[Review] The Studies of Lipid Phase Polymorphism in Model Membranes

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Funding: This research was funded by the CKWA start-up grant to the STEM Research Center.

Potential competing interests: No potential competing interests to declare.

Abstract

Polymorphic transitions in biological membranes are drawing more and more attention from the researchers working in the field of structure and function relationships in membranes of cells and subcellular organelles. In this review, we critically review the latest concepts on the effects of dynamic structure and polymorphic transitions in lipid phase on tentative physiological activities of biological membranes that may lead to rejuvenation of mitochondrial membranes. The role of non-bilayer lipid structures triggered by action of cationic membrane active peptides isolated from snake venoms and other sources in translocation of cationic proteins across membranes is also critically reviewed.

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Keywords: non-bilayer lipid structures, cationic membrane active proteins, polymorphic lipid transitions, model membranes.

Introduction

Polymorphic transitions of lipids in biological membrane such as transitions from the lamellar lipid phase to the non-bilayer lipid phase has been in a center of attention of membranologies since over seventy years. The exact functional role (s) of non-bilayer lipid structures is (are) not well understood, however it is now well documented that such processes as intermembrane exchange of lipids, membrane fusion and fission are mediated by non-bilayer lipid phase transitions. In this review we give a brief overview of research studies which led to discovery of non-bilayer lipid structures in model lipid and biological membranes and chronological development of various concepts that link non-bilayer structures to various activities of biological membranes and related physiological reactions. We also list cationic synthetically made proteins

and peptides and those from natural sources which induce formation of non-bilayer lipid phases which at different circumstances may lead to rejuvenation of cells or to apoptosis. We believe that this review would be of interest of experts working in pharmaceutical, biomedical, biotechnology and bioengineering areas of research.

Discovery of non-bilayer lipid phase

Formation of the body-centered cubic phase and hexagonal phase is triggered by divalent cations in hydrated soaps with carbonyl groups in a soap's polar head which was determined by using the small-angle X-ray diffraction by V. Luzzati's team in late 1960s [1][2]. Those were probably the first reports on the non-bilayer structures formed by lipid-like organic compounds. By the early 1970s it has been shown that hydrated lipids form not only a lamellar phase but also a variety of non-bilayer phases [3][1][4][5]. Formation of non-bilayer phases has been explained by the conical shape of non-bilayer lipids [3][5]. It was determined by ^{31}P-NMR that cardiolipin (CL) and phosphatidylethanolamine (PE), non-bilayer lipids in IMM, formed the H_{II} phase when hydrated in presence of divalent cations[5][6][7]. Hydrated monogalactosyldiacylglycerol (MGDG), a non-bilayer lipid of TM, also adopted the H_{II} phase as determined by X-ray diffraction [8]. By the end of previous century there was an abundance of data obtained showing that non-bilayer lipid phases can be easily generated in model membranes made of lipids isolated from TM [9]. Freeze-fraction electron microscopy data have revealed that keeping lipids isolated from TM with co-solutes of sugars or betaine [10] or treating TM lipids with a non-cryogenic low temperature [11] triggers the formation of an H_{II} phase. Coexistence of both bilayer and hexagonal phases was revealed by molecular dynamics simulation of TM lipids [12].

In late 1970s and early 1980s ^{31}P-NMR spectroscopy has been widely used in studies of polymorphic behavior of lipids isolated from mitochondrial membranes. Dispersion of PC isolated from rat liver mitochondrial membrane retained lamellar phase [13], but dispersion of PE isolated from the same membranes undergone lamellar-to-H_{II} transition in the 10-37 °C temperature range [13]. A dispersion of the total lipid mixtures from the rat liver mitochondrial membrane revealed coexistence of bilayer and non-bilayer lipid phases [13]. Model PC membranes enriched with CL or PE in solution with divalent cations contained hexagonal H_{II} phase coexisting with a lamellar phase[6][7]. By the late 1980s the existence of non-bilayer lipid phase has been widely documented but the perception that non-bilayer lipids, which are probably present in all biological membranes, are generally arranged in a lamellar phase in lipid membranes persisted until the beginning of 21st century [14].

