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E-mail: bjsir07@gmail.com

Phase Transition in Di-oleoylphosphatidylglycerol/Monoolein Membranes due to Interactions of Positively Charged Peptides at their Lipid Membrane-Interface

Shah Md. Masum[a]* and Masahito Yamazaki[b]

[a]Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh and [b]Department. of Physics, Faculty of Science, Shizuoka University, Japan

Abstract

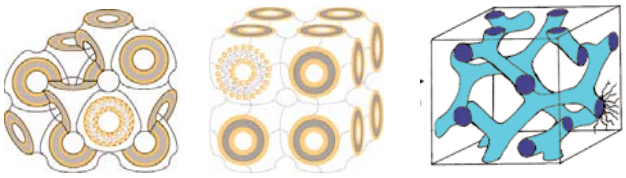
To elucidate the factors that induce phase transitions in biomembranes due to interactions of proteins/peptides at the lipid membrane-interface, the effects of positively charged peptides on the cubic phase (Q^{229}) of Dioleoylphosphatidylglycerol (DOPG)/Monoolein (MO) membranes were investigated. Small angle X-ray Scattering (SAXS) results revealed that 12 mol% DOPG/88 mol % MO membranes in excess water at 25^{o} C is body centered cubic phase of crystallographic space group *Im3m* (Q^{229}). In presence of peptide LLKKK, the lattices constant of Q^{229} phase was gradually decreased with an increase of peptide concentration and a phase transition from cubic (Q^{229}) to cubic (Q^{224}) phase occurred at *R*=0.080; (R= molar ratio of peptide to lipid). On the other hand the designed peptide WLFLLKKK and antimicrobial peptide Magainin-2 induced lamellar phase (L_{α}) in the same mixture membranes. These results indicate that the interactions of the these peptides with this mixture membrane are different: LLKKK induces electrostatic attractive interactions and that of WLFLLKKKK and Magainin-2 bound with the lipid membranes induce electrostatic repulsive interaction at the membrane-interface, might be the major factor inducing different phase transitions in 12 mol% DOPG/88mol% MO mixture membranes.

Key words: Antimicrobial peptide Magain-2, Dioleoylphosphatidylglycerol, Monoolein, Cubic phases, Small angle X-ray Scattering

Introduction

Cubic phases of biomembranes have attracted much attention in both biological and physico-chemical aspects (Seddon *et. al.*, 1995; Luzzati, 1997; Hyde *et. al*. 1997). Three dimensional arrangements of lipid bilayers similar to cubic phase structures have been observed in various cells by transmission electron microscopy (Hyde *et. al.*, 1997). The biomembrane cubic phases which includes Q^{224} (Schwartz's D surface) Q^{229} (P surface) and Q^{230} (G surface) have an infinite periodic minimal surface (IPMS), play important roles in membrane dynamics such as membrane fusion, control of membrane functions and intracellular various structures of membranes (Hyde *et. al.*, 1997).

To elucidate the physiological roles of biomembranes cubic phases and the mechanism of above phenomena, understanding the stability and phase transition of biomembrane cubic phases are important (Seddon *et. al.*,1995; Luzzati,. 1997 and Anderson *et. al.*, 1988).



Q^{224} Phase Q^{229} phase Q^{230} phase

Fig. 1. Cubic phases of biomembranes

Interactions of proteins with lipid membranes play important roles in the static and dynamic structures of biomembranes and also their functions. Many water soluble proteins can be bound with the lipid membrane reversibly and their binding depends on their concentration in the aqueous regions, conformation, and local net charge. As for example, myristoylated alanine-rich C kinase substrate (MARKS) and src ($pp60^{src}$) can be bound with the lipid membrane using both electrostatic attractive and hydrophobic interactions (Kim *et. al.*, 1994; Buser. *et. al.*, 1994). Recent biophysical studies indicate that the lipid membrane interface is composed of

*Corresponding author: E-mail: masumdhk@yahoo.com

hydrophilic head group, part of hydrophobic hydrocarbon chain and water molecules due to large thermal motion such as undulation and protrusion exert in biomembranes (Israelachvili, 1992; Nagle and Nagle, 2000). The interactions between the substances like short peptides or ions and the lipid membrane interface play a crucial role on membrane structures and their functions which is not well understood.

