Antimicrobial peptides modulate bilayer barrier properties using a variety of mechanisms of actions

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Various antimicrobial peptides (AMPs) are active in changing the biophysical properties of cell membranes. AMPs often interact with lipids and create AMP-lipid complexes which lead to the creation of protein-lined or lipid-lined well structured ion channels, less structured ion flowing pores, localized disorders or defects, etc. in membranes. Such special structures require various AMP specific mechanisms. The complexes often appear with various distinguishable structures. A few of them are, for example, linear β-helix created by e.g. gramicidin A, barrel-stave pore created by e.g. alamethicin, toroidal pore created by e.g. magainin, melittin, etc., lipidic channels created by e.g. ceramides (example of a non AMP-induced channel), defects created by e.g. gramicidin S, etc. In this minireview we shall address about the primary mechanisms which dictate the formation and function of the mentioned ion flowing events. The AMP effects on membrane depend mainly onophysical properties such as geometric, electrical, mechanical, etc. of membrane and AMPs. Also, the regulation of AMP or general membrane protein (MP) functions depend on the hydrophobic coupling between membrane and the integral AMPs/MPs. Specifically, the AMP-membrane hydrophobic coupling dependent regulation of membrane properties and AMP functions depend mainly on the electrostatic and mechanical energetic AMP-membrane couplings. Both of these energetics emerge from a common calculation of screened Coulomb interaction between AMPs and the host membrane. It is also important to mention that the electrostatic energetic coupling is found to be the dominant contributor while mechanical energetic coupling is found to serve as secondary role in the mentioned energetic coupling regulation of both membrane and AMP functions. Experimental, theoretical and computational results will be used to address the problems and support our remarks.

Keywords antimicrobial peptide; membrane protein; lipid membrane; ion channel; defects; screened Coulomb interaction

1. Introduction

Biological membranes are dynamic composite structures, composed of lipid bilayers together with embedded bilayer-spanning proteins that move in the plane of the membrane. This was originally proposed in the fluid mosaic membrane model [1]. A lipid bilayer’s primary function is to serve as a semi-permeable barrier for solute movement between different, membrane-separated fluid compartments. This barrier function depends on the bilayer’s hydrophobic core being a poor “solvent” for polar solutes. The bilayer permeability coefficient for solute $X$ ($P_X$) can be approximated as:

$$ P_X = \alpha_X \cdot \frac{D_X}{\xi}, $$

(1)

where $\alpha_X$ is the solute partition coefficient between the bilayer core and the aqueous phase, $D_X$ is the solute diffusion coefficient in the bilayer core (which varies little among small solutes [2]), and $\xi$ denotes the bilayer hydrophobic thickness (~30 Å for hydrocarbon-free bilayers [3,4], or 40-60 Å for hydrocarbon-containing bilayers [5]). Experimental results obtained for a wide variety of solutes show that $P_X$ is proportional to $\alpha_X$, as approximated by the solutes’ oil/water partition coefficient [2,6,7], and that the solute diffusion coefficient in the bilayer core is similar to the diffusion coefficient in bulk hydrocarbons ($10^{-6}$ to $10^{-5}$ cm$^2$/s [8]).

The primary function of the component proteins inside membrane is to catalyze the selective transfer of material and information across biological membranes. In the course of catalyzing this transfer, membrane proteins undergo conformational changes, namely: (a) the opening/closing transitions in ion channels [9-11] and (b) the shift in substrate binding site accessibility in conformational carriers and ATP-driven pumps [12]. To the extent that these protein conformational changes involve the protein/bilayer interface, they will perturb the bilayer immediately adjacent to the protein [13-17], cf. Fig. 1. That is, protein conformational changes involve not only rearrangements within the protein, but also interactions with the environment, particularly with the host bilayer.

The success of Eq. (1) in predicting small-molecule permeability coefficients naturally leads to the notion of lipid bilayers being thin sheets of liquid hydrocarbon, stabilized by the lipid polar head groups, as implied in the original formulation of the fluid mosaic membrane model [1]. If that were the case, one would expect that $|\Delta G_{\text{def}}^0| << k_BT$, in which case membrane protein function would be little affected by changes in bilayer properties – except in cases where the interfacial surface charge densities vary [18-20]. Here energetic cost, $\Delta G_{\text{def}}^0$ is the energetic cost associated with the
Structured ion passing pores, localized disorders or defects, etc. In formation of such special structures in a membrane create AMP-lipid complexes which lead to the creation of protein-lined or lipid-lined well-structured ion channels, less AMPs are active in changing various biophysical properties of cell membranes. They often interact with lipids and various classes of such events with a special focus on their membrane effects. We shall discuss the details of also fall in different classes due to the diversities in their structures and mechanisms. Based on our existing knowledge on various antimicrobial type membrane effects of a set of small size charge neutral drug molecules mainly used as chemotherapy drugs.

