EDO UNIVERSITY IYAMHO
Department of Biochemistry

BCH 316: Biomembrane

Instructor: Dr. Olulope Ajayi, email: olulope.olufemi@edouniversity.edu.ng

Lectures: Wednesday (2-3 pm), Friday (10 am-12 pm). LT6. Phone (+234)8063737930

Office hours: Monday-Friday (8.00 am-4.00 pm). Office: New College of Medical Sciences and Faculty of Engineering Office Block, Floor 2 Rm. AD 82

General overview of the Course

This course covers the central dogma of membrane biology (the fluid mosaic model), Membrane functions, types and composition: Lipid structure, properties and formation of the bilayer; protein and carbohydrates. Membrane structure and integrity. Membrane asymmetry and movements, diffusion, rotation and fluidity. Isolation and identification electron microscopy and marker enzyme assays. Introduction to receptor function: antigenicity of membrane components. Cell membrane and toxins, transport processes, action of polymyxin and ionophores. Introduction to neurotransmission. Membrane transport system- active versus passive transport systems. Transport of sugars and amino acids. Defence mechanism in parasites. Biomembranes of parasites.

Intended Learning Outcomes

At the end of this course, students should be able;

1. To define biomembrane
2. To list and discuss constituents of biomembrane
3. To discuss the functions of biomembrane
4. To discuss diffusion of biomembranes lipids
5. To discuss the roles of integral proteins
6. To discuss artificial membrane and its applications
7. To discuss membrane fusion
8. To explain the interaction of toxins with biomembranes
9. To describe defense mechanisms in parasites

Assignments: We expect to have 5 individual homework assignments throughout the course in addition to a Mid-Term Test and a Final Exam. Home works are due at the beginning of the class on the due date. Home works are organized and structured as preparation for the midterm and final exam, and are meant to be a studying material for both exams. There will also be 2 term

papers are expected to be written by individuals taking this course. This is aimed at broadening studentôs knowledge of the course.

Grading: We will assign 10% of this class grade to homeworks, 10% for the term papers, 10% for the mid-term test and 70% for the final exam. The Final exam is comprehensive.

Textbooks: The recommended textbooks for this class are as stated:
Title: Lehninger Principles of Biochemistry
Authors: David L. Nelson, Michael M. Cox
Publisher: Addison-Wesley 2nd edition
ISBN-13: 9781464126116

Title: Lippincottôs Illustrated Reviews Biochemistry

Author: Denise R. Ferrier

Publisher: Lipincott Williams & Wilkins

ISBN: 978-1-4511-7562-2

Title: Harperôs Illustrated Biochemistry. 28th edition

Authors: Robert K. Murray, Daryl K. Granner, Victor W. Rodwell

Publisher: McGraw Hill Lange

MAIN LECTURE

Topic: Membrane Biochemistry: Introduction

What are membranes?

Membranes are complex molecules composed of proteins, lipids and carbohydrate molecules. They are sheet-like enclosed structures consisting of an asymmetric lipid bilayer with distinct inner and outer surfaces. These sheet-like structures are formed spontaneously in water due to the amphipathic nature of lipids. The membranes contain numerous proteins that carry out specific functions. They are structures that define and control the composition of the space that they enclose.

Membrane Lipids

The major lipids in mammalian membranes are; Phospholipids, Glycosphingolipids and Sterol (Cholesterol)

The Phospholipids

There are two major classes of phospholipids; Phosphoglycerides and Sphingophospholipids.

Phosphoglycerides

They are the more common class of phospholipid and consist of a glycerol backbone to which two fatty acids are attached in ester and a phosphorylated alcohol. The fatty acid constituents are usually even-numbered carbon molecules, most commonly containing 16 or 18 carbons. They are unbranched and can be saturated or unsaturated with one or more cis double bonds. The simplest phosphoglyceride is phosphatidic acid

Sphingophospholipids

This is the second major class of phospholipids. Sphingophospholipids contain the sphingosine backbone rather than glycerol. Sphingomyelin is prominent in myelin sheaths. A fatty acid is attached by an amide linkage to the amino group of sphingosine forming a ceramide. The primary hydroxyl group (-OH) of sphingosine is esterified to phosphorylcholine forming sphingomyelin.

