

Modeling of the hEP1 receptor based on the crystallographic structure of β_2 -adrenergic receptor and its assessment with docking studies and molecular dynamics simulation

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ABSTRACT

Introduction: The human EP1 (hEP1) prostanoid receptor is a G-Protein Coupled Receptor (GPCR) which plays important physiological roles in some systems in the body like cardiovascular and immune systems and could be a very important target for drug design.

Materials and methods: To understand the molecular structure of hEP1 receptor, a homology model of the receptor was constructed from the 2.4 Å resolution crystal structure of human β_2 -adrenergic receptor (PDB code: 2RH1), using three different sequence alignments. The model including PGE₂ inside the active site was subjected to molecular dynamics simulation. Docking studies were performed for PGE₂ and 10 prostanoid analogs in the active site of the modeled receptor.

Results and Discussion: The structure of modeled receptor remained stable during the 10 nanosecond(ns) simulation. In the docking simulations a correlation of $r^2=0.74$ was observed between the K_i values and the docking scores of the prostanoid compounds. The structure which was modeled in the present study can be used in the structure-based drug design, helping the rational design of novel ligands for the hEP1 receptor.

Keywords: human prostanoid E1 receptor, G-protein coupled receptors (GPCRs), human β_2 -adrenergic receptor, homology modeling, flexible-ligand docking, molecular dynamics simulation

INTRODUCTION

The prostanoid receptors belong to the G-protein coupled receptors (GPCRs) which comprise a large family of integral membrane proteins. Their endogenous ligands, prostanoids, exert a variety of actions in various tissues and cells: from relaxation and contraction of various type of smooth muscles to modulation of neuronal activity, regulation of secretion and motility in the gastrointestinal tract, involvement in apoptosis, cell differentiation and oncogenesis (1, 2). Therefore it seems that the prostanoids generation pathway and their receptors are involved in a broad spectrum of diseases including cancer, inflammation and hypertension (1). For example the EP1 receptor, a subtype of the EP receptors, preferentially binds to prostaglandin E₂ and is described as a smooth muscle constrictor and fever inducer and is involved in fertilization in female and inflammatory pain (2). As a result the physiological roles assigned to the GPCRs family, like prostanoid receptors, are very important and

finding the structure of these receptors to design new drugs is one of the most demanding targets in biological studies. There are three crystal structures of members of GPCRs receptors available: β_2 -adrenergic receptor (PDB: 2RH1) (3), β_1 -adrenergic receptor (PDB: 2VT4) (4) and A_{2A} adenosine receptor (PDB: 3EML) (5). It seems that for improvement of the number of discovered receptor genes compared to the number of receptor structures, another approach has to be considered. As a consequence it has been proved appropriate to employ homology modeling in order to derive a model of the GPCR ligand binding site and to facilitate drug design process (6). Early attempts were focused on bacteriorhodopsin as a template for modeling the structure of the receptors (7). Since there is no significant sequence similarity between bacteriorhodopsin and GPCRs, conventional homology modeling techniques cannot be applied to this problem with any confidence (6). There are some studies about the use of the bovine rhodopsin structure as a template for homology

modeling (8-10). Another study carried out by using bovine rhodopsin as a template to generate the homology model of h β_2 adrenergic receptor and compared it with the crystallographic structure of h β_2 adrenergic receptor (11). The constructed model was well and in agreement with the crystallographic data. The aim of this study was to obtain the structure of the hEP1 receptor based on homology modeling, using the h β_2 -adrenergic receptor as a template, and assessment of the whole structure and binding site according to the mutagenesis data.

Methods

Molecular structures

The human prostaglandin E2 receptor EP1 subtype (PE2R1, 402 amino acids) sequence was obtained from SWISS-PROT database (accession number P34995). Human β_2 -adrenergic receptor crystal structure with 2.4 Å resolution (PDB code: 2RH1) was downloaded from Protein Data Bank and used as a template for comparative modeling of PE2R1.