Recent investigations of the effects of electrostatic interactions due to surface charges indicated that electrostatic interactions at the lipid membrane-interface due to incorporation of fatty acid (Oleic acid) (Aota Nakano *et al*, 1999) or negatively charged lipids such as dioleoylphosphatidic acid (Li *et al.*,2001) or partitioning of peptide (Masum *et al.*, 2003) to the neutral lipid membrane-interface induces phase transition between cubic phase and lamellar phase (L_α) and also between the cubic phases of different infinite periodic minimal surface (IPMS). Transformation of lamellar (MLV and LUV) to cubic phases membranes induced by low concentration of Ca^+ ions were observed in Dioleoylphosphati dylglycerol (DOPG) /Monoolein (MO) membrane (Trarek *et. al.*, 2005). Antimicrobial peptides with bactericidal and fungicidal activity have been isolated from a wide variety of microorganisms including amphibians, invertebrates, plants and mammals (Zasloff, M., 2002). The target of these peptides is to the lipid membrane regions of the bacteria and fungal cells. Among these antimicrobial peptides, magainin-2 isolated from African clawed frog *Xenopus laevis* has been extensively investigated (Zasloff, M., 1987). Magainin-2 has four positive charges and forms a a helix in the lipid membrane interface, lie parallel to the membrane surface (Tamba *et al.*, 2005). The present study focus on the interactions between the designed peptides and Magainin-2 which are positively charged and membranes containing negatively charged lipid. The possibility of phase transition from cubic to cubic phase and cubic to lamellar phase in Dioleoylphosphatidylglycerol (DOPG)/Monoolein (MO) membrane was also investigated.

Materials and Methods

MO (1-monooleyl-rac-glycerol) was purchased from sigma Chemical Co.(St. Louis, MO). 1,2- dioleoyl-sn-glycro-3-[phosphor-rac-(1-glycerol)] (DOPG) sodium salt was purchased from Avanti Polar lipids. Piperazine-1,4 bis (2-ethane-sulfonic acid) (PIPES) was purchased from Dojino Molecular Technologies, Inc. These chemical were used without further purification.

Preparation of lipid membrane

Appropriate amount of 10 mM PIPES buffer was added to the dry lipids mixture of MO and DOPG in excess water (50 mM Lipid concentration). The PIPES buffer containing lipid mixture was then vortexed about 30 sec at room temperature (~25 ^oC) several times. For SAXS measurement, pellets after centrifugation (13000 g, 30 min at 25 ^oC; Tomy, MR-150) of the lipid suspension were used. To investigate the interaction of peptide with the DOPG/MO-MLV membrane in the cubic phase, peptide in 10 mM PIPES buffer was added to the dry lipid mixture and vortexed. The vortexed suspension was then incubated at 25 ^oC using a temperature control incubator for 4 hr. After incubation, the suspension was centrifuged for 30 min at 25 ^oC and excess buffer was removed by suction. pellets of the lipids were collected in a thin glass capillary for the SAXS measurements.

Peptide Synthesis and Purification:

The peptide was synthesized by the FastMoc mehod using a 433A peptide synthesizer (PE Applied Biosystems, Foster City, CA). The Sequence of designed peptides is LLKKK and WLFLLKKK and that of Magainin-2 is GLFGAIAGFIENGWEGMIDG. These peptides have an amide block C-terminus. Peptides were purified by reversed phase high performance liquid chromatography (LC-10AD and SPD-10A, preparative column (10X250mm, 10 mm) and a C18 analytical Column (4.6 X 250 mm, 5 mm; Vydac/ The separation Group Inc., Hesperia, CA). The purified peptide was analyzed by ion-spray ionization mass spectrometry using a single quadrupole mass spectrometer (API 150EX, PE SCIEX, PE Applied Biosystems, Foster City, CA); ionization was performed at a flow rate of 5 mm/min. The measured masses of designed peptides LLKKK and WLFLLKKK peptides and that of Magainin-2 were 627.8 ± 0.1, 1074 ± 0.3 and 2465 ± 0.1 Da. respectively. These masses correspond to the molecular masses calculated from their amino acid compositions.

