Enhancing the Fight against Malaria: From Genome to Structure and Activity of a G-Protein Coupled Receptor from the Mosquito, Anopheles Gambiae.

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Abstract Enhancing the Fight against Malaria: From Genome to Structure and Activity of a G-Protein Coupled Receptor in the Mosquito, Anopheles Gambiae Grace Chitima Mugumbate Department of Chemistry, University of Cape Town, Private Bag, Rondebosch, 7701, South Africa. Submitted March 2010 G-proton coupled receptors (GPCRs) are excellent drug targets that occupy a central position in the physiology of insects and are involved in transmission of signal from the extracellular to the intracellular side of the cell. Adipokinetic hormone receptors (AKHRs) are GPCRs that mediate physiological functions of the neurohormones, adipokinetic hormones (AKHs) that regulate mobilisation of energy reserves during mosquito flight. Ligand binding to GPCRs depends on the three dimensional (3D) structures of the receptors but to date no crystal structures of insect GPCRs are available. This work focused on building molecular models of AKHR from the genome of the malaria mosquito, identifying its binding site and studying the conformational and structural changes during molecular dynamics of the active and inactive receptor. Homology modelling was used to build the helices based on the crystal structures of rhodopsin and beta2-adrenergic receptor (β2AR). The loops were built separately and joined to their respective helices. Molecular dynamics was used for conformational search of the loops. The two resulting 3D structures of the GPCR from the malaria mosquito had similar overall structures. However, the β2AR-based structure had an 'open' conformation in the extracellular region, whilst the rhodopsinbased model was 'closed'.

NMR restrained molecular dynamics was used to determine the solution conformation of AKH-I from Anopheles gambiae (Anoga_akh). Docking calculations of this peptide and the decapeptide, Del_CC, from the blister beetle showed that helices 2,3,5,6, and 7, and the extracellular domains defined the binding pocket of AKHR. The 'open' AKHR model provided easy access to the binding site and had higher affinity for the ligands than the 'closed' structure. During molecular dynamics, after binding of the agonist, the receptor binding pocket closed to protect the ligand. At the same time the intracellular region opened. Although conversion of the receptor from inactive to active state was slow with Anoga_akh, the receptor had a higher affinity for the ligand than for Del_CC as indicated by estimated free energy of binding, -47.3 kJ/mol and -38.5 kJ/mol respectively. The protein-ligand complexes were stabilised by an intense network of H-bonds, salt bridges and hydrophobic interactions. Tyr285 (H6) played an important role in binding Del_CC, whilst ILe106 (H2) was pivotal in binding Anoga_akh.

Since AKHR facilitates energy mobilisation during insect flight, knowledge of the 3D structure and binding pocket of the receptor from the malaria mosquito could lead to structure-based design of non peptide antagonists that prevent binding of AKH molecules. This would stop generation of energy for the mosquito to fly and pave the way for development of insect specific insecticides and reduction of transmission of malaria