Chemotherapy Drugs Thiocolchicoside and Taxol Permeabilize Lipid Bilayer Membranes by Forming Ion Pores

Md. Ashrafuzzaman1, M Duszyk2, J A Tuszynski1,3
Department of Oncology1, Cross Cancer Institute, University of Alberta, Edmonton, Canada
Department of Physiology2, University of Alberta, Edmonton, Alberta, Canada
Department of Physics3, University of Alberta, Edmonton, Alberta, Canada

E-mail: ashrafuz@ualberta.ca

Abstract. We report ion channel formation by chemotherapy drugs: thiocolchicoside (TCC) and taxol (TXL) which primarily target tubulin but not only. For example, TCC has been shown to interact with GABA_A, nuclear envelope and strychnine-sensitive glycine receptors. TXL interferes with the normal breakdown of microtubules inducing mitotic block and apoptosis. It also interacts with mitochondria and found significant chemotherapeutic applications for breast, ovarian and lung cancer. In order to better understand the mechanisms of TCC and TXL actions, we examined their effects on phospholipid bilayer membranes. Our electrophysiological recordings across membranes constructed in NaCl aqueous phases consisting of TCC or TXL under the influence of an applied transmembrane potential (V) indicate that both molecules induce stable ion flowing pores/channels in membranes. Their discrete current versus time plots exhibit triangular shapes which is consistent with a spontaneous time-dependent change of the pore conductance in contrast to rectangular conductance events usually induced by ion channels. These events exhibit conductance (~0.01-0.1 pA/mV) and lifetimes (~5-30 ms) within the ranges observed in e.g., gramicidin A and alamethicin channels. The channel formation probability increases linearly with TCC/TXL concentration and V and is not affected by pH (5.7 - 8.4). A theoretical explanation on the causes of chemotherapy drug induced ion pore formation and the pore stability has also been found using our recently discovered binding energy between lipid bilayer and the bilayer embedded ion channels using gramicidin A channels as tools. This picture of energetics suggests that as the channel forming agents approach to the lipids on bilayer the localized charge properties in the constituents of both channel forming agents (e.g., chemotherapy drugs in this study) and the lipids determine the electrostatic drug-lipid coupling energy through screened Coulomb interactions between the drug molecules and lipids. The strength of this electrostatic energetic coupling determines the stability of the drug-induced ion pores. These findings may elucidate cytotoxic effects of chemotherapy drugs and aid in the development of novel drugs for a broad spectrum of cancers and other diseases.

Introduction
Colchicine has a long history of use in immune-system diseases (1, 2). In 2009, colchicine won FDA approval as a stand-alone drug for acute gout and familial Mediterranean fever. It inhibits leukocyte-
endothelial cell adhesion (3) and T-cells activation (4) by binding to tubulin dimers, which prevents their polymerization into microtubules (MTs) (5). Due to increased rate of mitosis cancer cells are more vulnerable to colchicine poisoning than normal cells. The potential usefulness of colchicine in cancer chemotherapy is limited by its toxicity against normal cells. Colchicine shifts the dynamic equilibrium in MT’s towards disassembly by sequestering most of tubulin (6) and inducing slow disassembly of MT’s (7). This may account for the gradual change of the membrane action potential or threshold (increase) and the resting potential which, changed at modest concentrations of colchicine by only 2.5 mM, at higher concentrations (10 mM) was reduced by up to ~ 5 mV (6). Colchicine is also found to bind in the nuclear periphery which effects a constriction of the pore-complexes with concomitant inhibition of RNA egress and disordering of the nuclear membrane phospholipid bilayers (8). Since its FDA approval in 1992 taxol has been used for ovarian, lung, breast cancer, etc. (9) due to its stabilization of MTs (10) which makes it suitable for use in combination therapy (11). However, taxol’s poor solubility is a serious problem that requires conjugation with cremophor or albumin. A taxol-phospholipid liposome construct increases taxol’s antitumor efficacy by delaying tumor progression compared to the effectiveness of free taxol administered in cremophor (12). Taxol is also found to inhibit endosomal-lysosomal membrane trafficking by favorably inducing small dense vesicles over larger ones (13). This suggests that taxol affects the structural integrity of membranes by perhaps changing the lipid curvature profiles. Taxol’s effects on liposomes were elucidated using fluorescence, circular dichroism, differential scanning calorimetry, fluorescence polarization and X-ray diffraction (14) showing that taxol incorporated into liposomes penetrates into the acyl chain domain which alters physical properties e.g., the range of the phospholipid phase transition, lipid order parameter, fluidity etc. of both artificial and biological membranes. Both MT-stabilizing taxol and MT-disrupting colchicine were found not to affect the human platelet membrane lipid fluidity (15). The evidence of strong interactions of colchicine with lipids (16) also suggests that colchicine is a poorer candidate as an anti-cancer drug by being weakly accessible to tubulin. Although the anti-cancer efficacy of colchicine and taxol has been found to be affected by lipids, there is still a substantial lack of information about the effects of these molecules on cell membranes, especially in regard to the membranes transport properties which depend on physical, geometrical and structural aspects of membranes. Studying these effects will shed important light on off-target interactions of these chemotherapeutic agents and hence may lead to an improved drug design in the future.

