Structure of the Connexin 26 Gap Junction Channel at 3.5 Å Resolution

Intercellular signaling is fundamental to the complex biological functions of multicellular organisms such as neural transmission, immune reaction, or reproductive function. Gap junctions mediate the intercellular signal by connecting the cytoplasms of two neighboring cells [1]. A gap junction contains clusters of intercellular channels called "gap junction channel." End-to-end docking of two hemichannels, also referred to as "connexons," forms a gap junction channel. Each connexon is composed of six connexin subunits surrounding the central pore. There are 21 connexin (Cx) isoforms in human with highly conserved sequences but different physiological properties. They can form heteromeric (more than two different connexins in a connexon) or heterotypic (a gap junction channel with different connexons) channels, conferring further complex diversity. Gap junctions are known to be involved in a wide variety of biological processes such as the cardiovascular system, reproduction system, nervous system and auditory system [2].

The primary method for the three-dimensional structural analysis of the gap junction channel has been electron microscopy (EM) [3,4]. Although EM revealed a great deal of the structure of the gap junction channel, a high resolution structure where each amino acid could be distinguished has been essential for more detailed biochemical and physiological analyses. Here, we have determined the structure of Cx26 at 3.5 Å resolution by X-ray three-dimensional crystallographic analysis. The data sets were collected at beamline **BL44XU**.

The overall structure of the Cx26 gap junction channel is like that of tsuzumi, a traditional Japanese drum (Fig. 1). Since there are no obstructions through the pore, and our crystallization condition generally promotes the channel in its open state, the structure is considered as an open conformation.

The Cx26 monomer has four transmembrane helices (TM1-TM4), two extracellular loops (E1, E2), an N-terminal region (NT), a cytoplasmic loop (CL), and a C-terminal tail (CT) (Fig. 1). NT has an N-terminal helix (NTH) inserted into the pore. The anomalous signals from seleno-methionine derivative crystals definitely reveal the four-helical arrangement. Each connexin protein has three intramolecular disulfide bonds between two extracellular loops, which are essential for the formation of the intercellular channel. The anomalous signals from native sulfur atoms show the locations of the three disulfide bonds in the extracellular region.

Most of the intermonomer interactions are located at the extracellular side of the membrane region (Fig. 2). These residues, and also those of the intramonomer interactions, are conserved among connexin isoforms, suggesting that the manner of folding and oligomerization is conserved in the connexin family, as has been suggested by the similarity in cryoEM structures of two types of connexin (Cx43 and Cx26) [3,4]. The extracellular loops, E1 and E2, mediate the docking of hemichannels (Fig. 2). Through these interactions, E1 and E2 make a tight seal in the extracellular space, separating the channel interior from the outside environment.

The permeation pathway of the Cx26 gap junction channel consists of an intracellular channel entrance, a pore funnel, a negatively charged path, and an extracellular cavity (Fig. 3). The intracellular channel entrance is composed of the cytoplasmic parts of TM2 and TM3. Positive charge residues are concentrated in this region and surround the entrance, creating a positively charged microenvironment around the channel pore. The pore funnel is formed by six NTHs and is the constriction site of the pore. The negatively charged path is located at the TM1/E1 boundary and the channel is narrowed again here. The extracellular

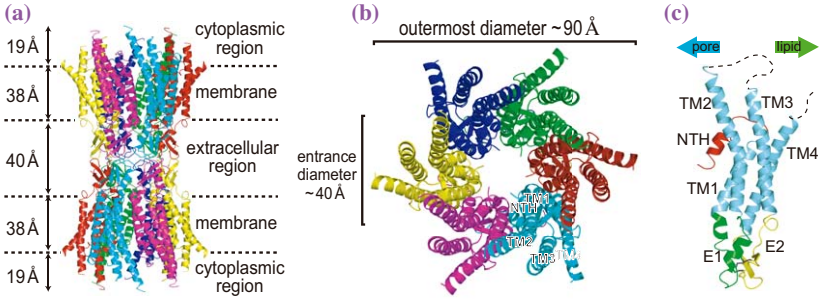

Fig. 1. Overall and monomer structures of human Cx26 gap junction channel in ribbon representation. **(a)** Side view of the Cx26 gap junction channel with the locations of plasma membranes and scale of each region. **(b)** Top view of the Cx26 gap junction channel representing the arrangement of the transmembrane helices and N-terminal helix. **(c)** Structure of the Cx26 monomer in ribbon representation.

