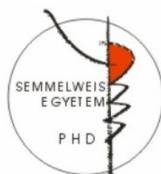


Structural analysis of histamine receptors and its application in drug design

PhD theses

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1 Introduction

The immune system plays an essential role in the homeostasis. In allergic diseases, however, the immune system recognizes harmless antigens as causative agents. Since the number of people suffering from allergy increases gradually, there is an unmet need for new, more potent antiallergic agents. Literature data suggest that histamine H1 and H4 receptors are potential therapeutic targets against allergy. H1 and H4 antagonists may be used separately or in combination representing an effective therapeutic option for allergy and other immunological diseases.

Currently, experimentally determined 3D structure of the histamine receptors is not available. On the other hand, homology models can be built by using the experimental 3D structure of a sequentially related protein, and the sequence alignment of the target and the template proteins.

Structure-based methods can be used effectively in several stages of drug design. For example, a structural protein model can be used to map the binding site, and explore novel sites for ligand design. These sites may provide opportunities increasing affinity and/or selectivity. Moreover, protein models of high quality can be used to identify novel ligands by virtual screening. In this case, promising compounds are selected from large databases by high throughput docking and the activity of these compounds is tested using *in vitro*

biological assays. *In vitro* hits can serve as suitable starting points for the development of novel, potent and selective ligands.

2 Objectives

We aimed to develop a relevant homology model of the human histamine H1 receptor (hH1R). We planned to use site-directed mutagenesis data from the literature to identify the binding site of the receptor. Furthermore, we aimed to dock known H1 antagonists to the binding site of the receptor. We proposed that we can identify novel interaction sites of the binding site by the analysis of the resulting ligand-receptor complexes.

We also aimed to develop a homology model of the human histamine H4 receptor (hH4R). After the identification of the binding site, we planned to dock known H4 actives to our receptor model. Then we aimed to analyze the applicability of hH4R models developed with different methodologies for virtual screening. Furthermore, our objective was performing a virtual screening on one of the developed hH4R models. For this purpose we collected more than 8.7 million compounds that were commercially available. After the virtual screening, we planned to test the H4 activity of the most promising compounds by biological tests.

3 Methods

Automatic sequence alignment was carried out by ClustalW 1.83. Homology models of hH1R and hH4R were built by MODELLER. As a template, we used the crystal structure of bovine rhodopsin determined in 2000 (PDB ID: 1F88). The models were validated by the available mutagenesis data from literature and by a set of structural validation programs (PROCHECK, WHATIF, PROSA, HARMONY). Ligand docking with flexible protein side chains was carried out by FlexiDock. FlexX was used for docking with rigid protein side chains. High throughput docking on the hH4R model was exclusively done using the “ClusterGrid” production grid system developed by the National Information Infrastructure Development Institute (NIIF). Binding poses were scored by the own score of FlexX as well as the scoring functions available in the CScore package (Sybyl). The H4 activity of the virtual hits were evaluated by radioligand binding assay on a SK-N-MC cell line stably transfected with hH4R.

4 Results

4.1 The human histamine H1 receptor (hH1R) model

4.1.1 Agonist binding and receptor activation

According to the available mutational data Asp107 (3.32) and Asn198 (5.46) have crucial roles in histamine binding, while Lys191 (5.39) is mainly responsible for receptor activation. We found that Asp107 (3.32) and Asn198 (5.46) are in favorable positions for anchoring histamine. On the other hand, Lys191 (5.39) is not able to form an H-bond with the imidazole N(1) of histamine. We speculate that after histamine binding Lys191 (5.39) can approach TM3. Consequently, the EC part of TM5 moves to the interior of the receptor, while the IC part, that is the G-protein binding site of hH1R, moves in the opposite direction. This can result in the activation of the receptor (Figure 1).

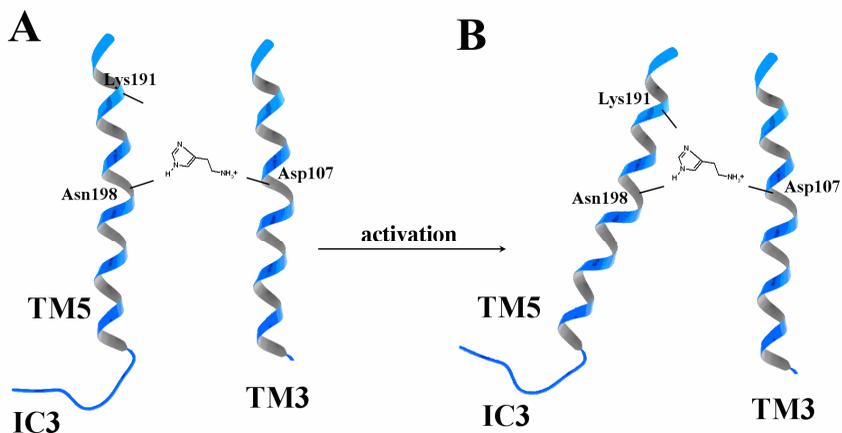


Figure 1. Proposed repositioning of TM5 in hH1R after histamine binding.