MT’s are hollow cylinders constructed from a heterodimer of two 55 kDa proteins known as α and β tubulin. The tubulin dimer locks a single molecule of GTP within the non-exchangeable nucleotide binding site of α tubulin (17) and an additional molecule of GTP binds the β-subunit but it can hydrolyze into GDP following polymerization (Figure 1) providing the conformational flexibility required during the polymerization-depolymerization cycle (18). MTs observed in vitro are composed of 11 to 18 individual protofilaments, whereas in vivo they are made up of only 13 protofilaments (19). MTs are involved in numerous critical, cellular processes including mitosis, cell motility, maintenance of cellular morphology, and the activity of cell surface receptors. (20, 21). MTs exhibit a process of constant polymerization and depolymerization called dynamic instability, allowing them to efficiently search for attachment sites (e.g. to the kinetochore of a chromosome (22)). Paclitaxel and its analog docetaxel, some of the most successful cancer chemo-therapy agents, bind to a luminal location of an MT and stabilize it (23-25). Hydrogen/deuterium exchange coupled with mass liquid chromatography demonstrated that increased rigidity in paclitaxel stabilized MTs was distinct from stabilization as a result of GTP-induced polymerization (26). A different drug site within β-tubulin was shown to bind colchicine, a water-soluble alkaloid (27) that binds only to free tubulin and not to MTs. Colchicine has extremely strong anti-mitotic activity but its use as a cancer treatment is limited since it binds tubulinindiscriminately.
Fig. 1: Paclitaxel, colchicine and Vinca binding sites on α/β tubulin where drug molecules are in green. From bottom to top, colchicine, paclitaxel and vinblastine from PDB files: 1SA0, 1JFF and 1Z2B have been superimposed and fit back onto the 1SA0 structure to obtain the relative positioning of each drug within the β tubulin monomer (cyan) and two α tubulin monomers (yellow) at the top and bottom of the frame. The GTP at the non-exchangeable and GDP at the exchangeable site are colored purple.

