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Giant MACPF/CDC pore forming toxins: A class of their own☆

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ABSTRACT

Pore Forming Toxins (PFTs) represent a key mechanism for permitting the passage of proteins and small molecules across the lipid membrane. These proteins are typically produced as soluble monomers that self-assemble into ring-like oligomeric structures on the membrane surface. Following such assembly PFTs undergo a remarkable conformational change to insert into the lipid membrane. While many different protein families have independently evolved such ability, members of the Membrane Attack Complex PerForin/Cholesterol Dependent Cytolysin (MACPF/CDC) superfamily form distinctive giant β-barrel pores comprised of up to 50 monomers and up to 300 Å in diameter. In this review we focus on recent advances in understanding the structure of these giant MACPF/CDC pores as well as the underlying molecular mechanisms leading to their formation. Commonalities and evolved variations of the pore forming mechanism across the superfamily are discussed. This article is part of a Special Issue entitled: Pore-Forming Toxins edited by Mauro Dalla Serra and Franco Gambale.

1. Introduction

Pore Forming Toxins (PFTs), also referred to as pore forming proteins, have the ability to breach cell membranes by forming pores in the lipid bilayer. These pores can be either lytic to the target cell, e.g. by osmotic flux, or the pores can mediate the translocation of proteins (typically toxins) into the cytoplasm of the target cell. PFTs are commonly categorised into two groups depending on the elements of secondary structure used to span the cell membrane [1,2]. α-PFTs utilise amphipathic α-helices to span the bilayer membrane whereas β-PFTs form amphipathic β-barrel pores. Regardless of the mechanism used, all PFTs are more generally characterised by their remarkable ability to change from water-soluble proteins into integral membrane proteins.

Exemplars of well studied β-PFTs include virulence factors produced by pathogenic bacteria such as the cytolytic Panton-Valentine leukocidins and α-hemolysin (from *Staphylococcus aureus*) [3,4], aerolysin-like toxins (from *Aeromonas hydrophila*) [5], and anthrax protective antigen (from *Bacillus anthracis*) [6]. In most β-PFTs, 7 to 9 monomers self-assemble in a ring-shape oligomer on the cell surface to form a transmembrane β-barrel pore typically less than 25 Å in diameter. In contrast, the Membrane-Attack Complex/PerForin (MACPF)/Cholesterol Dependent Cytolysin (CDC) superfamily form giant β-PFT up to 350 Å in diameter. This unusually large and diverse superfamily is found in all kingdoms of life and performs a wide variety of functions. For example, members of this superfamily are involved in vertebrate immunity [7,8], venom toxicity [9,10], development [11], neural development [12] and pathogen invasion/egress [13–15].

1.1. The CDC family of proteins

The CDC family of the MACPF/CDC superfamily is a group of toxins produced by a wide range of Gram positive bacteria. Extensive research has determined the role of important CDCs in pathogenicity and also highlighted a range of functions for the CDC pore. The CDC pneumolysin (PLY) is secreted by *Streptococcus pneumoniae*, the major cause of pneumonia and bacterial meningitis, and is suggested to directly mediate cell lysis [16]. Perfringolysin O (PFO, *Clostridia perfringens*) aggravates gas gangrene in synergy with the *C. perfringens* α-toxin [17]. Listeriolysin O (LLO, *Listeria monocytogenes*) enables phagosomal escape of the bacterium into the macrophage cytosol [18]. Finally, streptolysin O (SLO, *Streptococcus pyogenes*) is able to mediate translocation of a cytotoxic effector through the cell membrane without requirement for pore formation [19]. The function of CDCs is not solely limited to pore formation but extends to signalling events leading to a range of effects such as inflammation and adaptive immune responses (for a review see [20]).

Comprehensive studies have shed light on the central conundrum of the cytolysin pore formation: how do soluble monomeric CDCs self-assemble on the target cell surface to form an oligomeric membrane embedded pore? Although, the pores formed by CDCs had been studied using Transmission Electron Microscopy (TEM) since the 1970s [21], a

