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Review Article

Applications of Molecular Modeling in Endocrinology- Review Article

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Abstract

Modeling and simulation techniques are now widely used alongside with their experimental counterparts in order to complement them, or sometimes to bring first insights into the possible outcome of experiments. "Modeling" is a general word that could encompass a wide range of methods that could be applied to a variety of subjects and problems. Here, the use of selected molecular modeling methods has been described in the field of endocrinology, throughout of some concrete examples.

Keywords: Molecular modeling, Homology model, Docking, Endocrinology

Introduction

In scientific studies, models are built after simplification of the object or event they intend to represent, should be verified, and if necessary, modified. In many applications the trueness of the model is of less importance than its adequacy for the intended application (1). Here, some examples are presented as representative applications of the field of molecular modeling in endocrinology. These two fields are chosen with regard to their more concrete applications in medical sciences, and this is an overview that should be considered as a glance into this vast subject.

Molecular Modeling

Molecular modeling is related to structural bioinformatics and concerns static and dynamic representations of macromolecules and ligands structures and interactions in computers. Of the many subjects of molecular modeling, some examples are described involving protein modeling, and computational drug design (which includes QSAR methods, docking and virtual screening among others). Protein modeling is the prediction of a three-dimensional structure for a protein of which only the amino acid sequence is known. Either comparative modeling (homology modeling) is used, with the use of a similar protein structure (2), or secondary structure predictions and use of ab initio methods is performed, that could predict the three-dimensional structure of a protein directly from its amino acid sequence (3). Refinement of the obtained models _as well as access to a dynamical picture of the protein or protein/ligand interaction_ is sometimes done by using molecular dynamics simulations (4). For a more detailed description of current issues in pro-

tein modeling, interested readers are referred to a recent review (5). Docking methods are essentially computer algorithms that give information on the possible binding site and binding mode of a ligand into a macromolecule or about macromolecule interactions with each other, such as protein-protein interactions (6). Each docked compound is given a score which is used in order to select the potentially most active compounds (7). Virtual screening is the process of searching for potential ligands of a particular target between thousands or even millions of compound that are synthesized in silico (in computer) (8). QSAR (quantitative structure-activity relationship) is also used in defining effective structural properties of potential drugs: this method finds a suitable equation that would summarize the physicochemical properties of a set of ligands. This equation could then be used in order to predict the effectiveness of novel compounds (9).

Each of these techniques has its own uses and limitations, and it should be mentioned that in the processes of drug design, a combination of these methods is usually applied.

Protein modeling examples

Wolframin

In the study of transmembrane proteins such as wolframin, computational methods have been privileged, due to practical difficulties that exist in the preparation of this class of proteins (10). Usually, an approximation is given about the location of their transmembrane segments, and their overall topology, i.e. a two-dimensional model is built. Wolframin is involved in wolfram syndrome, for which more than 160 mutations have been detected at amino acid level (Lesperance MM. WFS1 Gene Mutation and Polymorphism Database). In order to give some insight into the possible effect of some novel mutations, topology prediction was done with the use of several algorithms, and the best model was chosen in accordance with experimental data (Fig.1). In these cases, the impact of single amino acid mutations could be hypothesized _to some extent_ based on their biochemical properties and putative locations (11).

CYP 21A2

One of the most common form of congenital adrenal hyperplasia, results from 21-hydroxylase deficiency, which is caused by mutations in the encoding gene CYP21A2 (12). Models of the protein have been built in several studies, and novel mutations introduced and studied in these structures (13-15).

