Our results provided an essential clue for examining the mechanism of ion permeation through potassium channels.

3P-205 1分子構造変化の可視化によって明らかになった KcsA カリウムチャネルの開閉ねじれ運動

Visualization of conformational changes of single molecular KcsA potassium channels revealed global twisting motions upon gating.

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In the last ten years crystal structures of potassium channels have revealed static pictures of their open and closed conformations. To examine how the channels undergo conformational changes during gating, we took the following method. KcsA potassium channel, which exhibits pH-dependent gating, was fixed on a glass plate with a gold nanocrystal attached to the other end. Synchrotron white X-rays were irradiated and a diffraction spot reflected from the nanocrystal was recorded in video rate. Motions of the channels were figured from the trajectory of the diffraction spots. At neutral pH where the channel kept closed, restricted Brownian motion was detected. At acidic pH where the channel underwent gating, twisting motion exceeding 20 degrees was observed, while the bending motion was within the range of 5 degrees. These motions were also observed in the channel fixed in the erect and sideways-laid orientations. This consistent dynamic picture viewed from different viewpoints demonstrated that the channels twisted around the ion permeation pathway upon gating. When the cytoplasmic domain was deleted, the twisting motions were also observed but were slightly enhanced. Moreover, in the presence of a channel blocker, tetrabutylammonium, which binds to the pore in the transmembrane domain, the twisting motion was stopped. From these results, we conclude that the KcsA channel exhibits global twisting motions upon gating with a tight mechanical coupling between the transmembrane and cytoplasmic domains.

3P-206 細菌機械受容チャネル MscS の細胞質ドメインの構造変化とチャネルゲーティングの関係

Role of conformational change in the cytoplasmic domain of bacterial

mechanosensitive channel MscS in its mechanogating

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The bacterial mechanosensitive channel MscS opens upon hypoosmotic shock to protect the cells from lysis. MscS forms a homoheptamer of a subunit composed of transmembrane (TM) and cytoplasmic (CP) domains. Recently we have shown that an electrostatic interaction between TM and CP domains is crucial for channel opening, based on the observation that D62N mutant, which loses TM-CP domains interaction, was hard to open (Nomura et al., Biophys. J., 2008). We also reported that CP domain swells up when MscS opens (Machiyama et al., Ann. Meeting of BJS, 2007), however, the relationship between the channel opening and CP domain swelling remains unknown. To address this issue, we constructed wild/F178C and D62N/F178C mutants of which cysteine residues were labeled with Alexa488 and Alexa568 randomly, and measured FRET efficiency to estimate changes in the diameter of CP domain at Phe-178 during channel opening. Both mutants showed decreases in FRET efficiency with a smaller value for D62N/F178C, indicating that the loss of TM-CP interaction decreases the degree of CP domain swelling. This is the first experimental evidence showing that the CP domain swelling by tension in the membrane has a crucial role in MscS opening via an electrostatic interaction between TM and CP domains.

3P-207 1分子構造・機能同時計測によるカリウムチャネル (KcsA チャネル) の開閉機構の解明

Simultaneous measurements of optical and electrical properties of potassium channel, KcsA

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To clarify the gating mechanism of ion channels, we have tried to simultaneously measure the functional and conformational changes of single ion channels. Here, conformational changes of the potassium channel, KcsA, were detected fluorescently. The crystal structure of KcsA was determined in 1998 and a lot of studies on its structure have been done, providing much structural information. At first, based on such information, several sites that are likely to regulate the gating were selected. And amino acids at these sites were exchanged for cysteine and were labeled with an organic dye molecule (tetramethylrhodamine-maleimide). Next, to detect conformational changes accompanied by gating, macroscopic fluorescence intensity was measured under activated (pH 4) and inactivated condition (pH 7). Five mutants labeled at the vicinity of a membrane or the cytoplasmic region had large fluorescence intensity difference between pH4 and pH7. From these results, these regions are likely to be in different conformation in open and closed state and dynamically change during gating. We will simultaneously measure its current and fluorescence intensity, and clarify the relationship between functional and conformational changes of potassium channel.

3P-208 マイクロアレイにおける α -ヘモリシンのポリヌクレオチド輸送特性

Transport Property of Polynucleotide Molecules on α -Hemolysin Nanopore Array
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In this paper, we examine the transport properties of polynucleotide molecules through α -hemolysin (α -HL) nanopores, a bacterial membrane ion channel, using a microfluidic chip. The α -HL heptamer forms a nanopore of 1.5 nm in diameter on a bilayer lipid membrane (BLM). Once formed, the nanopore remains open-state and passes a detectable ionic current through the membrane. Since the aperture is comparable to a molecular size, the ionic current will diminish by the penetrating molecule. The recent works focusing on this transport phenomena revealed that the nanopore may provide intensive information on the structure and composition of single polynucleotide molecules. We, therefore, fabricate the microfluidic, arrayed chip, targeting a parallel recording of the membrane ion-channel currents to realize high throughput analyses of the transport properties. By the simple three-step protocol, we achieve to form planar bilayer lipid membranes in an array format on the chip and to integrate the nanopores into the membranes at once. Here we performed the simultaneous monitoring of the nanopore currents on the microchip, where the signal consisted of stepwise currents from the nanopores and sharp spikes corresponding the transports of a nucleic acid polymer. The features of the blockading spikes successfully discriminate, for instance, the different concentration of the nucleic acid polymer by its frequency. The results promise the high potentiality of our microchip for high-throughput applications, and we report them in detail.

3P-209 電位依存性プロトンチャネルのプロトン透過の温度依存性

Temperature-dependence of Proton Permeation through a Voltage-Gated Proton Channel.

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The voltage-gated proton channel exists in phagocytotic cells to promote proton flux through the membrane during respiratory burst. The channel shows extreme