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Review

On the role of lipid in colicin pore formation

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Abstract

Insights into the protein–membrane interactions by which the C-terminal pore-forming domain of colicins inserts into membranes and forms voltage-gated channels, and the nature of the colicin channel, are provided by data on: (i) the flexible helix-elongated state of the colicin pore-forming domain in the fluid anionic membrane interfacial layer, the optimum anionic surface charge for channel formation, and voltage-gated translocation of charged regions of the colicin domain across the membrane; (ii) structure–function data on the voltage-gated K^+ channel showing translocation of an arginine-rich helical segment through the membrane; (iii) toroidal channels formed by small peptides that involve local participation of anionic lipids in an inverted phase. It is proposed that translocation of the colicin across the membrane occurs through minimization of the Born charging energy for translocation of positively charged basic residues across the lipid bilayer by neutralization with anionic lipid head groups. The resulting pore structure may consist of somewhat short, ca. 16 residues, trans-membrane helices, in a locally thinned membrane, together with surface elements of inverted phase lipid micelles.

Keywords: Anionic lipid; Born energy; Colicin; K^+ channel; Toroidal pore; Voltage-gating

Contents

Abbreviations: BLM, bilayer lipid membrane; CP, channel polypeptide (domain); ε, dielectric constant; FRET, fluorescence resonance energy transfer; L/P, lipid/protein molar ratio; NMR, nuclear magnetic resonance; SC, spontaneous curvature; TM, trans-membrane

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1. The colicin system

Because colicins must cross all or part of the *E. coli* cell envelope to exert their cytotoxic function, they provide a useful system for studies on (i) receptor-mediated protein import and (ii) lipid-mediated protein insertion into membrane bilayers, channel formation, and protein translocation across membranes. Receptor-mediated passage across the outer membrane [2] allows all colicins to enter the periplasmic space. Translocation across the cytoplasmic membrane allows the nuclease colicins to access their intracellular targets. Insertion into the cytoplasmic membrane allows channel formation by the pore-forming colicins, which is the topic of the present discussion.

2. X-ray structures of channel-forming colicins and C domains

Colicins are multi-domain proteins with highly asymmetric (elongated) structures. Colicin domains are believed to be independently responsible for functions of translocation (T) across the outer membrane and periplasmic space, receptor recognition (R) and cytotoxic (C) activities (channel formation in the cytoplasmic membrane, endonuclease activity in cytoplasm, etc). Translocation requires interaction of the T domain with components of bacterial Tol or Ton systems [3]. The recently published structure of colicin B [4] brought the number of solved structures of intact colicins to four (Fig. 1A–D). In all of these colicins the N-terminal segment lacks secondary structure (residues 1–61, 1–96, 1–66 and 1–91 in colicins B, E3, Ia and N, respectively). The crystal structures of the C-terminal domains responsible for cytotoxic activity for colicins A [5], E1 [6], E7 [7] and E9 [8], and colicin E3 coiled-coil receptor-binding domain docked to BtuB receptor [2] have been solved. Comparison of the structures of colicins B, E3, Ia and N reveals two distinctive interdomain configurations, summarized in (A, B) below.

(A) Ton-dependent colicin Ia (Fig. 1A) [9] and Tol-dependent E3 (Fig. 1D) [11] form elongate 210- and 140-Å structures, respectively. The N-terminal T domains and the C-terminal C domains in these colicins, despite being on opposite ends of the amino acid sequences, are brought to proximity by their connections to the N and C-termini, respectively, of the coiled-coil structure of the R domain (Fig. 1A and D). Most of the elongate structure of these colicins resides in the coiled-coils, which are 160 and 100 Å in length in colicins Ia and E3.

(B) Colicin B [4] and N [10]. Inter-domain configurations of the pore-forming colicins B (Ton-dependent; Fig. 1B) and N (Tol-dependent; Fig. 1C) are similar ("dumb-bell" shape), but are different from the pore-forming Ton-dependent colicin Ia and the Tol-dependent ribosomal endoribonuclease colicin E3. The pore-forming domain of colicin B forms the C-terminal globe of a dumb-bell. The N-terminal segment, separated by a 73-Å-long α-helix, forms another globe that contains the T and R domains [4]. The crystal structure of colicin N contains the R domain, and may ultimately turn out to be similar to colicin B, but at present its T domain has not been solved [10]. The R and C domains in colicin N are separated by a single 68-Å-long α-helix. Thus, the colicin B structure displays a different organization of functional domains, and a new structure–function concept, in which it appears that the functions of receptor-binding (FepA; [12]) and translocation [13] would be performed by the same globular domain.

(C) Structures of the C-terminal pore-forming domains. The soluble ca. 20-kDa pore-forming domains of colicins A [5] and E1 [6] (Fig. 1E and F), along with the structures of these domains in the intact colicins B, Ia and N, have the characteristic signature of a 10-helix globule. The average length of these helices (helices II–X) is 12 ± 3 residues (14 ± 3, 12 ± 2, 11 ± 3, 12 ± 3 and 13 ± 3 residues in helices II-X of colicins A, E1, Ia, B and N, respectively; Table 1), much shorter than required (ca. 20 residues) to span a standard membrane bilayer. The length of helix I in C domains, which is part of the long inter-domain helix, was not included in this calculation.

