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Dedicated to Professor Zeno Simon
on the occasion of his 80th anniversary

3D HOMOLOGY MODEL OF THE HUMAN PROSTANOID RECEPTOR hEP3 AND DOCKING STUDIES

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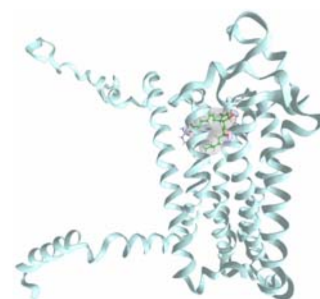
When crystal structure solvation of a biological target is hampered by different reasons, homology modeling is a reasonable solution to overcome these impediments. Using this technique, a homology model for the human prostaglandin-E2 (PGE2) receptor subtype 3 (hEP3) was built based on the X-ray structure of the human adenosine receptor A2a (PDB ID: 3EML). The stereochemical quality of model was verified and confirmed using the PROCHECK program. For the identification of the hEP3 binding site two applications from Schrödinger suite were used: SiteMap and Glide. The docking of the endogenous ligand (PGE2) in the hEP3 binding site, highlighted the key interactions with the important amino acids of the binding site. These results were confirmed by the mutagenesis experiments published in the literature. Thus, the validated hEP3 homology model can be further used in various computational studies in order to get a better understanding of its pharmacological implications.



INTRODUCTION

G protein-coupled receptors (GPCRs) are seven-helix transmembrane proteins which play an essential role in many vital body functions,[1,2] from sensory reception to the regulation of cell activity, movement, and death.[3] The regulation of signal transduction from extracellular environment to the internal metabolic system is the key role played by GPCRs. Many pathological conditions appear when disturbances in the regulation of signal transduction occur.[3] A large number of marketed drugs (>40%)[4] act on GPCRs, the most successful class of drug targets to date[5] (approximately 30% of all clinical drug targets). Another proof of the outstanding importance of the GPCRs is provided by the winning of Nobel Prize by Robert Lefkowitz and Brian Kobilka for major contributions to the field of this class of proteins.[6]

The prostanoid EP receptors which are activated by the endogenous ligand prostaglandin (PGE2) belong to the class A GPCRs superfamily and are classified in four subtypes: EP1, EP2, EP3 and EP4. The human prostaglandin-E2 (PGE2) receptor subtype 3 (hEP3) is the main receptor subtype that mediates PGE2 induced contractility in human pregnant myometrium at term.[7] Also, the hEP3 receptor mediates decreases in cAMP and inhibits some biological processes such as: neurotransmission, sodium and water re-absorption in kidney tubulus and gastric acid secretion.[8] Ras is another signaling pathway, involved in cancer pathology that can be activated by hEP3 receptor.[9] Increased biological data reveal that hEP3

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antagonist have a great potential for: the treatment of cardiovascular diseases,[10] inhibition of tumor growth and angiogenesi[11,12] thrombosis reduction[13-15] and also, brain damage reduction induced by inflammation and hypoxia-ischemia.[16] In this paper, we present the building of the 3D homology model for hEP3 receptor using as template the 2.6 Å resolution crystal structure of human adenosine receptor A2a. After the model refinement and evaluation, the identification of the binding site (the key amino acids involved in the interactions with the ligands) is a must. For this purpose the SiteMap[17,18] and Glide[19-21] software from Schrödinger suite were used. The obtained hEP3 homology model following by the docking procedure may provide important tools for understanding and guiding the design of the new ligands, agonists and/or antagonists.