Sterols

Cholesterol is the most common sterol in mammalian membranes. It is found in the plasma membranes of mammalian cells and to a lesser extent in certain organelles such as mitochondria, golgi complexes and also in nuclear membranes. Cholesterol intercalates among the phospholipids of the membrane with its hydroxyl group at the aqeous interface and the remainder of the molecule within the leaflet. Saturated fatty acids have straight tails, whereas unsaturated fatty acids, which generally exist in the *cis* form in membranes, make kinked tails. As more kinks are inserted in the tails, the membrane becomes less tightly packed and therefore more fluid.

Lipid bilayer: This is a thin bimolecular sheet of mainly phospholipid molecules that form the structural basis for all cell membranes. The amphipatic nature of phospholipids suggests that the two regions of the molecule (hydrophilic head and hydrophobic tail) have incompatible solubilities.

Membrane proteins

Proteins are the major functional molecules of membranes and consist of; enzymes, pumps, channels, structural components, antigens, receptors. They are vital components of the

membrane lipid bilayer. The hydrophilic nature of peptide bond is minimized by the helical structure of proteins. Their hydrophilic regions protrude at the inside and outside faces of the membrane but connected by a hydrophobic region that traverses the hydrophobic core of the membrane bilayer. Membrane proteins are of two types

Integral: They are located within the membrane

Peripheral: They are located on the surface of membrane

The Integral Proteins

They constitute a high percentage of membrane protein, interact extensively with the phospholipids and require the use of detergents for their solubilization. They span the membrane bilayer .They are asymmetrically distributed across the membrane bilayer. They are usually globular and are amphipathic

The Peripheral Proteins

They are bound to the hydrophilic regions of specific integral proteins. They do not directly interact with the hydrophobic cores of the phospholipids in the bilayer.

Functions of the Biomembranes

1. They keep toxic substances out of the cell
2. They contain receptors and channels that allow specific molecules, such as ions, nutrients, wastes, and metabolic products, that mediate cellular and extracellular activities to pass between organelles and between the cell and the outside environment
3. They separate vital but incompatible metabolic processes conducted within organelles.

Topic: Membrane Fluidity

Membrane fluidity is the ability of membrane to take on a more liquid-like or fluid arrangement. Membrane fluidity is a function of temperature and lipid composition

Temperature

The hydrophobic chains of the fatty chains can be aligned or ordered to provide a rather stiff structure in a bilayer lipid.

Increase in temperature causes the hydrophobic side chains to undergo a transition from ordered state to a fluid arrangement. The temperature at which the structure undergoes the transition from the ordered state to disordered state is called the transition temperature (Tm).

Lipid Composition

The multiple (unsaturated) bonds that exist in the cis configuration increase the fluidity of a bilayer by decreasing the compactness of the side chain. Cholesterol reduces the fluidity of membranes. Transition temperature is however indistinguishable at a high cholesterol-phospholipid ratio.

The fluidity of a membrane significantly affects its functions. As membrane fluidity increases, so does its permeability to water and other small hydrophilic molecules. The lateral mobility of integral proteins increases as the fluidity of the membrane increases.

Diffusion of membrane lipids

Flexibility is a notable feature of biomembranes. Membrane flexibility is their ability to change shape without losing their integrity and becoming leaky. It is a peculiar quality of biomembranes. Membrane flexibility is made possible by the non-covalent interactions among the lipid bilayers and the motions allowed to individual lipids. Sterols reduce membrane flexibility. The rigid planar structure of the steroid nucleus inserted between fatty acyl side chains reduces the freedom of neighboring fatty acyl chains to move by rotation about their fully extended conformation.

Types of diffusion of membrane lipids

1. Flip-flop diffusion/ transbilayer: This is uncatalyzed transverse diffusion
2. Catalysed transverse diffusion
3. Uncatalysed lateral diffusion

Flip-flop diffusion

Flip-flop occurs slowly within a temperature range of 20-40^{o}C. It requires that a polar/charged head group leave its aqueous environment and move into the hydrophobic interior of the bilayer. This type of movement becomes necessary during the synthesis of the bacterial plasma membrane, phospholipids are produced on the inside surface of the membrane and must undergo flip-flop diffusion to enter the outer leaflet of the bilayer. The flippases facilitates flip flop diffusion this provides transmembrane path that is energetically more favourable and much faster than the uncatalyzed movement