The two helices, VIII–IX, which do not have charged side chains, form a hydrophobic helical hairpin in the apolar core of the channel domains, a feature that is also found in the structures of the membrane-active domain of diphtheria

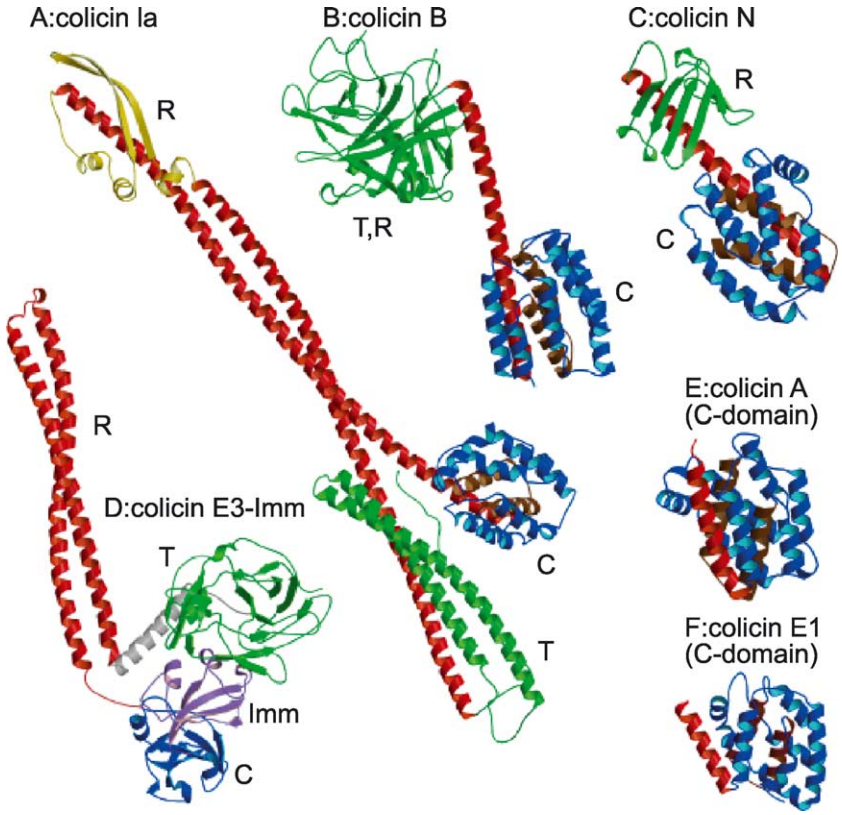

Fig. 1. Ribbon diagrams of the X-ray structure of colicin Ia (A), B (B), N (C) and E3 (D), and pore-forming domains of colicins A (E) and E1 (F). Pore-forming (cytotoxicity) domains are shown in blue and brown (hydrophobic hairpin); connecting single helix (B, C, E, F) or double-helix colicin E3 coiled-coil (A, D), red; translocation domain, green; immunity protein in colicin E3 (D), magenta.

toxin [14] and anti- and pro-apoptotic proteins of Bcl-2 family ([15–18]). This suggests that the function of this hydrophobic hairpin structure motif is to provide a trans-bilayer membrane anchor for the protein after interaction with the membrane surface.

Table 1
Lengths of defined α-helical segments of colicins in their soluble state

Helix	Helix length[a], residues				
	A	E1	Ia	B	N
I[b]	22	16	–	–	–
II	14	14	11	14	14
III	12	15	11	12	13
IV	12	11	10	11	12
V	11	12	15	7	10
VI	17	10	13	13	17
VII	12	11	7	12	13
VIII	18	12	12	12	13
IX	18	15	12	17	18
X	9	8	7	8	7+3[c]
A[d]			105		
B[e]			108	47	43
Avg II–X	14	12	11	12	13
Avg II–VII	13	12	11	12	13

[a] Helix assignment obtained from crystal structures of C domains of colicins A [5] and E1 [6], intact colicins Ia [9], B [4], and a colicin N fragment containing R and C domains [10].
[b] Helix I is part of long helix B, connecting C and R domains.
[c] Two helical segments downstream of the hydrophobic hairpin in colicin N.
[d] Helix connecting T and R domains [9].
[e] Helix connecting R and C domains [4,9].

