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Supported Bilayer Lipid Membrane: A Review

Review Article

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Abstract

Supported membranes represent an elegant route to designing well-defined fluid interfaces which mimic many physical-chemical properties of biological membranes. Growths in the applications of physical approaches in understanding and controlling lipid membranes have witnessed a rapid growth in recent years. The present reviews highlight some of the key challenges of cellular membranes and exemplify their utility in fundamental biophysical studies and technological applications.

Keywords: BLM; Model membrane; Membrane potential; Membrane conductance

Introduction

Membranes are vital components of all living systems forming the outer boundary of cell organelles which contain lipid and carbohydrate. The basic function of membrane is to define a boundary between or within cells and organelles. Many cellular processes depend on the membranes' selectivity and ability to separate different area and strongly regulate transport within and across membranes. Membrane can be natural (biological) or artificial (model) that mimic biological membrane. Lipids that organized into a bilayer, serve as a basic matrix for other constituents of biomembranes, namely, protein and carbohydrate. The lipid components in bio-membranes are zwitterionic phospholipids [1,2]. The complexity of biological membranes and their interactions with intra and extracellular networks make direct investigations difficult especially for specific ion transportation. Due to this reason; model membranes have played an important role in identifying and understanding property of bio-membranes [3].

During the last 20 years, phospholipids bilayers deposited onto solid substrates have been the most commonly used experimental model systems. These model systems are readily prepared by directly depositing lipid onto solid surfaces or immersing solid into lipid solution to yield large areas with excellent mechanical stability without losing their fluid nature. The combination of fluidity and stability on planar surfaces offer advantages over self-supporting membranes as it gives a chance to investigators to carry out experiments and use analytical methods that are difficult or impossible to be used with biological membrane [4,5]. Typically, suspended bilayer lipid membranes are formed on porous, synthetic substrates, including polycarbonate, teflon, nylon, alumina, silicon, glassy carbon, and metal. The formation of bilayer lipid membrane (BLM) on these supporting substrates relies on the hydrophobic interactions between the amphiphilic (both hydrophilic and hydrophobic) phospholipids molecules and water. The charged sites on bilayer lipid membranes play the crucial role in determining the magnitude of membrane potential as well as for many biological functions such as drug application [6,7].

The membrane potential derives ultimately from two factors: electrical potential and chemical potentials (diffusion). Electrical potential arises from the mutual attraction between particles with opposite electrical charges (positive and negative) and the mutual repulsion between particles with the same charge. Diffusion (chemical)

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potential arises from the tendency of particles to diffuse from regions of higher concentration to regions where the concentration is low [8].

Another electrical property of membrane is its conductivity that helps in the evaluation of thermodynamic activation parameters. Membrane conductivity refers to the capability of a cell to allow ions to permeate as well as accumulation of ions in the interior of the membrane. Both membrane potential and conductance can help in the investigation of ion transport properties of membranes [9].

In nature, ion transport plays a vital role in the intra-cellular movement. Different studies were done on ion transport across sBLM and the factors that affect it. For example, transport of ions such as Na^+, K^+ Mg^{2+}, Ca^{2+}, Cu^{2+} and Zn^{2+} can be affected with various drugs such as captopril, quercetin, cholicalciferon cyclosporin A, etc. [8,10-15].

Biological membranes

Biological membranes are basic components of all living systems. These are composed mainly of lipids and are known to be asymmetric in lipid composition of their leaflets. A model of biological membrane is bilayer lipid membrane consisting of two lipid monolayers sandwiched together in which the hydrophobic tails interact in the interior of the membrane and the hydrophilic head groups comprise both the outer surfaces [5].

Model membranes

Phospholipid bilayer membranes represent a useful model system to investigate basic aspects of the lipid bilayer which is components of biological cell membranes and, particularly, to study the ionic transport processes. The molecules that form the bilayer of cell membrane are Phosphatidylcholine (PC) and Phosphatidylserine (PS).