METHODS

The following protocol has been used to build the homology model of hEP3. The primary sequence for hEP3 was retrived from the UniProt protein sequence database (sequence ID: PE2R3_HUMAN).[22] In the initial step multiple sequence-structure alignments were done using LOMETS[23] and iterative template fragment assembly simulations were performed. In the end, the X-ray structure of human adenosine receptor A2a was chosen as template (PDB ID: 3EML).[24] The alignment of the two sequences and the building of the hEP3 homology model were achieved by employing the on-line platform, I-TASSER server.[25-27] In order to assess the stereochemical quality of the 3D structure, the resulted model was evaluated with PROCHECK[28] server. The Protein Preparation Wizard[29,30] from Schrödinger software package was used to refine the built model of hEP3. SiteMap[17-18] was employed in the identification of the possible binding sites of the homology model of hEP3. For a better understanding of the binding mode of the endogenous ligand (PGE2) to the receptor, Schrödinger`s Glide SP docking protocol was used to run docking simulations of the ligand at the receptor's site. The structure of the endogenous ligand – PGE2 was prepared for docking using LigPrep[31] utility. In this respect, bond orders were corrected and all possible tautomers, and ionization states at physiological pH of 7.4 were generated. Finally, the structure was energetically minimized using the OPLS_2005 force field.

RESULTS AND DISCUSSION

The 3D homology model of hEP3 was built based on the experimental coordinates of the crystal structure of human adenosine receptor (hA2a), PD code: 3EML. The I-TASSER server was chosen to generate the homology model because it was ranked as top server for protein prediction in many CASP (Critical Assessment of protein Structure Prediction) experiments. The output of the I-TASSER server included five atomic models, ranked based on cluster density. The best model was selected taking into account the confidence score,[32] predicted TM-score[33,34] and RMSD[35] for the first model. The homology models were obtained based on an alignment between the target sequence (PE2R3_HUMAN) and the template sequence (3EML) with the aid of LOMETS (Local Meta-Threading-Server). The conserved amino acids in the GPCRs superfamily must overlay in the alignment of the two sequences, and the gaps should not be arranged in the domains of major importance, *i.e.* the transmembrane regions (TMs). The alignment used for the building of the best homology model was checked based on these information and all the required criteria were fulfilled. Thus, the distribution of conserved amino acids on the seven transmembranes aligned is as follows: G1.49, N1.50 and L1.52 on TM1; L2.46, A2.47, D2.50 and L2.56 on TM2; A3.45, A3.47, D/E3.49 (D3.49 at Template_3EML and E3.49 at Model_EP3), R3.50, A3.53, I3.54 and R3.55 on TM3; W4.50, A4.55, L4.58, P4.60, L4.62 and G4.63 on TM4; L5.48, L5.51 and L5.59 on TM5; C6.47, W6.48, P6.50, L6.51 and I6.53 on TM6 and A7.38, L7.41, N7.45, P.7.50 and Y7.53 on TM7. The Ballesteros-Weinstein convention[36] was used for the numbering of the amino acids. The alignment is presented in Fig.1.

In the next step, the hEP3 homology model was stereochemically validated. The assessment of the model's quality was performed with PROCHECK server which highlights regions that may need further investigation.[28]

The validation step verifies: the Ramachandran map, the deviation from normality of torsion angles, bond angles, bond lengths, planar groups and distances between some atoms. Also, the main-chain and side-chain parameters were evaluated. The Ramachandran plot describes the rotation of Psi and Phi dihedrals, pointing to the allowed and disallowed regions of torsion dihedrals values, being one of the most important indicators of the quality of 3D protein

structures.[37] The evaluation process carried out with PROCHECK server showed that obtained homology model of hEP3 requires the improvement of its stereochemical parameters.

The refinement process was accomplished using the Protein Preparation Wizard.[29,30] Hydrogen atoms were added and possible disulfide bonds were generated; in the end the protein structure was minimized using default OPLS_2005 force field with backbone atoms held fixed.[33] The refined model is very good and the stereochemical validation of the model was certified also by Ramachandran map.