3. Relevant membrane properties

3.1. Dielectric constants

The static lipid bilayer has two defined dielectric regions: (i) a 30–35-Å-wide low dielectric region ($\varepsilon \approx 2$–4) of the membrane, consisting of the hydrocarbon lipid acyl chains. It is the low dielectric acyl chain region that determines the usual length of membrane spanning domains of integral membrane proteins, approximately 20 residues and 9 residues, respectively, for trans-membrane (TM) α-helices and β-strands. (ii) A~15-Å-wide polar "interfacial" region [19,20] consists of lipid polar head groups and bound water, and has an effective dielectric constant, $\varepsilon \approx 10$–20, intermediate between $\varepsilon \approx 80$ of the bulk aqueous phase and $\varepsilon \approx 2$–4 of the membrane acyl chain region. The low dielectric bilayer creates a very large energy barrier, the "Born charging energy" (E_B) for the entry or passage of electrical charge. $E_B \approx 40$ and 32 kcal/mol, for the terminal ionic group of Lys ($z=1$; radius, $r=2$ Å), and Arg, Asp or Glu ($r=2.5$ Å), respectively [21]. The physical processes by which proteins or peptide segments containing initially charged residues pass through this dielectric barrier in the

process of channel gating are poorly understood. One might say that the fundamental problem in the understanding of the mechanism of voltage gating of colicin channels, or more conventional ion channels, is that of understanding when and why the Born description does not apply to protein movement in membranes.

3.2. Surface potential, ψ_o

A lipid bilayer membrane with an anionic lipid content of 25–30 mol% in an ambient ionic strength of 0.1–0.2 M has a surface potential of ~−60 mV, calculated using the Gouy-Chapman formalism [22]. As discussed below, this surface potential is optimum for voltage-dependent pore opening of the colicin E1 channel domain (CPE1) in planar lipid membranes [23]. The interactions that trigger unfolding of membrane-bound CP(E1) arise at least partly from the ψ_o, as the rate of unfolding on the membrane surface increases with increasing $|\psi_o|$ [24].

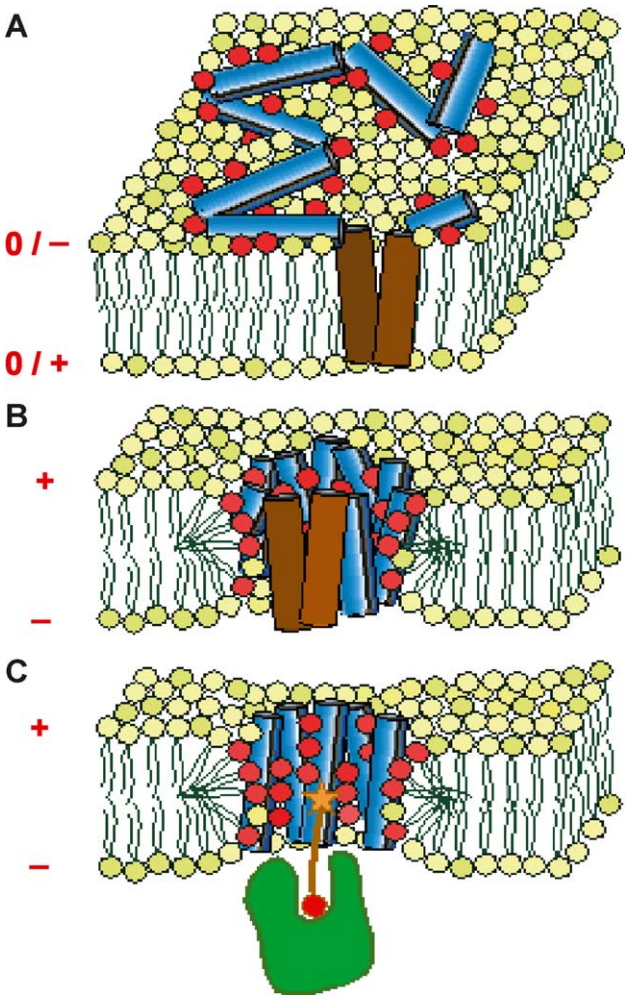

Fig. 2. Transition from closed pore (surface-bound flexible helical array) to open state of colicins may involve transient or permanent local formation of toroidal lipid-lined pores. (A) Closed state of pore after colicin binding to membrane or after pore-closing in the presence of a trans-positive potential. (B) Toroidal configuration of the open pore state. (C) Cross section of toroidal pore with an example of Cys (brown star) labeled through a 10–15-Å linker with biotin (red circle), and bound avidin (green). Helices in front of channel, shown in (B), are removed for visualization of pore interior. Anionic lipids that interact with basic side chains in the pore lining of the toroidal pore structure are shown in red.

3.3. Membrane fluidity

The unfolding of CP(E1) bound to dipalmitoyl-phosphatidylcholine liposomes was found to occur only above the defined lipid phase transition (melting temperature, ca. 41 °C) of these membranes [24]. This implies that some degree of lipid fluidity is necessary for unfolding in the membrane interfacial layer that precedes insertion into the bilayer region.

3.4. Membrane elasticity and curvature

The mechanical forces that govern elastic properties of lipid membrane, such as curvature stress and bilayer deformation, which result from hydrophobic mismatch, also modulate structure and functions of integral membrane proteins, including receptors and ion channels [25–27]. Protein import into and across the membrane bilayer is affected by lipid bilayer curvature [28–30]. In eukaryotic cells, the coiled-coil “BAR domain” of amphiphysin, which preferentially binds to highly curved negatively charged membranes, serves as a sensor of membrane curvature [31]. Changes in membrane mechanical properties result in formation of inverted hexagonal H(II) phase lipid domains (e.g., Figs. 2A, B), and other specialized lipidic structures that are not discussed here.