2. Membrane as a transporter: ion Channels or pores formed by AMPs or other biomolecules

A cell membrane’s primary role is to create barrier against materials to be transferring between cellular exterior and interior regions. As discussed earlier the presence of certain natural or artificial agents (during the time of treatment) like MPs, AMPs, etc., occasionally induce transient or stable transport properties into cell membranes. These induced properties are often found to be highly dynamic, time dependent and specific to the agents inducing them. The events also fall in different classes due to the diversities in their structures and mechanisms. We shall discuss the details of various classes of such events with a special focus on their membrane effects.

AMPs are active in changing various biophysical properties of cell membranes. They often interact with lipids and create AMP-lipid complexes which lead to the creation of protein-lined or lipid-lined well-structured ion channels, less structured ion flowing pores, localized disorders or defects, etc. In formation of such special structures in a membrane involving AMPs and lipids various mechanisms are observed which are mostly specific to AMPs. The complexes often appear with various experimentally distinguishable structures; a few of them are, for example, linear β-helix created by e.g. gramicidin A (gA), barrel-stave pore created by e.g. alamethicin (Alm), toroidal pore created by e.g. magainin, mellitin, etc., lipidic channels created by e.g. ceramides (example of a non AMP-induced channel), defects created by e.g. magainin S (GS), etc. We shall address about the structures of those mentioned membrane disrupting events and the primary mechanisms which dictate the formation and function of them. Based on our existing knowledge on various model studies we shall construct a complete platform to address the antimicrobial effects of a group of AMPs. All these membrane events not only follow certain structural complexities due to just their biophysical coexistence but that the phenomena follow complex energetics. We shall address the structural aspects here in details which will be followed by the energetic aspects in the next section.

2.1 Protein Lined Ion Channels in Membranes

In protein lined channels it is generally considered that channel forming peptides align themselves along the channels. Ions flow through the longitudinal axis of the channel between cellular exterior and interior regions and they most likely experience interactions with channel forming peptides. Only at the entry and exit levels of the channels ions are expected to experience interactions with lipids. Usually linear, cylindrical, etc., types of structures in channels are found in the class of protein lined channels. This class refers to highly ordered peptide structures in association with lipids in membranes. Lipids play important role which is mainly in the regulatory phase of the channels but the creation of such channels primarily depends on the properties (chemical, geometrical, electrical, etc.) of the channel forming peptides. The number and type of the sequences of amino acids and other constituents in the peptide, geometrical size (length, cross section, etc.) of the peptide, and charge properties of the participating atoms and the effective final charge of the peptide in the hydrophobic membrane environment, etc., play the crucial role in the construction of the protein lined
channels. Best examples of criteria exists in protein lined channels are found in well studied two ion channels namely gA and Alm channels which will be addressed here as examples.

gA channel is a dimer of two right-handed, \( \beta \)-6.3-helical subunits/monomers [32-34]. This channel is formed by reversible, trans-bilayer association of the subunits [35]:

\[
M_{\text{left}} + M_{\text{right}} \xrightleftharpoons[\kappa_{-1}]{\kappa_1} D,
\]

Here M and D represent gA monomer and dimer, respectively. The subscripts denote monomers residing in each bilayer leaflet. Here, \( \kappa_1 \) and \( \kappa_{-1} \) are two rate constants determining the channel appearance rate \( f_0 = k_1[M]^2 \); \([M]\) being the monomer concentration) and channel lifetime \( \tau = 1/k_{-1} \). A model demonstration has been presented in Figure 1. Peptides residing inside membrane occasionally approach to each other and depending on the bilayer environment associate with each other and make a dimer with a very short lifetime which spans over a considerable range in millisecond (ms) order and the range depends on the strength of the hydrophobic coupling between channel and the bilayer. It is important to mention that in this kind of channels only a dimer state is a stable structure for channel. No other channel states are noticed so far.