SAXS measurements

X-ray diffraction experiments were performed using nickel filtered Cu Kα X-rays (λ = 0.154 nm) from a rotating anode type X-ray generator (Rigaku, Rotaflex, RU-300) at 40 Kv and 200 mA. SAXS data were recorded using a linear (one dimentional) position sensitive proportional counter (Rigaku, PSPC-5) with a camera length 350 mm and associated electronics (multichannel analyzer, etc.; Rigaku). In all

cases, samples were sealed in a thin wall glass capillary tube (outer diameter 1.o mm) and mounted in a thermostable holder with a stability of ± 0.2° C (Kinoshita, 2001; Yamazaki, 1992)

Results and Discussion

Effects of LLKKK Peptide on the 12%DOPG/ 88%MO Membranes

The effects of LLKKK peptide on 12 mol% DOPG/ 88mol% MO membranes in excess PIPES buffer (pH 7.0) at 25° C were investigated. Under this condition, the membrane was in the body- centered cubic phase of space group *Im3m* (Q^{229} phase)(cubic aspect # 8)(International Tables for X-ray crystallography, 1985).

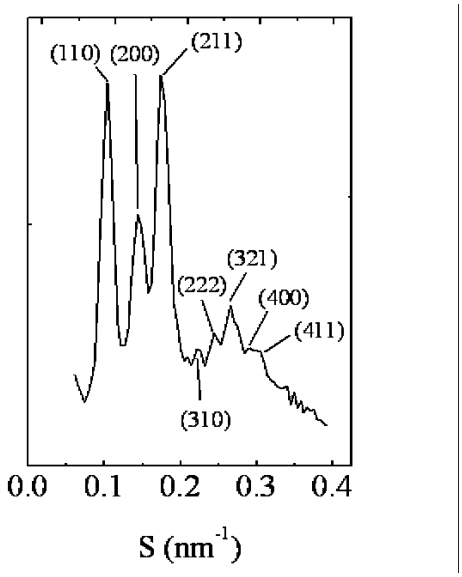


Fig.2: SAXS pattern of Q^{229} phase of 12%DOPG/88%MO in presence of LLKKK membrane (R=0.020)

Addition of small amounts of peptide LLKKK changed this cubic Q^{229} structure. Fig. 3 shows the SAXS pattern of 12%DOPG/88%MO/ LLKKK membrane (*R*=0.020; where *R* is the molar ratio of peptide to lipid). This SAXS pattern revealed several peaks had the spacing in Phase transition in the ratio of √2: √4: √6: √10: √12: √14: √16: √18. They were indexed as (110). (200), (211), (310), (222), (321), (400), and (411) reelections, indicating the membrane was in the Q^{229} phase. The reciprocal spacing, S, of the cubic phase is related to the lattice constant, a, by the equation S (h, k, l) = (1/a) $(h^2+k^2+l^2)^{1/2}$, where *h, k, l* are Miller indices. The lattice constant, a, of 12% DOPG/88%MO/LLKKK membrane (R=0.020) was 17.0 nm.

In contrast, as shown in Fig. 3 the SAXS pattern of the 12%DOPG/88%MO membrane containing LLKKK peptide (*R*=0.10) had spacing in the ratio of √2: √3: √4: √6: Ö8: √9: √10, indexed as (110), (111), (200), (211), (220), (221) and (310), reelections on a primitive cubic lattice of space group *Pn3m* (Q^{224} phase) (Cubic aspects # 4). The lattice constant of Q224 phase was 12.3 nm.

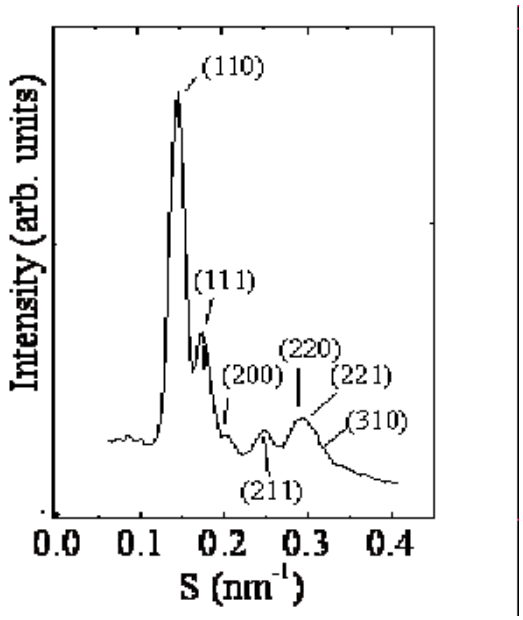


Fig. 3. SAXS pattern of Q^{224} phase of 12%DOPG/ 88%MO/WLFLLKKK membrane (R=0.10).

