Synopsis

The thesis entitled Structure Function Relationship in Membrane Lipids. Role of Headgroup-Hydrocarbon Chain Linkages has been divided into 4 chapters. Chapter 1 and 2 dealt with three different type lipid analogues to find out the role of the ester linkages in the glycerophospholipids, the most ubiquitous lipid present in mammalian cell membranes. In Chapter 3, the studies directed towards the cholesterol-lipid interactions in both cationic and zwitterionic lipid membranes to elucidate the issue of hydrogen bonding of 3β-OH group with carbonyl groups of the ester functionalities at the linker region of lipids in biomembranes. The Chapter 4 recorded the results of the studies towards the understanding of the interaction between dipalmitoylglycerol (DPG) and dipalmitoylphosphatidylcholine (DPPC) in their composite membranes.

Chapter 1. Section A. Synthesis, Thermotropic Behavior and Permeability Properties of Vesicular Membranes Composed of Cationic Mixed-Chain Geminally Anchored Surfactants

In order to elucidate the role of the linkage region that connects the polar headgroups with hydrophobic segments in a lipid monomer, cationic mixed-chain amphiphiles containing acyl and alkyl hydrophobic segments connected at the level of Me₂N⁺ headgroups 2a-d were synthesized. Related dialkyl dimethylammonium ion surfactants 1a-c and diacyl systems 3a-c were also synthesized. Despite mismatch in the connector region, amphiphiles 2a-d form bilayer vesicles like their dialkyl and diacyl counterparts as revealed by electron microscopy. Introduction of an ester connector function between the polar and hydrophobic parts raises the phase transition temperature (Tₘ), transition enthalpies and resistance to ion-permeation. Consideration of energy minimized conformations points toward the importance of differences in the depth of chain penetration into the putative bilayer.

Section B. Synthesis and Thermotropic Properties of Pseudoglyceryl trimethyl ammonium lipids with varying functionalities at the hydrocarbon chain – headgroup Linkages.

A series of pseudoglyceryl ammonium lipids have been synthesized to determine the role of the lipid connectors towards its membrane properties. Lipids with (O-acyl)-(C-)alkyl linkages 5a-5d and lipids...
with identical and mixed varieties of di-(O-acyl), 6a, (O-acyl)-(O-alkyl), 6b, (O-alkyl)-(C-acyl), 6c and di-(O-alkyl), 6d, were synthesized along with a lipid, 4, where the hydrocarbon chains are directly connected to the NMe₃⁺ headgroup.

$$\text{H}_2\text{C}^-\text{CH}_2^-\text{N}^+(\text{CH}_3)_3 \quad \text{R}_1 \quad \text{R}_2 \quad \text{Br}^-$$

4  \( R_1 = \text{C}_{17}\text{H}_{35}, \quad R_2 = \text{C}_{18}\text{H}_{37} \)

$$\text{H}_2\text{C}^-\text{CH}_2^-\text{N}^+(\text{CH}_3)_3 \quad \text{R}_1 \quad \text{R}_2 \quad \text{Br}^-$$

6a  \( R_1 = \text{OC}(=\text{O})\text{C}_{15}\text{H}_{31}, \quad R_2 = \text{OC}(=\text{O})\text{C}_{18}\text{H}_{37} \)
6b  \( R_1 = \text{OC}(=\text{O})\text{C}_{15}\text{H}_{31}, \quad R_2 = \text{OC}_{18}\text{H}_{33} \)
6c  \( R_1 = \text{OC}_{18}\text{H}_{33}, \quad R_2 = \text{OC}(=\text{O})\text{C}_{18}\text{H}_{31} \)
6d  \( R_1 = \text{OC}_{18}\text{H}_{33}, \quad R_2 = \text{OC}_{18}\text{H}_{33} \)

$$\text{H}_2\text{C}^-\text{CH}_2^-\text{O}^-\text{P}^-\text{O}(\text{CH}_2\text{H}^\text{N}^+(\text{CH}_3)_3 \quad \text{R}_1 \quad \text{R}_2 \quad \text{O}^- .$$