The Ramachandran plot of the refined 3D homology model of hEP3 is displayed in Fig. 2 and has the following distribution of non-proline and non-glycine amino acids residues: 90.5% residues are located in the most favored regions [A, B, L], 6.6% residues in additional allowed regions [a, b, l, p], 2.3% residue in generously allowed regions [~a, ~b, ~c, ~d] while only 0.6% of the residues are located in disallowed regions. The amino acid residues found in disallowed regions do not influence the quality of the model because they are not situated in the important areas (transmembrane domains). The refined hEP3 homology model is presented in Fig. 3.

The refined hEP3 model is intended to be used in docking studies and for this purpose the identification of the protein binding site is necessary. For this reason, SiteMap[17-18] was used to discover the possible binding site within the protein by identification of one or more regions that may be suitable for ligand binding. Thus, the contour maps (hydrophobic and hydrophilic) are generated. SiteMap[17-18] generates a score (SiteScore) for each discovered binding site, which is further used to evaluate the possible binding sites. The primary binding site identified by SiteMap has a SiteScore of 1.1, and it is located within the transmembrane bundle towards the extracellular side of the receptor (Fig. 3).

In order to characterize the binding mode of PGE2 in the active site, a docking procedure using Glide application from Schrodinger was performed. The grid box was centered on the active site and the size was set for a docked ligand to be smaller than 20 Å. Glide SP default protocol was used for the flexible docking of the PGE2 ligand. The best pose resulted from the docking experiment is presented in Fig. 4. PGE2, is involved in the formation of two interactions with two arginine residues as follows: a hydrogen-bond with an arginine residue from eLP2, R214, and a salt bridge with R333(7.40) found on helix 7. The carboxylic group of PGE2, negatively charged at physiological pH, is favorably placed in the vicinity of R333(7.40) amino acid to interact with its positively charged side-chain, while the 3-OH substitutent forms a hydrogen bond with the positively charged R214 from the eLP2. Also, the ligand is stabilized into the binding site by the presence of several hydrophobic amino acids residues, such as: V52, P55, V109, V110, I111, V113 and F328.

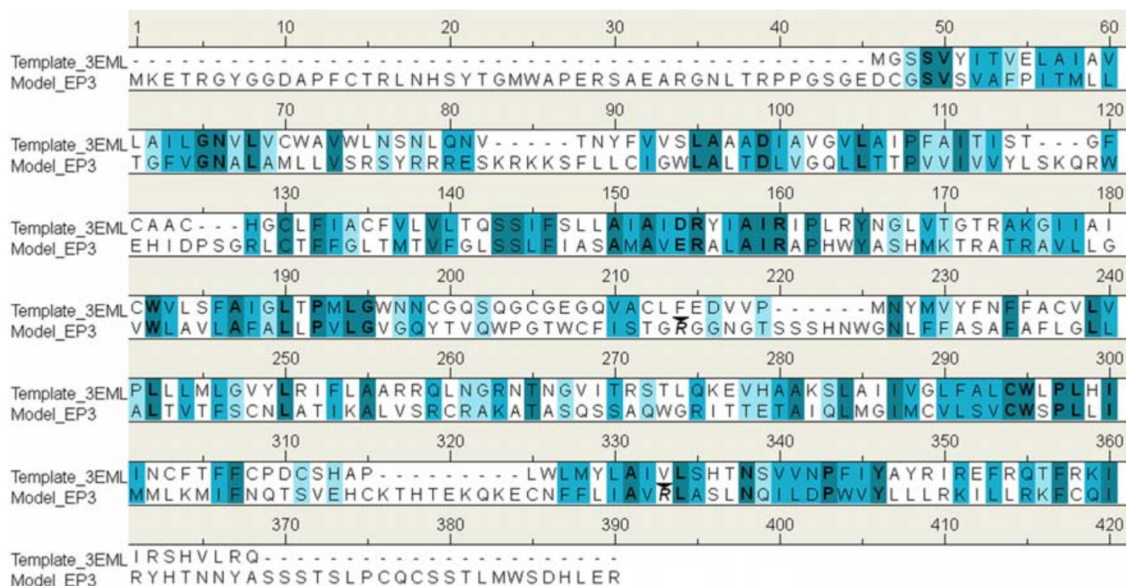

Fig. 1 – The sequences alignment of adenosine A2a (PDB ID: 3EML) and hEP3 (UniProt ID: PE2R3_HUMAN). Color code: Dark to light background – identical to low similar amino acids sequence; white – dissimilar amino acids; bold amino acids – highly conserved amino acids in the GPCR class; bold and caret amino acids – key amino acids in the ligand binding domain of hEP3.

