Hemolytic Lectin CEL-III Heptamerizes via a Large Structural Transition from \( \alpha \)-Helices to a \( \beta \)-Barrel during the Transmembrane Pore Formation Process*\(^{\text{a}}\)

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Background: The hemolytic lectin CEL-III self-oligomerizes upon binding to cell surface carbohydrates to form transmembrane pores.

Results: The CEL-III oligomer crystal structure revealed a membrane-inserted pore structure.

Conclusion: CEL-III forms a heptamer containing a long \( \beta \)-barrel as the pore structure, resulting from large conformational changes.

Significance: The heptameric structure of CEL-III provides insights into the action mechanisms of pore-forming proteins.

CEL-III is a hemolytic lectin isolated from the sea cucumber *Cucumaria echinata*. This lectin is composed of two carbohydrate-binding domains (domains 1 and 2) and one oligomerization domain (domain 3). After binding to the cell surface carbohydrate chains through domains 1 and 2, domain 3 self-associates to form transmembrane pores, leading to cell lysis or death, which resembles other pore-forming toxins of diverse organisms. To elucidate the pore formation mechanism of CEL-III, the crystal structure of the CEL-III oligomer was determined. The CEL-III oligomer has a heptameric structure with a long \( \beta \)-barrel as a transmembrane pore. This \( \beta \)-barrel is composed of 14 \( \beta \)-strands resulting from a large structural transition of \( \alpha \)-helices accommodated in the interface between domains 1 and 2 and domain 3 in the monomeric structure, suggesting that the dissociation of these \( \alpha \)-helices triggered their structural transition into a \( \beta \)-barrel. After heptamerization, domains 1 and 2 form a flat ring, in which all carbohydrate-binding sites remain bound to cell surface carbohydrate chains, stabilizing the transmembrane \( \beta \)-barrel in a position perpendicular to the plane of the lipid bilayer.

Many bacteria produce pore-forming toxins (PFTs),\(^{2}\) which are secreted as soluble proteins that bind to target cell surface receptors and oligomerize to form transmembrane pores in the lipid bilayer. Such a pore formation in the cell membrane disrupts various cellular functions or causes cell death. Among PFTs, \( \alpha \)-hemolysin and \( \gamma \)-hemolysin from *Saccharomyces aureus* (1, 2) have been extensively studied in terms of the structural changes that occur during pore formation in target cell membranes. Their pore formation processes proceed through binding to the cell membrane and self-association to form heptamers or octamers, followed by conformational changes to form a transmembrane \( \beta \)-barrel. Although both monomeric and oligomeric structures are available for \( \gamma \)-hemolysin, which enables a detailed examination of structural changes during pore formation, there is a paucity of information for other PFTs that are postulated to undergo large conformational changes on the cell membrane, leading to the formation of the membrane-inserted oligomers.

PFTs also exist in eukaryotes. The most well-known examples are complement proteins (C6–C9) and perforin in the immune systems of higher vertebrates. These PFTs contain membrane attack complex/perforin domains, which form membrane pores to lyse target cells or deliver other factors such as granzymes (3) into target cells to promote apoptosis. A mushroom lectin LSL is another example of a eukaryotic pore-forming toxin isolated as a hemolytic lectin (carbohydrate-binding protein) from the parasitic mushroom *Laetiporus sulphureus* (4). LSL is known to bind to Gal-containing carbohydrate chains as its receptors on cell surface. Interestingly, this protein shares structural similarities with the bacterial PFTs, aerolysin from *Aeromonas hydrophila* and \( \epsilon \)-toxin from *Clostridium perfringens*.