5a  \( R_1 = \text{OC}(=\text{O})\text{C}_{15}\text{H}_{31}, \quad R_2 = \text{C}_{18}\text{H}_{37} \)
5b  \( R_1 = \text{OC}(=\text{O})\text{C}_{17}\text{H}_{36}, \quad R_2 = \text{C}_{18}\text{H}_{37} \)
5c  \( R_1 = \text{OC}(=\text{O})\text{C}_{18}\text{H}_{31}, \quad R_2 = \text{C}_{18}\text{H}_{33} \)
5d  \( R_1 = \text{OC}(=\text{O})\text{C}_{17}\text{H}_{36}, \quad R_2 = \text{C}_{18}\text{H}_{33} \)

The vicinal disposition of the lipid hydrocarbon chains increases the thermotropic parameters compared to their geminal counterparts. The increase in the chain length at C(1) carbon atom influences more in determining the \( T_m \). The enthalpy of the main phase transition decreases with the increase in the C(1)-acyl-chain length whereas the C(2)-alkyl-chain length has an opposite effect. The phase transition enthalpy decreases moderately with the increase in the alkyl chain length at C(1) position. In case of entropy of the phase transition, it is also apparent that the lipids carrying C(1)-stearyl esters are having higher enthalpy of main phase transition than the other variety having C(1)-palmitoyl ester chain. When the chain length increases in alkyl-chain region, for a particular acyl-chain lipid, the entropy value decreases. The lower values of cooperativity units might be attributed for the headgroup-headgroup repulsions that expected to occur at the catonic lipid assemblies. Cast-film X-ray diffraction measurements and the calculated bilayer widths obtained by energy minimizations suggest the tilt angle decreases with the increase in hydrophobicity at the linker region on the replacement of each ester group by a hydrophobic CH₂CH₂ unit. The comparable results of some of the catonic lipids (6a-d) with their exact glycerophosphocholine analogues (7a-d) suggest the uniqueness of the pseudoglyceride lipids as a good putative model system of the naturally occurring glycerolipids of the biomembranes.
Chapter 2. Importance of Chain-Linkage Functionalities on the Properties of the Phosphatidylcholine Lipids in Bilayer Membranes

This section deals with the studies concerning the importance of the chain-linkage functionalities of the phosphocholine lipids in bilayer membranes. The most ubiquitous and extensively studied natural lipid, DPPC (7a), was considered to be the reference lipid which has two ester groups at the chain-backbone connector region. In one series of the new phospholipid, the acyl chain on C(2) position of the glycerol backbone has been replaced with an n-alkyl chain of appropriate length, leaving the acyl chain on C(1) position as in DPPC. Another lipid series were also prepared in which both the ester linkages of the fatty acyl chains in DPPC are replaced with C(1), C(2) – dialkyl chains of comparable lengths (9a-f and 10a-b) and their aggregation behaviors in water were examined. DLS and TEM studies and dye entrapments confirm the vesicle formation from the newly synthesized PC’s. Measurements of carboxyfluorescein efflux rates, from its vesicle entrapped states, clearly demonstrate the remarkable influence of chain-linkage types on controlling the transmembrane permeations. To elucidate the extent of hydration at the linker region, excited fluorophore lifetime measurements in various lipid vesicles were done. To explain the above observations, thermal transition properties of each type of lipid vesicles were examined independently by temperature dependent fluorescence anisotropy measurements and differential scanning calorimetry. Thermal transition data firmly establish the critical differences in lipid packing features in various vesicular assemblies. The observable differences in thermal properties of lipids were rationalized with the help of molecular modeling and X-ray diffraction measurements.

Chapter 3. Section A The effects of Cholesterol inclusion on the vesicular membranes of Cationic Lipids.