Although many studies investigated different aspects of the effects of the chemotherapy drugs in the inner cellular level, none addresses their function in the cellular wall. All the drug effects on the MT assembly depend highly on the fractional cellular intake of the drugs. The intermediate membrane wall and hydrophilic/hydrophobic boundaries in the drug pathways undoubtedly influence the ultimate effectiveness and cytotoxic effects of the anticancer drugs. Effects of the drugs on the membrane’s transport properties and the interaction between drug molecules and the membranes constituents are important but remain largely unexplored. The relatively small size of the drug molecules primarily suggests that their mechanisms are distinct from antimicrobial mechanism(s) of membrane permeabilization, e.g. formation of well structured long-lived protein-lined (28-30) ion channels. Otherwise, their amphiphile type effects would modulate the bilayer physical properties like e.g., triton X-100 and capsaicin effects (31) or transient defects disordering in the lipid layers as observed for the antimicrobial peptide gramicidin S (GS) (32). The goal here was to evaluate the two drug molecules thiocolchicoside (TCC) and taxol (TXL) (Fig. 2) for their effects on lipid model membranes using electrophysiology. Our results suggest that these drug molecules do not only permeabilize lipid bilayer membranes but also create certain structures through reorganization between lipid molecules and themselves. We provide evidence for the formation of stable ion pores/channels inside lipid model membranes involving these molecules whose characteristics are unconventional. That is, the discrete conductance events do not exhibit rectangular shapes in their current versus time plots (as seen in most antimicrobial peptides/membrane proteins-induced channels) but instead exhibit novel triangular
shape effects. We therefore propose that these chemotherapy drug-induced pores/channels create toroidal-type channel structures (33-37).

\[ \text{Fig. 2: Structure of TCC and TXL (Taxol-A).} \]

**Materials**

Phosphoethanolamine (POPE), phosphatydyleserine (POPS) and phosphatidylcholine (POPC) were from Avanti Polar Lipids (Alabaster, AL, USA), were used without further purification. \( n \)-Decane was 99.9\% pure from ChemSampCo (Trenton, NJ, USA). TCC was from ChemRoutes (Edmonton, Canada) and TXL was from Vector Lab (Burlington, Canada). Dimethylsulfoxide (DMSO) was from Burdick and Jackson (Muskegon, MI).

**Methods**

Planar lipid bilayers were formed by applying the lipid cocktail of POPE: POPS: POPC (5:3:2, v/v/v)/\( n \)-decane. The painting method was used to form membranes over a 150 \( \mu \)m septum of a bilayer cuvette. The working volume of bilayer cuvettes was 1 mL in both the \( cis \) (recording electrode) and \( trans \) (reference electrode) chambers. TCC and TXL stock solutions were prepared in DMSO (4 mg/mL), and were then further diluted (1 mg/mL) in standard buffer (0.5 M NaCl, 10 mM HEPES, pH 7.4) to be used in doing experiments. For pH experiments, the pH buffer was changed from HEPES to 2-(N-morpholino) ethanesulfonic acid (MES) for solutions at pH 5.7. TAPS (2-hydroxy-1,1-bis(hydroxymethyl)ethylamino-1-1 propanesulfonic acid)) was used to make the buffer (NaCl solution) at pH 8.4. After formation of the bilayer, we waited for about 1 hr and then tested the stability of the bilayer by applying transmembrane potential \( V=400 \) mV across it using both polarities. TCC or TXL stock solution was added to the \( cis \) chamber with continuous stirring for 10 min. The observed ion channel activity at reasonable \( V \) was recorded as traces.
Fig. 3: TCC and TXL (both at 90 μM) permeabilize lipid bilayer membranes by inducing nonzero current events. Here, pH=5.7, V=100 mV. Both traces were filtered at 20kHz but the lower one shows higher noise due to its presentation (current axis) at amplified scale. We also observed conductance events (data not shown) at a much lower range and for more biologically relevant (to cancer therapy) concentrations e.g. at drug concentration c_D=10 μM for both TCC and TXL but at V~200 mV or higher.