Figure-4 shows the dependence of lattice constant and type of phases in 12%DOPG/88%MO/LLKKK membrane as a function of peptide concentration. At R> 0.06, 12% DOPG/88%MO membranes were in Q^{229} phase. At R£0.080, 12% DOPG/88% MO/LLKKK membranes were in Q^{224} phase.

Thus, a phase transition from Q^{229} to Q^{224} phase occurred at *R* = 0.080. The lattice constant of the Q^{229} phase immediately before the phase transition (*R*=0.060) and that of the Q^{224} phase immediately after phase transition *R* = 0.080) were 13.7 nm and 12.3 nm, respectively

The ratio of their lattice constants (Q^{229}/Q^{224}) was 1.18, which is close to the theoretical value determined by the analysis of coexisting cubic phases based on the Bonnet transformation (Tenchov *et. al.,* 1998; Hyde *et al.,* 1984). This also supports that Q^{229} to Q^{224} phase transition in 12 mol% DOPG/88 mol% MO membranes. As a control experiment, effect of LLKKK peptide on 100% MO was performed. No change in lattice constant and phase structure

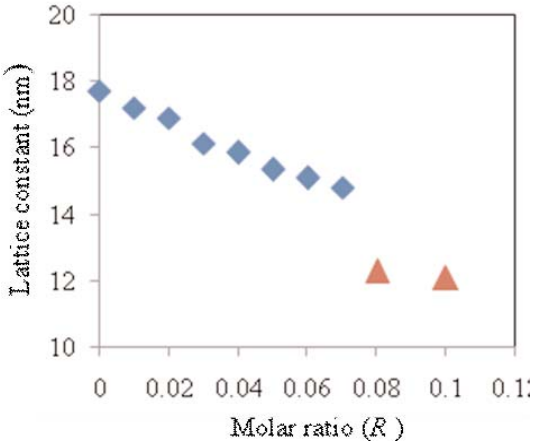


Fig. 4. Lattice constant and phase of 12 mol% DOPG/88 mol% MO membranes in presence of LLKKK peptide (u Cubic (Q^{229}) phase; s Cubic (Q^{229}) phase).

was observed (data were not shown). The SAXS results clearly show that LLKKK peptide decreased the lattice constants of Q^{229} phase and induced a Q^{229} to Q^{224} phase transition in 12% DOPG/88% MO membrane. It is reasonably considered that electrostatic repulsion between the negatively charges of DOPG at the membrane-interface of DOPG/MO membrane decreased owing to the electrostatic attractive interaction induced by positively charges of LLKKK peptide. At high salt concentration (NaCl), no phase transition was observed in these mixture membranes (Tarek *et al.*, 2005).

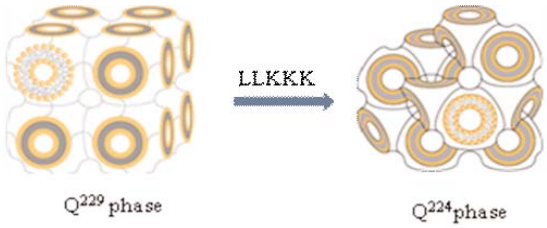


Fig.5. Phase transition in DOPG/MO membranes in presence of peptide LLKKK

Effects of WLFLLKKK and Magainin-2 on the Cubic phase (Q^{229}) of 12%DOPG/88%MO Membranes

We investigated the interactions of WLFLLKKK peptide and Magainin-2 with the 12 mol% DOPG/88 mol% MO mixture membranes and their effect on the phase transition of cubic phase obtained by these mixture membranes. The increase of WLFLLKKK peptide concentration (R=0.02), there has no significant difference in the lattice constant of Q^{229} phase.

However, at and above R0≥02, a new set of SAXS pattern were appeared where the three peaks are in the ratio of 1:2.

