Membrane proteins: structure and function

Explain, giving examples, how the structural features of membrane proteins are related to their functions.

Membrane proteins are protein molecules that have any kind of association with the membrane of a cell or organelle, and are normally split into two different groups: integral and peripheral membrane proteins. As stated by Earnshaw & Pollard (2002), integral proteins pass through the membrane, whilst peripheral proteins only relate to either the inside or outside surfaces of the lipid bilayer. Membrane proteins have a number of different functions, and their structures are well adapted for this. I have placed the membrane proteins into three categories (although they do overlap at times) – receptors and cell signalling; cell adhesion molecules, and transport proteins – and will go through each category explaining the relationship between structure and function.

Receptors and Cell Signalling

Cell communication is the main function of receptors, enabling cells to recognise what is going on around them, and allowing them to then act and respond accordingly. The signal transduction pathway is the procedure that takes place when a signal on a cell's surface is changed in a number of stages, into a certain cellular response, and it is in this pathway that receptors play their part. We are told by Earnshaw & Pollard (2002) they can be stimulated by a number of types of signalling molecules, for example, nutrients, neurotransmitters, hormones and growth factors, and through the signal transduction pathway, can produce a number of cellular responses such as the electrical potential of the plasma membrane, gene expression and enzyme activity. The majority of receptors on the target cell's surface

are transmembrane proteins (integral proteins that span the whole of the membrane). In general, receptors are very specific, and are shaped and structured in such a way that they will only bind to a certain ligand, and this makes sure that the receptor can only be activated by that type of ligand, resulting in a particular response.

Receptor tyrosine kinases (RTKs) are receptors that have a very high affinity for a large number of growth factors and hormones, such as epidermal growth factor (stimulates proliferation of some cell types), platelet-derived growth factor (stimulates, among others, survival of some cell types), and insulin (stimulates protein synthesis), confirmed by Albert et al. (2002). There are around 16 structural subfamilies, and most of them (but not all, for example the insulin receptor doesn’t) consist of a single polypeptide chain that spans the whole of the membrane. The section that does span the membrane tends to be composed of between 25 and 38 amino acids. The N-terminal region of the polypeptide chain, which can be quite large in size (it can have anywhere between 15-100 residues attached), is extracellular, and this is what binds to the ligands. The C-terminal region of the polypeptide chain is intracellular and this has the domains present that are responsible for the kinase activity of these receptors.

For there to be activation of the kinase domains on the C-terminal of the receptor, there must be a conformational change in the receptor. As the receptor is only one chain, it must form a dimer or a higher oligomer (this is where two or more receptor molecules come together; the process known as oligomerisation). Earnshaw & Pollard (2002) suggest that at the C-terminals there are several tyrosine residues, and when the dimmers are formed, they

are able to cross-phosphorylate each other – a process known as autophosphorylation. This increases the kinase activity of the domain and allows for easier binding between the docking sites and intracellular signalling proteins inside the target cell, stated by Albert et al. (2002). For the RTK to be activated, the ligand, whether it be a growth factor or hormone, normally has to bind to two adjacent receptor chains simultaneously. The insulin receptor is an example of a RTK. It was believed by Weissmann et al. (1975) that the insulin receptor only existed on the exterior surface of the membrane. However, more recent research tells us that this is incorrect. Albert et al. (2002) tells us that the receptor is a tetramer. This means that it has four polypeptide chains, two alpha and two beta. One alpha and one beta chain make up a subunit, and are linked by disulfide bonds. The two subunits are therefore identical and make up a stable dimer. The beta chains span the membrane and is connected to a cytoplasmic tyrosine kinase domain. The alpha chains form the insulin-binding extracellular domain. When insulin binds to these alpha chains, a conformational change is brought about on the kinase domains – they are brought together and autophosphorylation occurs. This stimulates kinase activity, and eventually leads to the increase of glucose uptake from the blood into muscle cells and adipose tissue.

G proteins (also known as GTP-binding proteins and GTP-ases) are also receptor proteins that are within the membrane and are involved in second messenger cascades. According to Albert et al. (2002) there are two distinct groups of G-protein: heterotrimeric G proteins and small GTP-ases, however, here I will focus on heterotrimeric G proteins.