Topic: Artificial membrane and membrane fusion

Artificial membranes are synthetically made membranes usually for separation purpose. They are produced from organic compounds i.e. cellulose nitrate or acetate, inorganic compounds i.e. alumina etc. Separation depends factors such as the chemical properties, physical properties, nature of separated particles and choice of driving force

Application of artificial membrane

1. Dehydrogenation of natural gas

2. Removal of microorganisms from products (dairy product)
3. Water purification-reverse osmosis,
4. Microfilteration: Membrane pore size (0.1-10μm) Process of separating material of colloidal size. Ultrafilteration. Membrane pore size (0.1-0.01 μm)
5. Dialysis

Advantages of artificial membranes

1. The possibility of varying the lipid content of the membranes. This allows systematic assessment of the effects of varying lipid composition on certain function
2. Purified membrane proteins/enzymes can be integrated into these vesicles in order to assess the required factors for the proteins to reconstitute
3. The environment of these systems can be rigidly controlled and systematically varied (e.g. ion concentrations, ligands)

Liposomes

They are small artificial vesicles of spherical shape that can be created from cholesterol and natural non-toxic phospholipids.

Their usefulness in drug delivery is as a result of its size, hydrophobic and hydrophilic characters. Liposomes can also be made to entrap certain compounds inside themselves. Examples are drugs and isolated genes. Liposomes can be used to distribute drugs to certain tissues. If certain antibodies to certain cell surface molecules could be incorporated into liposomes so that they would be targeted to specific tissues or tumors, the therapeutic impact would be considerable. DNA entrapped inside liposomes appears to be less sensitive to attack by nucleases.

Membrane Fusion

This is the coming together of two membranes without loss of continuity. Mechanisms involving membrane fusion are seen in exocytosis (release of neurotransmitters), endocytosis, cell division, fusion of egg and sperm cells and entry of membrane-enveloped virus into its host cell

Requirements for membrane fusion

1. They must recognize each other
2. Their bilayer structures become locally disrupted resulting in fusion of the outer leaflet of each membrane (hemifusion)
3. Their surfaces become closely apposed which requires the removal of water molecules normally associated with the polar head groups of lipids
4. The fusion process is triggered at the appropriate time or in response to specific signal
5. Their bilayers fuse to form a single continuous bilayer

Instances of membrane fusion

1. The entry of an enveloped virus (influenza virus) into a host cell
2. The release of neurotransmitters

The entry of an influenza virus into a host cell

The influenza virus is enveloped by a membrane containing hemagglutination (HA) protein. The virus enters the host cell by inducing endocytosis at a pH of about 5. The low pH causes a conformational change in the HA which exposes a sequence within the HA protein, this enables the protein penetrate the endosomal membrane. Thus, the endosomal membrane and the viral membrane are connected through the HA protein. The HA protein bends at its middle forming a hairpin shape, bringing its two ends together, this pulls the two membranes into close apposition, causes fusion of the viral membrane and the endosomal membrane

The release of neurotransmitters

Neurotransmitters are released at synapses when intracellular vesicles loaded with neurotransmitter fuse with the plasma membrane. This process involves a family of proteins called SNARES.

Types of SNARES

1. v-SNAREs: SNAREs in the cytoplasmic face of the intracellular vesicles
2. t-SNAREs: those in the target membranes with which the vesicles fuse
3. Other proteins involved: SNAP25 and NSF

During fusion, v- and t- SNAREs bind together and undergo a structural change that produces a bundle of long thin rods made up of helices from both SNARES and two helices from SNAP25. This structural change pulls the two membranes into contact and initiates the fusion of their lipid bilayers.

Topic: Communication through Biomembranes (Part 1)

Intended learning outcomes

1. To discuss the transport types in membranes
2. To discuss transporters

Transport types

1. Passive (Diffusion); Simple or facilitated via ion channels and transporters
2. Active transport

Passive transport (Diffusion)

Simple Diffusion

The passive flow of a solute from a higher to a lower concentration due to random thermal movement. It depends on factors such as the thermal agitation of the specific molecule, concentration gradient across the membrane i.e. transmembrane gradient of the substrate and the solubility of that solute in the hydrophobic core of the membrane bilayer.

Facilitated diffusion

The passive transport of a solute from a higher to a lower concentration mediated by specific protein transporter. Hydrophilic molecules that cannot freely pass through the lipid bilayer membrane do so passively by facilitated diffusion or by active transport. Facilitated diffusion is explained by the ping-pong mechanism. In the ping state, it is exposed to high concentrations of solute and molecules of the solute bind to specific sites on the carrier protein. Binding induces a conformational change that exposes the carrier to a lower concentration of solute (pong state).