Cationic proteins trigger formation of non-bilayer lipid structures

From early 1980s the research groups in biophysical membranology have begun employing a range of powerful physical methods to elucidate whether the membrane-active proteins from natural sources can induce polymorphic transitions in model membranes [15][16][17][18]. Several cationic proteins were found to trigger the bilayer to non-bilayer transitions in PC membranes enriched with CL [15][18]. One of them is cytochrome *c* (Cyt *c*), a small 12 kD water soluble peripheral protein of IMM that is involved in the respiratory chain as an electron carrier [18]. Cationic Cyt *c* is rich in lysine residues, and it

specifically binds to CL to induce non-bilayer lipid structures [15][19] and trigger pore formation [20] in CL-containing model membranes. It has been recently discovered that in non-polar environment Cyt *c* forms nanospheres with CL which possess lipoperoxidase activity [21]. Cyt *c* peroxidase is activated by reactive oxygen species [22]. In the oxidized form Cyt *c* can readily trigger apoptosome assembly [23]. It is possible that the bilayer to non-bilayer transition triggered by the Cyt*c* binding to CL, which in turn facilitates the peroxidase activity, plays a crucial role in initiating the intrinsic apoptosis pathway [24]. Mitochondrial creatine kinase is another potential cationic protein which may trigger non-bilayer phase formation in CL containing membrane. It has been shown that C-terminal lysine residues of creatine kinase drive its binding to membrane phospholipids [25]. Separate studies have shown formation of the creatine kinase induced CL clusters [26] and segregation of CL in phospholipid monolayers[27] which allow one to suggest that creatine kinase may trigger formation of non-bilayer phase in CL segregated areas of membrane. The very intriguing Szeto–Schiller tetrapeptides have been shown to rejuvenate mitochondrial functions and promote tissue regeneration at aging [28]. These tetrapeptides are made of basic lysine and hydrophobic amino acid residues and they specifically target CL in mitochondria and model membranes, and they are being studied as potential therapeutic agents to treat mitochondrial disfunction and diseases associated with aging [28]. It makes sense to investigate if the Szeto–Schiller tetrapeptides can induce formation of non-bilayer lipid phase in IMM as controlled lipid polymorphism in IMM could be an important step in the process underlying rejuvenation of aging mitochondria.

Cobra venom cytotoxins link membrane permeability with non-bilayer lipid phase

Cytotoxins CTI and CTII, membrane-active cationic proteins of 7 kD from the Central Asian cobra venom, have been widely used in studies to probe the structural organization of model lipid membranes [29][30][31][32][33][34][35][36][37][38][39][40][41][17][42]. Both cytotoxins phenocopy membranotropic properties of C8 subunit of the F_0 sector in bovine ATP synthase[43][34]. Both cytotoxins avidly bind to membranes enriched with acidic phospholipids, but do not interact with pure PC membranes [36][40]. It has been shown that CTII decreases angular anisotropy of the spin label EPR spectral signals in lipid films enriched with acidic phospholipids, but CTI demonstrates same ability only in lipid films enriched with CL, but not with other acidic phospholipids [36]. Both CTI and CTII triggered aggregation of liposomes and dehydration of membrane surface, but CTII induced intermembrane exchange of lipids and membrane fusion in membrane enriched with any kind of acidic lipids, while CTI was able to do the same only in membranes enriched with CL [29][30][32][33][34][35][38][39][41][17][44]. ^{31}P- and ^{1}H-NMR spectroscopy studies of the large unilamellar liposomes made of either PC+10mol% CL or PC+10mol% PS and treated with either CTI or CTII have shown that CTII induces formation of non-bilayer structures and increases membrane permeability of both PC+10mol% CL and PC+10mol% PS liposomes, while CTI induces formation of non-bilayer structures and increases membrane permeability only in PC+10mol% CL liposomes (Figure 1) [30][32][34].