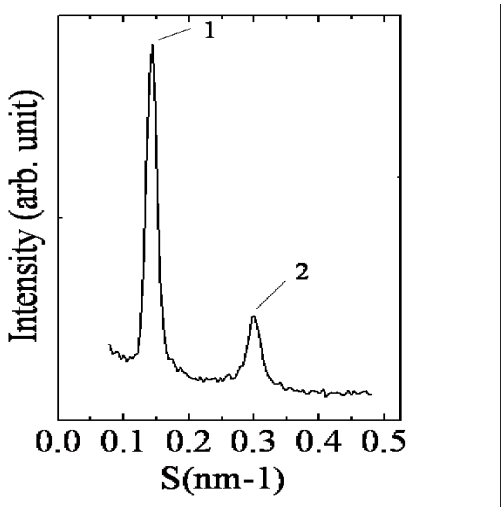


Fig.6. SAXS pattern of Lα phase of 12%DOPG/ 88%MO/WLFLLKKK membrane (*R*=0.05)

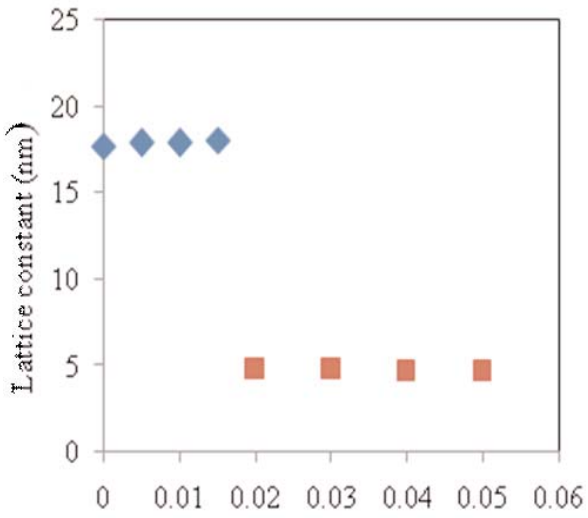


Fig. 7. Lattice constant/spacing and phase of 12mol% DOPG/88 mol% MO membranes in presence of WLFLKKK (u ¨; Cubic (Q^{229}) phase; n Liquid crystalline (Lα) phase).

This corresponds to lamellar liquid crystalline phase i.e Lα phase. The spacing of the lamellar phase is 4.6 nm.

On the other hand, the same effects observed in case of magainin-2 where the lattice constant of the Q^{229} phase of 12mol% DOPG/88 mol% MO magainin-2 concentration (*R*= ≥0.020) and a phase transition Q^{229} to Lα phase occurred at *R*=0.020. The spacing of Lα phase is constant (5.2 nm) upto *R*=0.10 membrane increased with an increase of

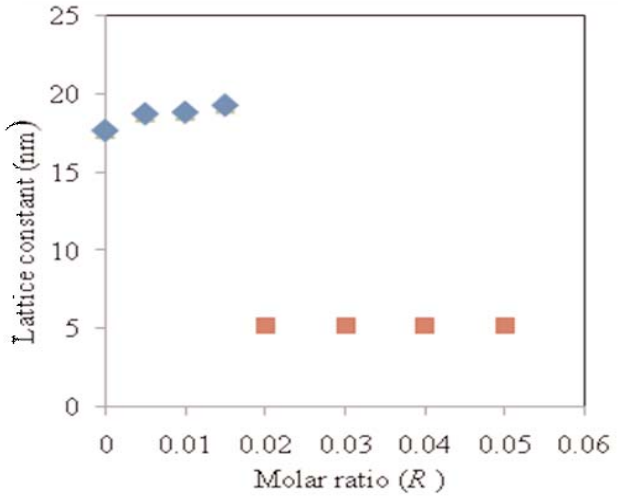


Fig. 8. Lattice constant/spacing and phase of 12 mol% DOPG/88 mol% MO membranes in presence of Magainin-2 (u¨; Cubic(Q^{229}) phase; n Liquid crystalline (Lα) phase)

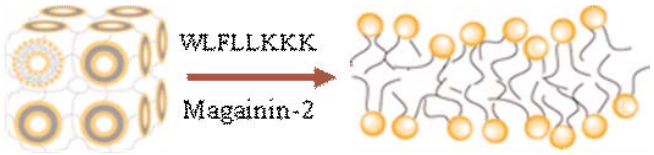
At present, there is no quantitative theory to explain the electrostatic interaction induced phase transition between Q^{224} and Q^{229} phases in biomembranes. It is reported that electrostatic interactions at the membrane-interface of neutral lipid MO membrane increased with an increase of surface charge density by incorporation of negatively charges DOPA lipid (Li *et al.*, 2001) or partitioning of positively charged peptide WLFLLKKK (Masum *et al.*, 2003) in MO membrane induced a phase transition from Q^{224} to Q^{229} phase. As the electrostatic interaction decreases with an increase of salt concentration (such as NaCl), the reverse phase transition occurs. These experimental results are useful to consider the mechanism of this phase transition. The increase of electrostatic repulsive interactions due to surface charges reduces the absolute value of spontaneous curvature, $|H_0|$, of the monolayer membrane inducing a phase transition from $Q^{224} \Rightarrow Q^{224} \Rightarrow L\alpha$ in Monoolein/ dioleoylphosphatidic acid (Li *et al.*, 2001; and in Monoolein/WLFLLKKK peptide membranes (Masum *et al.*, 2003). The spontaneous curvature of the monolayer membrane is characterized by a packing parameter, *V/Al*, Where *V* is the volume of the entire lipid molecule, *A* is the area of the lipid head group at the lipid-water interface and *l* is the length as follows (Marsh, 1996).