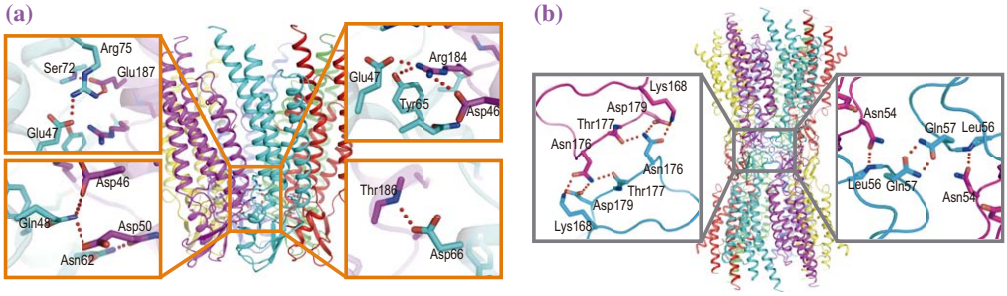

Fig. 2. Structural organization of Cx26 hexamer. **(a)** Intermonomer interactions in a connexon. Each interaction is shown in the magnified image. **(b)** Interactions between apposing connexons. Interactions in E1 and E2 are each shown in the magnified image.

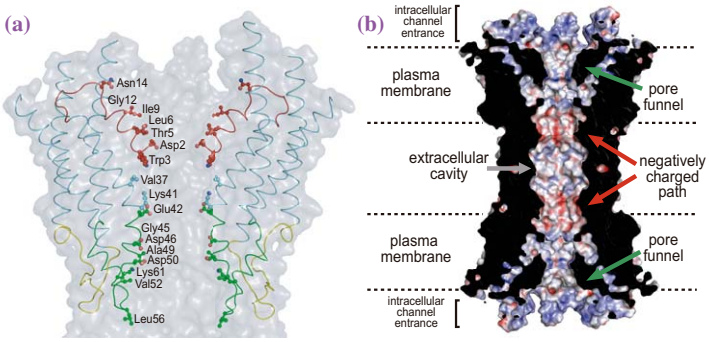

Fig. 3. Pore architecture of the Cx26 gap junction channel. **(a)** Side view of the Cx26 gap junction channel pore. Side chains of the pore-lining residues are drawn as sticks. **(b)** Surface potential distribution of the Cx26 gap junction channel interior.

cavity is formed by twelve portions of E1 from each subunit, creating a tight continuous inner wall of the channel in the extracellular region.

Six N-terminal helices form the "pore funnel" (Fig. 4). The circular hydrogen bond network at the bottom of the funnel stabilizes the pore funnel. The hydrophobic interactions draw the pore funnel onto the innermost wall of the channel, making it an open state (Fig. 4). Gap junction channels have the transjunctional voltage dependent gating mechanism [5]. A number of experiments using chimeras or substitutions suggest that NT, particularly the second amino acid residue, is the voltage sensor. Together with the recent EM-determined structure of the Met34Ala mutant, a novel mechanism of V_j-gating is suggested [4,6] (Fig. 4).

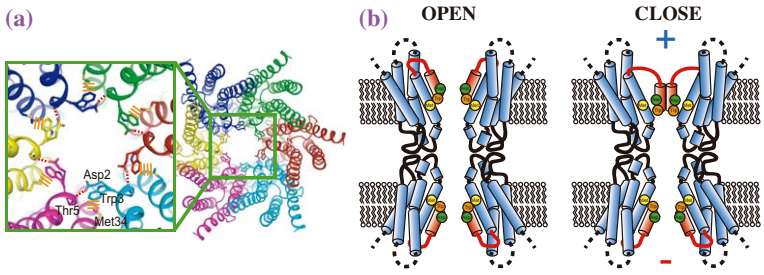

Fig. 4. Structure of the pore funnel and the plug gating model. **(a)** The six NTHs form a pore funnel, which is stabilized by a circular hydrogen bond network (red dashed lines) and attached to the inner wall of the channel by hydrophobic interactions (orange dashed lines). **(b)** Proposed plug gating model. The pore funnel senses the transjunctional voltage difference and forms a pore plug that blocks the channel.

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