Phosphatidylcholine molecule has a zwitterionic head group that consists of a negatively charged phosphate and a positively charged choline group (Figure 1). It is more abundant and inexpensive than other lipids. This molecule can form bilayers and is considered as a model of a biological membrane on substrate. It can also form a bilayer in aqueous solution spontaneously with the hydrophilic headgroups in contact with water and hydrophobic alkyl chains inside the bilayer (Figure 2).

The supporting substrate, like silicon, glass, quartz, metals, mica, polymers, etc., can be used to increase the strength and mechanical

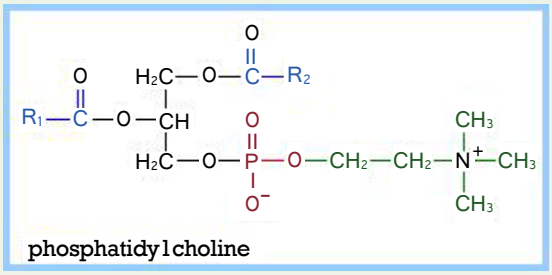

Figure 1: Structure of Phosphatidylcholine (http://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb1/part2/lipid.htm accessed on 11/12/2013).

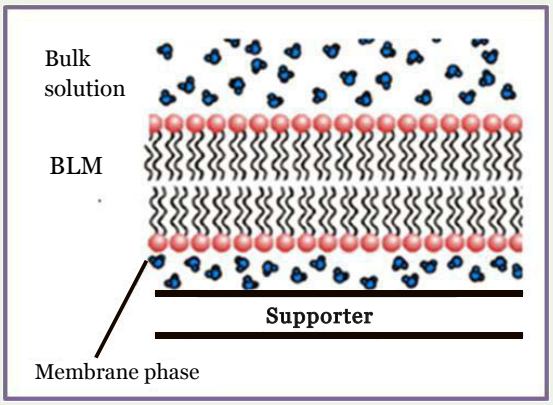

Figure 2: Diagram of solid supported phospholipids bilayer membrane [26].

stability of the BLM. The electrical properties of lipid bilayers have been characterized by experimental techniques such as fluorescence microscopy, X- ray diffraction, Impedance Spectroscopy, electron microscopy, atomic force microscopy, nuclear magnetic resonance spectroscopy, potential and conductance measurement, etc. [16,17]. All of the above techniques strongly depend on the physico-chemical conditions, such as ionic strength, pH of the aqueous solvent, temperature, besides the structural arrangement of the hydrophobic lipid matrix. Different techniques such as, potential measurements, titration techniques, conductance measurements, and electrokinetic measurements have been applied to study the ionization of lipid headgroups. The majority of electrokinetic studies have been performed at lipid vesicles in aqueous electrolyte solutions. This gives information on the lipid density, diffusion, the lipid headgroups, alkyl chain orientation, the electrostatic potential, the dipole moment of the membrane, and ion binding to the lipid membrane [18-22].

Electrical Properties of Membrane

Membrane potential

Membrane performance can be characterized by measuring the membrane potential and conductance. By definition, the membrane potential is the potential difference that is generated between two solutions of the same electrolyte at different concentrations separated by a charged membrane within two compartments [23]. The membrane potential can be expressed by the difference between internal and external potential in the membrane [24]. However, it is clear that, as a result of concentration gradient across the membrane, the concentrations of counterions at the membrane-solution interfaces at both sides show variations during the membrane potential measurements. It has been also reported in the literature that membranes with none uniformly distributed fixed charges can have different ion selectivity compared to membranes with uniformly distributed charges. Membrane potentials in cells are determined primarily by factors such as concentration of ions on the inside and outside of the cell and the permeability of the cell membrane to these ions [25]. The membrane potential data helps in the evaluation of the magnitude of effective fixed charge density which is one of the selectivity parameters charged membranes [26].