Multiple sequence alignment

The N-terminal and the third extracellular loop of the h β_2 -adrenergic receptor were ignored for the alignment to the hEP1 receptor sequence. Sequence alignments were performed using three distinct methods. First alignment (ALIGN-I) was produced by the sequence alignment of h β_2 -adrenergic receptor and hEP1 using ClustalW (12). Multiple sequence alignment between h β_2 -adrenergic receptor and the 41 prostanoid receptors from all species called ALIGN-II. The TM segments of the hEP1 and those of h β_2 -adrenergic receptor were aligned as indicated in the GPCR database (<http://www.gpcr.org>). For the extra-transmembrane regions, the ClustalW 1.83 was utilized using a gap penalty of 10 and employing BLOSUM62 weight matrix. The obtained alignment was called ALIGN-III.

Homology modeling

A homology model of EP1 with the homologous h β_2 -adrenergic receptor based on the different sequence alignments, as described above, was made by using MODELLER version 9.2 (13). From the alignments, 3D models containing all non-hydrogen atoms were obtained automatically using the method implemented in MODELLER. Based on the sequence alignments, MODELLER extracts a large number of spatial restraints from the template structures and builds a molecular model of the query protein. Of the 1000 models generated with MODELLER, the one corresponding to the lowest value of the probability density functions (pdf) and fewest restraints violations was used for further analysis. An *ab initio* method implemented in the MODELLER that has been demonstrated to predict the conformations of loop regions was used to build the loops. The models

constructed based on ALIGN-I, -II and -III were named as MODEL-I, II and III respectively. Then the DOPE profile and the Ramachandran plot for each model were obtained using MODELLER and PROCHECK (14), respectively. The intracellular loop 3 as a problematic region in the MODEL-II was subjected to an *ab initio* loop modeling procedure implemented in the MODELLER and the best resulting model was called as MODEL-IV.

Molecular dynamics simulations

All further calculations were carried out with the GROMACS (15) simulation package using the ffgmx force field at constant temperature (300 K), pressure (1 bar) and number of particles. Solvent (i.e. water and ions), lipid, protein and ligand were coupled separately to a temperature bath, with a coupling constant of $T=0.1$ picoseconds (ps).

PGE₂, as a ligand, was placed into the active site region of MODEL-IV of hEP1 receptor according to the hydrogen bond contacts determined in point mutation analysis. A minimization step was subjected to the ligand and the protein. The resulting protein/ligand model was used in the following MD. A topology for PGE₂ was obtained from the PRODRG server (16). The lipids were described by using a previously developed topology file (Tieleman, see <http://moose.bio.ucalgary.ca>).

Protein/ligand model was inserted at the center of the POPE bilayer with its long axis normal to the membrane-water interface. The α -helical domain of the receptor was placed at the same level as the lipid bilayer, and the eighth short helix was placed at the polar interface of the membrane. Overlapping lipid and water molecules were discarded to avoid poor van der Waals interactions. Then twenty eight chloride ions were added to the solution in order to ensure neutrality of the entire system that comprised the receptor, 251 POPE, 12424 water molecules and 28 chloride ions (a total of 53485 atoms). Periodic boundary conditions were applied in all three directions of space.

A common approach adopted to equilibrate the simulation box by putting the restraints on the protein and the ligand, while the lipids and the water molecules optimize their interactions with the protein (17). This first part of the molecular dynamics was held for 2 ns. Temperature and pressure of the system were controlled by their couplings to the reference values of 300 K and 1 bar using time constants of 0.1 and 1.0 ps respectively. After minimisation of the complete system the restraints were removed stepwise with two times 100 ps MD simulation. Finally a 10 ns molecular dynamics was carried out on the entire system. The run parameters were the same as above.