This process is reversible and the net flux across the membrane depends upon the concentration gradient.

Rate of entrance of solutes into cell by facilitated diffusion is determined by the

1. Concentration gradient across the membrane
2. Amount of carrier available
3. Affinity of the solute-carrier interaction
4. Rapidity of the conformational change for both the loaded and the unloaded carrier

Factors affecting diffusion of a substance

1. Concentration gradient across the membrane. Solutes move from high to low concentration
2. The electrical potential across the membrane toward the solution that has the opposite charge. The inside of the cell usually has a negative charge
3. The permeability coefficient of the substance for the membrane
4. The hydrostatic pressure gradient across the membrane. Increased pressure will increase the rate and force of the collision between the molecules and the membrane
5. Temperature. Increased temperature will increase particle motion and thus increase the frequency of collision between external particles and the membrane
6. Facilitated diffusion involves certain transporters or ion channels. A multitude transporters and channels exist in biological membranes that route the entry of ions into and out of cells

Active transport

This is the transport of a solute across a membrane against a concentration gradient. It requires energy (frequently derived from the hydrolysis of ATP).

Resemblance of Passive and active transport with substrate-enzyme interaction

1. There is a specific binding site for the solute
2. The carrier is saturable, so it has a maximum rate of transport
3. There is a binding constant (Km) for the solute and so the whole system has a Km
4. Structurally similar competitive inhibitors block transport.

Transporters

Are membrane proteins that speed the movement of a solute across a membrane by facilitating diffusion. They are classified into carriers and channels

Carriers

Catalyze transport at rates below the limits of free diffusion, bind substrates with high stereo specificity, are saturable just like enzymes and function as monomeric proteins

Channels

They show less stereospecificity than carriers. They are usually not saturable. They generally allow transmembrane movement at higher rates than carriers. They are oligomeric complexes of several identical subunits. The permeability of a channel depends on the size, extent of hydration and extent of charge density on the ion

Transport systems

Uniport system: Moves one type of molecule bi-directionally

Co-transport system: the transfer of one solute depends upon the stoichiometric simultaneous or sequential transfer of another solute

Symport system: moves two solutes in the same direction. Examples are the proton-sugar transporter in bacteria and the Na^+ -sugar transporters (for glucose and certain other sugars) and Na^+ -amino acid transporters in mammalian cells.

Antiport system: move two molecules in opposite directions (eg, Na^+ in and Ca^{2+} out).

Ion channels

They are very selective, in most cases permitting the passage of only one type of ion (Na^+, Ca^{2+}, etc).

Properties of Ion Channels

1. They are composed of transmembrane protein subunits.
2. Most are highly selective for one ion; a few are nonselective
3. They allow impermeable ions to cross membranes at rates approaching diffusion limits.
4. They can permit ion fluxes of 106ï 107/s.
5. Their activities are regulated.
6. The main types are voltage-gated, ligand-gated, and mechanically gated.
7. They are usually highly conserved across species.
8. Mutations in genes encoding them can cause specific diseases.
9. Their activities are affected by certain drugs.

Ionophores

Are molecules that act as membrane shuttles for various ions. They contain hydrophilic centers that bind specific ions and are surrounded by peripheral hydrophobic regions. This allows the molecules to dissolve effectively in the membrane and diffuse transversely therein. Aquaporins are proteins that form water channels in certain membranes.

Topic: Communication through Biomembranes (Part 2)

Intended learning outcomes

1. To discuss active transport system

2. To discuss endocytosis and exocytosis

Active Transport System

This transport system requires that molecules are transported against concentration gradients. They require energy which can come from hydrolysis of ATP, electron movement and light. About 4 major classes of ATP-driven active transporters have been recognized (P, F, V and ABC transporters). Examples of the P class is the Na+K+ ATPase and Ca2+ATPase of muscle. The second class is referred to as F-type (example is mt-ATP synthase. The V-type active transporters pump protons into lysosomes and other structures. ABC transporters include Cystic fibrosis transmembrane regulator protein (CFTR protein), a chloride channel implicated in the causation of cystic fibrosis. Multidrug resistance-1 protein (MDR-1 protein) which pump a variety of drugs, including manyanti-cancer agents out of the cells