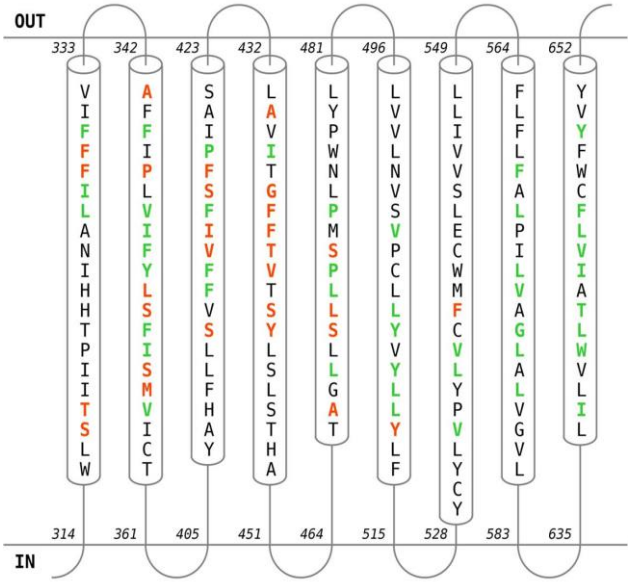

Fig.1: Schematic representation of a simple structure prediction for trans-membrane segments wolframin. Prediction was made with the use of HMMTOP server (www.enzim.hu/hmmtop)

In the case of an insertion mutation (962_963A), the model was able to show that both mutations would affect the heme-binding part of the protein and result in an incomplete P450 (13) (20). Associating experimental and modeling studies, the effect of K121Q mutation was assessed both in vitro and in silico, with the experimental data indicating a lower enzyme activity and decreased substrate affinity. The model revealed then the position of the mutated amino acid to be located in a helix possibly important in heme binding (14). An insertion of three valines at codon 71 was studied by a molecular dynamics simulation (15), using a previously reported model that had been useful in

explaining structural effects of about 60 disease-linked mutations (16). The simulation suggested the generation of a more unstable structure of the mutant protein (15).

CYP 17A1

In a close context, modeling of cytochrome P450c17 (a 17A-hydroxylase/17, 20-lyase) has been performed in multiple studies, and the effects of structural modifications resulting from mutations (ending in sexual infantilism, amenorrhea, and pseudohermaphroditism) (17-21) has been done. Another potential use of these models could be in the design of structure-based inhibitors of the enzyme, potentially effective in prostate cancer (22). With a three-dimensional structure of the enzyme, docking of new ligands and molecular dynamics simulations was performed, in order to design other potential inhibitors such as inhibitors of type 5 17_beta-hydroxysteroid dehydrogenase, which would also be effective in prostate cancer (23).

MHCII

Higher risk of developing Hashimoto's thyroiditis (a type of autoimmune thyroid diseases) has been found to be associated with specific regions of HLA (human leukocyte antigen, the major histocompatibility complex in human) (24). A modeling of HLA located a set of amino acids (Y26, Y30, Q70, and K71) positioned in the HLA-DR peptide-binding pocket that are associated with the disease. An interesting side-result of the modeling work was the finding of a difference between the structure of the disease-associated pockets in human and mouse suggesting that results derived from mouse models could not be readily extrapolated for human Hashimoto's thyroiditis (25). As a previous study had shown the importance of R 74 in HLA DR peptide binding pocket in Grave's disease (26), targeting this region has been suggested to have potential therapeutic effect (24).

TPO

Many cases of congenital hypothyroidism are related with thyroid peroxidase mutations (27). In a case of goitrous congenital hypothyroidism, a mutation of Q660E was observed; a protein model of the mutant was generated and submitted to molecular dynamics simulation. Electrostatic binding energy calculation showed the possibility of less interaction between the heme group and the mutant protein (28). In a rather different context, a model of the same protein was used to locate the putative amino acids that would be targeted by antibodies generated in autoimmune thyroid diseases (29).

Thyrotropin receptor

Thyrotropin receptor (TSHR) is a G-protein coupled receptor (GPCR), whose three-dimensional model has been used to clarify the potential roles of its various important regions. Experimental data was first gathered and checked with regard to the importance of the mutated residues, then focused modeling of particular segments such as extracellular loops and segments interacting with ligands (30, 31) and intracellular loops possessing a role in interacting with G-protein was performed (32, 33).

