Molecular dynamics simulations studies of Gonadotropin Releasing Hormone(GnRH) and GnRH Receptor

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Abstract

Sexual maturation of human cells in ovaries and prostate is closely linked to the secretion of Gonadotropin Releasing Hormone (GnRH). The hormone binds to receptors (GnRH receptors) in the surface of the cells and instigates an extensive biochemical cascade that leads to production and release of luteinizing hormone (LH) and the follicle-stimulating hormone(FSH). Sequence analysis data classify GnRH receptors (GnRHR) as part of the G protein-coupled receptors (GPCRs). Structural data from GPCRs show the presence of a seven trans-membrane helical bundle. The lack of any structural data about GnRHR, impedes the rational design of agonist or antagonist GnRH peptides or non-peptide mimetics for use in the treatment of infertility and hormone dependent cancer. In this study, we employ these common structural and sequence characteristics for the construction of the homology model of GnRH receptor. Structural information from the humanB2-adrenergic receptor as well as rhodopsin has been used in order to create a theoretical model of GnRHR. Furthermore, molecular dynamics (MD) simulations have been used to refine the proposed model and investigate the impact of the bilayer membrane in GnRHR conformation. Additionally, MD simulations were implemented for the analysis of the conformations of GnRH in aqueous solution. The results support the proposed model in the literature regarding the bent conformationof the hormone. The presence of a β -turn in the conformation of the peptide has been shown to be paramount in the binding of GnRH to the receptor and its subsequent activation.

Results

GnRH Receptor Homology Model.

The initial model for the receptor was based on the crystal structures of human β 2-adrenergic receptor (PDB ID: 2rh1), bovine (PDB ID: 3dqb) and squid (PDB ID: 3ayn) rhodopsins. The model of GnRHR was superimposed on the C α , N and C backbone atoms with homology models based on other rhodopsin/GPCRstructures. The comparison between the proposed model in this study with those reported in the GPCR database, reveals the structural similarities, despite the differences in the templates employed. The comparison of the models is also employed in order to verify the positioning of conserved residues Asn^{2.50(87)}, Asp^{2.61(98)}, Asn^{2.65(102)}, Lys^{3.32(121)} and Asp^{7.49(319)}. Our model positions the specific residues with orientation inside the helical bundle as reported in the literature [1]. Residues Asn^{2.50(87)} and Asp^{7.49(319)} are oriented so that their side chains point towards each other inside the helical bundle. The particular orientation has been proposed to stabilize the conformation of GnRHR *via* the formation of a water mediated bridge between the residues.

ECL2 loop refining and modeling.

ECL2 is highly variable in the different members of the family and flanks the trans membrane domains TM4 and TM5. ECL2 has been implicated in ligand binding and it has been proposed that regulates ligand entry into the receptor [2]. The disulfide bond between Cys¹¹⁴ and Cys¹⁹⁶ has been proposed to drive the proper folding and activation of GPCRs.

GnRH molecular dynamics (MD).

GnRH has been isolated as a decapeptide from hypothalamic cells. Post translational modification leads to the modification of the C- and N-termini residues [3]. The MD simulations of GnRH, performed in this study in aqueous environment, have shown the presence of a bent conformation for the hormone at positions 5-8. The analysis of our simulations shows the presence of three distinct clusters during the MD simulation (Figure 1). The data derived from the MD production run are in accordance with studies suggesting that the presence of a β -turn between residues 5 to 8 is paramount for the function of GnRH (Figure 1). Furthermore, our simulations

have shown that the bent conformation of the hormone is retained through the simulation time from the moment of its formation.

Conclusions

GnRH has been implicated in various regulatory pathways in the human organism [4]. Any imbalances in the production of GnRH or its binding to GnRH receptor leads to the development of various cancers [5]. The conformational analysis of GnRH provides the relevant structural information for the design of analogues that further stabilize the bent conformation around residues 5-8 of the hormone. In combination with the development of a reliable model for GnRH receptor we can implement MD simulations to further analyze the interactions in the GnRH-GnRH receptor complex. Thus, it is possible to provide valuable information for the rational design of peptide analogues and non-peptide mimetics with improved binding affinity and greater stability [6,7].

Figure 1: Superimposition of the backbone of the three representative GnRH clusters reported in the MD simulation. The bend is appeared between the residues 5-8.

Methods

Homology model. The homology modeling process was performed with MODELLERv9.17 software.

Loop modeling. Loops ICL1-3, ECL1 and ECL3 were not considered for loop modeling/refinement. The SuperLooper2 web server (<http://proteininformatics.charite.de/ngl-tools/sl2/start.php>) was implemented for the modeling of ECL2.

Molecular Dynamics (MD). All MD simulations were performed using the AMBER14 software package.

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