Plasma membrane Na+-K+- ATPase

This is an important enzyme that regulates the intracellular concentration of Na+ and K+. Usually, cells maintain a low intracellular Na+ concentration and a high intracellular K+ concentration coupled with a net negative electrical potential inside. ATPase is the pump that maintains these ionic gradients and is activated by Na+ and K+. It pumps Na+ out and K+ into cells. The ATPase is an integral membrane protein containing a transmembrane domain allowing the passage of ions and cytosolic domains that couple ATP hydrolysis to transport. It has catalytic centers for both ATP and Na+ on the cytoplasmic side of the plasma membrane with K+ binding sites located on the extracellular side of the membrane.

The phosphorylation by ATP of three Na+-binding sites on the cytoplasmic surface of the cell induces a conformational change in the protein leading to transfer of 3 Na+ ions from the inner to the outer side of the plasma membrane. 2 molecules of K+ bind to sites on the protein on the external surface of the plasma membrane resulting in dephosphorylation of the protein and transfer of the K+ ions across the membrane to the interior. Therefore, 3 Na+ ions are

transported out for every 2 K+ ions entering. This creates a charge difference between the intra and extracellular compartments, making the intracellular compartment more negative. ATPase is inhibited by cardiac drugs such as oubain and digitalis. The Na+-K+-ATPase can be coupled to various other transporters such as those involved in transport of glucose

Endocytosis

This is a process by which cells take up large molecules. It is a mechanism for regulating the content of certain membrane components e.g. hormone receptor. It can be used to study how cell function. DNA transfection depends on endocytosis. It is responsible for the entry of DNA into the cell.

Endocytosis requires energy (usually from ATP), Ca^{2+}, and contractile elements in the cell (possibly the microfilament system). Endocytotic vesicles are generated when segments of the plasma membrane invaginate, enclosing a small volume of extracellular fluid and its contents. The vesicle pinches off when the fusion of plasma membranes seals the neck of the vesicle at the original site of invagination. This vesicle fuses with other membrane structures thereby ensuring the transport of its contents to other cellular compartments. Most endocytotic vesicles fuse with primary lysosomes to form secondary lysosomes which contain hydrolytic enzymes hence, are specialized organelles for intracellular disposal

Types of Endocytosis

1. Phargocytosis
2. Pinocytosis

Phargocytosis

Phargocytosis involves the ingestion of large particles such as viruses, bacteria, cells or debris and granulocytes. It occurs only in specialized cells such as macrophages. Macrophages may ingest 25% of their volume in an hour

Pinocytosis

This is a property of all cells that leads to the cellular uptake of fluid and fluid contents.

Types

1. Fluid-phase pinocytosis. It is a non selective process in which the uptake of a solute by formation of small vesicles is simply proportionate to its concentration in the surrounding extracellular fluid. Fibroblasts are examples, they internalize their plasma membrane at about one third the rate of macrophages
2. Absorptive Pinocytosis. This is a receptor-mediated selective process primarily responsible for the uptake of macromolecules for which there are fixed numbers of binding sites on the plasma membrane. The vesicles formed are derived from invaginations that are coated on the cytoplasmic side with a filamentous material called

coated pits. Absorptive pinocytosis of extracellular glycoproteins requires that the glycoproteins carry specific carbohydrate recognition signals. These recognition signals are bound by membrane receptor molecules which play a role analogous to that of the LDL receptor. A galactosyl receptor on the surface of hepatocytes is helpful in the absorptive pinocytosis of asialoglycoproteins from circulation. Moreover, acid hydrolases taken up by absorptive pinocytosis in fibroblasts are recognized by their mannose 6-phosphate moieties. Viruses which cause hepatitis, poliomyelitis and AIDS initiate their damage by absorptive pinocytosis. Moreover, iron toxicity also begins with excessive uptake due to endocytosis

Exocytosis

This releases certain macromolecules from the cell to extracellular space. It is involved in membrane remodeling when the components synthesized in the golgi apparatus are carried in vesicles to the plasma membrane. Signal for exocytosis is usually hormone which when it binds to a cell-surface receptor, induces a local and transient change in Ca^{2+} concentration. Ca^{2+} triggers exocytosis.