Docking procedure

Three-dimensional structure of PGE₂ and 10 other

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ALIGN-I
hb2adr DEWVWVGMGIVMSLIVLAVFGNVLVITAIKFERLQTVNYPFITSACADLVMLGAVVFFGAHILMKMWTFG---NFWCFEFTSIDVLCVTASIEITLCVIAVDRYFAITSPFKYQSLITKPKARVILIMVWIVSGLTFL 139
hEP1 P-IFSMTLGAVSNLALALLA-----QAAGRLRRRSAAATFLLFVASLLATDLAGHVIIFG---ALVLRLYTAGRAPAGGACHFLGGCMVFFGLCPILLGCGMAVERCVGVTRPFLHAAARVSVARARLALAAVALVALL 133
      TM1 TM2 TM3 TM4
hb2adr PFGMHVYRATHQEAINECYAETCCDFFTNQAYAIASSIVSFYVPLVIMVYVSRVFEAKRQL-----KFCLEKHKALKTGLIMGTFTLCWLPFFIVNIHVHVIQDNIIRKEVYIL 250
hEP1 PLARVGRVYELQYPGTWCFIPLGPPGWR-----CALLAGLFASLGLVALLAALVNCNLS-GLALLRARWRRRRRPPASGPDSSRRRWGAHGPRASASSASSIASASTFFGGSSRSGSARRARAHDEMVMVQVLGIMVVC 269
      TM4 TM5 TM6 TM7
hb2adr LNWIGVNSGFNPLIYCRSPDFRIAFQELLCL
hEP1 ICWS-----PMLVLA--LAVGWSSTSL
      TM7
ALIGN-II
hb2adr DEWVWVGMGIVMSLIVLAVFGNVLVITAIKFERLQTVNYPFITSACADLVMLGAVVFFGAHILMKMWTFG-NFWCFEFTSIDVLCVTASIEITLCVIAVDRYFAITSPFKYQSLITKPKARVILIMVWIVSGLTFL 136
hEP1 LPFISMTLGAIVSNLALALLAQAAGRL-----RRRSAAATFLFVASLLATDLAGHVIIFGALVLRLYTAGR----APA--GGACHEFLGGCMVFFGLCPILLGCGMAVERCVGVTRPFLHAAARVSVARARLALAAVALVAL 131
      TM1 TM2 TM3 TM4
hb2adr SELPIQMHVYRATHQEAINECYAETCC-----DFFTNQAYAIASSIVSFYVPLVIMVYVSRVFEAKRQL-----KFCLEKHKALKTGLIM 219
hEP1 VALAVALLPLARVG-----RYELQYPGTWCFIPLGPPGWRQALLAGLFASLGLVALLAALVNCNLSGLALLRARWRRRR-RRRPPASGPDSSRRRWGAHGPRASASSASSIASASTFFGGSSRSGSARRARAHDEMVMVQVLGIM 266
      TM4 TM5 TM6 TM7
hb2adr GFTFLCWLPPFFIVNIHVHVIQD-----LIRKEVYILLNWIGVNSGFNPLIYCRSPDFRIAFQELLCL
hEP1 VVSCICWSMPLV-LVALAVG-----GWSSTSLQRFLFLAVRLASWNLDFWVYILLRQAVLRQLRLRLLP
      TM6 TM7
ALIGN-III
hb2adr DEWVWVGMGIVMSLIVLAVFGNVLVITAIKFE--RLQ---TVNYPFITSACADLVMLGAVVFFGAHILMKMWTFG-NFWCFEFTSIDVLCVTASIEITLCVIAVDRYFAITSPFKYQSLITKPKARVILIMVWIVSGLTFL 136
hEP1 AVPPSGASPALEIFSMTLGAVSNLALALLAQAAGRLRRRSAAATFLLFVASLLATDLAGHVIIFGALVLRLYTAGR--GRAPAGGACHEFLGGCMVFFGLCPILLGCGMAVERCVGVTRP-----LLHAAARVSVARARLALAAVAL 136
      TM1 TM2 TM3 TM4
hb2adr SELPIQMHVYRATHQEAINECYAETCCDFFTNQAYAIASSIVSFYVPLVIMVYVSRVFEAKRQL-----RQL-----KFCLEKHKALKTGLIM 205
hEP1 VALAVALLPLARVG-----RYELQYPGTWCFIPLGPPGWRQALLAGLFASLGLVALLAALVNCNLSGLALLRARWRRRRRPPASGPDSSRRRWGAHGPRASASSASSIASASTFFGGSSRSGSARRARAHDEMVMVQVLGIM 262
      TM4 TM5
hb2adr LKHKALKTGLIMGTFTLCWLPFFIVNIHVHVI-----QDNIIRKEVYILLNWIGVNSGFNPLIYCRSPDFRIAFQELLCL
hEP1 AHDVEMVQVLGIMVVCICWSMPLV-LVALAVG---SSTSLQRFLFLAVRLASWNLDFWVYILLRQAVLRQLRLRLLP
      TM6 TM7