4. Colicin insertion into membranes

4.1. Prerequisites for pore formation

4.1.1. Helix elongation

With an average length of 12 residues (Table 1), the defined α-helical segments of the soluble colicin C domains, including CP(E1), are too short to span a membrane bilayer of standard thickness. Therefore, one of the structure changes that must occur upon binding of CP(E1) to the membrane is an elongation of the helices that will ultimately span the membrane. Upon binding to the membrane, the helices of CP(E1) were found to elongate by approximately 30% as determined by circular dichroism and infra-red spectroscopy [32]). Such helix elongation should be a general property of pore-forming toxins that ultimately form a TM structure, in which at least four helices span the membrane as α-helices. However, increased helicity was not found for the 174-residue channel domain of colicin Ia (CP(Ia)) bound to lipid membranes (POPC/POPG, 3:1, mol/mol; L/P=75), measured by solid-state NMR [33]). It was suggested in the latter study that the absence of helix elongation for the CP (Ia) upon binding is a consequence of

the presence of long helical segments in the soluble CP(Ia). However, the average length of the helices of soluble CP(Ia) is not larger than those of CP(E1), but rather slightly smaller (Table 1). Thus, an alternative explanation of the absence of helix elongation in this experiment may be the high P/L ratios necessary for NMR measurements [33]. This may result in insufficient space for helix elongation [34]. When L/P<65 in liposomes of 100-nm diameter, there is almost no free lipid, as can be calculated from the ratio of the area subtended on the membrane surface by a molecule of P178, and a phospholipid, which are approximately 4200 and 65 $Å^2$, respectively [32]. However, recent NMR data show similar effects at higher L/P ratios (M. Hong, pers. comm.).

4.1.2. Conformational flexibility

The models for CP(E1) in the initial surface-bound state and the final membrane-inserted open channel state (Fig. 2A and B) [32] imply that helix elongation and membrane insertion of the CP domain require large-scale rearrangement within the plane of the interfacial layer and in the direction orthogonal to it. Significant freedom of motion in the membrane interfacial layer should be a prerequisite for the protein translocation needed for insertion into the bilayer from surface-bound state, and one rationale for the requirement of membrane fluidity (Section 2C). This conformational freedom has been demonstrated by FRET detection of reversible thermal melting and change of inter-residue distances of membrane-bound CP(E1) [32], and a FRET study of the pathway of CP unfolding on the membrane surface, whose rate on a time scale of tens of milliseconds was dependent on membrane fluidity and anionic liquid content [24]. The requirement of conformational mobility of the colicin for membrane insertion and cytotoxicty in vivo explains the inhibition of channel formation by disulfide cross-linking of colicin A [35] and colicin E9 [36]. Solid-state NMR analysis of the molecular dynamics of CP(Ia) in the membrane-bound and soluble state indicated that the motional amplitudes of the backbone and side-chains, and the conformational mobility, are increased in the membrane-bound state [34].

4.1.3. Trans-membrane distribution of positively charged residues; the cis (inside)-positive rule

The orientation of integral membrane proteins (caveat) can be predicted from the asymmetric distribution of basic residues on the *cis* and *trans* side of the membrane [37], where *cis* (*trans*) refer, respectively, to the side from, and to, which the polypeptide is inserted (directed). The observed phenomenon is that, statistically, there are more positively charged residues on the *cis* than on the *trans*-side of an inserted or assembled integral membrane protein. The basis of the rule is the energetic difficulty of translocating basic residues across the low dielectric hydrophobic core of membrane bilayer [21]. This rule is usually discussed in the context of the assembly of integral membrane proteins. Because it applies to proteins exported from the cytoplasm in bacteria and imported into mitochondria, where the signs of the membrane potential are opposite to that of the direction of import, this rule for membrane protein assembly appears to be independent of the TM potential. It is not clear that it applies to voltage-gated domains of toxins and colicins.

4.1.4. Membrane thinning

Small membrane-active peptides added at high concentration to liposome membranes can cause membrane thinning. The insertion of peptides such as the 23-residue peptide, magainin-2, causes thinning of synthetic lipid bilayers, measured by lamellar X-ray diffraction, which can facilitate spontaneous peptide insertion [38]. The extent of bilayer thinning is 1 Å at a peptide/lipid molar ratio of 1:100 and 2 Å at 1:60. This effect could be relevant in liposome experiments with colicin CP domains. On the one hand, the channel domain/lipid molar ratio is smaller, typically 1:500 in liposome experiments with CP(E1) [32]; however, the amino acid/lipid molar ratio is probably similar to that for magainin. Protein-induced membrane thinning may not be a factor in planar bilayer experiments where protein/lipid ratios are very small. However, significant bilayer thinning could be associated with lipid-dependent toroidal-based formation of the colicin pore (Section 4).

