7tm helix protein rhodopsin and bacteriorhodopsin comparison

Compare and contrast the 7TM helix proteins rhodopsin and bacteriorhodopsin at the molecular and cellular levels.

Rhodopsin and bacteriorhodopsin both belong to the G-protein coupled receptor (GPCR) family. They each have 7 alpha helical transmembrane domains which are embedded in their respective membranes. The two proteins are related but not identical and therefore contain many similarities as well as differences. Structurally the proteins are similar, while functionally, aside from being light-sensitive proteins and using light to initiate their processes, these two distinct proteins have a range of differences.

Rhodopsin is a eukaryotic protein and it is the main photoreceptor pigment contained within the discs of the outer segment of vertebrate rod photoreceptors amongst other supporting proteins. Rhodopsin molecules have very high sensitivity to light and are the pigment responsible for enabling vision in dim light conditions and monochromatic vision in the dark. Exposure of rhodopsin to light causes the pigment to be photobleached, this initiates the transmission of the optical signal. The full regeneration of the human rhodopsin molecule occurs within approximately 45 minutes. Bacteriorhodopsin is a prokaryotic protein present in archaea. It is a light-dependent proton pump which is used to carry out phototrophy. Energy from light is utilised to move protons out of the cell, across the membrane, forming a large concentration gradient and making the inside of the cell up to 10000 times more alkali than outside. The subsequent proton gradient is converted into chemical energy. Both rhodopsin and bacteriorhodopsin maximally absorb light of the wavelength around 500nm which is the green colour range.

Both rhodopsin and bacteriorhodopsin contain a retinal chromophore, although the two chromophores are not identical and rather, are isomers of each other. The retinal chromophore is comprised of a β-ionone ring bound to a polyene chain. In both cases the retinal chromophore is positioned horizontally within the membrane. Rhodopsin is formed of the protein opsin and the reversibly and covalently bound co-factor 11-cis-retinal, which is the photo-reactive chromophore. Opsin consists of 7 transmembrane α helices which are connected by polypeptide loops which occlude the agonist binding site. There is an 8 th helix present in opsin however this is not transmembranous and plays a regulatory role in rhodopsin. The Æ -amino group of lysine 296 within the opsin molecule is covalently bound to the aldehyde group of retinal by a protonated Schiff base in a central cavity between the 6 th and 7 th helix.

Bacteriorhodopsin is formed of the protein bacterio-opsin which is bound to all-trans-retinal in a covalent and reversible manner. Bacterio-opsin, as in rhodopsin, is formed of 7 transmembrane α helices connected by polypeptide loops. The Æ -amino of lysine 216 within bacterio-opsin is covalently bound to the aldehyde group of retinal. Again this interaction occurs within a central pocket on the 7 th helix. Rhodopsin exists as a monomer within the membrane of rod cells whereas bacteriorhodopsin in its wild type state is in a trimer of 3 identical protein chains each rotated 120° relative to the others. Bacteriorhodopsin and rhodopsin have no detectable sequence homology to one another, however due to the similarity of their tertiary structures it is believed they are evolutionarily related.

In vertebrate rod cells light induces phototransduction by interaction with rhodopsin. Light is absorbed very efficiently by retinal due to its polyene tail. The retinal absorbs a photon which causes it to undergo an isomerisation reaction to the activated all-trans-retinal configuration from its initial 11-cis-retinal configuration. In order to accommodate the altered shape of the all-trans-retinal the rhodopsin molecule undergoes a series of relaxations which includes the movement of helices 5 and 6 outwards creating a cavity, this movement is around 5Å. Upon the absorption of a photon by the retinal chromophore the rhodopsin molecule is converted to photorhodopsin within 200 femtoseconds. The second intermediate forms within picoseconds following irradiation and is called bathorhodopsin, this has all-trans bonds which are distorted. The next intermediate is lumirhodopsin which forms within nanoseconds. Lumirhodopsin is converted to metarhodopsin I within milliseconds. During these steps the protonated Schiff’s base remains unchanged. Finally metarhodopsin I is converted to metarhodopsin II, within milliseconds, this causes the Schiff base to become deprotonated. Neuronal excitation is initiated by metarhodopsin II activating transducin, an associated G protein, triggering a second messenger cascade with cyclic guanosine monophosphate (cGMP), activating the visual phototransduction pathway. The activation of transducin is triggered by the exchange of GDP to GTP on the α subunit of transducin. Activated transducin binds and removes the inhibitory subunits which inactivate cGMP phosphodiesterase. This process activatescGMP phosphodiesterase which subsequently hydrolyses cGMP. Hydrolysis of cGMP reduces the cellular levels of cGMP which leads to inactivation of the cGMP-gated cation channels in the cell membrane. This causes the photoreceptor cells to become hyperpolarised altering the rate of