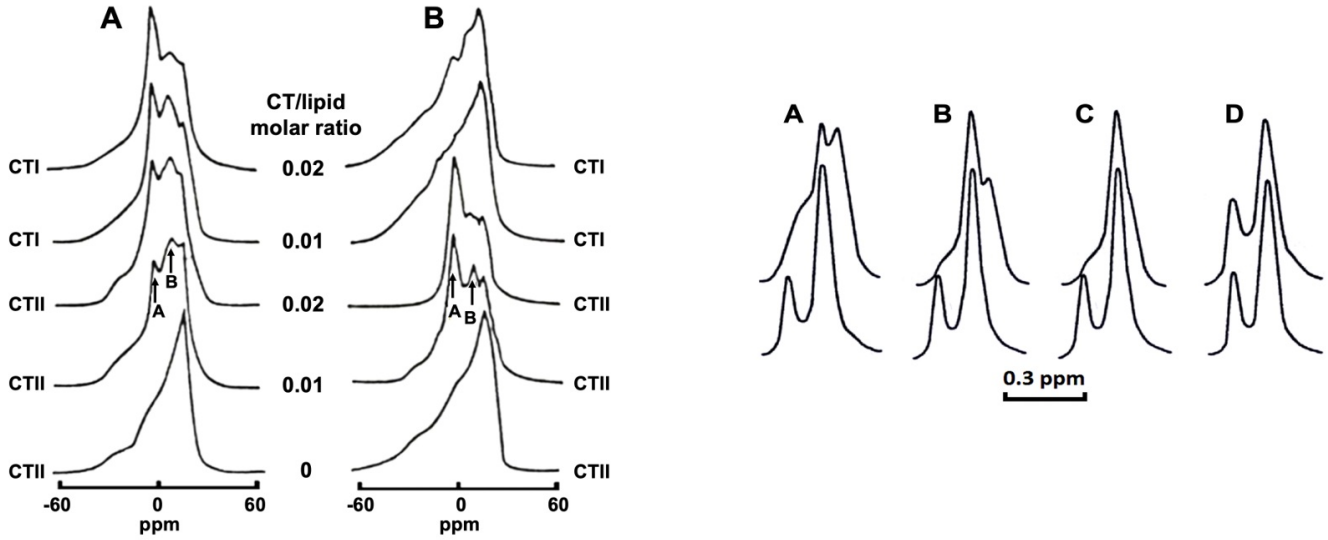

Figure 1. ^{31}P-NMR spectra on the left derived from large unilamellar liposomes of PC+10mol% CL (**A**) and PC+10mol% PS (**B**) treated with cytotoxins at given CT/lipid molar ratios. Signal A belongs to phospholipids with rapid isotropic mobility that exchange with lamellar phase within 10^{-2}–10^{-4} s. Signal B belongs to phospholipids with immobilized movement that do not exchange with lamellar phase within 10^{-2}–10^{-4} s. ^{1}H-NMR spectra on the right derived from the $N^{+}(CH_3)_3$ groups of PC in large unilamellar liposomes composed of PC+ 10 mol% CL (**A** and **B**) or PC+ 10 mol% PS (**C** and **D**) in the presence of $K_3[Fe(CN)_6]^3$ treated with either CTII (**A** and **C**) or CTI (**B** and **D**) at a cytotoxin/lipid molar ratio of 0.02. This figure is modified from [32].

The results of a recent study of interaction of CTI and CTII with a model outer mitochondrial membrane (OMM) that employed ^{31}P- and ^{1}H-NMR spectroscopy, molecular dynamics and Autodock simulation [28] were analyzed in conjunction with the results of previous studies on CTI and CTII interaction with model cell membrane and IMM [30][32][34][35][45], and a molecular mechanism of translocation of cobra cytotoxins via cell membrane, OMM and to IMM has been suggested (Figure 2). According to the suggested mechanism, cytotoxin binds via its hydrophobic and basic residues to the cell membrane surface and at the same time cytotoxin attracts a membrane of neighboring cell to form an intermembrane junction in form of inverted micelle with the cytotoxin in its center (Figure 2, A), which is an intermediate step in the process of membrane fusion. After fusion of cell membranes linked by intermembrane junction, cytotoxin settles inside of inverted micelle in a single cell membrane (Figure 2, B). Due to high surface curvature, inverted micelle transforms to the bilayer and cytotoxin is released into cytosol where cytotoxin targets CL on surface of OMM and then forms intermembrane junction between OMM and lysosomal membrane (Figure 2, C). After the fission between OMM and lysosomal membrane, cytotoxin settles in the inverted micelle in OMM and then eventually gets into the intermembrane space (Figure 2, D) where cytotoxin forms another intermembrane junction between OMM and IMM (Figure 2, E). After the fission between OMM and IMM cytotoxin settles in inverted micelle in IMM (Figure 2, F) and from there cytotoxin may translocate into matrix. At very low concentrations cytotoxin enhances plasticity of IMM and facilitates formation of intra-cristae membrane compartments which increase ATP synthase activity [8][34][37]. However, at higher concentrations cytotoxin disrupts mitochondrial integrity and functions [34][37].