4.2. Colicin insertion

4.2.1. Time course

The initial events associated with colicin insertion into membranes have been studied through the interaction of CP(E1) with liposome membranes. The interaction is triggered by utilizing an acidic pH~4 to (a) create attractive electrostatic interactions between cationic CP(E1) (effective binding charge, +7) and liposome membranes with a physiological (30 mol%) anionic lipid content [39,40], and (b) to facilitate unfolding of the protein at the membrane surface [41]. The sequence of the initial events is membrane binding, unfolding, helix elongation, and insertion into the membrane [30]. Thus, unfolding precedes insertion, and helix extension does not require insertion. These kinetic data imply that unfolding, helix extension, and insertion of CP(E1) are closely coupled, but sequential, events. The net result is an unfolding of the globular channel domain into an extended flexible helical network in the 10–15-Å-thick membrane interfacial layer (Fig. 2A).

4.2.2. Membrane surface potential tuned for colicin channel formation

It was proposed that the anionic lipid content of the membrane and the resulting surface potential are critical for the conformational freedom of membrane-bound CP(E1). Compensatory changes of ionic strength and anionic lipid content associated with large amplitude channel current demonstrate that the colicin channel current is observed over a narrow range of the membrane surface potential, whose value, −60 mV, corresponds to that in many biological

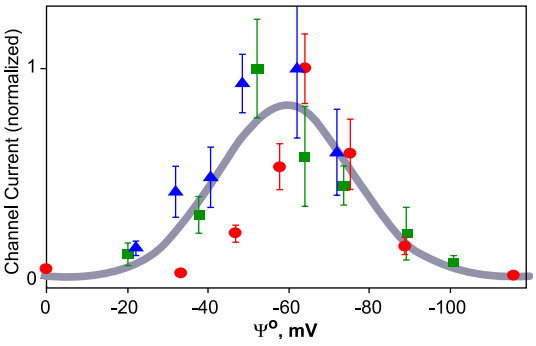

Fig. 3. Macroscopic steady-state current in planar bilayers as a function of surface potential, calculated from Gouy-Chapman theory. Data were normalized relative to the amplitude of the maximum current for different ionic strengths; i.e., at 25, 30, and 70 mol% DOPG for 0.1 (circles, red), 0.3 (squares, green), and 1.0 M (triangles, blue) KCl, respectively.

membranes. Thus, the surface potential appears to be "tuned" [42]. At optimum tuning, where the channel current mediated by CP (E1) is maximum (Fig. 3), $\psi_o^{max}=-60$ mV, which corresponds to a favorable free energy of electrostatic interaction of the channel domain with the membrane surface, $\Delta G_{el}\approx -10$ kcal/mol. The interaction energy of the bound polypeptide with the membrane surface is, in first approximation, proportional to the membrane surface potential, ψ_o. As a bound polyvalent cation, the channel polypeptide has a larger affinity for lipid head groups than do monovalent ions.

4.3. Translocation-import of colicin Ia

4.3.1. Protein import is integral to the mechanism of voltage gating; trans-membrane folding pattern of the colicin Ia channel (Fig. 4)

Gating of CP(E1) was known to involve import of at least 40 residues of the protein from the membrane surface into the hydrophobic core of the bilayer [41]. Much more information on the nature of the inserted channel has come from experiments using *cis*- and *trans*-side streptavidin trapping of biotin-labeled single cysteine mutants of CP(Ia) studied in planar lipid bilayers [43] (11,19): (i) Using CP(Ia) that was biotin-labeled at the apex (residue 594) of the Helix VIII–IX hydrophobic hairpin, this system has provided proof for (i) voltage-independent *trans*-side penetration of the VIII–IX hairpin [44]. (ii) The voltage-gating event consists of translocation to the *trans*-side of a streptavidin-accessible domain, residues Leu474–Tyr541, a long 68-residue peptide segment consisting of helices II–V in the soluble CP(Ia). The translocated segment is bounded by up- and downstream TM helices, defined by residues Asn454–Leu474 and Tyr541–Arg560, downstream from the hydrophobic hairpin anchor, Ileu574–Ileu612. Including the bordering TM helices, the biotin–streptavidin experiments show that approximately 115 residues of the colicin Ia channel domain can be translocated across the membrane by the voltage-gated opening of the channel. It has also been possible to demonstrate voltage-dependent translocation of an additional nine-residue heme-agglutinin peptide or highly charged FLAG (DYKDDDDK) epitope, inserted after Ile508 [45].

Voltage-dependent translocation of a 70-residue segment of CP(A) across the membrane was also demonstrated for colicin A [46]. Moreover, it was shown that an 86- or a 134-residue hydrophilic protein inserted into the translocated segment can be moved to the *trans*-side and is functionally active there [46] (Fig. 4).