Neurotransmission

Membranes of the neurons have an asymmetry of inside-outside voltage (electrical potential). They are electrically excitable as a result of the presence of voltage-gated channels. Hence, appropriate stimulation by a chemical signal mediated by a specific synaptic membrane receptor, opens the channels in the membrane to allow the rapid entry of Na+ or Ca^{2+} so that the voltage difference rapidly collapses and the segment of the membrane is depolarized. The gradient is quickly restored by the action of the ion pumps present in the membrane. When large areas of the membrane are depolarized in this manner, the electrochemical disturbance propagates in wave-like form down the membrane, generating a nerve impulse.

Myelin sheets, formed by Schwann cells, wrapped around nerve fibers provide an electrical insulator that surrounds most of the nerve and greatly speeds up the propagation of the wave (signal) by allowing ions to flow in and out of the membrane only where the membrane is free of the insulation (at the nodes of Ranvier). The myelin membrane is highly rich in lipids, this confers a tremendous insulating property on it. Demyelination and impaired nerve conduction are features of certain diseases such as Multiple sclerosis and Guillain-Barr syndrome

Glucose transport

The entrance of glucose into the cell is the first step in energy utilization. A number of glucose transporters are involved depending on each tissue. Glucose enters the erythrocyte by facilitated diffusion called GLUT1. In the skeletal muscle and adipocytes, glucose enters by a specific transport system enhanced by insulin. Glucose transport in the small intestine involves the binding of Na+ and glucose to different sites on a Na+-glucose symporter located at the apical surface. Na+ moves into the cell down its electrochemical gradient and drag glucose with it.

Therefore, the greater the Na+ gradient the more glucose enters and if Na+ in extracellular fluid is low, glucose transport stops. In a bid to ensure a steep Na+ gradient, this Na+-glucose symporter is dependent on gradients generated by the Na+-K+- ATPase which maintains a low intracellular Na+ concentration. The transcellular movement of glucose in this case involves one additional component; a glucose uniporter: which allows the glucose accumulated within the cell to move across the basolateral membrane

Topic: Isolation of sub-cellular organelles

Intended learning outcomes

1. To review cellular organelles
2. To discuss the process involved in the isolation of cellular organelles

Sub-cellular organelles

Organelles are organized structures within the cell e.g. the nucleus, mitochondria, ribosomes, golgi bodies, endoplasmic reticulum etc. They are separated by centrifugation based on the difference in their size, density, shape

Steps in the isolation of cell organelles

1. Homogenization
2. Sub-cellular fractionation by centrifugation
3. Marker assays

Homogenization

This is the disruption of cells under conditions that prevent deterioration aimed at isolation of morphologically intact and functionally active organelles and microsomal fractions. It is the first step in the isolation of sub-cellular organelles. It involves breaking the cell membrane

Common homogenization techniques

1. Dounce homogenization: crushing of cells between two revolving solid surfaces
2. Filteration: cells are forced through smaller pores in a filter
3. Grinding: where cells are ground by swirling with glass beads
4. Sonication: where cells are bombarded with ultrasonic vibrations
5. Solubilization: in which cell membranes are dissolved by detergents such as triton X-100

Enzyme digestion is also used to remove cell-wall constituents. The method of choice depends on the type of tissue to be homogenized and the specific purpose of the experiment. During homogenization, isotonic sucrose is added to the homogenization buffer to prevent osmotic rupture of organellar membranes. After homogenization, the homogenate is spurn at low speed to remove any intact cells along with large cellular debris. This is followed by subcellular fractionation

Differential centrifugation

This is the movement of subcellular particle in a centrifugal field. This movement (sedimentation) results from the interaction between a particleôs weight, the resistance it encounters in moving through a suspension medium and the relative centrifugal force exerted on the particle. Under a given centrifugal force, particles that are relatively large or dense will sediment more rapidly than particles that are smaller and lighter. The order of sedimentation is typically from most to least dense; nuclei, mitochondria, lysosomes, plasma membrane, endoplasmic reticulum and contractile vacuoles. It precedes density-equilibrum centrifugation

Density-equilibrum centrifugation

subcellular organelles are layered on a density gradients and subjected to a very high centrifugal force. The density gradient is formed by layering increasing concentration of sucrose solutions in a centrifuge tube. Other solutions such as cesium chloride, Percoll can be used. These two solutions when spun spontaneously set up a density gradient, thereby alleviating the need manually layer sucrose solutions of varied concentration.