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Membrane conductance

Membrane conductivity is the measurement of a cell membrane's potential for both permeation and resistance to being permeated in which membrane can have both permselectivity and permeability property [8]. It is an electrical term meaning the reciprocal of resistance that mainly depends on concentration and temperature of bathing electrolyte. There are three types of membrane conductivity that are measured; the first one is the ability of all solutes such as water and solute to permeate a cell wall without resistance. The second form of membrane conductivity is semi-permeable, in which the membrane is impermeable to solutes and absorbed interior of cell membrane but permeable to biological molecules such as water. The last form of conductivity is impermeable, in which no form of solute can permeate but absorbed interior of cell membrane [9].

Although the electrical properties of lipid bilayers, which are of fundamental importance in many areas of biology, have been characterized by means of different experimental techniques such as, electron microscopy and electrochemical impedance spectroscopy (EIS), the basic mechanism of the ion translocation and ion permeation responsible for the observed, relatively low conductance is not yet completely understood, and the fact that the electrical conductance reflects different averaged processes at a macroscopic level [10,11]. The conductance of biological membranes is much higher, typically by several orders of magnitude than model membrane. The reason is that there are all kinds of ion channels and other pores penetrating the membrane and allowing additional currents to flow. This is due to the more selective behavior of biological membrane towards ion found around it [12]. We can obtain the values of activation parameter such as change in entropy, enthalpy and free energy from membrane conductance which indicate permeability as well as permselectivity of model membrane. The earlier studies showed that low value of free energy is related to spontaneous flow of ions into the model membrane and high value of entropy results in high magnitude of membrane conductance [15].

Ion Transport across Lipid Bilayer and Natural Membranes

Phospholipids bilayer membranes represent a useful model system to study basic aspects of passive ionic transport across the lipid bilayer components of biological cell membranes [12]. In the simplest version of the transport model of ions in layered or confined structures, ions must diffuse up to the membrane, adsorb, cross the membrane pore, desorbs, and finally diffuse away on the other side of the membrane (Figure 3). Such a transport is basically dependent on the size of permeate species as well as on the extent of permeate-fixed charge group interactions. Among the different steps describing the overall transport of an ion across the lipid membrane, the adsorption and desorption processes are particularly dependent on the chemical potential and voltage drops in the polar and non polar regions and on the thickness of the double layer at the surface of the charged membrane. The flux of cations and, to a lesser extent, anions can be qualitatively justified by a diffusion process through short-lived, water-filled pores formed as consequence fluctuations in the lipid organization that allow ions to pass throughout the bilayer [13].

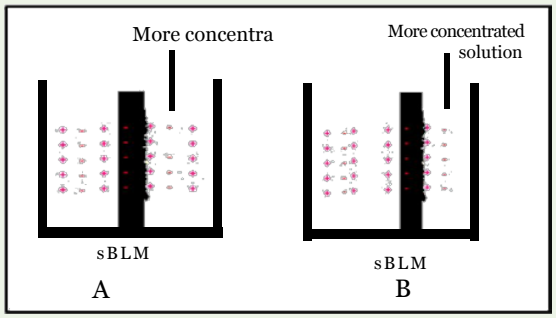

Figure 3: Transport of ion across model membrane; A) movement of ion from high concentration to lower; B) after cation transported towards lower concentration

Ion transport across biomembranes is associated with various biofunctions, such as, metabolism, photosynthesis, neurotransmission [27]. The fluid on both sides of the membrane contain high concentrations of mobile macro ions such as, sodium (Na^+), potassium (K^+), chloride (Cl^-), calcium (Ca^{2+}) and micro ions such as Mn^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, and Co^{2+} [8,28]. These ions are transported across cell membrane, but anions such as bicarbonate, sulfate, oxalate, formate, etc., are not transported easily, probably, because they consists nuclei of different elements and they are highly hydrated as a result of which these species face difficulty in getting transported across sBLM. [8,13,29-30].