Mineral corticoid receptor

Pseudohypoaldosteronism type 1 is related with mutations of the gene encoding the mineralcorticoid receptor. Clinical studies, experimental and modeling techniques were used in order to generate mutant receptors, and allow a closer observation of mutated residues. In this case, a crystal structure of this receptor was used (34), where the E972G mutation seemed to disturb a hydrogen bond networking that would change the location of a helix involved in ligand binding (35).

Glucokinase

Numerous activating and inactivating mutations have been reported for glucokinase, leading to hyperinsulinemic hypoglycemia and diabetes mellitus respectively. The enzyme, which converts glucose to glucose-6-phosphate, is allosterically regulated, and based on its structure a precise visualization of the missense mutations locations is possible (36). As example, the V62M mutation, located in the vicinity of the activating allosteric site, is active in vitro, but causes hyperglycemia in the patients bearing this mutation. It is the struc-

ture and binding properties of the enzyme that may be affected, which are leading to hyperglycemia via complex mechanisms resulting in defect of its regulation (37).

pVHL

von Hippel-Lindau (vHL) disease is related to abnormality of the VHL gene, whose product (pVHL) has been characterized and crystallized. pVHL makes interactions with Elongin B and C, and this complex is part of a ubiquitin protein ligase. The available crystal structure allows the assessment of the effect of single mutations, as in the case of R167W. This arginine residue is conserved among species, and its mutation could lead to some disruption into the structure of pVHL, and subsequently, its interaction with Elongin C (38).

Examples of docking, QSAR, and virtual screening

GPR40 (FFAR1)

GPR40 ligand was identified to be medium to long chain fatty acid, and this receptor, alongside with related GPCRs GPR41 and 43 has been since considered as a drug target. Overexpression of GPR40 in transgenic mice had led to enhanced insulin secretion in presence of high levels of glucose, which is suggestive of potential therapeutic benefits upon activation of this receptor (39). A variety of agonists have been identified for GPR40 (40-43), and models of the receptor have been created in order to assess potentially important residues in ligand-receptor interactions (44, 45). In these studies, virtual screening methods are applied in order to discover new ligands for the receptor (2,600000 compounds were screened in this case, resulting in 6 hits) (42), docking methods could be used in order to position the ligand in its putative binding pocket, and molecular dynamics simulations follow in order to get a dynamic picture of residues involved in ligand-receptor interactions (44, 45).

Thyrotropin receptor

Antagonists of thyrotropin receptor (TSHR) could be proposed as potential therapeutic for therapeutic agents for the treatment of Graves' disease (46). Based on the similarity of this receptor with the luteinizing hormone/ chorionic gonadotropin receptor (LHCGR), a small molecule that was previously identified as partial agonist of that receptor (47) was tested for the thyrotropin receptor (31). Docking testing of this thienopyrimidine molecule performed on a model of TSHR resulted into detecting a putative binding site. This small molecule proved to be also a partial agonist of TSHR in experimental studies, but with lower potency compared with LHCGR (31). In a subsequent study, another model of the complex between TSHR and the partial agonist was used, novel compounds were synthesized and one compound found to be a moderate specific antagonist of the receptor. Docking of that compound into the receptor suggested that it would act by blocking the access of agonists to deeper regions of the binding pocket (46).

Androgen receptor

In conditions such as prostate cancer and alopecia, antagonists of the androgen receptor could be considered as therapeutics. Multiple non-steroidal compounds have been reported as antagonists of this receptor including structures containing indole (48), thiohydantoin (49, 50), benzopyran (51), benzonitrile (52, 53), and phenylpyridine (54). QSAR models have been formulated with a general view on antagonists (55) and for specific structures, for which the QSAR model has been utilized in a virtual screening to discover potential lead compounds (54).

