Twisting Conformational Changes of Single KcsA Potassium Channel upon Gating using Diffracted X-ray Tracking

Ion channels are signal transduction molecules that selectively permeate ions and generate electrical signals through turning on and off of ion permeation pathways. Channels respond to various stimuli, such as membrane voltage and chemical substances. With sensor domains, channel molecules transduce various stimuli into conformational changes of ion permeation pathways, leading to ion-conductive (open) and non-conductive (closed) states. Single-channel current recordings have elucidated the random nature of opening and closing transitions (gating; Fig. 1(a)). Genes of potassium channels exist from bacteria to humans and constitute one of the largest gene family. Ten years ago, the crystal structure of a potassium channel from *Streptomyces lividans* (KcsA channel) was resolved (Fig. 1(b)). The three-dimensional structure provided the architecture of the ion permeation pathway and insights on how ions are selectively permeated. Crystal structures of several other types of potassium channel have been obtained. However, the mechanism underlying the gating has not been elucidated from static images of potassium channels.

Here, we applied the diffracted X-ray tracking (DXT) method to examine conformational changes of single KcsA potassium channels upon gating [1]. Gold nanocrystals were produced (20 nm × 5 nm), which elicit single diffraction spots from the (200)-plane upon irradiation of X-rays. KcsA channels were mutated to have four reaction sites for secure binding of a nanocrystal. Diffraction spots from the nanocrystals were recorded at a video rate, which represents the trajectory of the conformational changes of the channel molecules [2]. To observe the channels from a fixed viewpoint, channels were attached to a glass plate in the same orientation. Here, an upright orientation was taken by attaching the extracellular loop of channel to the plate. A gold nanocrystal was bound to the cytoplasmic domain of the channel and white X-rays (**BL44B2**) were irradiated normal to the glass surface (Fig. 2(a)). In this configuration, radial motions of diffraction spots represent the bending of the channel molecule along the longitudinal axis, and circumferential motions of the spots correspond to rotational changes of the channel around the axis (Fig. 2(b)). KcsA channels show pH-dependent gating, which were exploited in this study to contrast the changes in motion in a different gating status.

When channels were in the closed state at neutral pH, diffraction spots moved radially. This represents bending motions of channels in the range of a few degrees. When channels were actively gated at acidic pH, diffraction spots moved circumferentially (Fig. 3(a,b)). These motions of the spots correspond to the rotation of the channel around the longitudinal axis. Random clockwise and counter-clockwise rotations were recorded. We found that the range of the rotations was several tens of degrees, which are unexpectedly large values. The direction of the rotational motions was sometimes reversed during recordings. We call this type of motion as twisting. This is the first observation of a large twisting in the conformational change of channel molecules upon gating.

The twisting of the channel was confirmed by observing the motion from different viewpoints. The channel was oriented upside-down (Fig. 2(c)), in which the channel molecules were attached to the glass plate through the cytoplasmic domain and the nanocrystal was attached to the extracellular loop. Again, similar twisting motions were detected. In another experiment, channels were laid sideways. The channel was attached to the glass plate at the N-terminus and the nanocrystal was attached to the

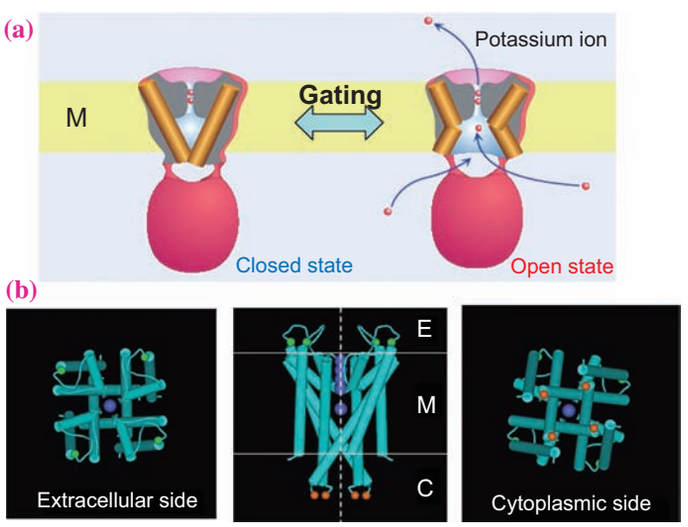

Fig. 1. KcsA potassium channel. **(a)** Cartoons for gating transition of channels. **(b)** Architecture of KcsA potassium channels for transmembrane domain. The potassium channels are tetrameric and composed of two transmembrane helices for each subunit. Inner helices form a bundle crossed at the intracellular side. Dark blue spheres are potassium ions, red spheres are the binding sites for the nanocrystal and green spheres are sites for attaching the glass plate.