CEL-III is a eukaryotic PFT that has been purified as a Ca\(^{2+} \)-dependent hemolytic lectin from the marine invertebrate *Cucumaria echinata* (sea cucumber) (5–7). Although this lectin exhibits binding affinity for carbohydrates containing GalNac and Gal at nonreducing ends, it also shows hemolytic and cytotoxic activities by forming pores in target cell membrane, which may contribute to defense against this animal’s predators. CEL-III is composed of two ricin B-chain-like carbohydrate recognition domains (domains 1 and 2) and a C-terminal domain (domain 3) that is responsible for oligomerization in target cell membranes. CEL-III binds to specific carbohydrate chains on the target cell surface via five carbohydrate-binding sites in domains 1 and 2. This binding to the cell surface is assumed to promote conformational changes of domain 3 to...
expose its hydrophobic region including two α-helices (H8 and H9), which then leads to self-oligomerization and concomitant insertion into the cell membrane to form transmembrane pores (7, 8). As shown for other PFTs, detailed analyses of the oligomerization mechanisms, including large conformational changes, are very important in elucidating the actions of PFTs. However, information that describes structural transitions during the oligomerization process is limited. This absence of structural data is due to the difficulties associated with the crystallization of the oligomeric forms of PFTs. We have previously found that CEL-III can oligomerize upon binding disaccharides containing β-galactoside structures, such as lactose (Galβ1–4Glc) and lactulose (Galβ1–4Fru) in aqueous solution under high salt concentration and high pH conditions. The size of this soluble oligomer was estimated to be a hexamer or heptamer on SDS-PAGE, suggesting its structural similarity with the pore-forming oligomers in the erythrocyte membrane (7). We have recently crystallized this soluble oligomer in the presence of the detergent n-dodecyl-β-D-maltoside (DDM), and found the presence of a 7-fold rotational symmetry in its oligomeric structure (9). In the present study, we have determined the crystal structure of this CEL-III oligomer, which reveals the heptamer transmembrane pore structure. Compared with the monomeric structure, an extensive secondary structural change of α-helices to β-strands is suggested to play an important role in the pore formation process in target cell membrane.

**EXPERIMENTAL PROCEDURES**

*Purification of CEL-III—Specimens of *C. echinata* were collected from the Sea of Genkai, Fukuoka, Japan. CEL-III was purified from the body fluid of *C. echinata* by chromatography using lactose-Cellulofine, GalNAc-Cellulofine, and Sephadex G-75 columns, essentially as described previously (5, 10).

*Preparation of the CEL-III Oligomer—*CEL-III oligomers were prepared as described previously (7) with the following modifications. Purified CEL-III monomer (2 mg/ml) was incubated in the presence of 1 M NaCl, 100 mM glycine-NaOH buffer (pH 10), 10 mM CaCl$_2$, and 100 mM lactulose at 25 °C for 2 h. After oligomerization, the protein was dialyzed against 10 mM Tris-HCl (pH 7.6), 150 mM NaCl and then solubilized with 0.1% DDM in the same buffer. The solubilized CEL-III oligomers were concentrated to 12 mg/ml using a 100-kDa cutoff membrane (Vivaspin; GE Healthcare). For stabilization, the CEL-III oligomers were prepared as described previously (7) with the following modifications. Purified CEL-III monomer (2 mg/ml) was incubated in the presence of 1 M NaCl, 100 mM glycine-NaOH buffer (pH 10), 10 mM CaCl$_2$, and 100 mM lactulose at 25 °C for 2 h. After oligomerization, the protein was dialyzed against 10 mM Tris-HCl (pH 7.6), 150 mM NaCl and then solubilized with 0.1% DDM in the same buffer. The solubilized CEL-III oligomers were concentrated to 12 mg/ml using a 100-kDa cutoff membrane (Vivaspin; GE Healthcare). For stabilization, the CEL-III oligomers were incubated in 10 mM Tris-HCl (pH 7.6), 150 mM NaCl, 10 mM CaCl$_2$, 100 mM lactulose, and 0.5% DDM at 20 °C for 1 h.

**Small-angle X-ray Scattering**—Small-angle x-ray scattering measurements were done on the beamline BL-10C at the Photon Factory (Tsukuba, Japan). A wavelength of 1.488 Å and a specimen-to-detector distance of 958.8 mm were used, and all measurements were carried out at 25 °C in a temperature-controlled cell holder. The times for small-angle x-ray scattering analysis of CEL-III monomer and oligomer in the presence and absence of DDM solutions (concentration of approximately 3 mg/ml) were 120, 300, and 1200 s for each measurement. The radius of gyration ($R_g$), forward scattering intensity normalized with respect to the protein concentration ($I(0)/C$), molecular mass, and maximum particle dimension ($D_{max}$) (Table 1) were calculated as described in a previous paper (11).