Alm channels form barrel stave pores [36, 37]. In this type of pore the peptides align in a way such that it seems they make a cylinder where the peptides stay on the surface of the cylinder. Many conductance states depending on the number of monomers involved in forming the cylindrical channel is possible. A model diagram is proposed and explained [31] in Figure 2. Here the channel is assumed to be formed due to inter monomer binding. The channel also experiences hydrophobic couplings with the lipid monolayers in its both longitudinal terminals. Unlike gA channels where we observe only one ordered gA dimer state there is no unique cylindrical Alm channel state as evidence. Alm channels with various cylindrical states are possible. Perhaps a transition between different cylindrical channel states also is a reality [31]. These are modeled in the diagram [31] in light of the earlier proposed models [36, 37]. The readers will learn more about the stability, energetics, and regulation of the channel conformational states in next section.

**Figure 1:** Bilayer deforms at the bilayer gA channel coupling area which incurs an energetic cost (see next section). The upper panel shows a lipid bilayer without any integral membrane protein. Lower panel shows a bilayer with integral gA monomers and dimers of different lengths. When gA channels are formed inside a lipid bilayer experiencing a transmembrane potential difference between two sides, the bilayer conducts a current pulse with a specific average pulse width (gA channel lifetime) and height (gA channel conductance) depending on the gA channel type (the number of amino acids in the structures of gA monomers). Two types of gA monomers are schematically structured here to produce two gA channels of different lengths \( l \). \( d_0 \) is the unperturbed thickness of the bilayer.

**Figure 2:** A model diagram is proposed and explained [31] in Figure 2. Here the channel is assumed to be formed due to inter monomer binding. The channel also experiences hydrophobic couplings with the lipid monolayers in its both longitudinal terminals. Unlike gA channels where we observe only one ordered gA dimer state there is no unique cylindrical Alm channel state as evidence. Alm channels with various cylindrical states are possible. Perhaps a transition between different cylindrical channel states also is a reality [31].
Figure 2: Barrel stave model for Alm channel formation inside lipid bilayers [31]. Cylindrical rods are schematic diagrams for Alm monomers in three-dimensional view (in two-dimensional view along the cylinder axis they appear as circles). Top panel shows three-dimensional (3d) view, middle panel shows the transition between different conduction pores of Alm channels and the two lower panels (two-dimensional (2d) view of the channels only from membrane surface where Alm monomers are seen only along their longitudinal direction so they appear as circles) represent the two possible mechanisms how inter-channel conduction level transformation happens. In the bottom panel we consider that the monomers already exist in a structured form of Alm channel where the pore radius changes by reorganization of the channel forming monomers. The other 2d view illustrates a possible model of Alm channel formation and transformation between different conduction levels where the pore radius increases by addition of monomer(s) from the surrounding space where monomers randomly move into the channel. The reduction in the pore radius occurs by releasing the monomers from the cylindrical surface of the channels. Both of the models in 2d views are valid explanations of the upper 3d structures of Alm channels. Taking three monomers in the 0th conductance level is an arbitrary choice but our reverse calculation using experimental values of cylindrical Alm pore conductances and the theoretical values of the cross-sectional areas of different Alm pores hint that 3 monomers perhaps form the 0th conductance level. Faded circles and bonds in 2d views are shown to distinguish their inactivity in the channel’s conduction mechanism. This model diagram has been published in refs. [31, 38].
2.2 Lipid Lined Ion Channels in Membranes

In lipid lined channels it is generally considered that channel forming peptides interact with lipid membrane and the complex creates the alignment of lipids along the channels. Ions flow through the opening between cellular exterior and interior regions and perhaps through the lipid regions avoiding the peptides involved in creating channels. The lipid alignment causes an opening which may look like a long cylindrical lipid aligned channel where the membrane thickness may not dramatically change. The other possibility is that the membrane thickness slowly vanishes at the channel opening which can equivalently be considered as a broken membrane condition. In many investigations this broken membrane structure has been predicted as a model for the lipid lined channels. Figure 3 explains schematic diagram of this condition. This kind of structures are found to be induced by both AMPs e.g. magainin[39, 40], melittin[41], colicin[42], etc., or by other non-antimicrobial agents e.g. the recently discovered pores by chemotherapy drugs thiocolchicocide(TCC) and taxol(TXL) molecules [38]. The magainin, melittin and colicin induced toroidal pores can also be considered as protein-lined channels because here the peptides are always associated with the lipid headgroups result in the lipid monolayers bending continuously inward, so that the pore is lined by both peptides and lipid headgroups.