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Figure 1. Alignment of hEP1 and $\text{h}\beta_2$ -adrenergic receptor (hb2adr) by three different methods. ALIGN-I and II are between hEP1 and between 41 prostanoid receptors from all species with β_2 -adrenergic receptor respectively (by ClustalW). ALIGN-III extracted from global alignment of all proteins belongs to rhodopsin-like family. Seven TMs are depicted by underlined letters.

prostanoid analogs, including all hydrogen atoms, were constructed and optimized using Polak-Ribiere conjugate gradient algorithm and AMBER95 force field implemented in HyperChem (HyperCube Inc., Gainesville, FL). Docking calculations with GOLD (Genetic Optimization of Ligand Docking) version 3.0.1 (18) were performed using default parameters. The binding site of MODEL-IV definition was based on the known SAR data and site-directed mutagenesis information and all amino acid residues within 10 Å from the center constituted by the binding site. Visual inspection was performed to confirm that all important amino acids were included in the defined binding site. Each molecule was docked 100 times and the top-ranked pose was retained for further analysis.

Molecular images and animations

All the molecular images and animations were produced by using VMD (19) and rendered by Tachyon ray tracer. Schematic two-dimensional representations of the docking results were produced using LigPlot (20).

RESULTS

Multiple sequence alignment and homology modeling

All three alignments were employed in the protocol have covered most of the conserved residues (Figure 1). The ALIGN-I, II and ALIGN-III were entered to the protocol, modeling process was employed for these three sets of alignments and models constructed which are called as MODEL-I, MODEL-II and MODEL-III respectively.

Based on the sequence alignments, MODELLER extracts a large number of spatial restraints from the template structures and builds a molecular model of the query protein. The resulting output was a homology model of hEP1. The alignment of the hEP1 and β_2 -adrenergic receptor identified

in the GPCR database and obtained from multiple sequence alignment between β_2 -adrenergic receptor and the 41 prostanoid receptors from all species. Also models which were generated by the alignment among all prostanoid receptors and β_2 -adrenergic receptor were almost as compatible as alignment between two of these proteins

The models were distinguished by comparison of the pdf, stereochemical quality of the models, with PROCHECK, and DOPE (Discrete Optimized Protein Energy) scores as summarized in table 1. Evaluation of the stereochemical quality of the MODEL-II with PROCHECK showed that no residue is in the disallowed regions of the Ramachandran plot (Figure 2). DOPE, which is implemented in the MODELLER, was used to assess the energy and the quality of the models as a whole. Because MODEL-II had the lowest deviation relative to β_2 -adrenergic receptor and the best stereochemical quality, it was chosen for further refinement step.

Molecular dynamics simulation

The seven transmembrane helices reached to a C α atom RMSD of nearly 6 Å from the starting structure (Figure 3A). Analysis by GROMACS command, g_energy confirmed that the total system energy dropped to its final value during MD simulation after 500 ps (Figure 3B). Overall, the seven TM segments maintained their α -helical secondary structures during 10 ns simulation. The hEP1 receptor (MODEL-IV) and its ligand inserted in the hydrated lipids bilayer after 10 ns MD simulation is shown in figure 4. The key interactions of ligand PGE₂ and the prostanoid receptors have been highlighted by site-directed mutagenesis. These include a strong hydrogen bond between the carboxylic group of PGE₂ and some hydrogen donors in the upper part of the TM7 oriented inside the protein canals which are formed by TM1, TM2, TM3 and TM7. In the MODEL-IV, Arg338 and Phe334 in TM7 were involved in

Table 1. Comparison of the model fitness criteria among the three models.

Model	PDF	PROCHECK analysis		DOPE score
		Residues in most favored regions	Disallowed regions	
MODEL-I ^a	1406	90.9%	0.4%	-25.44
MODEL-II ^b	1169	94.3%	0%	-31.009
MODEL-III ^c	1329	90.2%	0.7%	-31.951

^aBased on EP1/ β_2 -adrenergic alignment.