**Crystallization, Crystal Stabilization, and Derivatization**—For crystallization, sitting drops of 4–8 μl of sample were mixed with 2 μl of a solution containing 100 mM sodium acetate (pH 4.2), 100 mM CdCl$_2$, and 30% PEG400 and were equilibrated by vapor diffusion against the same solution. Crystals grew within 2 weeks. For stabilization and cryoprotection of the crystals, the mother liquor was replaced by a cryostabilization solution of 100 mM sodium acetate (pH 4.2), 0.1 M CdCl$_2$, 10 mM CaCl$_2$, 100 mM lactulose, and 35–45% PEG400. For heavy atom derivatization, crystals were soaked in a buffer containing 100 mM sodium acetate (pH 4.2), 0.1 M CdCl$_2$, 10 mM CaCl$_2$, 100 mM lactulose, 35% PEG400, 0.5% DDM, and 2 mM K$_3$PtCl$_4$ for 12 h at 20 °C.

**Data Collection, Structure Determination, and Refinement**—NATIVE1 data were collected at beamline i03 of the Diamond light source (Harwell, UK), and native2 and Pt-derivative data were collected on BL-5A at the Photon Factory (Tsukuba, Japan). Data indexing, integration, and scaling of native1 were done on the program HKL2000 (12), and that of native2 and the Pt-derivative were carried out with the CCP4 (13) programs Mosflm (14) and SCALA (15). Data collection statistics are summarized in Table 2. All data sets belonged to space

| **TABLE 1** Structural parameters of monomeric and oligomeric CEL-III by small-angle x-ray scattering |
|-----------------|---|--------|----------------|
| **CEL-III** | $R_g$ | $I(0)/C$ | Molecular mass |
| Monomer | 24.2 | 45.9 (1)$^a$ | 4.7 × 10$^{14}$ | 75 |
| Oligomer | 92.6 | 954 (20.8) | 9.8 × 10$^{15}$ | 277 |
| Oligomer + 0.5% DDM | 61.7 | 332 (7.25) | 3.4 × 10$^{15}$ | 177 |

$^a$ Values in parentheses indicate the ratio of $I(0)/C$ to that of the monomer.

| **TABLE 2** Data collection and refinement statistics |
|-----------------|-----------------|-----------------|-----------------|
| **Crystal type** | Native1 | Native2 | Pt-derivative |
| **Data collection and processing statistics** | | | |
| Space group | C2 | C2 | C2 |
| Unit cell dimension (Å) | | | |
| $a$ (Å) | 219.8 | 218.3 | 217.5 |
| $b$ (Å) | 228.7 | 230.7 | 231.6 |
| $c$ (Å) | 133.0 | 133.6 | 133.7 |
| $β$ (°) | 127.1 | 126.9 | 126.8 |
| Wavelength (Å) | 0.9763 | 1.000 | 1.000 |
| Resolution (Å)$^b$ | 53.5–2.90 | 50.0–3.30 | 50.0–4.50 |
| | (3.06–2.90) | (3.36–3.30) | (4.58–4.50) |
| Total reflections | 441,835 | 557,027 | 213,116 |
| Unique reflections | 113,863 | 79,710 | 31,095 |
| $R_{ave}/R_{free}$ (%) | 7.1 (2.0) | 21.3 (5.1) | 14.7 (4.3) |
| Redundancy | 3.9 (3.9) | 7.0 (7.0) | 6.9 (6.7) |
| Completeness (%) | 98.6 (99.3) | 98.7 (100) | 99.5 (99.9) |
| Number of HA sites | 10.0 (41.1) | 9.6 (48.5) | 12.6 (47.4) |

$^b$ The values in parentheses are for the highest resolution shell.

$R_{ave} = \frac{\sum|I_{obs} - I_{calc}|}{\sum I_{obs}}$ and $R_{free} = \frac{\sum|I_{obs} - I_{calc}|}{\sum I_{obs}}$, where $I_{obs}$ is the observed intensity and $I_{calc}$ is the average intensity of multiple observations of symmetry-related reflections.
Crystal Structure of the Pore-forming CEL-III Heptamer

FIGURE 1. **Crystal structure of CEL-III heptamer.** Side (A) and bottom (B) views of the CEL-III heptamer are shown in ribbon representation. Each subunit is shown in a different color. Lactulose molecules bound to CEL-III are shown in orange sticks. Ca$^{2+}$ and Mg$^{2+}$ ions are indicated in green and light blue spheres, respectively.