Heterotrimeric G proteins have 7 membrane domains and three subunits present on the inner surface of the cell membrane: Ga (this holds the binding site for GDP and GTP), Gß, and G?. Kimball (2007) suggests that when the G protein is in an inactive state, GDP is bound to Ga. When a ligand, for example a hormone, attaches itself to the G protein receptor on the outside of the cell membrane, there is a conformational change in the receptor. This causes a change in shape in Ga, which causes GTP to replace GDP. Gß and G? are dissociated from Ga, and form a dimer, whilst the activated Ga activate the effector molecule. Deactivation of the G protein require hydrolysis of GTP, producing GDP and inorganic phosphate, and the return of the GßG? dimer.

Cell Adhesion Molecules

Cell adhesion molecules (CAMs) are glycoproteins, and can either be cell-to-cell or cell-matrix adhesion molecules, according to Albert et al. (2002). There are some CAMs that are calcium dependent, and others that are calcium independent. Cadherins are calcium dependent and are present in vertebrate tissues. The hydrophobic alpha helix pass through the membrane only once and are anywhere between 700-750 amino acids in length. It is believed that the extracellular region of the polypeptide is folded into around 5 cadherin repeats and between each repeat are calcium ions which lock the repeats together, forming stiff rod-like structures. These are able to interact and attach themselves to other molecules of the same kind (known as the homophilic mechanism) on adjacent cells by acting like an interlocking molecular zipper. Cadherins not only ensure that cells within tissues are firmly held together, they also indirectly link the actin cytoskeletons of the

cells they join together. The cadherins have a cytoplasmic tail that, with the help of intracellular anchor proteins catenins, bind to the actin, and everything is firmly kept in place. There are many different types of cadherins, for example N-cadherin (found in neurons) and P-cadherins (found in the placenta). The best known cadherin is E-cadherins, found in adheren junctions in epithelial tissues and they are the first cadherins expressed during mammalian development. If the E-cadherin is not present or expressed, the strength of cellular adhesion in a tissue is decreased, meaning cells are able to move around. This is not at all good if cancer has formed, as its ability to cross the basement membrane and invade near tissues is increased.

Integrins are slightly different to cadherins. They are calcium independent and are heterophilic CAMs – able to bind to a number of different ligands, according to Weissmann et al. (1975). They are heterodimers, and have two polypeptide chains that span the membrane once: one alpha and one beta. The N-terminals of each polypeptide chain protrude 16nm above the outside of the membrane, and this is what associates with the ligand. These regions of the polypeptides are made of different domains. The alpha chain has three domains folded in a similar way to immunoglobulins, the beta chain has four epidermal growth factor (EGF) like domains. Bound to these chains are divalent cations, and it is believed that they interact with the acidic regions on the ligand and ensure its binding. The cytoplasmic tails of the integrins are able to interact with a number of different signalling proteins (linked by paxillin proteins), and this helps with its function of signal transduction.

These ‘ tails’ are linked to actin filaments via talin and vinculin proteins (different proteins to what are used with cadherins).

It is thought by Weissmann et al. (1975) that many calcium independent CAMs contain one or more immunoglobulin (Ig) like domains that are characteristic of antibody molecules. Neural cell adhesion molecule (NCAM), present in most neurons and skeletal muscle, is a good example of a calcium independent CAM. It binds to other cells in a homophilic mechanism, and it is believed that there are at least 27 forms of NCAM. The polypeptide has a large extracellular region which folds into five Ig-like domains, held together by disulfide bonds. Sialic acid is present on the some forms of NCAM, and, due to its negative charge, it stops cell adhesion, and this is important in cell migration and invasion.

