Alpha and Beta Estrogen Receptors: Molecular Modelling and Conformational Analysis through Molecular Dynamics

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Motivation

Estrogens, a most important group of steroidal hormones, regulate sexual differentiation and functions, preside bone construction, remodelling and homeostasis, lipoproteins synthesis, learning and memory functions. They protect the central nervous and cardiovascular system from the risk of degenerative diseases. In women, after menopause, these functions are seriously impaired; as a consequence, it is of deep interest to find a compound which could substitute the endogenous estrogens without eliciting the dangerous side effects that seem to be associated with the traditional Hormone Replacement Therapy (HRT), e.g. mammary and uterine cancer. In this view, it seems important to design compounds, of steroidal and non-steroidal origin, that can discriminate between the two human Estrogen Receptor (ER) isoforms: ERα and ERβ. Actually, the ERβ isoform seems to be principally correlated with the non-sexual functions of the estrogenic compounds, while the ERα form is suspected to be more correlated to the cancerogenic side effects of HRT. These are the reasons for the present investigation of the dynamical behaviour of the ERα- and ERβ-LBD models in explicit water, in different conformations (agonist and antagonist), in the presence or absence of their endogenous agonist ligand 17-β-Estradiol (E2). This was achieved by Molecular Dynamics (MD) protocols and Essential Dynamics (ED) analysis.

Methods

The starting structures of ERα and ERβ Ligand Binding Domains (LBD) were 1G50[1] (α-LBD bound to E2, agonist, at 2.90Å) and 1ERR[2] (β-LBD bound to RAL, antagonist, at 2.60Å). The lacking β structures, in agonist (BsuA) and antagonist (BsuAa) conformation, have been generated by Homology Modelling using the Swiss-Model server provided with the template of the corresponding α structure, the ERβ-LBD sequence was derived from the 1QKM[3] PDB structure. E2 was then rigidly docked to 1G50 and BsuA models, using the AutoDock package. The models were then optimized through a Molecular Mechanics (MM) protocol of energy minimization, through which the ER-LBD structure was released gradually. 1.MM on side chains with backbone frozen, in vacuo. 2.MM with the whole protein frozen, in water. 3.MM on the side chains with the backbone frozen, in water. 4.MM on the whole system, protein and water. The constraints applied to the ligand were the same as those applied to the protein side chains. This optimization was performed by the GROMACS package. The resulting models were submitted to the PDB-Procheck package, ADIT, to check their structural consistency. Six models were optimized: 1G50, BsuA, 1ERR, BsuAa, all apo-receptors, and 1G50-E2 and BsuA-E2, complexes. On these structures NVTMD simulations have been performed using the GROMACS package, after a 60ps thermalization during which the temperature was gradually increased to 300K. The MD runtime has been of 12ns for 1G50, BsuA, 1ERR, BsuAa and 3ns for 1G50-E2, BsuA-E2. A specific topology file for E2 has been built for the GROMACS force field, as non standard data exist for such a ligand. These simulations were analysed by the ED method to point out the principal components (PCs) of the molecular motion.

Results

All the simulation trajectories resulted to be reliable and significant as indicated by the cosine content test (cc), the cc is a measure of the similarity of the trajectory to a random diffusion. BsuA model was found to be the most flexible structure, interacting colser with the ligand, whose presence reduced drastically the structure mobility. 1G50 was found to be more loosely bound to the E2 ligand, so that the ligand presence influenced less severely the structure. As expected, the maximum flexibility of the structure is mapped in the peripheral loops of the protein, but it is

differently distributed in each system. The accessibility of the binding site got reduced along the simulation in the absence of the ligand, while it kept constant in its presence. The presence of the ligand, which is essentially non-polar, causes an increase in the hydrophobic surface area accessible to the solvent, in relation to the hydrophilic one. The hydrogen-bonding network of the ligandprotein interaction, was differently characterized in 1G50 and BsuA. In the first case, the number of H-bonds varies from zero to four, while in the second case one or two bonds are present during the whole simulation. This correlates well with the observed minimum distance between the ligand and the protein. The behaviour of the models is quite well parted between the agonist and antagonist conformations, so that, from the point of view of motion, 1G50 and BsuA could be clustered together, as well as 1ERR with BsuAa. This difference in behaviour is particularly enhanced in the values of the parameters characterising the H12. Actually, these topological parameters are conserved along the simulation in the case of antagonist conformations, while rigid movements are wider for agonist conformations.

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References

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