Pore Formation Mechanism of Staphylococcal Pore-forming Toxin

Pathogenic bacteria express pore-forming toxins (PFTs) to attack host cells. PFTs are expressed as soluble monomeric proteins, which assemble to prepore oligomer on the target cells. After forming prepore, conformational change occurs, and then the pore is formed. Although the crystal structures of monomer and pore have been determined, the detailed mechanism was unclear because the high-resolution structure of prepore was unknown. In this study, we determined the crystal structure of the prepore of staphylococcal PFTs, which showed an invisible transmembrane region with rigid other parts. Based on the structural information and biochemical data, we propose a two-step transmembrane pore formation mechanism.

PFTs are important proteins universally used in a wide range of organisms to attack target cells. Eukaryotes use PFTs as immune proteins [1], whereas pathogenic bacteria secrete them to kill blood cells [2]. PFTs are expressed as water-soluble monomeric proteins, which assemble on the target cell membranes to form a ring-shaped nonlytic oligomeric intermediate, called prepore [3]. At the prepore stage, the transmembrane pore is not yet formed. After forming the prepore, a marked structural change occurs to form membrane-inserted pores.

PFTs secreted by *Staphylococcus aureus*, a major cause of hospital- and community-acquired infections, are the best studied PFTs. Most of the staphylococcal PFTs consist of two different polypeptides, in which four molecules of each component alternately assemble in a circular pattern. The crystal structures of the monomer and the pore have been determined, and a structural model of the prepore was constructed by superposing the structure of the monomer onto that of the pore [4-6]. Based on these structures, a feasible pore formation mechanism was proposed. However, the mechanism had a serious drawback, i.e., protomers of the prepore model show steric hindrance. Therefore, the actual pre-pore structure should differ from the model, and it was necessary to determine the practical structure of prepore to elucidate the mechanism of β-barrel pore formation. However, since prepore is unstable and it proceeds immediately to form the pore, crystallization of prepore was considered to be impossible.

In the present study, we successfully determined the crystal structure of the prepore-state oligomer of staphylococcal PFT using a mutant which forms stable prepore [7]. The revealed structure was octamer as observed for the previous pore structure, in which two components alternately and circularly assemble. However, the β-barrel region showed a striking difference, i.e., the bottom half of the β-barrel corresponding to the transmembrane region was completely invisible, whereas the upper half showed clear electron density (Fig. 1). No significant structural differences were observed in other regions. These observations indicate that, in prepore, only the transmembrane region is disordered, whereas an octameric assembly similar to the pore is formed. The stem region, which crashed into the adjacent protomer in the previous prepore structure model, extruded to form the upper half of the β-barrel. Consequently, the revealed prepore neatly avoided the steric hindrance.

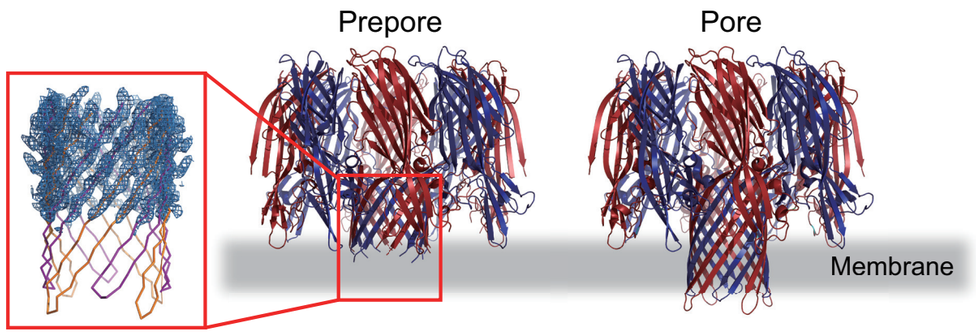

Figure 1: Structure of prepore and pore. The electron density of the β-barrel region of prepore is also shown.

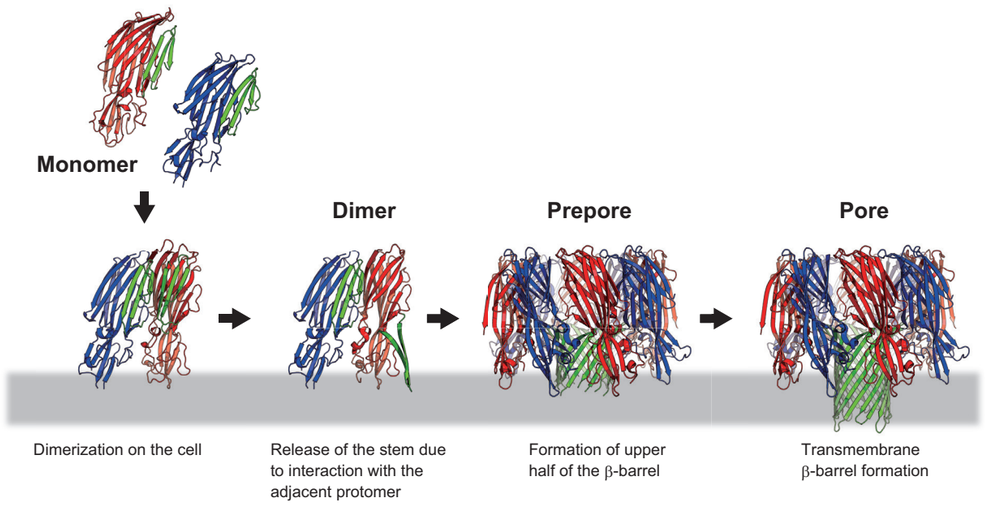

Figure 2: Pore formation mechanism revealed by this study.

To confirm that the observed structural change occurs on erythrocytes, we constructed six cysteine mutants of the β-barrel region, and fluorescence emission spectra of the fluorescent dye conjugated on the introduced cysteine were measured. The results showed that the difference between prepore and pore is the environment around the bottom half of the β-barrel, which is highly consistent with the results of crystal structure analysis.

The flexible transmembrane region had a glycine-rich sequence. To evaluate the role of these glycine residues on the flexibility of this region, several mutants with substitution of Pro for Gly were constructed and their hemolytic activities were compared. The results showed that flexibility at the junction between the trans- and extramembrane regions plays a pivotal role for a marked conformation change between monomer to pore.

From all these observations, we propose the following two-step β-barrel pore formation mechanism (Fig. 2). First, soluble monomers assemble to dimer on the cell membrane. Then, the stem region of one protomer is extruded due to the approach of the adjacent protomer. Next, four dimers assemble to octameric prepore, in which the upper extramembrane half of the β-barrel is formed. In this state, the bottom transmembrane half is not yet formed. Finally, the flexible transmembrane half folds into a rigid membrane-spanning pore. The β-barrel of staphylococcal PFTs is commonly quite stable, and was considered as a rigid structure unit. Therefore, it is surprising that extra- and transmembrane halves of the β-barrel are formed independently. Owing to the structure analysis of the prepore in this study, the atomic structures of monomer, prepore and pore of staphylococcal PFTs were revealed. The two-step β-barrel pore formation mechanism based on these actual structures reasonably explains the pore formation process with no stereochemical discrepancies.

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