^bBased on prostanoid receptors/ β_2 -adrenergic alignment.

^cBased on the rhod-like GPCR alignment.

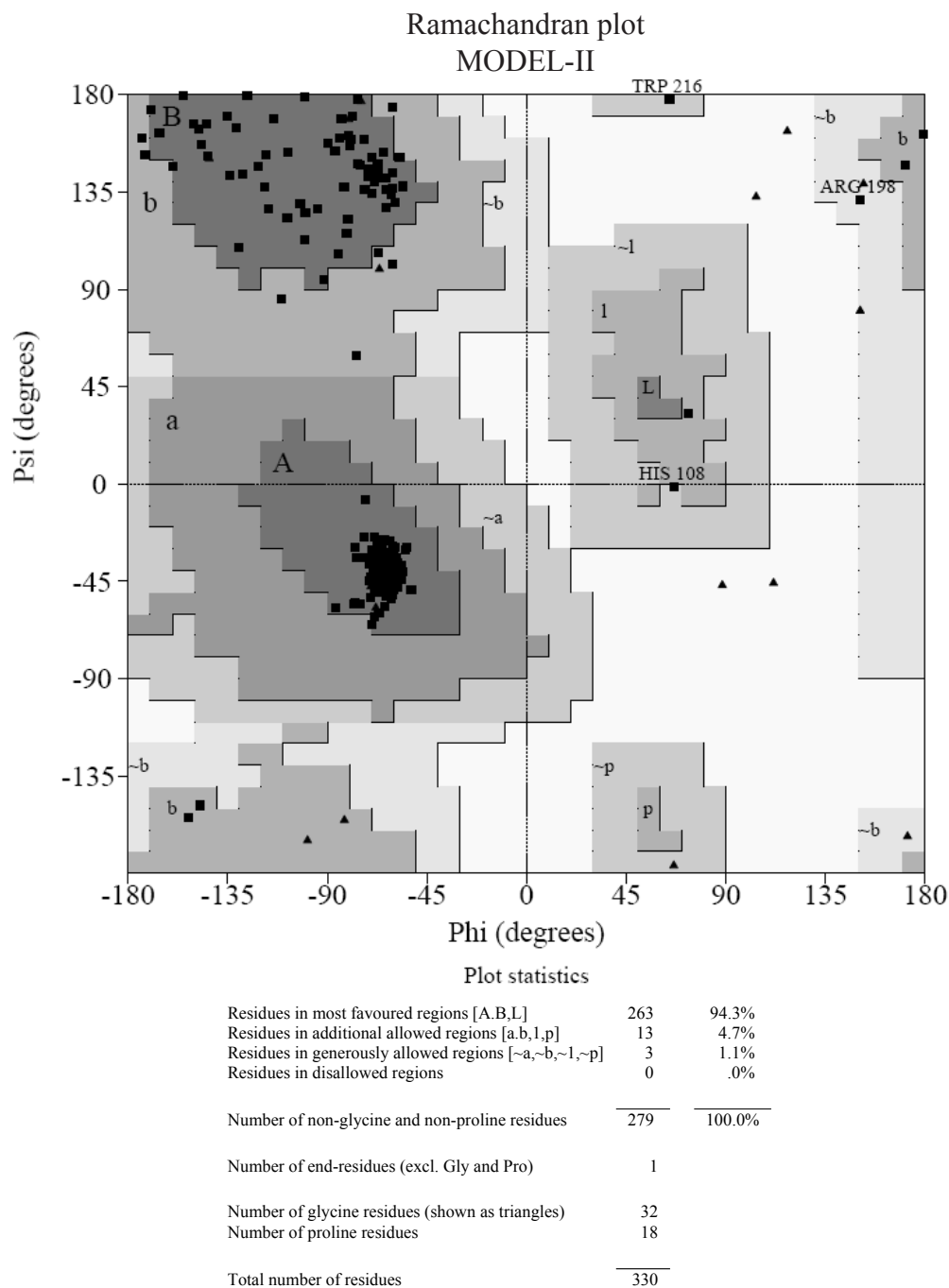


Figure 2. Ramachandran plot analysis of the hEP1 receptor model (MODEL-II).