4.3.2. The problem of the structure of the colicin Ia pore

These experiments demonstrate convincingly that C domains of the pore-forming colicins contain a general protein translocation capability. However, the intra-membrane structure of a presumed monomeric colicin channel poses a major problem. The structure of the open channel is particularly enigmatic, as discussed more completely elsewhere [47]. The assumption that the channel of the intact colicin or the pore-forming domain is monomeric in planar bilayers leads to a structurally incomplete view of the channel. This assumption is based on titration of ionic solute efflux in liposomes [48] and of channel current in bilayers [49], and the "one-hit" nature of the cytotoxic effect in vivo [50]. For the intact colicin, the monomeric open channel is proposed to consist of four TM helices, two of which are hydrophobic helices VIII–IX of the membrane anchor, and two hydrophilic or amphiphilic helices frame the large translocated domain [45,47]. The TM components of the C-terminal channel domain alone are believed to consist of only three TM helices, one–two hydrophilic helices and the

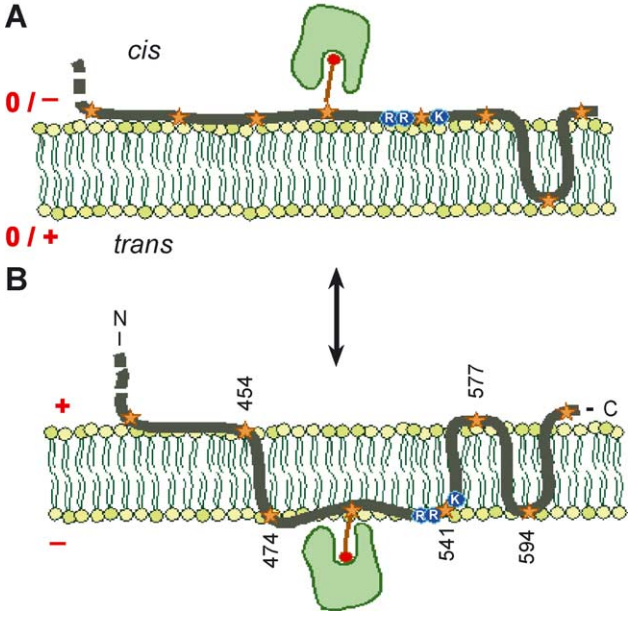

Fig. 4. Detection of voltage-dependent translocation of a large part of the pore-forming domain of colicin Ia to the *trans*-side of the membrane, using biotin–streptavidin complex formation. Gating changes carried by Arg534, Arg537, Lys544 are blue [1].

two hydrophobic helices of the anchor [51], as is the T domain of diphtheria toxin in the open channel state [52]. It is difficult to visualize the structure of a three-helix channel, and there is no precedent for it, either from structures or biochemical–biophysical analysis. A possible involvement of lipids in the translocation pathway and the ion-conducting channels of pore-forming colicins is considered below (Ref. [53]; Section 6).

5. Concepts for colicin translocation derived from the structure of the K^+ channel

Unlike colicins, K^+ channels are integral membrane proteins with a relatively rigid structure. Nevertheless, recent advances in the study of K^+ channel structure in closed and open states revealed voltage-gating features that could be relevant to the mechanism of action of other channel-forming proteins including colicins.

From the X-ray structure studies of the KcsA bacterial voltage-gated K^+ channel, it was concluded that it is formed by four subunits that encircle a central ion conducting pathway [54,55]. Each subunit has two TM helices and a tilted pore helix that runs half way across the membrane. The arrangement of the two TM helices shows one to be internal in the channel structure, i.e., near the ion pathway, and the other is outside. The negatively charged C-terminus of the pore helix dipole is pointed to the ion pathway.

5.1. Ion-selectivity filter

Unique features of the selectivity filter of the K^+ channel prevent the passage of Na^+ ions, but allow K^+ to be conducted across the membrane. The presence of a water-filled cavity near the middle of the membrane and oriented helix dipoles allows K^+ to remain hydrated to overcome electrostatic repulsion during K^+ entry and to be stabilized at the membrane center [54]. Thus, the effective dielectric constant seen by a K^+ ion in the channel in the center of the membrane is $\gg 2$, the value associated with the hydrocarbon lipid acyl chains (see Section 3A). In the selectivity filter, water is replaced by eight protein oxygens arranged similarly to the oxygens of the water of the hydrated K^+ in the central cavity. An excessively high affinity of K^+ to its binding sites is prevented by presence of several closely spaced K^+.

5.2. Voltage-gating

Comparison of the crystal structures of KcsA and MthK, Ca^{2+}-gated K^+ channel, which were crystallized under conditions that favor closed and open (in the presence of Ca^{2+}) conformations, respectively, implies that the gating of K^+ channel is coupled to large conformational changes, so that the inner helices obstruct the pore in the closed state and expand the pore diameter in the open state [56].