Results

Fig. 3 shows considerable conductance events observed when a V is applied across a lipid bilayer membrane doped with TCC or TXL. A lipid membrane itself is generally nonconducting to ions but in the presence of TCC or TXL different current levels were seen. The long-time records indicate that time independent/random appearance of current events was induced by both compounds. The stability of different current levels varied significantly. The point count plot shows that the conductance events do not correspond to discrete current level(s) with any discrete values of their conductance. The current levels appeared with all possible conductance which is similar to the GS induced conductance events (32). Although no major qualitative difference between TCC and TXL-induced membrane permeabilization was observed, TXL-induced current levels cover relatively lower conductance range than those for TCC. Fig.4 shows the change of activity (A) with the change of V or TCC/TXL concentration (c_D) in the aqueous phase bathing the membranes. A linear progression relation between A and V or c_D was seen before A approached 1.0 at very high V and c_D. Although at very high V and c_D the membrane seems to show spontaneous nonzero resistance against ion flow, there are no standard lower or upper cut off values of both V and c_D to initiate or saturate respectively the appearance of conductance events. The lower/upper cut off values for V can be increased/decreased by decreasing/increasing the value of c_D and vice versa.
**Fig. 4:** TCC or TXL-induced channel activity $A$ increases proportionally with both the $V$ and drug concentration $c_D$. Here Activity ($A=\sum A_i/(\sum A_i + A_{nc})$) is defined as the ratio of the total point count in all conducting phases relative to the sum of the total point counts of both conducting phases and the nonconducting phase. $A_i$ and $A_{nc}$ are total point counts in the conducting phase $i$ and the nonconducting phase. pH 5.7.

In Fig. 5 short-time records of drug-induced current events show the appearance of independent conductance events. Any current event can be due to independent conductance event or due to a combination thereof. Current event therefore does not represent any controlled or reproducible parameter to address the nature of the induced conducting pore across membranes. To understand it we need to find independent conductance events, few of which are shown in Fig. 5. Note that the independent conductance events appear with triangular shapes meaning conductance of any single event is not constant but it increases/decreases spontaneously over the time interval comparable to the ‘lifetime’ of any specific conductance event. To the best of our knowledge, the appearance of triangular conductance events is unique. The heights of the peak points of different conductance events are also different. We observed random spontaneous transitions between different current levels within a discrete conductance event during its lifetime. The discrete conductance events detected here were found to be roughly characterized by conductance ~0.01-0.1 pA/mV and lifetimes ~5-30 ms. For comparison, we have also shown the nature of conductance events observed in two other well studied channels namely gramicidin A (gA) and alamethicin (Alm). Like conductance events in channels
formed by integral membrane proteins or antimicrobial peptides both gA and Alm, conductance events appear with rectangular shapes meaning transition between discrete current levels is transient or extremely abrupt. In gA channels only a back-and-forth transition between two current levels due to gA dimer/monomer states while in Alm channels many transitions between different conductance states consisting of varied numbers of monomers participating in the formation of channels are found. Importantly in both of these structurally different channels the transition between different current levels corresponding to discrete states is totally transient. The spontaneous transition between different random current levels in TCC/TXL-induced conductance events is therefore an important novel finding.

Fig. 5: The upper panel shows triangular-shape conductance events induced by 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 (32). 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.

**Fig. 6**: A, gA channels deform lipid bilayer’s resting thickness at the cost of a bilayer deformation energy originating from electrostatic interaction between lipids and gA monomers to compensate the hydrophobic mismatch (\(d_0-l\)) between bilayer thickness (\(d_0\)) and gA channel length (\(l\)). With channel formation bilayer conducts a current pulse with an average pulse width (channel lifetime \(\tau \sim \text{ms}\)) and height (channel conductance \(\sim \text{pA}\)) depending on gA monomers forming channels and lipids forming bilayers. Two types of monomers have schematically structured two different channels here. B, In Alm channels cylindrical rods represent monomers on a ‘barrel stave’ pore. Transition between
different Alm conductance pores by addition/release of monomer(s) from/to the surrounding vicinity is shown in lower panel. C, Chemotherapy drugs TCC and TXL form toroidal-type ion channels which raises the possibility of spontaneous change of the pore radius due to the complex interaction and/or hydrophobic coupling between the lipids and the pore inducing drug molecules (TCC, TXL) as observed experimentally by us.