Figure 3: Chemotherapy drugs TCC and TXL induced proposed toroidal-type ion pores in a lipid bilayer membrane possibly associated with a spontaneous change of the pore cross section. This novel model is explained in a recent article[38].

2.3 Lipidic Channels/Pores in Membranes

Here we shall describe about an ion channel which is formed by lipids inside membranes. Although lipids primarily exist across the lipid layers in a membrane ceramides behave differently. Ceramides form channels called ceramide channels due to special organization of ceramides in phospholipid membranes. Ceramide channel is also an example of a lipidic channel. A ceramide is a lipid molecule composed of the amino acid sphingosine and a fatty acid. Ceramides exist in great concentrations in the plasma membrane of a cell and act as signaling molecules for a number of cellular functions. Ceramides may also have a role in certain pathological states, including cancer, obesity, diabetes, inflammation, etc. Understanding of ceramide organization in membrane is therefore an important field which has important medical interests. A model structure of ceramide channel is presented here in Figure 4. From the model diagram it is clear that the ceramide channels look more like Alm’s barrel stave pore but have no resemblance with the structures of other channels like toroidal or β-helical types described earlier. That means the lipid membrane adjusts with the complex of ceramide molecules at the channel membrane interface and creates no vanishing of the membrane thickness like what is proposed in the case of toroidal pores. Detailed understanding about the mechanism of the lipidic channels is still in the ‘yet to discover’ phase but some insights are already found in the research papers published by many groups especially a few mentionable here [43-45].
Figure 4: These model diagrams on the C_{16}-ceramides in membranes (ceramide channel) were provided by Professor Marco Colombini which was recently published [43]. Permission from the publisher has been achieved to use the figure. Besides this paper earlier papers of the group e.g. [44, 45] can also be read to understand details about the ceramide channel phenomena in lipid membranes.

(A) The channel is slightly tilted to illustrate the columns that span the membrane, each consisting of six ceramide monomers. The pore is lined by hydroxyl groups. The hydrocarbon tails are oriented parallel to the plane of the membrane. The columns are arranged in an antiparallel fashion so that the carbonyl oxygen of the amide linkage (red) is only visible in every other column. The pore diameter of this 48-column channel is 10 nm.

(B) A model of a segment of a smaller ceramide channel showing how it might interface with the phospholipid membrane. Note the slightly hourglass shape of the pore and the distorted phospholipids (lighter colors) that cover the hydrocarbon chains of the ceramides at the end of the channel. The structure of this interface is an illustration of the results reported from molecular dynamic simulations by the same group [45].
2.4 Defects in Membranes

We have so far in this section addressed how membranes can be forced to compromise with the lipid created bilayer’s electrical insulation properties through inducing some complexes of the participating antimicrobial peptides, lipids, specific biomolecules, etc. In all of these cases the structure of the complexes takes some specific forms which are understandable and can be modeled. But there exists some other ways a few agents can create no such ordered structures but appear to create disorders inside membranes. These disorders, often referred as ‘defects’ may occasionally be compared to events that are responsible for creating conductance properties in a non-channel fashion inside membranes. As they are random disorders it is hard to schematize them due to their varied ways of appearances. But attempts are taken by researchers to model them using scientific analogy. In-plane diffusion model of Bechinger or similar models [46, 47] can be considered as examples. In this Bechinger model, the insertion of antimicrobial peptides into phospholipid bilayers are considered to disorder the hydrocarbons chains of adjacent phospholipid molecules, creates locally thinning of the bilayer and increases the cross-sectional area per phospholipid molecule. This process finally leads to local disturbances in bilayer packing and leads to increased bilayer permeability. Such bilayer perturbation requires minimal peptide aggregation, which would be both entropically and electrically unfavorable. Yet, these regions of instability may eventually overlap due to lateral diffusion within the membrane, thereby producing transient “openings” of a variety of sizes. Evidence of such defects is recently discovered by us while investigating the membrane effects of a small AMP GS [48]. This GS-induced destabilization of the phospholipid bilayer would be expected to be enhanced with the insertion of additional peptide molecules and with increasing transmembrane potential, as was observed. Also the bilayer properties, participating lipids, hydrocarbons all were found to play a concomitant role in the induction of the AMP induced defects or non-channel conductance events in the study by us discovering a novel AMP mechanism of action [48].