The gating-linked conformational change and transmembrane movement of one of the TM helices are clearly relevant to the problem of colicin channel gating. As in the case of colicin Ia, when a voltage-gated channel opens, charged amino acids, the "gating charges", move through the membrane in the TM electrical field, coupling electrical work to channel opening [57]. The gating charge of the voltage-gated K^+ channel is equivalent to almost 14 charges moving entirely across the TM voltage difference. Most of this charge is attributable to four Args per subunit, a total of 16. In the crystal structure of KvAP, the voltage-gated K^+ channel, the pore structure is surrounded by α-helical voltage sensors [58]. It is proposed that these gating charge Args are located on hydrophobic helix-turn-helix voltage sensor paddles which "appear as though they could move within the membrane at the protein–lipid interface when the channel opens" (Fig. 5) [59,60]. TM movement of the Arg-rich segments was detected by a biotin-avidin assay derived from, and analogous to that used in the colicin Ia assay (IV, C). Biotin was tethered to the segment at various positions, and effects of avidin were assessed at positive (open state) and negative (closed state) potentials applied to planar bilayers with incorporated K^+ channels. K^+ channel conductance or channel openings were affected by avidin in several constructs for both states. For two

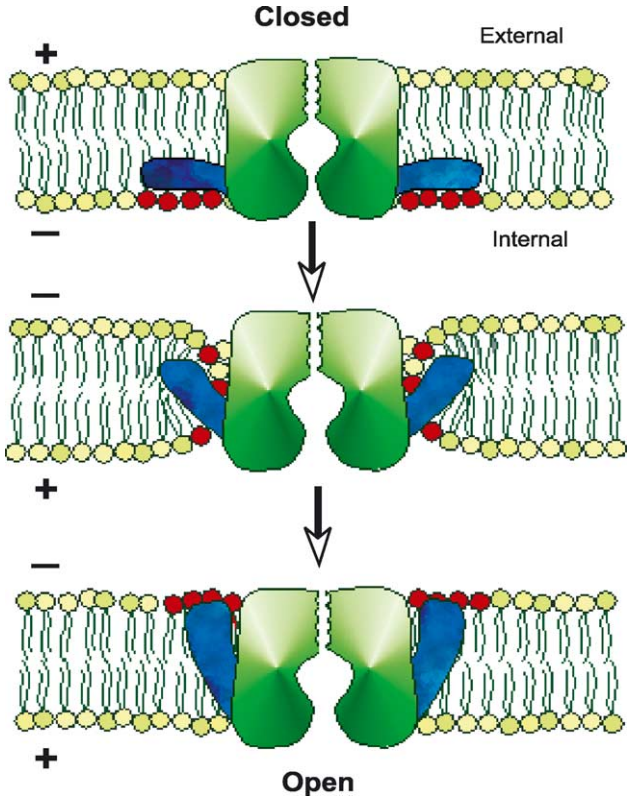

Fig. 5. Voltage-gating of K^+ channel may involve transient formation of local non-bilayer lipid structures around a translocated voltage-sensing segment (blue) to decrease the energetic cost of insertion of the gating charge (Arg) into the bilayer.

positions of biotin, the effect of avidin was observed in open and closed states, implying that the biotin-tethered segment can be dragged across the entire membrane, from the intracellular side in the closed conformation to the extracellular side in the open channel state. A proposed mechanism of voltage sensing is that the electrical field pulls on a helix-turn-helix structure with four Args on the channel's perimeter to induce a pore conformation change [59,60]. Another example of voltage-dependent gating is the MscS, mechanosensitive channel. It features a similar helix-turn-helix structure with arginine residues on its perimeter [61].

If the proposed movement of an Arg-loaded segment across membrane is valid and it occurs through the lipid–protein interface, the long-held assumption that charged amino acids must be shielded from the nonpolar environment of the membrane would be reevaluated [60]. The hydrophobic and electrostatic interactions can balance each other so that the delocalized charge on an Arg can be drawn into the membrane at protein–lipid interface. There are alternative descriptions of the mechanism of K^+ channel voltage-sensing [62], where the trans-membrane movement of the Arg-rich helix is considered as sliding within the protein assembly in a focused TM electrical field.

5.3. Anionic lipid dependence

Refolding of the KcsA tetramer from monomers depends on lipids. The KcsA tetramer is more stable in a lipid bilayer compared with detergent micelles. The strongest stabilizing effect was observed with phosphatidylethanolamine, the bilayer-distorting lipid [63]. However, stabilization does not require the presence of negatively charged lipids, while the presence of phosphatidylglycerol or other negatively charged lipids might affect the kinetics of refolding. Moreover, anionic lipids appear to be obligatory for ion conductance through this channel [27]. Channel activity of KcsA is only seen when it is reconstituted into liposomes containing negatively charged lipids, which might influence the gating properties of the channel including conformational changes in TM helices [56].

The idea that the anionic lipid head groups could provide compensatory charge for the movement of Arg-loaded segment associated with channel gating comes immediately to mind (Fig. 5). A similar mechanism could be involved in the colicin voltage-gating mechanism, explaining the ability of a large hydrophilic peptide segment to move across the membrane hydrophobic core (Fig. 2), and the steep dependence of channel conductance on anionic lipid content [42].

6. A toroidal model for the colicin pore

The model of a toroidal lipid pore was proposed to explain the mechanism of cytotoxic activity of antimicrobial peptides (Shai–Matsuzaki–Huang model; Ref. [64]). It has been discussed in the literature as an intermediate step in the 'carpet' model [65,66]. However, we note that the carpet model does not require any precursor membrane-inserted state. The toroidal model suggests direct involvement of lipid in the formation of membrane pores by the peptides. It is alternative to a traditional "barrel-stave" or "helical bundle" model [67–69], which proposed that pore walls are formed exclusively of peptide α-helices, and the conducting pore results from the aggregation of amphiphilic α-helical monomers.