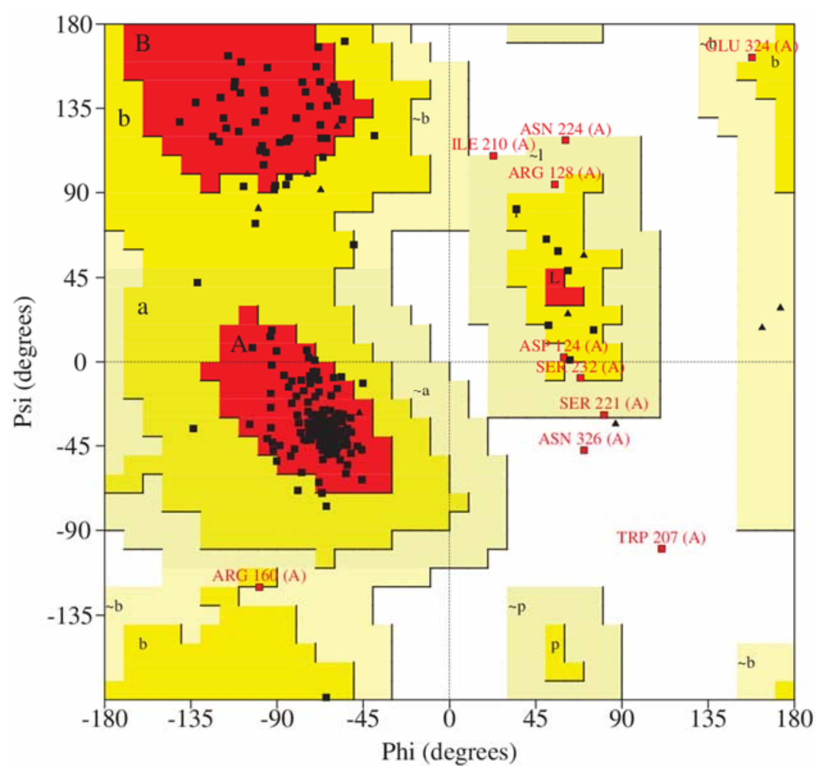

Fig. 2 – Ramachandran map for the hEP3 homology model showing that 90.5% of the Psi and Phi dihedral angles are clustered in the sterically allowed regions.

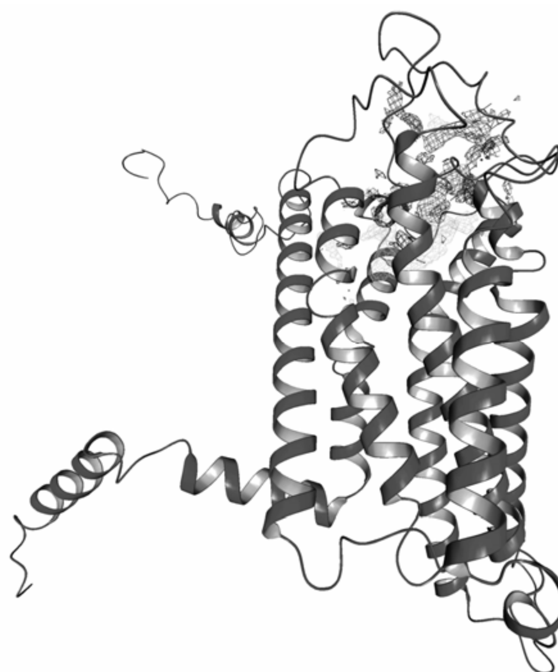

Fig. 3 – 3D structure of hEP3 obtained by homology model; the binding site found with SiteMap to be the most probable is depicted as grid surface; hydrogen-bond acceptor map – black mesh; hydrogen-bond donor map – dark gray mesh; hydrophobic map – light gray mesh.