During centrifugation, organelles initially layered on the density gradient will sediment until they arrive at the region of the gradient where the density of the suspension is equal to their own

Marker enzymes

Indicate the presence of an enzyme. Acid phosphatase is located in the lysosomes, succinate dehydrogennase is located in the mitochondria. ATP synthase is present in the inner mitochondrial membrane. Galactosyl transferase is present in the golgi apparatus. Glucose-6-phosphatase is present in microsomes. Galactosyl transferase is present in the Golgi apparatus. This helps to monitor where each enzyme activity is found during a cell fractionation protocol. Marker enzymes also provide information on the biochemical purity of the fractionated organelles. The presence of unwanted marker enzyme activity in the preparation indicates the level of contamination by other organelles. The degree of enrichment for the desired organelle is determined by the specific activity of the target marker enzyme. Electron microscopy is generally used as a final step to assess the preparationôs purity and the morphology of the isolated organelle

Question

Discuss the isolation of subcellular organelles

Topic: Cell membranes and toxins

Intended learning outcomes

1. To discuss the mechanisms by which toxins interfere with the hostôs cell membrane

2. To discuss the defense mechanisms in parasites

3. To discuss mode of action of polymyxins

Cell membranes play significant roles in the interaction of pathogenic microbes with the host cells. About 50% of the membrane volume is made up of transmembrane, peripheral and lipid-linked proteins arranged in a bilayer. Membrane lipid is important in providing cellular signaling, membrane trafficking as well as membrane microdomain organization. Infectious organisms rely on the multifunctional role of lipids to modulate cell processes to ensure their survival

Toxins

Toxins are powerful pathogenicity factor produced by macro and microorganisms which mediate drastic interactions of the pathogens on the organismôs host. Classically, bacteria toxins are divided into endotoxins and exotoxins. Endotoxins: membrane compounds of gram negative bacteria which elicits inflammation in the host. Exotoxins: secreted proteins which act locally and at distance of the bacteria colonization site. The invasion of cells by pathogens/toxins is marked by binding to carbohydrate moieties exposed by a lipid or a protein in the plasma membrane of target cells. Cholera toxins binds with its ɓ-subunit to the ganglioside GM1 in the intestinal cells, Pseudomonas aeruginosa attaches to respiratory cells by binding to asialoGM1 and asialo-GM2 through type IV pili. Influenza A virus initiates its uptake by binding to sialic acids in the host cell membranes. Glycosphingolipids are important for self-induced endocytosis of toxins and viruses. Phosphatidylinositol-phosphate (PIP) is also important for the uptake of pathogens. They are essential components of the cell membranes involved in signaling events; vesicle trafficking. One strategy by which invasive bacteria manipulate the PIP is the translocation of effector proteins, which act as phosphatidylinositol phosphatases. A second option to interfere with the PIP metabolism is the engagement of specific host cell receptors

Shigella dysenteriae

It secretes two types of enterotoxins; Shigella toxins I and II. In humans they cause serious complications in the GIT such as haemolytic colitis. The binding affinity of the ɓ-subunit of Shiga toxin (StxB) and cholera toxin (CtxB) to individual Gb3 and GM1 molecules respectively is very low. But the cooperative binding of multiple lipid molecules markedly increases the apparent affinity of the toxin to its receptor. After binding, Stx is internalized by clathrin-dependent as well as clathrin-independent endocytosis. Cholera toxin has been found to be associated with caveolae and is efficiently endocytosed into cells devoid of caveolin-1

Defense mechanisms in parasites

The interplay between parasite survival strategies and the host defense mechanisms is the basis for the relationship that exists between the host and the infecting parasite. Parasites have devised strategies for their survival while in the host; the parasite aims at propagating within the host and be transmitted to subsequent host, while the goal of the host is to limit the infection. Parasites have evolved mechanisms for evading the immune system of the host. Below are some of the mechanisms

Protozoan immune evasion strategies

Antigenic variation

In Plasmodium, different stages of the life cycle express different antigens. In trypanosomes, there is a whole range of variant specific surface groups (VSG) which protects the underlying membranes.