Transport of iron in natural membrane

Iron compounds have been classified as water soluble (ferrous sulfate, ferrous gluconate, ferrous lactate, ferrous ammonium sulfate), poorly water soluble but soluble in diluted acids (ferrous fumarate, ferrous succinate, ferric sacharate), poorly soluble in water or acid solutions (ferric pyrophosphate, ferric orthophosphates) and protected compounds (hemoglobin). Ferrous sulfate is the most common type of iron supplement. Other available forms include ferrous fumarate, ferrous succinate, ferrous gluconate, ferrous lactate, ferric ammonium citrate, ferrous glycine. Most of the time iron can be taken by human body in the form of ferrous rather than ferric because it is easily absorbed by cell membrane and soluble at physiological condition which is difficult for ferric [31].

Transport of iron across a cell membrane is important to human beings. But its transport as well as absorption can be affected by drugs such as anti-acids, Aspirin, Aminosalicylic acid Cholestyramin, Colestipol, Amino salicylic acid, Allopurinol, Acetohydroxamic acid which is taken for different disease such as, anti-inflammatory, tuberculosis and others [32]. Iron can be absorbed by the enterocyte in the duodenum and upper jejunum in the apical membrane with in different form.

First, consider absorption of iron in the ferrous and ferric forms (example, iron salts). Dietary Fe^{3+} (ferric) forms are converted to the Fe^{2+} (ferrous) forms in the stomach. This reduction is greatly promoted by the presence of H^+ and dietary ascorbic acid. The great advantage of this conversion is that the ferrous form as compared to the ferric form is much more easily released from the organic ligands to which

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it is bound and stays soluble [33]. Ferric iron precipitates at pH >3 (as found in the duodenum) and is not available for absorption from such precipitates. Ferrous iron remains soluble up to pH values of about 7.5 and is available for absorption. In addition at the low gastric pH, some substances (example, some amino acids) can bind with ferric iron to form a soluble chelate from which iron can be absorbed in the duodenum [34]. So the ideal situation is either reduction of ferric iron to ferrous iron, which can soluble at physiological pH in the duodenum, or the formation of soluble chelates from which ferric iron can be readily released at the apical membrane of the duodenal enterocyte. Both these processes are facilitated by a low gastric pH. The absorption pathway in the membrane involves a ferrireductase which converts any free ferric iron into the ferrous form. The ferrous form is then transported across the membrane and easily absorbed [34,35].

Second, consider the absorption of haem. Dietary haemoglobin and myoglobin is degraded releasing haem. Haem is soluble in the alkaline duodenal contents but almost insoluble at a pH < 6. It is readily absorbed as an intact metallo-porphyrin by the mucosal cells by a process involving a haem receptor. Haem is broken down in the enterocyte by haem oxygenase releasing the Fe^{2+} and easily undergo absorption [35,36]. Different vitamins facilitate absorption or transport of iron in cell membrane; for example, Vitamins A and C improve absorption as well as transport of iron. As the previous study shows there was a significant increase in iron absorption when both vitamins were present in diet [36,37].

Effect of Drug on Membrane Transport

Drugs can bind to lipid membranes and potentially modulate the physical properties of that membrane. However, the cell membrane is a carefully balanced environment and any changes inflicted upon its structure by a drug molecule must be considered in conjunction with the overall effect which may affect the function and integrity of the membrane [38].

Cyclosporine A (CsA) is a drug which is used to prevent graft rejection after organ transplantation. Its interactions with phosphatidylcholine and cholesterol can affect transport of ion across cell membrane by affecting porosity, selectivity, and can also modulate peripheral lipid-protein interactions [39,40]. Most of the time molecular targets for drugs are proteins (mainly enzymes, receptors and transport proteins), pores of fixed sites and nucleic acids. The interaction of a drug with a macromolecular target involves a process known as binding. There is usually a specific area of the macromolecule where this takes place, and this is known as the binding site. Typically, this takes in the form of a hollow or canyon on the surface of the macromolecule allowing the drug to sink into the body of the larger molecule [41].