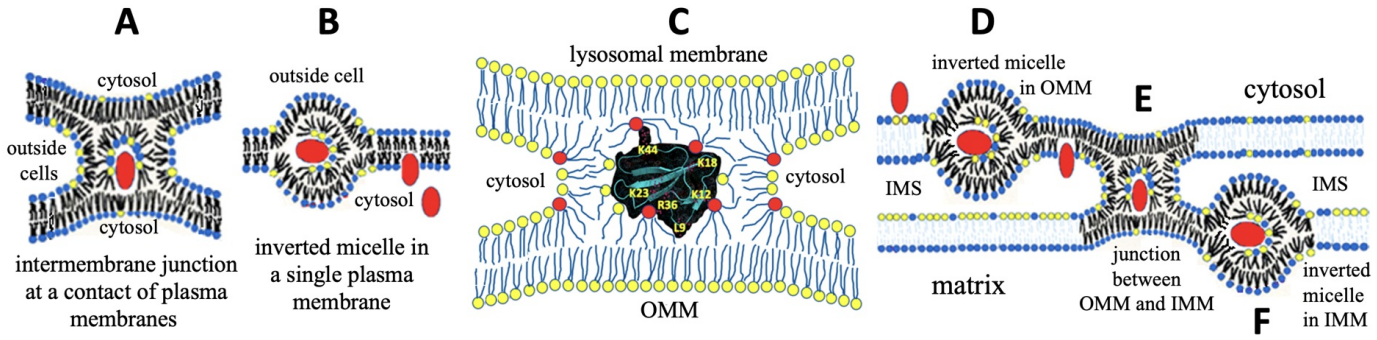

Figure 2. A – Intermembrane junction with CTII (shown red) formed at a contact of two plasma membranes has phospholipids immobilized by CTII which are responsible for ^{31}P-NMR signal B in Figure 7. **B** – After fusion of cell membranes CTII settles in an inverted micelle in a single membrane and due to the high surface curvature, the micelle transforms to the bilayer to release CTII into cytosol. **C** – Intermembrane junction with CTII at a contact of OMM and lysosomal membrane. Amino acid residues (a.a.r.) of CTII (given in cartoon representation) K23, R36 and K12 bind to OMM surface and L9 penetrate to the hydrophobic region of OMM. A.a.r. K44, K18 and a.a.r. K5, K35 on another side of CTII attract acidic lipids of neighboring lysosome to form an intermembrane junction. **D** – After fission between OMM and lysosomal membrane CTII settles in inverted micelle in OMM, then CTII is released into intermembrane space (IMS). **E** – CTII via attraction of CLs of OMM and IMM forms another intermembrane junction between OMM and IMM. **F** – After fission between OMM and IMM, CTII settles in inverted micelle in IMM. At high CTII concentration, these events disrupt mitochondrial integrity and functions, but at very low concentration CTII facilitates mitochondrial energetics. In **A**, **B**, **D**, **E** and **F** head groups of CLs are colored yellow and head groups of PCs are colored blue. In **C** head groups of CLs are colored red and head groups of PCs colored yellow. This figure is modified from [32][28].

Melittin – a cardiolipin targeting peptide

Melittin is a small membrane-active peptide from bee venom of 2,84 kD with anti-cancer properties[46][47][48]. Melittin is probably the only cationic membrane-active peptide that can bind to the pure PC membrane which is shielded by $N^+(CH_3)_3$ groups exposed toward solution on the membrane surface[49][40]. However, melittin is far more active in binding to and perturbing the structure of membrane enriched with acidic phospholipids than binding to pure PC membrane [46][47][48]. This explains higher cytotoxicity of melittin to cancer cells, which have acidic lipids on the outer surface of cell membrane, than to healthy cells, which have mostly PC molecules on the cell outer surface [47]. It is believed that melittin, similarly to snake venom cytotoxins, has evolved from body's membrane-active proteins that regulate functions of cell membranes and membranes of organelles through induction of changes in dynamics and structure of lipid phase of membrane [42][48]. Similarly, to cytotoxins, melittin triggers formation of non-bilayer lipid phase in cardiolipin-containing membranes [46][26], which at high melittin concentrations disrupts mitochondrial membrane integrity and function, but at low concentrations it is believed that melittin may increase mitochondrial membrane plasticity to rejuvenate mitochondrial functions [46]. Interestingly, short-changed alcohols facilitate melittin's membranotropic activity by making membrane surface interface more conducive for melittin action [48].

Conclusions

The plethora of experimental data on existence of non-bilayer lipid phases in model membranes suggests that non-bilayer lipids have an important role in physiology of biological membranes. In addition, correlation of experimental findings on potential of cationic membrane-active proteins (isolated from natural sources and synthetically made) on triggering non-bilayer lipid phases with the physiological reactions caused by these proteins in cells, tissues and organisms demonstrates not only the roles of non-bilayer lipids in controlling important body processes in health and disease but also opens a promise in potential use of cationic proteins in treatment of pathologies associated with abnormal rise or deficiency in non-bilayer lipid phase in biological membranes. We believe this mini review may attract more attention to the roles of non-bilayer phases in energy transducing membranes and will facilitate more rigorous research on elucidation of new important details in mechanisms of fundamental bioenergetic systems such as thylakoid and inner mitochondrial membranes.

Author Contributions: The work was initiated by E.S.G. and Y.H. has conducted research studies on relevant literature published over the last 60 years. E.S.G. has written the first draft of the manuscript and Y.H. contributed extensively by reviewing and editing the manuscript. Both authors participated in the final editing of the manuscript and agreed to the published version of the manuscript.

Funding: This research was funded by the CKWA start-up grant to the STEM Research Center.

Conflicts of Interest: The authors declare no conflict of interest.

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