Discussion and conclusions

Two chemotherapy drugs (TCC and TXL) were investigated for their effects on membrane’s electrical conductance due to the formation of special structures inside or across membranes. Erdal et al. has recently reported that a large number of potential anticancer drugs induce p53 protein-independent apoptosis and that lysosomal membrane permeabilization is a mediator of many such responses (38). Our discovery of both membrane permeabilization and the formation of ion pores/channels due to TCC and TXL sheds new light on the cytotoxic effects and other interactions exhibited by these chemotherapy drugs. Stimulation of conductance events due to chemotherapy drugs may also lead to the discovery of a novel type of ion channel formed by compounds whose primary mode of action involves cytoplasmic proteins. All lipid membrane permeabilizing membrane proteins or antimicrobial peptides have been found to form ion channels in which transitions between different current levels happen instantaneously. Only the drugs TCC and TXL induced single conductance events with a current versus time plot exhibits triangular shapes. This indicates that the induced pore radius spontaneously changes back-and-forth between smaller and bigger pores with all possible cross sectional areas in between. The spontaneous change of the TCC/TXL pore conductance immediately rules out the possibility of channel formation which might look like linear gA type, barrel stave Alm type, etc. channels. Although the point count plots of the long time current traces (Fig.3) resemble those observed for GS which induce defects inside membranes (32), a fine tuning of the single conductance events (Fig.5) suggests that the events are long-lived, not transient like those observed for GS (32). No such back-and-forth spontaneous transitions between different non-zero current levels as observed in a chemotherapy drug-induced discrete conductance event (Fig.5) are found in GS-induced defects which shows instantaneous/transient conductance events between zero conducting states only. TCC/TXL events appear with somewhat similar characteristics as Alm conductance events with back-and-forth transitions, though instantaneous, between different non-zero current levels (Fig.5). Our results, however, can neither rule out nor support the possibility of a mechanism which is somewhat similar to the so-called in-plane diffusion model of Bechinger (39, 40). Here, the bilayer disrupting molecules have been found inserted into the phospholipid bilayer disordering the hydrocarbon chains of the adjacent phospholipid molecules, locally thinning the bilayer, leading to local disturbances in bilayer packing and an eventual formation of toroidal type pores (Fig.6). TCC/TXL induced conductance events do not appear to be random defects (e.g., like GS-induced defects) or like other protein lined pores/channels (e.g. gA induced linear β-helix, Alm induced barrel-stave pore etc.), but rather more like events which correspond to a special structure induced inside membranes by forming a TCC/TXL and lipid molecule complex. Here we assume that lipids are forced to line across pore openings (Fig.6) and TCC/TXL molecules sit behind the head group region near the hydrocarbon chains of the lipids (14). Moderate dependence of channel forming activity on cD and V (linear) (which is much lower than the 2.6 power dependence on the identical parameters in the cases of protein-lined channels (41)) also provides support for the TCC/TXL channel (Fig. 6). This allows the membrane to be doped with higher amounts of the channel forming molecules at a moderate energy cost as the molecules just penetrate into the lipid headgroup-hydrocarbon chain interface. Only this type of broken membrane structure can ensure the spontaneous change of the pore’s cross sectional area which leads to the formation of triangular conductance events with spontaneous change of pore conductance. In the case of protein-lined channels the membrane’s resting thickness never vanishes, rather shows some kind of bilayer deformation from its resting thickness as was predicted in another
mechanosensitive channels study (42). The chemotherapy drug induced channel formation effects were also investigated changing the pH of the aqueous phase and unlike other protein-lined channels e.g. gA channels (43) and Alm channels, which report substantial phenomenological effect of pH changes, we observe almost no considerable effect of pH (Fig. 7). This suggests two main conclusions: (i) formation mechanism of chemotherapy drug induced channels is different from other conventional protein-lined channels and (ii) the investigated chemotherapy drug molecules appear to be equally effective in both normal cellular membranes and cancer cell membranes with different pH environments (44). Both of these points together make proper sense considering that the chemotherapy molecules are, on a broader scale, charge neutral while other channel forming peptides are usually charge bearing with specific (helical, beta sheet etc.) structures.