4.1.2 Identification of novel interaction sites for antagonist binding

Mutational data suggest a crucial role for Asp107 (3.32), Trp158 (4.56), Phe432 (6.52) and Phe435 (6.55) in antagonist binding. According to our model, we established that these residues are in favorable position to form interactions with ligands. We identified several novel amino acids at the binding site: Tyr108 (3.33), Phe184 (5.32), Phe190 (5.38), Phe199 (5.47), Phe424 (6.44), Trp428 (6.48) and Tyr431 (6.51). The role of these residues in ligand binding was not described in the literature so far.

4.1.3 Binding mode analysis of known H1 antagonists

Four known H1 antagonists (mepyramine, acrivastine, desloratadine, loratadine) were docked successfully to the binding site of the hH1R model by FlexiDock. Acrivastine, a second generation, zwitterionic antihistamine formed two ionic interactions with the side chains of Asp107 (3.32) and Lys191 (5.39) (Figure 2).

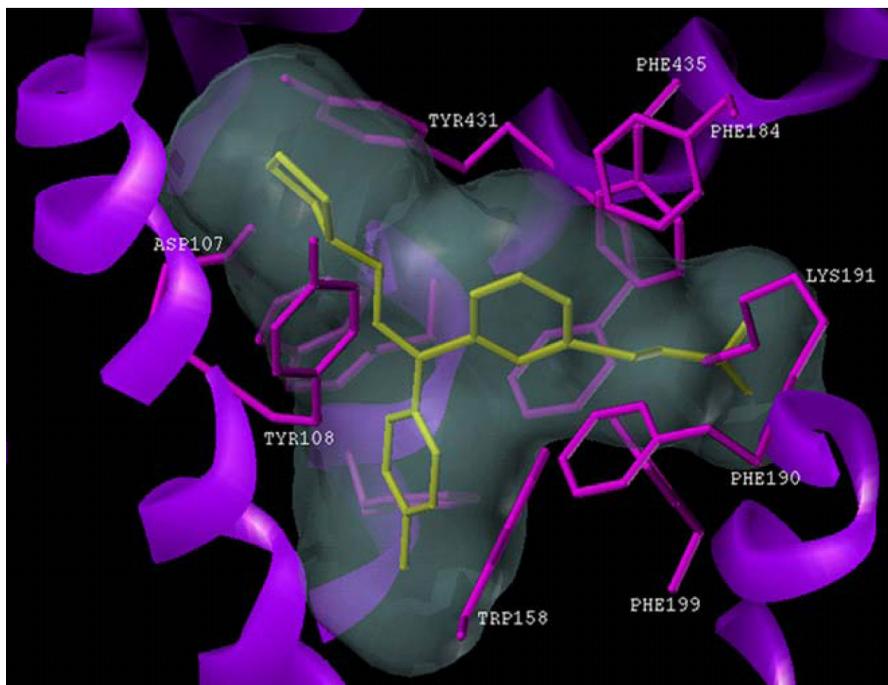


Figure 2. Proposed binding mode of acrivastine at the hH1R binding site.

4.2 The human histamine H4 receptor (hH4R) model

4.2.1 Agonist binding and receptor activation

Based on docking calculations, ligand-receptor complex optimizations and analyses of interacting molecular surfaces combined with experimental data from Shin et al. we identified a novel binding mode of histamine. In this binding mode, differing significantly from that previously reported, the protonated ethylamine side chain and imidazole N(3)-H group of histamine form interactions with the side chains of Glu182 (5.46) and Asp94 (3.32), respectively (Figure 3).

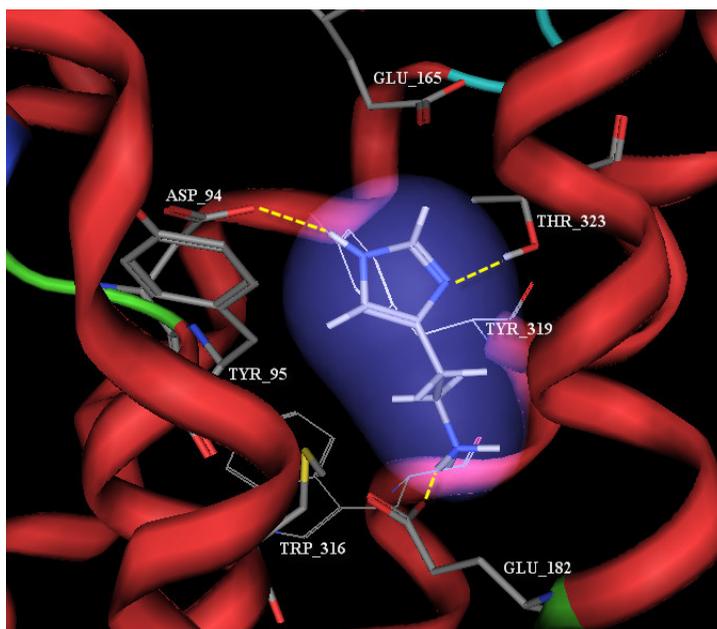


Figure 3. Proposed binding mode of histamine at the hH4R binding site.