P21 activated kinases inhibitors

Inhibiting various protein kinases is a proven general anti-cancer therapeutic mean, which has been recently highlighted in the treatment of endocrine tumors (56). P21 activated kinases (PAK) are also being considered as interesting targets in this regard. These possess important roles in diverse cellular processes, and have been shown to be also part of the downstream effectors of phosphoinositide-dependent kinase 1 (57). Their active-

tion could also contribute to cell survival (58). Thus, small molecule inhibitors of PAK have been studied (59). However, due to the difficulty of designing selective drugs toward ATP-binding pockets, efforts are now directed to the design of compounds that would target the allosteric binding site of the enzyme, and act specifically on this enzyme (60, 61). In this regard the enzyme structure is used in order to assess inhibitors mode of action, and refine their structures to obtain better compounds (62).

Cholesterol esterase inhibitors

Cholesterol esterase (CEase) catalyzes the hydrolysis of cholesterol ester which produces free cholesterol and inhibitors of this enzyme could be lowering cholesterol agents, as this has been shown in hamsters (63). As examples of inhibitors, isocoumarin-based molecules (64) and carbamate derivatives (65) could be named. Concerning the latter structures, classical QSAR studies have been performed for various derivatives (65-68). An interesting approach has been a more detailed analysis of the process, by dissociating the enzyme-inhibitory complex formation into two steps, which could result into a more precise view of the mechanistic details of the inhibitory process (65).

11 beta hydroxysteroid dehydrogenase type 1 inhibitors

Inhibitors of 11beta-hydroxysteroid dehydrogenase type 1 have been shown to be effective in mouse models (69) and recently in human (70) as antidiabetic agents that improve glucose metabolism. This enzyme catalyzes the conversion of cortisone to the active cortisol, and thus its inhibition, resulting in lower glucocorticoid activity, is suggested to be of use in metabolic and cardiovascular diseases (71). A variety of inhibitors have been designed and tested for this enzyme (72, 73). As usual in these research projects, docking of known chemicals into the enzyme structure would lead to insights on their binding modes and lead to the design of potentially more effective compounds (74). In the case of this enzyme, several pdb files are available which contain co-crystallized inhibitors (e.g. 3DQ5,3D4N,3-D3E,3BZU,2BEL).

Alpha-glucosidase and alpha-amylase inhibitors

A way to lower post-prandial hyperglycemia is the inhibition of enzymes that are involved in carbohydrate digestion. Acarbose has been the first of these agents to be commercialized as a drug, followed by miglitol and voglibose (75-77). An interesting finding has been the fact that these compounds may also possess preventive potential in the development of diabetes (78) and obesity (79), while increase of secretion of GLP1 has also been observed in the case of voglibose (80). Side effects of these drugs include gastro-intestinal problems, and mainly flatulence and diarrhea (81, 82). Multiple studies have been directed toward the characterization of other effective alpha-glucosidase inhibitors, many from natural sources (e.g. (83, 84)) and others based on synthesized structure (85). With the recent crystallization of the human alpha-glucosidase with inhibitors (86), structure-based design of inhibitors will finally become possible. Acarbose could also inhibit mammalian alpha-amylase (87), and protein extracts of white kidney bean have been shown to be inhibitors of this enzyme (88), and capable of antidiabetic effect in rats (89). Although there is still no alpha-amylase inhibitor officially marketed as antidiabetic/antiobesity agent , multiple studies report small molecules inhibitors of this enzyme (90-93), which have also been shown to be effective in vivo, in an animal model (94). Three-dimensional structure of alpha-amylase is available, but as to the inhibitors, proteinaceous ones have been mainly co-crystallized with the enzyme, which prompts the use of modeling techniques in order to provide an idea about the interactions between small molecule inhibitors and the enzyme (92, 93), and design of novel compounds. Molecules showing dual inhibitory activity toward both glucosidase and amylase (95) could also be of interest.

Conclusion

Modeling techniques are now widely used in various areas of medical sciences, ranging from clinical practice to more basic fields (e.g. assessing the

impact of a missense mutation on a protein structure). Given the advent of more powerful computer technologies, this highly interdisciplinary subject will continue to develop and propose new applications to the medical community.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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