neurotransmitter release and resulting in neuronal signalling. At each step of this process the signal is amplified. Deactivation of metarhodopsin II occurs by interaction with rhodopsin kinase and arrestin. Rhodopsin kinase phosphorylates the activated GPCR and arrestin desensitizes it. Regeneration of the rhodopsin pigment to its original state must occur in order to allow phototransduction to occur again. Multiple serine and threonine residues at the carboxyl terminal of metarhodopsin II are phosphorylated, this is catalysed by rhodopsin kinase, arrestin then binds to the altered metarhodopsin II preventing further interaction with transducin. The α subunit of the transducin re-associates with the βÆ´ subunits and its in-built GTP-ase activity hydrolyses the GTP to GDP, this returns the cGMP phosphodiesterase to its inactive state. cGMP is synthesised from GTP by guanylate cyclase increasing the cGMP levels to reopen the cGMP gated channels and this allows restoration of the cell to its original state.

In bacteriorhodopsin, like in rhodopsin, it is the retinal molecule which absorbs a photon and changes conformation. The initial all-trans-retinal configuration is converted to 13-cis-retinal. This isomerisation causes the bacteriorhodopsin molecule to undergo a conformational change, via a number of intermediates, to accommodate the change in retinal structure. This change of conformation subsequently alters the proton pumping action of bacteriorhodopsin. Upon absorption of a photon the all-trans-retinal photoisomerises to 13-cis-retinal, this is almost the exact opposite of the cis-retinal to trans-retinal configuration change that occurs in rhodopsin. In bacteriorhodopsin the initial photo-isomerisation results in the intermediate J600, as in rhodopsin this first isomerisation occurs in a femtosecond

timescale. J600 subsequently becomes K590 within 5 picoseconds. The next conversion of the K590 intermediate to the L550 intermediate happens within 2 microseconds. This conversion results in stronger hydrogen bonding between aspartate 85 and the protonated Schiff base within the extracellular channel. Conversion of L550 to the extracellular M410 intermediate occurs within microseconds, this process involves the translocation of a proton from the protonated Schiff base to the proton acceptor aspartate 85, within the extracellular channel, this takes microseconds. In order for the proton transport to occur out of the cell the Schiff base cannot be reprotonated by aspartate 85, otherwise no proton transport could occur. Instead, the access of the Schiff base must be altered to prevent reprotonation by aspartate 85. Next the extracellular M410 is converted to cytoplasmic M410, this allows the Schiff base to be deprotonated from the outside of the cell membrane and reprotonated from the inside of the cell and hence this change of access for the M410 intermediate from extracellular to cytoplasmic allows unidirectional proton transport out of the cell. This alteration repositions the access for the Schiff base from extracellular to intracellular. The cytoplasmic M410 is then converted to the N560 intermediate Following the earlier change in access for the Schiff base, it can then be reprotonated from aspartate 96 which is located within a channel from the cytoplasm to the membrane. This process occurs within milliseconds. The aspartate 96 is then reprotonated from the cytoplasm to allow the Schiff base to be reprotonated multiple times and allow the cycle to continue. To ensure one-way proton transport the Schiff base must be accessible to either aspartate 96 or aspartate 85 during different stages of the cycle and therefore the positioning of the Schiff base must be switched dependent on which

aspartate requires access and the stage of the cycle, aspartate 85 needs access to deprotonate the Schiff base on the extracellular side and aspartate 96 is used to reprotonate the Schiff base from the cytoplasm. Aspartate 85 and aspartate 96 are now both protonated in the N560 intermediate and this leads to a subsequent thermoisomerisation reaction. During this thermoisomerisation The N560 intermediate is converted to the O640 intermediate. In this step the activated 13-cis-retinal configuration is thermally isomerised back to the original all-trans-retinal configuration. To complete this cycle the aspartate 85 must be deprotonated. The final step involves the conversion of the O640 intermediate back to bacteriorhodopsin. The Schiff base is again repositioned switching the access to it back from the cytoplasmic side back to the extracellular side of the membrane, aspartate 85 is then deprotonated to restore the bacteriorhodopsin molecule back to its original state which facilitates the continual pumping of protons out of the cell across the membrane. The protons in the extracellular matrix are allowed to flow back into the cell via ATP synthase down the concentration gradient, this allows the synthesis of ATP which provides the energy to power the archaea cell.

There are a large number of differences ranging from the amino acid sequences to the functions of bacteriorhodopsin and rhodopsin, however in spite of these vast differences there are still many similarities including the 3 dimensional structure and the presence of a photon absorbing retinal chromophore. These similarities have led to the 2 proteins being grouped together in the Structural Classification Of Proteins and they provide relevant

structural information about other 7 transmembrane proteins in order to make structural predictions and associations about other similar proteins.