We found that two agonists (histamine and OUP-16) form complementary interactions with Asp94 (3.32), Glu182 (5.46) and Thr323 (6.55), whereas JNJ7777120 interacts with Asp94 (3.32) and Glu182 (5.46) only.

4.2.2 Enrichment tests

We analyzed the applicability of six hH4R models developed by different methodologies for virtual screening by enrichment tests. We found that different inactive sets have only marginal effect on the highest achievable enrichment factors. On the other hand, the ligand used for optimizing the receptor model, the pharmacophore constraints and the different scoring functions applied in high throughput docking had all significant effect on the results.

4.2.3 Virtual screening

The database containing 8.7 million 3D-structures of small molecules was screened virtually on one of the developed hH4R models by FlexX. After the virtual screening we selected compounds for *in vitro* testing by two different methods using visual inspection and automatic filtering. In summary, 255 virtual hits were evaluated by radioligand binding assay. We identified sixteen compounds with

significant H4 activity representing an overall hit rate of 6.3 %. (Figure 4).

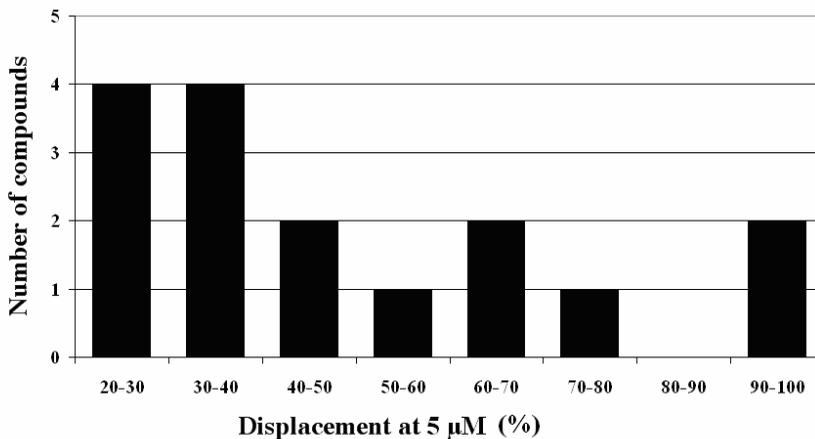


Figure 4. Distribution of the activities of the *in vitro* hits.

5 Conclusions

1. We have developed the structural model of hH1R by homology modeling. We analyzed the role of amino acids at the binding site that were proved to take part in agonist or antagonist binding. We identified several aromatic residues (Tyr108 (3.33), Phe184 (5.32), Phe190 (5.38), Phe199 (5.47), Phe424 (6.44), Trp428 (6.48), Tyr431 (6.51)) in suitable positions for antagonist binding. These novel interaction sites can be exploited in the design of new H1 antagonists.

2. We built the homology model of hH4R that were refined with considering ligand informations, docking and subsequent optimization. After the docking and optimization we found that two H4 agonists (histamine, OUP-16) form interactions with Asp94 (3.32) and Glu182 (5.46) as well as Thr323 (6.55). On the other hand, the H4 antagonist JNJ-7777120 forms interactions with Asp94 (3.32) and Glu182 (5.46) only. We demonstrated that histamine binds to hH4R in a different conformation that was previously proposed in the literature. Further investigations are needed to confirm this novel binding mode as well as the role of Thr323 (6.55) in ligand binding and receptor activation.
3. Six different hH4R models were evaluated by enrichment tests using twentyfive different scoring function combinations and three inactive molecule sets. We found that ligand information can significantly influence the performance of the models in virtual screenings. On the other hand, the application of different inactive sets did not considerably affect the maximal achievable enrichment factors. According to the calculated enrichment factors some of our hH4R models are suitable for virtual screening, and therefore can be used to identify novel H4 ligands.
4. We carried out a virtual screening on one of our hH4R models by docking more than 8.7 million 3D-structures of small

molecules. To the best of our knowledge, this is one of the largest structure-based virtual screenings, where the virtual hits were confirmed by an *in vitro* assay. Moreover, this is the first structure-based drug design study reported on the hH4R. After the virtual screening, we identified several novel ligands with significant H4 affinity. These scaffolds can serve as starting points in the development of potent and selective H4 ligands in future.

Basic publications related to the theme of the PhD thesis

Kiss, R.; Kovári, Z.; Keserű, G. M. Homology modelling and binding site mapping of the human histamine H1 receptor. *Eur. J. Med. Chem.* **2004**, *39*, 959-967.

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Other publication related to the theme of the PhD thesis

Jójiárt, B.; **Kiss, R.;** Viskolcz, B.; Keserű, G. M. Activation Mechanism of the Human Histamine H4 Receptor – An Explicit Membrane Molecular Dynamics Simulation Study. *J. Chem. Inf. Model.* **2008**, *48*, 1199-1210.

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Várkondi, E.; Pintér, F.; **Kiss, R.;** Schwab, R.; Breza, N.; Örfi, L.; Kéri, G.; Peták, I. Biochemical assay-based selectivity profiling of clinically relevant kinase inhibitors on mutant forms of EGF receptor. *J. Recept. Signal Transduct. Res.* **2008**, *28*, 295-306.

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