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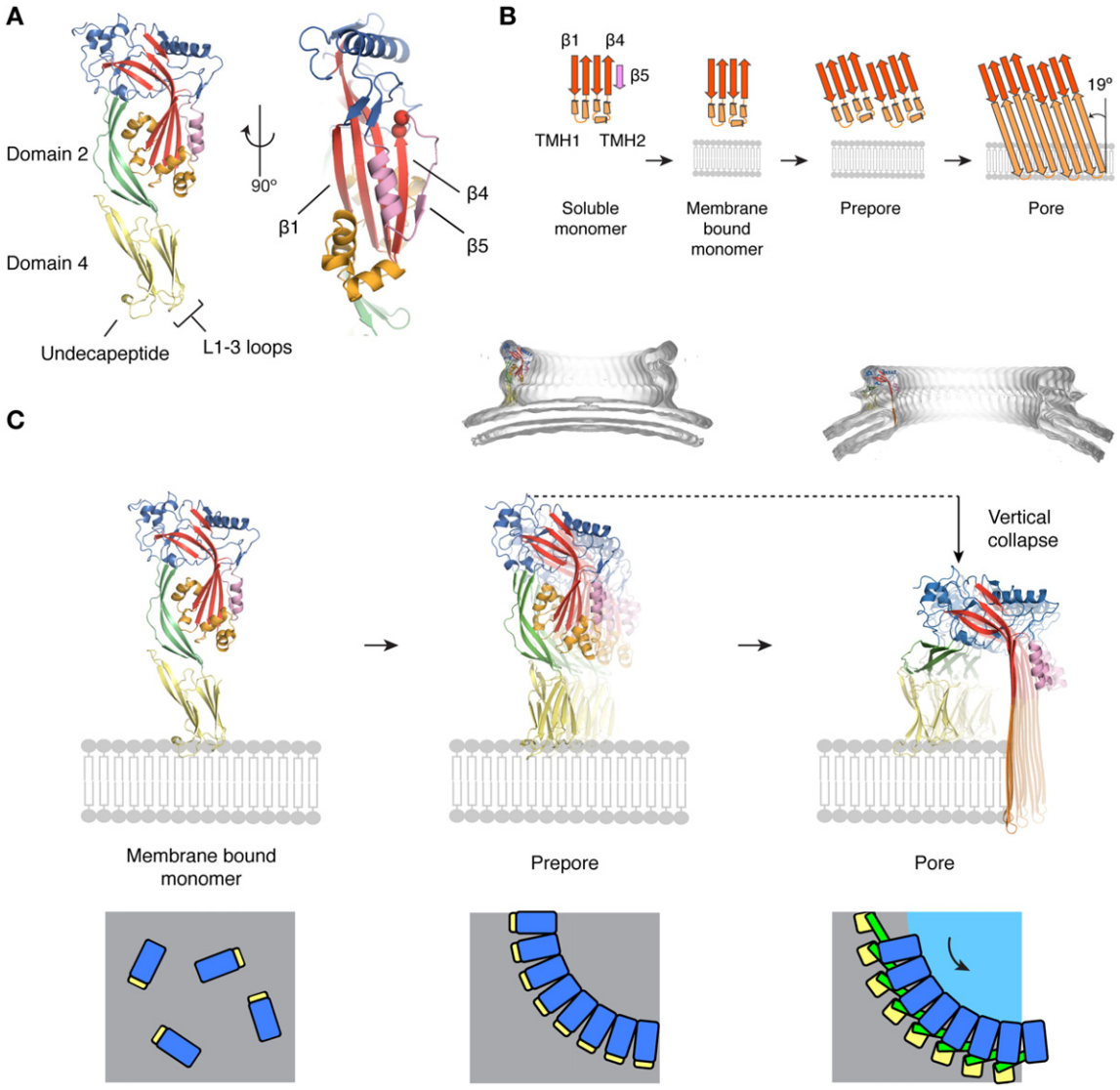

Fig. 1. The key steps of CDC pore formation. A. Crystallographic structure of the archetypal CDC PFO (PDB ID: 1PFO) [22]. The MACPF/CDC domain, previously identified as Domain 1 and Domain 3, comprises the characteristic central bent β-sheet (red) sandwiched by the TMH regions (orange). Strand β5 and the neighbouring α-helix are in pink. The remainder of the domain is in blue. The pair of conserved glycines is shown as red spheres. Domain 2 is composed of a non-contiguous elongated and twisted β-sheet. Domain 4 (*Ig* fold) is in yellow; the membrane binding L1–3 loops are indicated. B. Schematisation of the unfurling and insertion of the TMH regions. In the soluble state the TMH regions (orange) are present as folded α-helices. The central β-sheet (red) is expanded by strand β5 (pink), which is displaced upon membrane binding. In the prepore complex the central β-sheet is postulated to establish oligomeric contacts compatible with the final pore (strands β1 and β4). The TMHs also experience some degree of flexibility and unfolding. In the final pore the TMH regions insert into the bilayer membrane as amphipathic β-hairpins with a modest tilt of 19° to form the final pore. The up to 200 β-strands that compose the β-barrel extend the central β-sheet. C. Mechanism of CDC pore formation. After membrane anchoring by the Domain 4 loops (left panel), 30 to 50 subunits oligomerise (centre panel) through the flat faces of the MACPF/CDC domain to form the circular prepore complex. The dimensions of the individual subunits in the prepore resemble the monomeric structure. Finally, conversion to the pore (right panel) follows the vertical collapse of the MACPF/CDC domain most likely by coordinated domain movements that see Domain 2 rotate towards the bilayer surface. This brings the MACPF/CDC domains at the required height for TMH insertion as a giant transmembrane β-barrel. The pneumolysin prepore and pore maps are represented as grey surfaces [29], the corresponding protein models are from [73]. Bottom panel: Schematisation of CDC pore formation viewed from the top. The re-orientation of Domain 2 likely results in the coordinated rotation of the ring of MACPF/CDC domains. Only a quadrant of the ring is represented for clarity.

major breakthrough came in 1997 with the X-ray crystal structure of the CDC PFO, which provided the first atomic-resolution insights into the CDC family [22] (Fig. 1A). The narrow elongated molecule was identified as being composed of four domains (Fig. 1A). Together, the non-contiguous Domains 1 and 3 form a globular domain. The linker domain, Domain 2, connects Domain 1 to the Immunoglobulin-like (*Ig*-like) Domain 4 (Fig. 1A). Following the determination of the PFO structure, biophysical experiments demonstrated that not one but two regions of Domain 3 inserted into the membrane [23,24].

Over the last 15 years, biochemical and biophysical studies have defined a general three-step mechanism of CDC pore formation [28,29]. Firstly, the secreted monomeric CDC molecules bind the target cell surface via recognition of a membrane receptor. Thirty to fifty monomers then assemble into an oligomeric, ring-shaped, prepore complex [25,26]. Finally a massive conformational change permits formation of a giant transmembrane β-barrel pore 250 to 350 Å in diameter.

Independent biophysical studies have also demonstrated that a 40 Å vertical collapse of the prepore accompanies conversion of the prepore to the membrane embedded pore [27–29]. Additionally, two bundles of α-helices per subunit unfurl from Domain 3 and refold to contribute two amphipathic β-hairpins (4 β-strands, Fig. 1B) to the pore [23,24]. Thus, a ring of 30 to 50 CDCs will form a giant β-barrel composed of 120 to 200 β-strands.