2.5 A comparable analysis of the Electrical Conductance States representing Membrane Permeability

In Figure 5 we have presented our electrophysiology results on current traces recorded across phospholipid/n-decane membranes doped with channel forming antimicrobial peptides or chemotherapy drug molecules to show as examples of current traces through ion channels. The AMP –induced channels are found to be transporting currents with distinguishable amplitudes considering their different conformational states and rectangular current events in current versus time plot are found in these cases (see e.g. both linear β-helical gA and barrel stave Alm channel currents). The transitions between different current states are transient meaning the transition takes no time. While the current traces through TCC and TXL induced proposed toroidal channels show no clear constant current amplitudes that might represent for any specific conductance state. The triangular current events in these cases rather represent some channel/pore whose cross sectional area changes back-and-forth freely with the change of time. Unlike transient current transitions in AMP channels the transition of current in chemotherapy drug channels is a time dependent phenomenon. AMP channels undergo structural transitions between distinguishable structures e.g. gA’s monomer and dimer states (Figure 1) and Alm’s different states depending on the participating Alm monomers in its barrel stave pore which determine the cylindrical channel’s distinguishable cross sections (Figure 2). Each distinguishable structure represents a discrete current level in a single AMP channel conductance state. As all distinguishable structures are pretty stable we observe stable amplitudes in all corresponding current levels. Totally different current structure is observed in the case of chemotherapy drug channels. The spontaneous change of current amplitude clearly suggests for no specific structure of channel with a constant geometrical dimension. Only the broken membrane model (see the two-dimensional view in Figure 3) can support the idea of a time dependent continuous change of pore cross sectional area. This is a novel discovery by us [38]. The lipidic channels created by e.g. ceramides represent no different than barrel stave pore type current transitions (for details see [43-45]). But the current traces dedicated to representing defects inside membranes (Figure 6) show another novel behavior. Sudden spikes with apparently no specific ‘amplitude and stability’ show conductance events inside membranes [48]. These spikes certainly represent for no stability of any distinguishable structure or any specific stable complex created by the antimicrobial peptide GS with lipids. Unlike the certain presence of discrete peaks at certain values of conductance (representing certain stable structures of channels) in the point count versus conductance plots in gA and Alm channels we observed no discreteness in the point count plots of the current traces through both chemotherapy drug channels and GS induced defects.
Figure 5: Electrical Conductance States Determining the Membrane’s Transport Properties Induced by Ion Channels. The upper panel shows triangular-shape conductance events induced by chemotherapy drugs TCC and TXL, both at 90 μM, pH=5.7, V=100 mV. Both traces were filtered at 20 kHz but the lower one shows higher noise due to its presentation (current axis) at an amplified scale. In a high resolution plot (shown in the right side of the arrow) of a single event only with showing individual points (in Origin 8.5 plot) we observe all points (open circle) with increasing and decreasing, respectively, corresponding values of conductance at both left and right lateral sides of the chemotherapy drug induced triangular conductance events. The lower panel (A) illustrates rectangular-shape conductance events in gA and Alm channels [31, 48]. gA channel activity was recorded at 200 mV and Alm at 150 mV. Traces representing gA and Alm channel activities in phospholipid bilayers were recorded at filter frequencies 2 kHz and 20 kHz, respectively. A lower filter frequency for traces representing gA channel activity is alright because of the channel’s relatively higher stability. In (B) the point count plots of the current traces through gA and Alm channels peak at discrete values of conductance.
Figure 6: Electrical conductance states determining the membrane’s transport properties induced by defects [48]. GS-induced ion conductance events in zwitterionic phosphatidylcholine/n-decane bilayers with 1.0 M NaCl, pH 7.0 on both sides. (A) and (B) show long-time (11 s) and short-time (1 s) current traces of GS-induced ion conductance events, respectively. (C) all point conductance level histograms constructed from the long-time traces (A). Two peaks (C) at 0 pA/mV and around 1 pA/mV respectively represent the baseline conductance of the “unperturbed” bilayer and the conductance levels of the GS-induced ion conductance events. Readers who are interested in knowing the details of the GS effects on various model membranes may read the paper by Ashrafuzzaman et al. [48].

From the analysis of the current traces (Figures 5 and 6) we can conclude that membrane transport properties appear with various distinguishable properties of currents flowing through the conductance events and that the structures of the conductance events appear with varied types depending on the properties of the participating agents and the membranes. A few of them have been addressed here. Due to the limited scopes we have used only these few examples here. We wish to present a complete picture in another platform.