Some drugs react with the binding site and become permanently attached via a covalent bond. However, most drugs interact through weaker forms of interaction known as intermolecular bonds. These include electrostatic or ionic bonds, hydrogen bonds, Vander Waals interactions, dipole-dipole interactions and hydrophobic interactions. It is also possible for these interactions to take place within a molecule that called intermolecular bonds. None of these bonds is as strong as the covalent bonds that make up the skeleton of a molecule, and so they can be formed, and then broken again. This means that equilibrium takes place between the drug being bound and unbound to its target. The binding forces are strong enough to hold the drug for a certain period of time to let it have an effect on the target, but weak enough to allow the drug to depart once it has done its job. The length of time the drug remains at its target will then depend on the number of intermolecular bonds involved in holding it there. Drugs having a large number of interactions are likely to remain bound longer than those that have only a few. The relative strength of the different intermolecular binding forces is also an important factor. Functional groups present in the drug can be important in forming intermolecular bonds with the target binding site. If they do so, they are called binding groups. However, the carbon skeleton of the drug also plays an important role in binding the drug to its target. The specific regions where this takes place are known as binding regions [42].

Vitamin B6, which is called pyridoxine, is one of vitamins B complex. Physician advice vitamin B complex for management of anemia because it increases absorption of different vitamins for human body, all vitamins B help the body to convert food (carbohydrates) into fuel (glucose), which is burned to produce energy. Vitamins B complex are necessary for healthy skin, hair, eyes, liver and body metabolize fats and protein. They also help the nervous system function properly. Vitamin B6 also helps the body to make several neurotransmitters, chemicals that carry signals from one nerve cell to another. It is essential for normal brain development and function, and helps the body make the hormones serotonin and norepinephrine and melatonin (which helps regulate the body clock) [43]. It is important for the proper functioning of enzyme reactions, for amino acid synthesis, metabolism of fatty acids, synthesis of prostaglandins, manufacture of all amino acid, dopamine, epinephrine as well as in the conversion of tryptophan to vitamin B3, and for the transportation of magnesium and zinc across cell membranes. It is important for the health and proper functioning of the immune system, the skin and mucous membranes.

Along with vitamins B12 and B9 (folic acid), B6 helps control levels of homocysteine in the blood. Homocysteine is an amino acid that associated with heart disease. B6 is also necessary for the production of red blood cells and cells of the immune system, its deficiency includes muscle weakness, nervousness, irritability, depression, difficulty concentrating, and short-term memory loss, skin problems, cracking of the lips and tongue, anemia, peripheral neuropathy, insomnia, and depressed immune function [44] . Pyridoxine is also used to prevent or treat a nerve disorder caused by certain medications (such as isoniazid), to treat certain hereditary disorders (such as xanthurenic aciduria (genetic disorder), cystathioninuria (metabolic disorder). and in the production of DNA and RNA, the body's genetic material [43-45]. Even though this drug has so many advantages, little information is available on its effect on iron transport across sBLM.

The effect of combined pyridoxine and riboflavin deficiency on tissue iron content was studied in rats, which were given in the form of diet. Iron concentration of pyridoxine and riboflavin deficient rats was lower. The obtained results suggest that riboflavin and pyridoxine

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deficiency can damage the absorption and utilization of iron [46]. Finally understanding the effect of drug in which it interacts with cell membrane has critical importance in pharmacological science which used to know the toxicity as well as its efficiency. Embedment of the drug molecules into the hydrophobic region of lipid bilayer decreases the rate of the performance of lipid and consequently, reduction of membrane free volume. Experimental work on model membrane has demonstrated that the membrane properties can strongly affected by the presence of membrane associated molecules as well as the presence of drug. Examples of parameters that can be affected by drug-membrane interactions include the conformation of acyl groups, the membrane surface and thickness, the membrane potential, the porosity behavior and the membrane fusion proteins [47].

Conclusion

Drugs affect the selective transport of ions across membrane. Supported bilayer lipid membrane has been found to become more selective in the presence of drugs as a result the membrane potential gradually increased with decrease in drug concentration. In addition the effective charge densities become higher in the presence of this drug. It would be possible to increase the selectivity behavior of model membrane towards specific cations in the presence of a given drugs with incorporating of ion channel that increasing both permeability and permselectivity properties model membrane which is special property of membrane.

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