As the level of antigen increases, a small fraction of the population switches to producing a coat of a new VSG with an antigenic character circulating antibodies are no longer able to recognize

Strategy of avoidance

Some pathogens reduce antigenicity so that they are not recognized as foreign. The Ŭ2-macroglobulin is present on the surface of adult schistosomes and has a potent anti-protein activity. If attached to a molecule with the appropriate conformation, it may also act as aproteinase inhibitor preventing breakdown of parasite tissues. Plasmodium lives inside Red Blood Cells (RBCs) which have no nucleus, when infected, it is not recognized by immune cells. Other stages of Plasmodium live inside liver cells. Plasmodium ookinetes develop in serosal membrane & are beyond reach of phagocytic cells (hemocytes). Leishmania parasites and Trypanosoma cruzi live inside macrophages

Enzymatic role

Enzymes are produced that are capable of inactivating reactive oxygen intermediates resulting from the host inflammation defense systems. The antioxidant enzymes counteract oxidative burst. Some of the enzymes are superoxide dismutase, catalase, glutathione peroxidase, glutathione S- transferase (GST). Glutathione S- transferase is located in the external tegument of schistosomes

Shedding or replacement of surface

Example: Entamoeba histolytica.

Immunosupression

Manipulation of the immune response e.g. Plasmodium.

Anti-immune mechanisms

Leishmania produce anti-oxidases to counter products of macrophage oxidative burst

Helminth immune evasion mechanisms in the vertebrate host

Large size

It is difficult for immune system to eliminate large parasites. Primary response is inflammation to initiate expulsion, often worms are not eliminated.

Coating with host proteins

Tegument of cestode & trematode worms, is able to adsorb host components e.g. RBC Ags, thus giving the worm the immunological appearance of host tissue. Schistosomes take up host blood proteins, e.g. blood group antigens & MHC class I & II molecules, therefore, the worms are seen as ñselfò.

Molecular mimicry

Synthesis of surface proteins similar to host proteins by the parasite which are unrecognized as foreign. In tapeworm, synthesis of antigen that resemble blood group of MHX antigen of the host. Depression of the host immune response

Anatomical seclusion

Trichinella spiralis can live inside mammalian muscle cells for many years.

Shedding or replacement of surface

Examples; hookworms, trematodes

Immunosupression

Manipulation of the immune response. High burdens of nematode infection often carried with no outward sign of infection.

Polymyxins

They are antibiotics produced by Paenibacillus polymyxa. There are two main types; polymyxin B and polymyxin E (colistin). They are usually used in treating gram negative bacterial infections. They however have little or no effect on gram positive bacteria. This is because the cell wall of gram positive bacteria is too thick to permit access to the membrane. Their neuro and nephrotoxicities make them last line therapy.

Summary and Conclusion

This course has informed students on the central dogma of membrane biology, functions, types and composition of the biomembrane. It also covered lipid structure, properties and formation of bilayers. It also covered the isolation and identification electron microscopy and marker enzyme assays. Introduction to receptor function: antigenicity of membrane components. Cell membrane and toxins, transport processes, action of polymyxin and ionophores. Introduction to neurotransmission. Membrane transport system- active versus passive transport systems. Transport of sugars and amino acids. Defence mechanism in parasites. Biomembranes of parasites.

Interaction and Questions

1. What is membrane fluidity?
2. Explain the physiological roles of integral protein
3. Write an essay on Immunoglobin-like proteins
4. Discuss the isolation of subcellular organelles
5. What is endocytosis and exocytosis?
6. Describe the active transport system
7. List the advantages of artificial membranes
8. What is membrane fusion?
9. Mention two instances of membrane fusion
10. List other applications of artificial membrane
11. Distinguish between active and passive transport
12. What are the factors that affect diffusion of a substance?
13. What are transporters?
14. What are the differences between channels and carriers?

Bibliography/ Further Reading

1. Leninger Principles of Biochemistry
2. Harpers illustrated Biochemistry
3. Padh, H. 1992. Organelle isolation and marker enzyme assay. Pages 129ï 146, *in* Tested studies for laboratory teaching, Volume 13 (C. A. Goldman, Editor). Proceedings of the 13th Workshop/Conference of the Association for Biology Laboratory Education (ABLE), 191 pages
4. Dr. Michele Loewen. Proteins and enzymes, ultracentrifugation. 24 pages. www.cbr.nrc.ca/loewen/Home.html
5. http://www.sfu.ca/biology/courses/bisc318/2015%20pdfs/lecture_33_Apr-02_Evasion%20of%20immunity.pdf