3. Energetics behind the creation of Membrane Transporting Events Ion Channels or Pores

Protein-lined ion channel lipid bilayer coupling – An energetic perspective

Protein-lined ion channels are formed as a result of a very complex direct interaction between channel forming monomers (MPs or AMPs) and lipids. gA and Alm are the two most studied ion channels which form β-helical [32-34] and barrel-stave [36, 37] pores, respectively, across lipid bilayer membranes (see Figures 1 and 2). In both cases the channels couple with the membrane’s two adjacent monolayers at two longitudinal edges of the channels. We shall here address how the channel bilayer energetic coupling regulates integral channel functions [31].

In bilayer-spanning channel formation the association of two trans-bilayer gA monomers is governed by the dimerization coefficient: 

\[ K_D = [D]/[M_g]^2 = k_1/k_-1 = \exp\left\{-(\Delta G_{prot}^0 + \Delta G_{def}^0)/k_B T\right\}, \]

where \([M_g] \) and \([D] \) are monomer and dimer concentrations; and \(k_1 \) and \(k_-1 \) are rate constants follow from equation (2). Here, \(k_B \) and \(T \) are the Boltzmann...
constant and absolute temperature, respectively. Since the bilayer deformation energy $\Delta G_{\text{def}}^0$ is sensitive to the hydrophobic mismatch ($d_{0\text{-}l}$) between bilayer thickness ($d_0$) and gA channel length ($l$), the bilayer responds to its deformation by imposing a restoring/channel-dissociation force $F_{\text{dis}}$ on the edges of a channel. Increasing $F_{\text{dis}}$ is reflected in a decreasing $\tau$ and channels become molecular force transducers [30]. Within limits, the channel structure is invariant when the lipid bilayer thickness is varied [49], meaning that the gA channels are more rigid than the host bilayer. All-atom molecular dynamics simulations of gA in bilayers [50]show how lipid head groups organize themselves in the region of hydrophobic free length $d_{0\text{-}l}$. Potential-of-mean-force calculations [51] suggest that transmembrane protein interactions are regulated by a hydrophobic mismatch equivalent to $d_{0\text{-}l}$. The calculation of $F_{\text{dis}}$ has been a long-standing challenge. Based on the so called theory of elastic bilayer deformation [52] $\Delta G_{\text{def}}^0$ has been found to be approximately changing as a quadratic function of $d_{0\text{-}l}$[52, 53] but subsequent developments [54-58] resulted in the introduction of the lipid intrinsic curvature $c_0$ (whose positive and negative changes correspond to increases and decreases of the hexagonal lipid phase, respectively, and any such local curvature profile generally controls the lipid packing energy profiles in bilayers) (details in ref. [59]) into the expression for $\Delta G_{\text{def}}^0$ which is now considered to be changing as a quadratic function of $d_{0\text{-}l}$ and intrinsic curvature $c_0$ ($\Delta G_{\text{def}}^0 = H_B d_{0\text{-}l}^2 + H_X d_{0\text{-}l} c_0 + H_c c_0^2$), consequently, $F_{\text{dis}}$ is found to be linearly dependent on $d_{0\text{-}l}$ and $c_0$ [30, 60]:

$$F_{\text{dis}} = -\left(\frac{\partial}{\partial \Delta G_{\text{def}}^0}\right)_{d_{0\text{-}l}, c_0} = 2H_B d_{0\text{-}l} + H_X c_0$$

The calculation of $F_{\text{dis}}$ has been found to be approximately changing as a quadratic function of $d_{0\text{-}l}$[52, 53] but subsequent developments [54-58] resulted in the introduction of the lipid intrinsic curvature $c_0$ (whose positive and negative changes correspond to increases and decreases of the hexagonal lipid phase, respectively, and any such local curvature profile generally controls the lipid packing energy profiles in bilayers) (details in ref. [59]) into the expression for $\Delta G_{\text{def}}^0$ which is now considered to be changing as a quadratic function of $d_{0\text{-}l}$ and intrinsic curvature $c_0$ ($\Delta G_{\text{def}}^0 = H_B d_{0\text{-}l}^2 + H_X d_{0\text{-}l} c_0 + H_c c_0^2$), consequently, $F_{\text{dis}}$ is found to be linearly dependent on $d_{0\text{-}l}$ and $c_0$ [30, 60]:

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The screened Coulomb interaction model perfectly describes the general bilayer regulation of membrane protein functions. This publication [31] also correctly describes why the well accepted so called theory of elastic bilayer deformation model [52-58] is totally unacceptable due to its inability to explain important general phenomena related to the membrane regulation of membrane protein functions. A brief explanation on this issue will also automatically come here while describing the screened Coulomb interaction model.