Small unilamellar vesicles formed from four cationic lipids (A - D) in the absence and the presence of varying amounts of cholesterol have been studied using fluorescence polarization and \(^1H\)-
NMR techniques The fluorescence polarization data clearly indicate that the packing order in the cationic lipid bilayers are affected by inclusion of cholesterol. This effect appears to be present also with a lipid that is devoid of any formal linkage region. Thereby the interaction of this lipid with cholesterol through hydrogen bonding is not feasible. When the interactions of cholesterol with different types of cationic lipids in excess water have been examined in multilamellar dispersions using $^1$H-NMR spectroscopy, the conclusions arrived from fluorescence polarization measurements are supported. Detailed spectroscopic interpretations were derived from the comparison of respective cationic lipid spectra before and after the addition of various amounts of cholesterol. In all the cases, the CH$_2$ proton NMR line widths respond to the addition of cholesterol to vesicles. Hydrophobic association of the lipid and cholesterol appear to cause restriction of the chain (CH$_2$)$_n$ groups motions, leaving the terminal CH$_3$ groups relatively mobile. Finally, a rationale of the cholesterol-cationic lipid association was presented on the basis of energy-minimized structural drawings.

Despite the fundamental differences in specific structural features, polar character and mutual lipid monomer-monomer interactions within vesicles, the effects of inclusion of cholesterol in cationic lipid vesicles seem to be remarkably similar to that observed with cholesterol-phospholipid covesicles. The mutual hydrophobic association between cationic lipid and cholesterol appears to be more important than any possible hydrogen-bonding interaction between lipid and cholesterol.

Section B. Molecular Interactions between Cholesterol and various Lipids in Bilayer Membranes. Role of Hydrocarbon Chain-Polar Headgroup Linkage.

Four designed lipids are employed in the present study. Two of them are cationic and two have phosphatidylethanolamine (PC) as head group. Unlike, dipalmitoylphosphatidylethanolamine (DPPC), the other lipids employed herein do not have any ester linkage connecting polar headgroup with the hydrocarbon chains. As a result studies of cholesterol interaction employing the lipids devoid of any headgroup-chain linkage offer useful information about the role of 3β-OH of cholesterol in its association with lipid. Small unilamellar vesicles formed from each of PC and cationic lipids in the absence or the presence of varying
amounts of cholesterol were studied using fluorescence anisotropy method. These data clearly indicate that the order in the lipid bilayer packing is strongly affected upon inclusion of cholesterol. This effect is similar irrespective of the electrostatic character of the lipid employed. The interaction of cholesterol with various lipids have been further examined in their fluid states of multilamellar vesicular dispersion. The influence of cholesterol inclusion on vesicular dispersions were also examined by 1H-NMR spectroscopy and the influence on the bilayer depth, in presence of cholesterol, was examined upon casting the dispersions followed by X-ray diffraction studies. Effect of cholesterol on the efflux rates of entrapped carboxyfluorescein from the phospholipid vesicles was determined. Taken together, these investigations clearly suggest that despite differences in their structural features, and mutual inter-monomeric associations, the effects of cholesterol incorporation are remarkably similar. This emphasizes the importance of hydrophobic interaction between lipid and cholesterol and demonstrate that hydrogen bonding between lipid linkage region 3β-OH of cholesterol is not necessary to bring about the cholesterol induced effects.

Chapter 4. Dipalmitoylglycerol analogues for the Understanding of the Interaction of 1,2-diacetylgllycerol in DPPC membrane.