Fig. 7: TCC pore/channel activity A is independent of pH of the membrane bathing aqueous phase. 

$c_d=50 \mu g/mL$, $V=100 \text{ mV}$.

Energetics behind the creation of chemotherapy drug induced membrane transport events ion pores

A theoretical explanation on the causes of chemotherapy drug induced ion pore formation and the pore stability can be found by analyzing the recently discovered general pictures of energetics behind the functions of protein-lined ion channels gA and Alm (45). gA and Alm are the two most studied ion channels which form β-helical (46-48) and barrel-stave (28, 29) pores, respectively, across lipid bilayer membranes (see Figure 6). 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 (45).

In bilayer-spanning channel formation the association of two trans-bilayer gA monomers is governed by the dimerization coefficient: $K_D=[D]/[M_g]^2 = k_1/k_{-1} = \exp\{-\Delta G^0_{prot} + \Delta G^0_{def})/k_B T\}$, where $[M_g]$ and $[D]$ are monomer and dimer concentrations; and $k_1$ and $k_{-1}$ are rate constants (43). Here, $k_B$ and $T$ are the Boltzmann constant and absolute temperature, respectively. Since the bilayer deformation energy $\Delta G^0_{def}$ is sensitive to the hydrophobic mismatch $(d_0-l)$ between bilayer thickness $(d_0)$ and gA channel length $(l)$, the bilayer responds to its deformation by imposing a restoring/channel-dissociation force $F_{dis}$ on the edges of a channel. Increasing $F_{dis}$ is reflected in a decreasing $\tau$ and channels become molecular force transducers (43). 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 \( d_0-l \). Potential-of-mean-force calculations (51) suggest that trans-membrane protein interactions are regulated by a hydrophobic mismatch equivalent to \( d_0-l \). 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 \( d_0-l \) (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 \( d_0-l \) and intrinsic curvature \( c_0 \) (\( \Delta G_{\text{def}}^0 = H_B(d_0-l)^2 + H_X(d_0-l)_c + H_c c_0 \)), consequently, \( F_{\text{dis}} \) is found to be linearly dependent on \( d_0-l \) and \( c_0 \) [43, 60]:

\[
F_{\text{dis}} = -\left( -K \partial / \partial \left( (d_0-l) \Delta G_{\text{def}}^0 \right) \right) = 2H_B \cdot (d_0-l) + H_X \cdot c_0,
\]

where \( H_B, H_X \) and \( H_c \) are phenomenological elastic constants. Using specific ‘elastic parameters’ in a fluid-like membrane is a good first-order approximation that works well within the limitations of a linear theory. However, in order to extend the applicability of the theory to a nonlinear regime, we propose to use the screened Coulomb interaction approximation (45). I shall briefly describe this published screened Coulomb interaction model which perfectly describes the general bilayer regulation of membrane protein functions. This publication (45) 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 (45), considering \( l < d_0 \), 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 participating in this chain that their interactions exist in an identical dielectric environment. The general form of the screened Coulomb interaction is

\[
V_\text{sc}(\vec{r}) = \int d^3 k \text{Exp}(i \vec{k} \cdot \vec{r}) V_\text{sc}(\vec{k})
\]

whose Fourier transform is (61)

\[
V_\text{sc}(\vec{k}) = \frac{V(\vec{k})}{1 + 2\pi k_\parallel T n}
\]