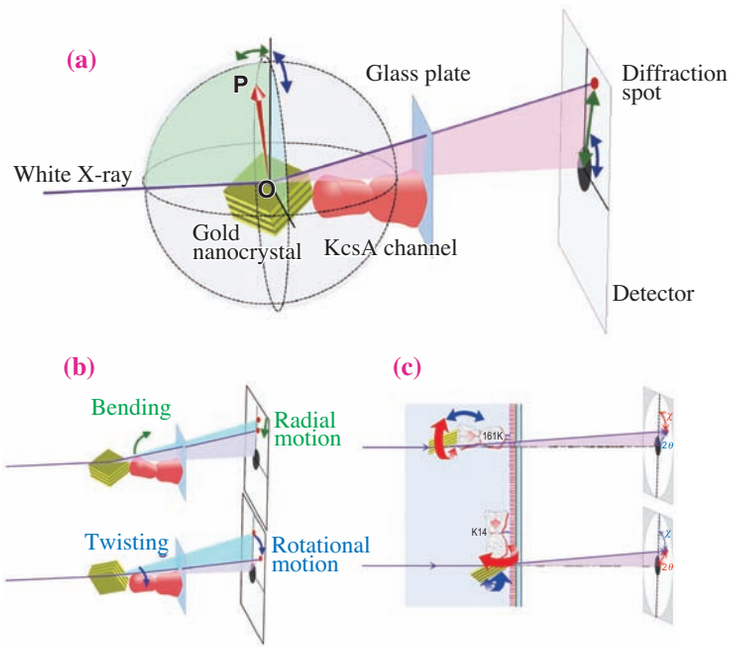

Fig. 2. DXT method. **(a)** Experimental configuration of DXT measurement. The channel was fixed on the glass plate in the upright position. White X-rays were irradiated normal to the glass surface and the diffraction spots were recorded on the detector, which was placed 100 mm from the sample. **(b)** Geometrical relationships of channel motions in real space and spot motions in image plane. **(c)** Different orientations of channel on plate. The upper cartoon shows that the channels were fixed in the opposite orientation. The lower cartoon shows the channel laid sideways.

cytoplasmic domain. In this orientation, the twisting of the channel should result in radial motions of the diffraction spots rather than circumferential motions. We found that no circumferential motions of the diffraction spots were observed even in the actively gated state, and radial motions were enhanced. These results are consistent with the geometrical conversion of the conformational changes of the channels in real space and the motions of the diffraction spots in the image plane. The underlying conformational changes of the channel seem to be invariant, even when the patterns of the motions of the diffraction spots differed significantly from different viewpoints.

In this study we traced the trajectories of the conformational changes of channel molecules upon gating. The clockwise and counter-clockwise twisting of the channel can be related to the helical bundle architecture of the permeation pathway. In the closed conformation, the helical bundle is crossed at the cytoplasmic side, which prevents the permeation of ions. The unwinding of the helical bundle, which allows ions to permeate, was detected as twisting motions (Fig. 3(c)). The twisting mode of conformational changes may prevail for gating of ion channels.

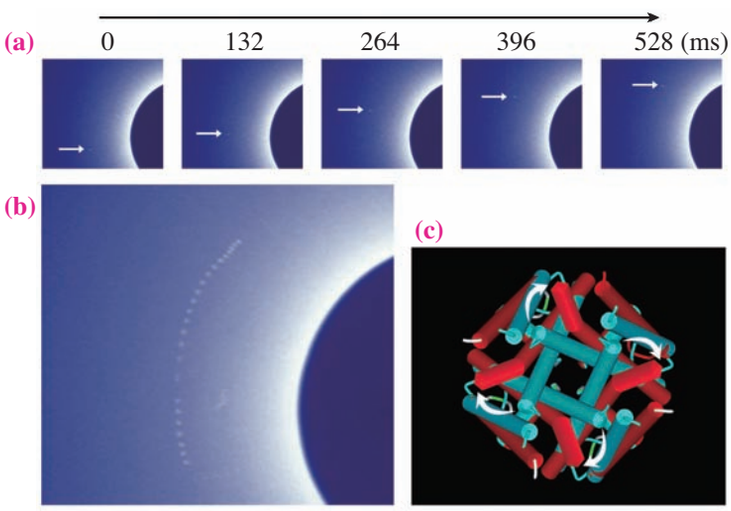

Fig. 3. Trajectories of diffraction spot upon gating. **(a)** Time series of image frames. **(b)** A trajectory of a spot recorded at a video rate was superimposed (24 frames) on an image frame. **(c)** KcsA channel viewed from intracellular side. Blue represents the closed structure and red represents the open structure. Concerted motions of helices lead to twisting of the channel structure.

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