

Analysis of Three-Dimensional Structure and Subunit Mismatch in Staphylococcal γ -Hemolysin Heteroheptameric Transmembrane Pore

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Staphylococcal γ -hemolysin (Hlg) is a two-component cytolysin secreted by *Staphylococcus aureus*. Hlg is illustrative molecules for the study of the assembly and membrane insertion of transmembrane proteins and have a unique characteristic of being composed of two separate water-soluble proteins, Hlg1 of 34kDa and Hlg2 of 32kDa⁽¹⁾. We have been revealed two components of Hlg assemble alternately on the membrane to form ring-shaped heteroheptameric transmembrane pores in a stochastic manner with subunit stoichiometries of 3:4 and 4:3^{(2), (3)}. However, three-dimensional (3-D) structure of Hlg pore has not been clarified yet. In this study, we performed construction of 3-D structure of the Hlg pore by using CAD, based on high-resolution transmission electron microscopy (TEM) images taken at various angles. Furthermore, quantification analysis of the subunit mismatch in the pore revealed that several subunits were arranged out of order from the regular heptagon, which will be due to the unique characteristic of heteroheptameric structure.

Hlg pores were isolated from Hlg-treated human erythrocytes with sucrose density gradient ultracentrifugation after solubilization with SDS at 20°C. The purified pores were stained with 1% phosphotungstic acid and examined under a Hitachi electron microscope H-8100 at an acceleration of 100kV. For measurement of subunit mismatch, the centers of each subunit in 60 sample pores were determined with image analysis and the lengths between centers placed side by side were measured. The ratio of length between each monomer against the averaged length among all subunits in a pore was defined as mismatched ratio. The longest mismatched length was numbered as L1 and other subunits arranged in the counterclockwise direction from L1 were numbered as L2, L3, L4, L5, L6 and L7. The 3-D structure of the pore was constructed with 3-D computer aided design software Pro/ENGINEER based on the size and shape of the subunits in TEM images with mismatched length.

I) The analysis of all samples shows the mismatched ratio of L1 is bigger than others (Fig.1). In a heptameric structure formed by alternately assembling of Hlg1 and Hlg2 with subunit stoichiometries of 3:4 or 4:3, there is only one part with identical monomers laying next to each other⁽³⁾. Therefore, L1 would be the length between identical monomers placed side by side, and the mismatched length will be due to the difference of binding forces between the different monomers and the identical monomers.

II) The superior region of the complex is the cylindrical structure with diameter and height are about 10 and 7 nm, respectively. In the cylinder bottom, the funnel-shaped structure is formed, that is, each monomer bended inside with angle of approximately 50 degrees and a small cylindrical structure with outer/inner diameters of 4.5/1.9 nm is attached (Fig.2). Because this inner diameter is similar to the functional diameter of the pore measured with polyethylene glycol⁽²⁾, the funnel structure is expected to be the transmembrane region, acting as a substantial pore.

[References]

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Acknowledgments

This work was partially supported by a Grobal COE Program Grant of the World Center of Education and Research for Trans-disciplinary Flow Dynamics from Tohoku University.

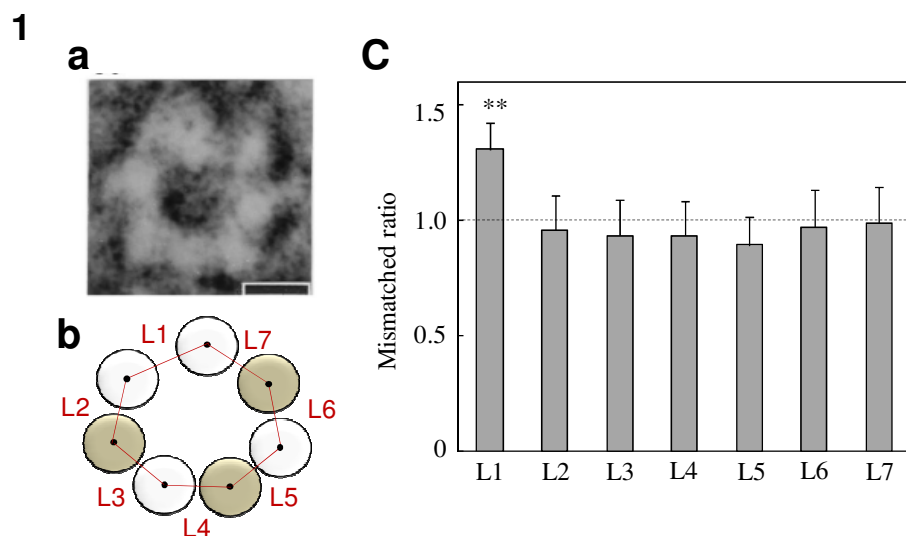


FIG. 1. Mismatch analysis in length between the centers of each subunits. (a) Typical TEM image of the Hlg pore at top view. The bar shows 3 nm. (b) Diagram of the Hlg pore with mismatched length. Identical subunits are shown as same color (i.e. white or yellow). (c) The mismatched ratio was calculated by normalized each length based on the averaged lengths among all subunits in a pore. Error bars show standard deviation. Post-hoc multiple comparisons were conducted using Turkey-Kramer's test, with the level of statistical significance taken as $P<0.05$.

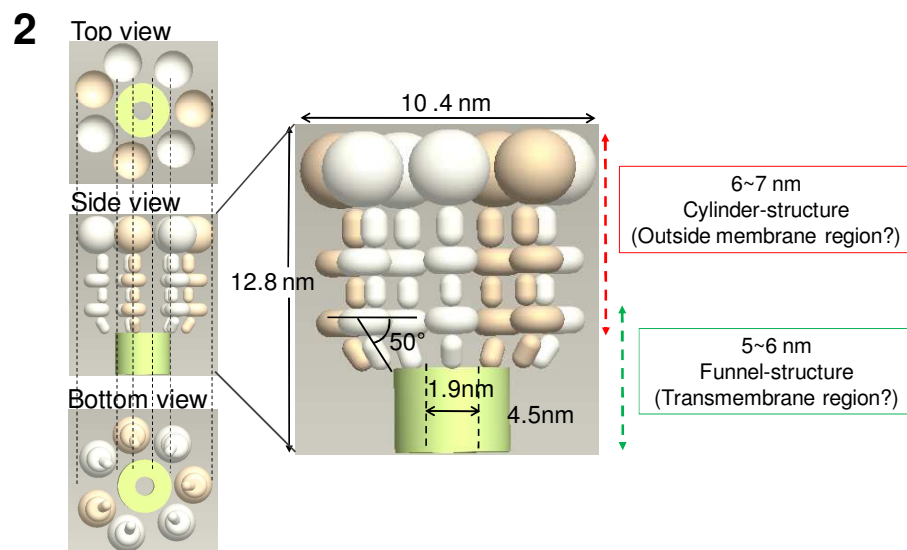


FIG. 2.

Schematic representation of the Hlg pore constructed with CAD. Identical subunits are shown as same colour (i.e. white or yellow). The bottom region (green color) indicates the estimated functional pore.