Transport Proteins

These are proteins that transport substances either in a cell (intracellular), through fluid outside the cell (extracellular) or through membranes. I will be focusing on the transport proteins that are associated with the membrane and there are a number of different types of proteins that transport substances: carriers, channels and pumps. Earnshaw & Pollard (2002) state that carrier proteins are one polypeptide chain that span the membrane around 12 times, and this is backed up by experiments that prove between 60-70% of the polypeptide chain is alpha helical – enough to create 12 helical membrane-spanning regions. However there are exceptions to this, for example carriers present in mitochondria and chloroplasts are only half as long in length, and form homodimers to make up the length. Both the N- and C-terminal are cytoplasmic. Carrier proteins provide reversible, passive

pathways for specific solutes to cross the lipid bilayer down their concentration gradient. The protein has two conformational states. The first is when the binding sites of the protein is exposed on the outside of the membrane. When a specific substance binds with it, it takes in the substance and retains it, whilst the shape of the protein itself changes (into its second conformational state) and the substance is now facing the inside of the cell. Carrier proteins, use the ion/substrate gradient as a source of energy to perform other jobs, for example driving other substances up their concentration gradient. Glucose transporters (GLUT) are an example of membrane carriers, and according to Professor Whitley (2008), there are five members of the glucose transporter family: GLUT1 through to GLUT5. Although they are all present in different tissues (GLUT2 present in liver and pancreatic ß cells; GLUT4 present in muscle and fat cells) they all passively transport glucose in and out if cells (they are bidirectional transport). They have 12 membrane spanning domains, with both the N- and C-terminals on the cytoplasmic side. They are driven by concentration gradients, and these gradients are maintained by phosphorylation.

Channels are found in the majority of epithelial cells, though not in those of skeletal muscle and the nervous system. They are ion specific and transport ions at a much faster rate than carrier proteins. These channels are able to open and close in a regulated way, stated by Earnshaw & Pollard (2002), and once open, due to electrical and concentration gradients, ions are able to pass quickly across the membrane. Any changes in the activity of the channel will lead to rapid electrical signals to excitable membranes of nerves and muscles, as the electrical potential is controlled by the movement of

ions through the channels. The majority of channels consist of multiple subunits. An example of a channel is a K+ channel, KcsA, and it has four identical subunits, each containing two transmembrane helices. On the cytoplasmic side of the membrane the transmembrane helices are very close together, however on the extracellular side, to allow for the selectivity filter and pore helices, they are more spread out. The pore goes all the way through the membrane, with a narrow part that is specific in all K+ channels. It is formed by three residues in a certain sequence (GYG), and this pore is just wide enough to allow a K+ ion to pass through. Pumps, also known as primary active transporters, use energy to transport solutes up their concentration gradient. There are many types of pumps carrying out very similar functions. Bacteriorhodopsin is a protein used by Archaea that uses light energy to move protons out of the cell. The proton gradient formed is then used as energy. Stated by Earnshaw & Pollard (2002) there are two-dimensional crystalline patches within the plasma membrane containing the bacteriorhodopsin. It has seven alpha helices that cross the membrane and these have one molecule of retinal buried in them. When a photon is absorbed the retinal molecule changes shape, which in turn leads to the conformational change of whole protein, and therefore the proton pumping action.

So far the transport proteins I have looked at have been transporting substances across the membrane. However transport through the extracellular fluid is just as important, and it is believed glyophorin does this. Glycophorin is present on the outer surface of a red blood cell, and is a glycoprotein. According to Albert et al. (2002) it has around 131 amino acids,

with the majority of the mass on external side of the lipid bilayer, and passes through the membrane only once. It also has a large number of oligosaccharide chains attached to the extracellular regions. The N-terminal is extracellular, whilst the C-terminal is intracellular. It was believed, as stated by Weissmann et al. (1975) that the function of glycophorin was either to carry sugar molecules in the blood, or to help keep stability and shape within the red blood cell, as it tends to exist as a homodimer and the hydrophobic regions are strong anchors. However, Albert et al. (2002) state that although there being nearly one million glycophorin proteins in each red blood cell, their function is not yet known. Difference in opinion may be due to the fact that experiments done in recent times have been able to cancel out what was originally believed in 1975.

Conclusion

Through research I have found that membrane proteins may all be built from the same building blocks (amino acids) and have the same basic structure – primary, secondary, tertiary and quaternary structures – but can develop into complex structures that are extremely specific to their function. There are so many different functions that are carried out by membrane proteins, and these are only successful due to the specificity of the proteins – the particular way the protein is folded, coiled, how they interact with other molecules and the bonds that are present within them.

References

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