In the screened Coulomb interaction model[31], considering $l\leq d_{0\text{a}}$, the channel extends its Coulomb interaction towards lipids sitting on the bilayer’s nearest resting thickness. A gA channel directly interacts with a nearest-neighbor lipid by Coulomb forces and this lipid interacts directly with the next-nearest-neighbor lipid but this second lipid’s interaction with the channel is screened by the channel’s nearest-neighbor lipid. The interaction between the third-nearest neighbor and the channel is screened by the lipids in between. An assumption has been made regarding all lipids interaction with the channel is screened by the channel’s nearest-neighbor lipid. The interaction between the third-nearest neighbor and the channel is screened by the lipids in between. An assumption has been made regarding all lipids interaction with the channel is screened by the channel’s nearest-neighbor lipid. The interaction between the third-nearest neighbor and the channel is screened by the lipids in between.

$$V_{sc}(\vec{r}) = \int d^3k \exp\{i\vec{k}\cdot\vec{r}\} V_{sc}(\vec{k})$$

whose Fourier transform is [61]

$$V_{sc}(\vec{k}) = \frac{V(\vec{k})}{1 + \frac{V(\vec{k})}{2\pi k_B T n}}$$

where $V(k)=(1/\epsilon_0\epsilon)q_g q_l k^2$ is the direct Coulomb interaction between gA monomer (charge $q_g$) in a channel and the nearest-neighbor lipid. $k=2\pi/r_{LL}$, $r_{LL}$ is the average lipid-lipid distance [62] which has been assumed to also be the distance between the channel’s longitudinal edge and the nearest lipid head group and has been considered here (for simplicity) to correspond to a certain lipid type only. In reality this may also change due to many parameters e.g., variations in the membrane’s electrical conditions, the presence of hydrocarbons within, etc., $n$ is lipid density $\sim 1/60 \text{ Å}^2$. Obviously, $k_BT=1.38 \times 10^{-23}$ Joule/K (300 K). Here, $\epsilon_0$ is the dielectric constant in vacuum and $\epsilon_r$ (~2) is the relative dielectric constant inside the membrane [63].

The strong binding energy between two monomers with identical charge profiles in a gA channel inside membranes ($U_{g,g}$) is due to the Lennard-Jones and Coulomb potentials which is supported by earlier work on the derivation of an attractive interaction potential between charges of the same type (contrary to the generally expected repulsive interaction) in solution [64] and due to arguments presented in ref. [38]. A change of gA channel stability is mainly due to the change of gA channel bilayer coupling energy ($U_{g,\text{bilayer}}$) even though the total binding energy is given by $U(r)=U_{g,g} + U_{g,\text{bilayer}}$. Here, $U_{g,\text{bilayer}}$ is a $1^\text{st}$, $2^\text{nd}$, etc. order term in the expansion of $V_{sc}(r)$ for the hydrophobic mismatch to be filled by single, double etc. lipids representing $1^\text{st}$, $2^\text{nd}$, etc. order screening, respectively. $\Delta G_{\text{prot}}^0$ and $\Delta G_{\text{def}}^0$ are proportional to $U_{g,g}$ and $U_{g,\text{bilayer}}$, respectively.
and gA monomers although at $d_0=0$ ($\Delta G_{\text{prot}}^{0}$) any fluctuation in $\Delta G_{\text{prot}}^{0}$ may appear as a channel function regulator, too. A complicated computational program using Mathematica 7 has revealed that $\Delta G_{\text{def}}^{0} = \text{Exp} \{d_0-l\}$ which suggests that $F_{\text{def}} = \text{Exp} \{d_0-l\}$, different from that found in the elastic bilayer consideration that is $F_{\text{def}} = \text{Exp} \{d_0-l\}$ (mentioned earlier). The gA channel lifetime $\text{Exp} \{-\Delta G_{\text{def}}^{0}/k_BT\}$ is a function of the gA channel lifetime $\text{Exp} \{-\Delta G_{\text{def}}^{0}/k_BT\}$ and the gA channel lifetime $\text{Exp} \{-\Delta G_{\text{def}}^{0}/k_BT\}$ (for details see ref. [30]), where $\lambda$ is the distance two gA monomers move apart to reach the dimer/monomer transition state [64]. Slight differences in the bilayer thickness gA channel length mismatch dependence of the theoretical trend of gA channel lifetime appear to depend on whether we use the expression for $F_{\text{def}}$ from the screened Coulomb model ($-\exp(d_0-l)$) or the elastic bilayer model ($-(d_0-l)$) in the case when $c_0$ is assumed to be unchanged. We have clearly addressed that screened Coulomb interaction model is certainly needed over elastic bilayer model to add the much needed anharmonic terms into the bilayer deformation energy [31]. The interaction energy originating from the localized charge properties of the lipids and peptides on the channels mainly contribute to the cause of bilayer channel coupling and the bilayer elastic property originated harmonic energetic term helps the bilayer-channel coupling to be stabilized. Screened Coulomb interaction model serves both of the purposes. The consideration of the bilayer channel harmonic coupling due to the bilayer elasticity can somehow represent the main bilayer channel coupling energetic rather serves some secondary support. This has been wrongly interpreted by many groups during almost three decades doing research in membrane protein functions.

