Modelling the interaction of steroid receptors with organic polychlorinated compounds

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Motivation

The organic polychlorinated compounds like dichlorodiphenyltrichloroethane (DDT) with its metabolites and polychlorinated biphenyls (PCBs) are present in atmospheric particulate as persistent contaminants. They have been recognized to have detrimental health effects both on wildlife and humans acting as endocrine disrupters (EDC) due to their ability of mimicking the action of the steroid hormone thus interfering with hormone response. There are several experimental evidences that they bind and activate human steroid receptors. Despite the growing concern about the toxicological activity of EDC, molecular data of the interaction of this compounds with biological targets are still lacking. In order to better understand the ability of EDC to bind in the receptor hormone binding pocket, we have simulated by docking approach the molecular models of the complexes of estrogen, progesterone and androgen receptors with DDT and PCB family compounds.

Methods

To model the receptor-ligand complexes, the coordinates of human estrogen ? and b (hER?, hERb), progesterone (hPR) and rat androgen (AR) receptors in complex with either the corresponding steroid hormone or with antagonists, were obtained from the Protein Data Bank (PDB entries : 1G50: ER? with estradiol, 3ERT:ER? with 4-hydroxytamoxifen, 1QKM: hERb with genistein, 1A28:hPR with progesterone, 1I37: AR with dihydrotestosterone). Molecular structures of the EDC ligands (namely p,p-, o,p-DDT, p,p-, o,p-DDE, p,p-o,p-DDD, PCB, PCB-OH) were built and energy minimized with MacroModel (Schrödinger LLC), the resulting geometries were re-optimized with semi-empirical quantum mechanic calculations, using the Hamiltonian AM1 as implemented in Spartan (Wavefunction Inc.) and atomic charges were calculated. Docking simulations were performed with Autodock 3.05, that takes into account the ligand flexibility (Morris, G. M., et al. 1998, J.Comp.Chem., 19: 1639-1662), using the Lamarkian Genetic Algorithm (LGA). The method was tested by reproducing crystallographic complexes using a blind approach to explore the entire protein with a grid encompassing the whole surface. Binding interactions for the DDT and PCB ligands were studied using a smaller grid including the ligand pocket region. Further refinement were obtained by use of the program QXP (McMartin C, Bohacek RS. 1997, J.Comp.Aid.Mol.Des., 11:333–344) that includes receptor side chains flexibility.

Results

The blind docking procedure was successful in reproducing the X-ray complexes (RMSDs from 0.49 to 0.6 Å.). These results gave confidence to investigate only the pocket region to evaluate the binding conformation of EDC for which no experimental data was available. Autodock simulations generated several ligand conformations that were ranked on the basis of the docking energy parameter calculated by the program. The free energies of binding

corresponding to the lowest docking energy values for each ligand were used to select the final model. The DDT and its metabolites (DDD, DDE) and the hydroxylated PCB metabolite (PCB-OH) docked with free energies of binding in the range -8,30 to -5.26 Kcal/mol while PCB was in the range -7.19 to -2.15 Kcal/mol. In all cases the lowest energies corresponded to conformations located in the buried hydrophobic cavity corresponding to the hormone steroid pocket. However, in progesterone receptor a second binding site was detected where all ligands, but PCB, were docked with a binding energy comparable to the hormone steroid site. This finding is in agreement with literature data that report the existence of two progesterone binding sites for this receptor (Helguero LA et al. J.Steroid Biochem. Mol. Biol. 84, 2003: 9-14). An alternative binding pocket was also detected for androgen receptor for which no experimental data are reported. If this result would be confirmed by experiments, it could prove the predictive value of our investigation. In general, DDT isomers and its metabolites and PCB-OH bind with an affinity which is 3 or 4 order of magnitude lower than natural ligand. PCB has a greater variability and in a few cases (3ERT, 1QKM) binds with very low affinities. Autodock results were refined with QXP to analyze to a deeper extent the energetic contribution of different conformations. A similar trend of binding energies was observed. Careful inspection of binding interactions showed that in all cases, except for PCB-OH, residues involved are mainly hydrophobic and some of them are common to all ligands. None of the hydrogen bonds stabilizing the hormone binding are conserved except for PCB-OH. Our results allow to describe a pattern of interactions for EDC ligands to steroid receptors suggesting the requirement of a large hydrophobic cavity to accomodate these chlorine carrying compounds. Although the affinity is lower than for natural hormones, their action can be brought about by a possible synergistic effect.

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