where \( V(\vec{k}) = (1/\epsilon_0 \epsilon_r) q_c q_d i / k^2 \) is the direct Coulomb interaction between gA monomer (charge \( q_c \)) in a channel and the nearest-neighbor lipid. \( k = 2\pi / r_{12} \), \( r_{12} \) 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 ~1/60 Å\(^2\). Obviously, \( k_\parallel T \approx 1.38 \times 10^{-23} \text{ Joule}/K \left( 300 \text{ K} \right) \). Here, \( \epsilon_0 \) is the dielectric constant in vacuum and \( \epsilon_r \approx -2 \) 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 \( U_{gA} \) is due to the Lennard-Jones and Coulomb potentials which is supported by earlier work on the derivation of an effective 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. (45). A change of gA channel stability is mainly due to the change of gA channel bilayer coupling energy ($U_{g,bilayer}$) even though the total binding energy is given by $U(r) = U_{g,bilayer} + U_{g,bilayer}$. Here, $U_{g,bilayer}$ is a $1^{st}$, $2^{nd}$, etc. order term in the expansion of $V_{sc}(r)$ for the hydrophobic mismatch to be filled by single, double etc. lipids representing $1^{st}$, $2^{nd}$, etc. order screening, respectively. $\Delta G_{prot}^0$ and $\Delta G_{def}^0$ are proportional to $U_{g,bilayer}$ and $U_{g,bilayer}$, respectively. $F_{dis}$ therefore originates from mechano-electrical properties of membrane and gA monomers although at $d_0$$l$ ($\Delta G_{def}^0$$0$) any fluctuation in $\Delta G_{prot}^0$ may appear as a channel function regulator, too. A complicated computational program using Mathematica 7 has revealed that $\Delta G_{def}^0$~$\exp\{d_0$$l$\} which suggests that $F_{dis}$$\approx\exp\{d_0$$l$\}, different from that found in the elastic bilayer consideration that is $F_{dis}$$d_0$$l$ (mentioned earlier). The gA channel lifetime $\tau$$\approx\exp\{\Delta G_{def}^0/k_BT\}$$\approx\exp\{-\Delta G_{def}^0/k_BT\} \ (for$ details see ref. (43)), where $\lambda$ is the distance two gA monomers move apart to reach the dimer/monomer transition state (64). Slight differences in the bilayer thickness gA channel length mismatch dependence of the theoretical trend of gA channel lifetime appear to depend on whether we use the expression for $F_{dis}$ from the screened Coulomb model (almost $d_0$$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 (45). 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 nohow represent the main bilayer channel coupling energetics 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 (45). The model also shows that $\Delta G_{def}^0$$\approx (q_1/q_g)^s$, $s$=1, 2, etc. for $1^{st}$, $2^{nd}$, 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. (45).

The above explanations generally suggest that the energetic coupling between channel forming agents and the lipids on the bilayer due to primarily their localized electrical properties regulate the stability and other functions of ion channels/pores. The localized charges emerge perhaps due to the mutual polarizing effects of the localized charges in the atomic level when both lipids and pore forming agents approach to each other even though sometimes the overall net charges of both may be zero. Like some of the zwitterionic lipids chemotherapy drugs despite their having net zero charges therefore are likely to show localized charges as they approach to each other to form ion pore (Figure 6C). In this situation any empirical calculation of the force field finds partial charges on each atom in the drugs (65-68). Each drug then falls under the influence of screened Coulomb interactions with the lipid head group chain. The sum of all these screened interactions between all drugs and all lipids in the drug-bilayer interaction region make the effective binding energy which should follow the general picture of energetics as applied to both gA and Alm channels. 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 bilayer
channel electrostatic energetic coupling arising apparently from the effective localized charge profiles (equation (1)) of both lipids and channel forming peptides (45) or biomolecules like chemotherapy drugs. Other platforms will be used to present those groundbreaking discoveries.

In conclusion, chemotherapy drugs TCC and TXL both permeabilize lipid bilayer membranes by forming ion channels with a new type of conductance events with spontaneously changing channel conductance. This study sheds new light on the molecular mechanism of chemotherapy drugs both across the cell’s membrane and in the cell’s cytoplasm. This understanding may help find novel drugs to treat cancer and other diseases occurring both in the inner cellular and membrane regions.

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