The screened Coulomb interaction model also explains the lipid curvature and charge effects on the regulation of membrane protein functions. Negative $c_0$ is found from this model to linearly destabilize gA channels, which agrees with experimental observations [31, 38]. The model also shows that $\Delta G_{\text{def}}^{0} \approx \frac{9}{qL} \pi \rho L$, $s=1, 2, \text{etc.}$ for $1^s$, $2^s$, etc. order screening, respectively, suggesting that channel formation is harder in bilayers containing charged lipids.

Besides gA channel the screened Coulomb interaction model also explains all general aspects of membrane regulation of Alm channel functions. Interested readers should find this analogy from refs. [31, 38]. We are now checking the applicability of the model towards explaining other membrane disrupting events like toroidal pores, defects, etc. and the analysis hints to be positive. Our ongoing molecular dynamics simulations suggest that there exist strong distance dependent electrostatic and van der Waal’s interactions ($-\kcal$/mole) between peptides (gA, Alm) or chemotherapy drugs (TCC, TXL) and membrane constructing phospholipids (manuscript in preparation by Ashrafuzzaman, Tseng and Tuszynski). These simulation results also validate our discovery that there exists strong electrical channel energetic coupling arising apparently from the effective localized charge profiles (equation (3)) of both lipids and channel forming peptides or biomolecules [31]. Other platforms will be used to present those groundbreaking discoveries.

4. Discussion

Membrane’s transport properties have been found to depend on physical structure and charge profiles of membranes, integral MPs, AMPs etc. and other membrane stabilizing constituents like hydrocarbons, membrane’s dielectric environment, etc. We have provided analysis of the theoretical energetic bilayer channel coupling as well as experimental results on electrical recordings demonstrating the membrane permeabilization due to the effects of three AMPs representing different structural classes and two chemotherapy drug molecules. These findings offer new insights into the function of MPs and membrane’s electrical properties and other relevant properties. We have found at least two qualitative processes namely ion channels with different structures and defects by which lipid bilayer membrane’s insulating properties get compromised. This may lead to a new understanding how drugs that target intra-cellular structures may also change the membrane properties. The energetic bilayer-channel coupling has been described using the screened Coulomb interaction model. This generalized theoretical model covers the consideration of both of the mechanical and electrical interaction energies between MPs and the lipid bilayers. We consider that the screened Coulomb interaction plays the most important role in the channel bilayer interactions and the bilayer’s inherent elastic properties help the membrane to deform near the channel interface to create physical channel bilayer binding. The interaction energies due to the localized charge profiles of the lipids and the integral peptides serve as the cause behind the membrane deformation near channels. Our discovery of screened Coulomb interaction model perhaps allows us to state that the understanding of MP/AMP functions and related drug design targeting at those MPs/AMPs inside cell membranes requires serious or primary consideration of the localized charge profiles of both MPs/AMPs and lipids. This review has allowed an explanation of the problem of the bilayer channel mismatch regulation of channel functions. We have also described newly reported effects of chemotherapy drug molecules on the membrane structure involving the formation of toroidal-type ion pores. A new type of membrane permeabilizing transient event ‘defect’ has also been briefly addressed. We hope that a deeper understanding of the function of MPs and change of membrane’s transport profiles due to the action of AMPs and chemotherapy drug molecules will help develop novel drugs for various health related issues involving membrane properties. These may range from bacterial infections to abnormal protein structures in various cancer cells. This mini review will provide enormous amount of focused information which will help develop scientific and technological explorations.
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