In the toroidal model, the lipid layer bends back on itself like the inside of a torus [70]. The bending is the result of a lateral expansion of the head groups relative to the acyl chains. The lateral strain in the monolayer is reduced by insertion of amphiphilic α-helical peptides in the head group layer. Alternatively, peptide insertion would cause the lateral strain and induce lipid monolayer bending. The evidence in favor of toroidal pore formation by these peptides is based on the sensitivity of their pore-forming activity to lipid curvature. It has been shown that lipids with positive spontaneous curvature (SC) stimulate, while lipids having negative curvature inhibit pore formation by magainin and syringomycin [71,72].

As shown in numerous studies, the dimensions of pores formed in the bilayer lipid membrane by antimicrobial peptides and toxins are large. They can allow passage of such bulky molecules such as carboxyfluorescein and calcein. According to neutron-scattering data, the radius of the water-filled pore formed by magainin is 18–19 Å [70].

The toroidal pore concept has also been proposed as a mechanism of action of large toxins belonging to the actinoporins, e.g., equinatoxin II [73] and sticholysins [74]. The influence of membrane curvature was shown to be opposite for sticholysin compared to magainin, suggesting that the presence of a small amount of cardiolipin, a strong inducer of negative SC, augments the efficiency of toxin pore formation because of the presence of a negative curvature region in the plane of the membrane around the toroidal pore. According to the toroidal pore structure, the phospholipids in such a pore would exhibit negative curvature in a dimension parallel to the bilayer plane but positive curvature along the bilayer normal [75]. The balance between positive and negative curvature would depend on the pore size, with larger pores having predominantly positive curvature. Thus, the size of the pore can be predicted from the dependence of the peptide pore-forming activity on the lipid SC.

The formation of toroidal pores by antimicrobial peptides requires their oligomerization on the membrane surface [76,77]. An oligomeric structure of the pore was also proposed for larger peptides like equinatoxin (M_r of about 20,000) that has a 30-residue amphipathic helix on its N-terminus [73]. Small antibacterial peptides do not form regular single channels in planar bilayer lipid membranes [78–80]. This is explained by the unstable structure of a toroidal pore formed in the absence of covalent cross-

linking of α-helices. In this case, pore formation requires a sufficient amount of peptide to be bound to the membrane surface. By connecting the α-helices in colicin CPs by the loops, nature creates a structure in which a single molecule (10 helices in the C domain) is sufficient to form a membrane pore, eliminating concentration restrictions. Another advantage of such structure is the presence of a hydrophobic hairpin, which probably serves as a nucleus for both (i) a hydrophobic core in the soluble state and (ii) cooperative voltage-gated pore formation.

A toroidal pore model was also proposed for pro-apoptotic proteins Bax and tBid [81–83]. In these studies, nonlamellar lipids with positive intrinsic curvature, such as lysophospholipids, promoted membrane permeabilization by Bax, whereas lipids with negative intrinsic curvature, such as diacylglycerol and phosphatidylethanolamine, inhibited membrane permeabilization. Surprisingly, lysophosphatidylcholine and other agents promoting positive membrane curvature have been observed to inhibit membrane leakage induced by tBid [84]. Bax and other Bcl-2 proteins display structural similarity to colicin channel-forming domains.

It is suggested that the monomeric C domain of colicins can form a toroidal pore (Fig. 2B). In agreement with this, pore-forming activity of CP(E1) was sensitive to the SC of lipids present in planar bilayers (Ya et al., manuscript in preparation). Lipid molecules would intercalate between α-helices so that polar head groups participate in the formation of a pore wall. A hairpin pair of hydrophobic α-helices (VIII–IX), practically lacking polar amino acid residues, might not be included in the pore wall, because it should not have the appropriate affinity. The hairpin may play a role of a stabilizing factor that preserves or maintains the orientation of the channel in the course of its switching on and off upon voltage application. The inverted micelle basis of the toroidal structure for the pore would allow helices that fit into the pore to occupy a TM configuration with a helix length somewhat less than the canonical 20 residues. This would be a consequence of membrane thinning associated with the toroidal pore (Fig. 2B). Thus, the average length of TM helices lining the pore would be approximately 16 residues, the 12-residue average length in the soluble CP domain (Table 1) plus the 30% increase detected upon binding to the membrane (Section 4A). It is predicted that the number of such helices associated with these pores will be greater than the three to four inferred from the biotin–streptavidin labeling experiments with CP(Ia) (Fig. 4). A model for the biotin–streptavidin interaction, in which biotin extends into the channel lumen, is shown (Fig. 2C).

During the last administration, it was proposed “in desperation” that lipids might contribute to the colicin Ia channel structure [45]. After 4 more years, it would appear that the limits of desperation have been reached, and it is considered “not unlikely” that lipids are involved in the structure of the colicin Ia channel domain [53].

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