RESULTS AND DISCUSSION

Preparation and Crystallization of CEL-III Oligomers—CEL-III forms oligomers in aqueous solution upon binding of β-galactoside–containing carbohydrates, such as lactose and lactulose, under high pH and high salt concentration conditions (7). These oligomers show a size on SDS-PAGE similar to those formed in the erythrocyte membrane during the hemolytic process. This suggests that CEL-III undergoes similar structural changes even in solution, triggered by the binding of specific carbohydrates, which may mimic the initial step of the action of CEL-III on target cell membranes. Although the soluble oligomers showed a band of 270 kDa, which corresponds to a hexamer on SDS-PAGE, their actual size in solution was estimated to be approximately 1 MDa. This size corresponds to a 21-mer by small-angle x-ray scattering measurements (21), suggesting that hexamers or heptamers further associate to form trimers through relatively weak interactions. These weak interactions were disrupted by detergents such as Triton X-100 and SDS (11). For crystallization of CEL-III oligomers, DDM was found to yield good crystals, which diffracted to 2.9 Å resolution (9). The sizes of the CEL-III monomer and oligomers determined by small-angle x-ray scattering are presented in Table 1. Whereas CEL-III monomer and the oligomer in the absence of DDM gave molecular masses of 4.7 × 10$^3$ Da and 9.8 × 10$^3$ Da, respectively, which are comparable with previous data (11, 21), the addition of 0.5% DDM resulted in a decrease in size to 3.4 × 10$^3$ Da. These results suggest that the dissociation of large oligomers to core oligomers occurs through the disruption of hydrophobic interactions between core oligomers. The crystals of CEL-III oligomers were prepared in the presence of 0.5% DDM by the sitting drop vapor diffusion method, and they were used for the x-ray diffraction data collection.

**Overall Structure of CEL-III Heptamer**—The crystal structure of the CEL-III oligomer was solved at 2.9 Å resolution using single isomorphous replacement with anomalous scattering method for phase calculation with Pt-derivative crystals. As shown in Fig. 1, CEL-III oligomer forms a drawing-pin-like heptamer with a height and diameter of 115 and 175 Å, respectively. There is a long 14-stranded β-barrel, which forms a pore when inserted in the target cell membrane. Although domains 1 and 2 of the heptamer maintain the basic monomeric β-barrel structure running along the 7-fold symmetry of the heptamer, which may form a pore when inserted in the target cell membrane. Although domains 1 and 2 of the heptamer maintain the basic monomeric β-barrel structure running along the 7-fold symmetry of the heptamer, which may form a pore when inserted in the target cell membrane. Although domains 1 and 2 of the heptamer maintain the basic monomeric β-barrel structure running along the 7-fold symmetry of the heptamer, which may form a pore when inserted in the target cell membrane. Although domains 1 and 2 of the heptamer maintain the basic monomeric β-barrel structure running along the 7-fold symmetry of the heptamer, which may form a pore when inserted in the target cell membrane. Although domains 1 and 2 of the heptamer maintain the basic monomeric β-barrel structure running along the 7-fold symmetry of the heptamer, which may form a pore when inserted in the target cell membrane. Although domains 1 and 2 of the heptamer maintain the basic monomeric β-barrel structure running along the 7-fold symmetry of the heptamer, which may form a pore when inserted in the target cell membrane. Although domains 1 and 2 of the heptamer maintain the basic monomeric β-barrel structure running along the 7-fold symmetry of the heptamer, which may form a pore when inserted in the target cell membrane. Although domains 1 and 2 of the heptamer maintain the basic monomeric β-barrel structure running along the 7-fold symmetry of the heptamer, which may form a pore when inserted in the target cell membrane. Although domains 1 and 2 of the heptamer maintain the basic monomeric β-barrel structure running along the 7-fold symmetry of the heptamer, which may form a pore when inserted in the target cell membrane.
the β-barrel. In addition, three aromatic residues, Phe-314, Trp-353, and Phe-336, exist at the upper (Phe-314 and Trp-353) and bottom (Phe-336) regions of the outer surface of the β-barrel. Such a distribution of aromatic residues in membrane-penetrating β-barrels (aromatic belt) was suggested to be involved in the interaction with the lipid head groups (24, 25).

In addition to the interactions between domain 3 from each protomer, carbohydrate-binding domains 1 and 2 also stabilize the pore structure by forming a large outer ring on the cell surface (Fig. 1A). In the crystal structure, their carbohydrate-binding sites are occupied by lactulose molecules added for the oligomerization process (Fig. 4). All of the carbohydrate-binding sites are located on the undersurface of the outer ring, facing the postulated cell membrane, as shown in Fig. 1A. This suggests that the transmembrane pore in the lipid bilayer may be stabilized by the outer ring composed of domains 1 and 2 binding to cell surface carbohydrate chains. An electrostatic potential analysis shows that the lower surface of the outer ring has a strong negative potential (Fig. 5). This is partly because of acidic residues coordinating 35 Ca$^{2+}$ ions, which are essential for the recognition of galactose residues. The inner surface of the pore shows a characteristic distribution of electrostatic potentials, in which the lower two-thirds and upper one-third have strong positive and negative potentials, respectively.