Four analogues of 1,2-dipalmitoyl glycerol (DPG) molecularly defined architecture (13-16) were synthesized and characterized to their analytically pure form. In the first two analogues the modification was done at the polar headgroup of DPG, by removal of oxygen atom from OH-group (13) and then the CH2OH-group was replaced by H-atom (14). These two analogues were expected to highlight upon the molecular interactions with the DPPC brought about from OH-group of 1,2-DPG molecule. In the next two analogues (15 and 16) the variation was done at the hydrophobic chain-backbone the linker region by systematic replacement of each OC(=O) group by two CH2 units keeping the length of the hydrophobic chains from the backbone nearly the same.
The membrane aggregates obtained from the binary mixtures of each of the DPG-analouges with DPPC were studied by fluorescence anisotropies due to doped probe DPH (1,6-diphenylhexatriene) to get informations about the hydrophobic chain ordering and the microenvironment around the probe sitting in the membrane interior. The effect on lamellar widths and the phase polymorphism upon incorporation of various mol % of DPG analogues in DPPC membranes were characterized by cast film X-ray diffraction and $^{31}$P-NMR experiments respectively. In addition the effects on the enzymatic (PLA$_2$) activities were measured to assess the nature of interaction that prevail between a modified DPG with DPPC molecules in membrane.

Taken together, these studies suggest the following:

1. The diacylglycerols (DG) affect the lamellar structures formed by the pure phospholipids in model- and natural membranes due to the change in the van der Waals interactions at the hydrophobic part of the membrane and also due to the influence exerted on the repulsive forces acting at the choline headgroups. The different tendencies of partitioning of DGs in fluid and solid-like domains influence the main phase transition temperature, $T_m$, of the composite membranes. The DGs which increase the $T_m$ partition into the 'solid-like' domain and those decrease the $T_m$ partition in 'fluid' domains. 1,2-DPG does not perturb the order of the fatty acyl chains neither above nor below the phase transition interval. In the case of DOG or short-chain DGs, e.g. DCG (1,2-Decaproyl glycerol), partition more towards the fluid-like domain and thus reduces the $T_m$.

2. On modification of the headgroup of DPG, DPPD and DPEG, the fluidizations of the membrane at below the $T_m$ and an increases in the $T_m$ were observed. The reduction of the hydrophilic character of the DPG-headgroup destabilizes the DPG/DPPC membrane packing environment at the gel-phase. The increase in $T_m$ and the rigidification of the composite membranes above the regular phase transition temperature of DPPC suggest the hydrophobic effect of the chains of mDs plays similar role as in the case of DPG/DPPC membranes.
3. On modification of the linker functionalities by the replacement of the each of the ester groups with a hydrophobic segment like CH₂CH₂ group, reduces the interfacial hydration structure that exists in DPG/DPPC systems. The subtle variation in linker zone of DPG from hydrophilic to hydrophobic environment fluidize at gel-phase and increase in Tₘ due to increase in intermolecular separation of DPPC molecules and increase in their dynamic freedom on the membrane leaflet.

4. Apart from the new phase formation by DPPD, DPEG and HPPD in DPPC membrane, their immiscibility might increase the local concentration of PLA₂ reaction products and induces higher activity of PLA₂ on composite with DPPC. The better mixing of HD with DPPC, as confirmed by ³¹P-NMR, the apparent isotropic HD/DPPC solid-like phase dilute the local concentration of the PLA₂ reaction products at the reaction domain and effectively moderates the PLA₂ activity.

List of Publications.


3. Molecular Interactions between Cholesterol and various Lipids in Bilayer Membranes Role of Hydrocarbon Chain-Polar Headgroup Linkages Bhattacharya, S and Haldar, S Communicated to Langmuir

4. Effect of mutation of Hydrocarbon Chain - Glycerol Backbone Linkages in Phospholipid Membranes Bhattacharya, S and Haldar, S manuscript under preparation

5. Molecular interaction between 1,2- dipalmitoylglycerol with Dipalmitoylphosphatidylcholine membranes through 1,2-DPG analogues Role of Glycerol backbone and connector functionalities Bhattacharya, S and Haldar, S manuscript under preparation

6. Thermotropic behavior of Cationic lipid vesicles from Pseudoglycend skeleton- Effect of Chain Linkage functions Bhattacharya, S and Haldar, S manuscript under preparation