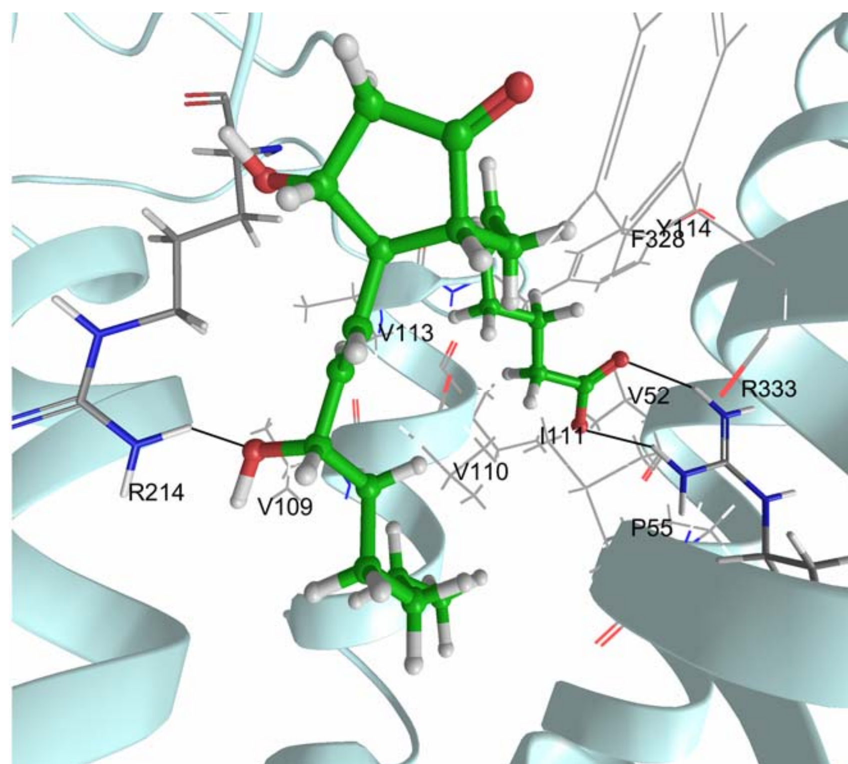

Fig. 4 – The alignment of PGE2 in the binding pocket of the hEP3 receptor. PGE2 structure is depicted with sticks and balls; the amino acids interacting with ligand are depicted with sticks, while the amino acids forming the hydrophobic environment for the ligand are represented with lines.

Our results are fully supported by previously reported studies, which have identified the key amino acids involved in ligand binding to the hEP3 receptor using site-directed mutagenesis experiments. Accordingly, the mutation of R7.40 to alanine abolished [^{3}H]PGE2 binding to the receptor, suggesting this residue is involved in ligand binding and transduction processes,[39-40] while other studies have shown that R214 and S211, from the second extracellular loop of (eLP2), are responsible for the ligand recognition.[41]

CONCLUSIONS

This study shows the steps followed for the building of a qualitative 3D homology model of hEP3 receptor. The "qualitative" term refers not only to the good stereochemical parameters of the generated model but also to the validation of the model by means of conclusive data that are in good agreement with the experimental ones obtained by site-directed mutagenesis studies. One of the assumptions was "that the ligand-recognition site might differ from the final ligand-binding site".[41] Our study provides a new hypothesis according to which the binding site may be an extended one, and includes both amino acids from transmembrane domains (particularly, R7.40 from TM 7) and amino acids from the second extracellular loop (especially R214). In the light of the presented results, we believe that the obtained 3D homology model of hEP3 can be a useful tool in further studies for the rational drug design.

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