**Structural Transition during Heptamerization**—The structural transition of the CEL-III monomer to its heptamer during the hemolytic process requires extensive conformational changes in domain 3. Domain 3 of monomeric CEL-III contacts the side of domains 1 and 2 with its two α-helices (H8 and H9) accommodated in the cleft between them (Fig. 3B). Upon heptamerization, the heptamer of CEL-III is stabilized by a large outer ring composed of domains 1 and 2 binding to cell surface carbohydrate chains. The outer ring may provide additional stability to the transmembrane pore in the lipid bilayer.
terization, it dissociates from domains 1 and 2, transforming to the bundle and stem regions that self-associate to form a central membrane-spanning axis (Fig. 3A). During such a heptamerization process, the two \(\alpha\)-helices transform to two long \(\beta\)-strands that further assemble into the 14-stranded \(\beta\)-barrel. This is a remarkable difference from conformational transitions observed for other \(\beta\)-PFTs, as represented by \(\alpha\)-hemolysin and \(\gamma\)-hemolysin from \textit{S. aureus} (1, 2), which form \(\beta\)-barrels in the membrane through the assembly of the pre-stem regions made of a \(\beta\)-hairpin that has originally formed a three-stranded \(\beta\)-sheet on the side of a soluble monomer. As we reported previously (8), domain 3 is found to oligomerize spontaneously once it is cleaved from domains 1 and 2. This finding suggests that oligomerization of CEL-III is triggered by the exposure of an internal face of domain 3 to the solvent, which may be promoted by the conformational changes induced by binding of domains 1 and 2 to the carbohydrate chains as CEL-III receptors on the cell surface. It seems possible that such conformational changes are induced by lateral movements of glycoproteins or glycolipids, to which domains 1 and 2 are bound. In addition, internal structural changes in domains 1 and 2 (Fig. 4, B and C) may also be involved in the dissociation of domain 3, supported by the finding that binding of a specific carbohydrate such as lactulose and lactose under high pH and high salt conditions promoted heptamerization of CEL-III (7).

As shown in Fig. 6, conformational changes of domain 3 during heptamerization occur in the structural units, which are referred to as terminal (residues 284–291 and 420–432), scaffold (residues 292–308, 362–386, and 401–419), wrapping (residues 387–400), and stem (residues 309–361) regions (Fig. 6B). Whereas residues 284–291 in the terminal region are situated 16 Å apart from residues 420–432 in monomeric CEL-III (Fig. 6A), after dissociation of domain 3 from domains 1 and 2, residues 284–291 connected to domain 2 are trailed along the...
movement of domain 3 and approach residues 420–432. By contrast, the stem region, originally composed of two \( \alpha \)-helices, three short \( \beta \)-sheets, and loop structures in monomeric CEL-III, converts to a long 2-stranded \( \beta \)-sheet (\( \beta \)-hairpin), which contributes to the 14-stranded \( \beta \)-barrel as a transmembrane pore formed by the heptamer. This transition includes a drastic secondary structural switch from \( \alpha \)-helices to \( \beta \)-sheets and a concomitant increase in main chain hydrogen bonds from 30 to 54, among which 28 are between adjacent protomers (Fig. 7, A and B). The wrapping region (residues 387–400) comprising two \( \beta \)-strands and a connecting loop also changes largely its conformation and hydrogen bond pairing (Fig. 7, C and D).