$$|H_0| = 1/R_0 \frac{cV/Al - 1 - ((cV/Al) - 2cV/Al + c)^{1/2}}{(1- c)/l}$$

Where c is constant: $\pi/(2\sqrt{3}) < c < 2\pi/(3\sqrt{3})$. From the equation, it can be seen that decrease of average value of *V/Al* increases the absolute value of monolayer curvature. On the other hand, increase of average value of *V/Al* decreases the absolute value of monolayer curvature. In the mixture membrane of DOPG/MO/LLKKK, electrostatic attractive interactions occurred between the positive charges of LLKKK peptides and Negative charges of DOPG lipids.

As a result, the average head group area A reduces which decreases the average value of *V/Al*. Thereby, the increase of $|H_0|$ attributives by the decrease of electrostatic interaction at the membrane surface. Moreover, the electrostatic attractive interactions decreases the lattice constant of the cubic phase membrane which indicated that the water content of the cubic phase membrane decreases.

In mixture membrane of DOPG/MO/ WLFLLKKK, it was observed that the lattice constants of the cubic phase of 12% DOPG/ 88% MO were almost same with small increase of peptide concentration and a phase transition from cubic(Q^{229}) to Lα phase occurred instead of Q^{229} to Q^{224} phase. This result indicated that the interactions between peptide WLFLLKKK and membrane interface of DOPG/MO are quite different than that of LLKKK peptide. The reason is that WLFLLKKK peptide contains tryptophen and phenylalanine which have high interfacial hydrophobicity and can be bound with the membrane interface of monoolein membrane due to hydrophobic interactions (Masum *et. al*, 2005).



Cubic (Q^{229}) phase (Lα phase)

Fig. 9. Cubic to lamellar liquid crystalline phase (Lα phase) transition in 12 mol%DOPG/88 mol%MO membranes

The small decrease of lattice constant is due to attraction between positively charged of WLFLLKKK and negatively charged of DOPG lipid. However, increase of WLFLLKKK peptide concentration i.e more peptide bound with the membrane interface, dominated the electrostatic repulsive interaction rather than attractive interaction at the membrane interface which induced a cubic to Lα phase transition instead of Q^{229} to Q^{224} phase in 12mol% DOPG/88 mol% MO membranes.

Thus the average value of the head group area, *A* increases the average value of *V/Al*, which reduces the absolute value of spontaneous curvature of monolayer membrane, $|H_0|$. This finding is similar as the results obtained by Li *et al.*,

2001 and Masum *et al.*, 2003. The authors demonstrated that the increase of electrostatic repulsive interactions due to surface charges reduces the absolute value of spontaneous curvature, $|H_0|$, of the monolayer membrane inducing a phase transition from $Q^{224} \Rightarrow Q^{229} \Rightarrow L\alpha$ in Monoolein/ dioleoylphosphatidic acid (Li *et al*., 2001; and in Monoolein/WLFLLKKK peptide membranes (Masum *et al.*, 2003). The other is that the electrostatic repulsive interactions increase the lattice constant of cubic phase membrane i.e increase of water content inside the cubic phase membranes. Several theoretical studies demonstrated that increase of water content induces a phase transition from Q^{224} to Q^{229} phase due to change of curvature elastic energy of the membranes (Chung, H. *et. al,* 1994; Turner D. C. *et. al.*, 1992; Templer, R. H *et. al.*, 1998).

Conclusion

From the above discussion, it can be concluded that the change in absolute value of spontaneous curvature of monolayer membrane owing to electrostatic interactions at the membrane interface is one of the determinant inducing phase transitions in biomembranes.

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