Although the terminal, wrapping, and stem regions accounting for 72% of the total residues in domain 3 undergo extensive structural changes, the remaining (scaffold) region basically retains its original conformation during the heptamerization process. The surface of the scaffold region is mostly buried inside the CEL-III monomer, becoming exposed to make significant interactions with adjacent protomers after heptamerization. It seems likely that the scaffold region plays a central role in constructing a heptameric ring in an early stage of the heptamerization process. Formation of the heptameric ring through scaffold regions between adjacent protomers is followed by the conformational changes in the wrapping and stem regions to form the \( \beta \)-barrel structure that inserts into the lipid bilayer. The importance of the scaffold region in pore formation has been suggested by the finding that the site-directed mutagenesis of Arg-378 to alanine resulted in an almost complete loss of hemolytic activity of CEL-III (26). In the heptameric structure, Arg-378 forms ionic and hydrogen bonds with Asp-373 and Asn-369, respectively, between the scaffold regions of adjacent protomers (Fig. 8 A) to stabilize the ring structure. It seems very likely that the formation of the ring structure composed of the scaffold region precedes the formation of the \( \beta \)-barrel in the lipid bilayer. When domain 3 in monomeric CEL-III is fitted to those of the heptameric structure so that their corresponding scaffold regions are superimposed, no collision occurs among these domains (Fig. 8 B). This fact also supports the assumption that a heptameric ring as a prepore (27, 28) may be formed prior to the \( \beta \)-barrel formation by the stem region after binding to the target cell surface. After prepore formation, the transmembrane pore composed of the \( \beta \)-barrel may be formed through a number of hydrogen bonds between adjacent \( \beta \)-strands, leading to a stable heptamer structure that is resistant to heat and detergent treatments (7).

**Proposed Mechanism of Pore Formation**—Based on these findings, we propose a spontaneous pore formation mechanism of CEL-III (Fig. 9 and supplemental Movie S1) comprising four steps: 1) binding to the specific carbohydrate chains on the cell membrane, 2) domain movements, 3) heptamerization, and 4) \( \beta \)-barrel formation. In step 1, CEL-III monomers bind to Gal/GalNAc-containing carbohydrate chains, such as those of glycolipids, on the target cell membrane via the five carbohydrate-
binding sites in domains 1 and 2 (Fig. 9A). In step 2, movement of the domains exposes the internal surface of domain 3. This structural change exposes the scaffold region that is buried in monomeric CEL-III (Fig. 9B) and induces self-association through their exposed hydrophobic surfaces (step 3, Fig. 9C). In step 4, the 14-stranded $\beta$-barrel is formed by association of 2-stranded $\beta$-sheets contributed from each protomer (Fig. 9D). During this $\beta$-barrel formation process, 26 hydrogen bonds in the two $\alpha$-helices and three short $\beta$-sheets disappear in a protomer, whereas 50 hydrogen bonds are newly formed in the two long amphipathic $\beta$-sheets (Fig. 7, A and B). This gives a net increase of 24 hydrogen bonds per protomer, and such an increase may provide the driving force for the extensive structural changes of the stem region. The pore structure of heptameric CEL-III shows conspicuous differences from those of the other $\beta$-PFTs, whose three-dimensional structures are known; the CEL-III heptamer contains a long $\beta$-barrel of 75 Å in length, whereas those of other $\beta$-PFTs are typically $\sim$50 Å in length. Additionally, the CEL-III heptamer forms a large flat ring of a diameter of 175 Å comprising domains 1 and 2, which contains 35 carbohydrate-binding sites. Such a large ring contacting the surface of the cell membrane might be advantageous for stabilizing the long membrane-spanning $\beta$-barrel perpendicularly embedded into the lipid bilayer.

Comparison with Other Proteins—Although CEL-III shows similarities in the size of pores with $\alpha$-hemolysin (1), $\gamma$-hemolysin (2) from S. aureus, Vibrio cholerae cytolysin (29), aerolysin from A. hydrophila (30), and the anthrax toxin from Bacillus anthracis (28), its secondary structural change from $\alpha$-helices to $\beta$-strands to form a membrane-spanning $\beta$-barrel rather resembles those of the cholesterol-dependent cytolysin family proteins and membrane attack complex/perforin domains (27, 31), which form larger pores. These proteins are produced by various pathogenic Gram-positive bacteria or cytotoxic T cells and natural killer cells in mammalian immune systems, respectively. These proteins also form pores composed of membrane-spanning $\beta$-barrels, which are derived from $\alpha$-helices in soluble monomeric form. Besides these proteins, secondary structural changes from $\alpha$-helices to $\beta$-strands are also known to be a critical step for the action of the proteins responsible for conformational diseases, such as Alzheimer, Parkinson, and prion diseases (32), which are assumed to be mediated by the deposition of aggregates resulting from structural conversion from $\alpha$-helices in soluble proteins to insoluble $\beta$-sheet aggregates. Further detailed analyses for the structural changes of CEL-III during heptameric pore formation should provide important clues not only to the action mechanisms of PFTs, but also to the mechanism of aggregation of the proteins responsible for conformational diseases.

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