Table 2. Docking scores and K_i values for prostanoid compounds.

Compounds	K_i^a (μM)	Docking Score ^b
9-Deoxy-9-methylene-prostaglandin E2	0.007425	46.51
Iloprost	0.009702	48.74
16-Phenoxy- ω -tetranor-prostaglandin E2	0.01683	49.49
M&B 28767	0.4752	49.04
15-keto-Prostaglandin E2	2.376	48.55
Prostaglandin E2 methyl ester	1.683	50.23
U46619	17.82	47.09
15(R)-Prostaglandin F2 α	49.5	45.7
Prostaglandin K2	49.5	43.89
Thromboxane B2	49.5	45.04

^aExperimental K_i values by Ungrin et al.

^bThe structure docked with the GOLD score.

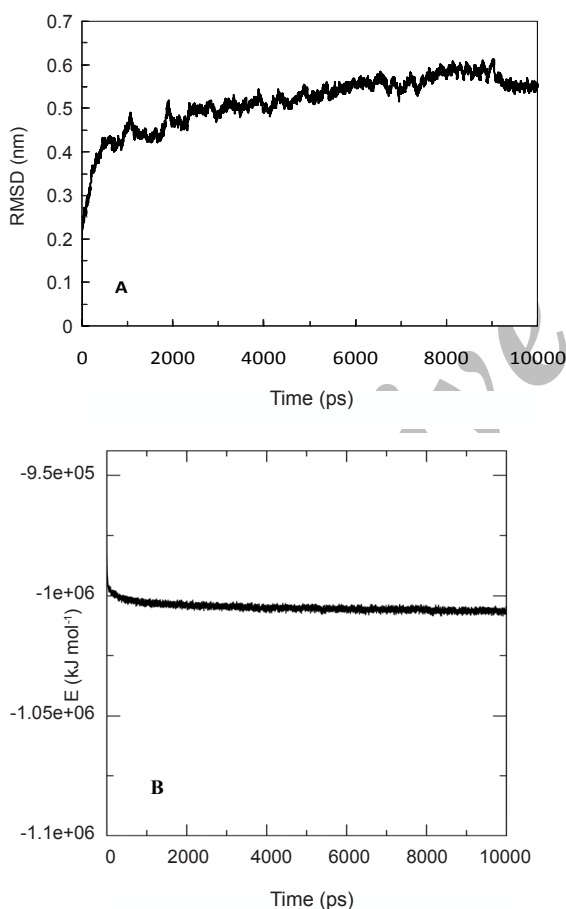


Figure 3. Evolution of the RMSD of the protein's $C\alpha$ (A) and Potential energy fluctuation of the system (B) during 10 ns MD simulation.

interaction with PGE₂ carboxylic moiety.

Docking of the prostanoid analogs

In order to investigate the prediction power as well as the characteristics of the binding site of the model

of this study, docking analysis was performed on PGE₂, as a potent analog of the hEP1 and some other prostanoid analogs (Table 2). PGs have two structural features, a cyclopentane ring and the side chains, and the receptors are supposed to recognize both of these structures and stabilize the ligand binding.

The putative binding domain of the prostanoid receptors lie within the upper half of the transmembrane-spanning region and this pocket is formed mainly by the first, second and seventh transmembrane segments, of which the former two are involved in the recognition of the ring structure and the latter in that of the side chains.

PGE₂ is located between the TM1, 2, 3, 7 helices and is covered by the EL2. The hydrophilic and hydrophobic interactions in the predicted binding mode of PGE₂ to hEP1 receptor are shown in figure 5. Two important hydrogen bond interactions were identified between the agonist PGE₂ and hEP1 receptor of which one is between 1-COOH group on the α chain of PGE₂ and Arg338 and Phe334 in TM7 of receptor. The other hydrogen bond interaction involves 9-C=O of the cyclopentane ring of PGE₂ and Gly193 (EL2). Also 15-OH of the ω chain has tendency for hydrogen bonding to Thr100, Ala101 and Gly102 (the first three residues of second helix).

Also table 2 shows the K_i of nine other prostanoid compounds, which was reported earlier by Ungrin et al. (21) and their corresponding docking scores, using GOLDScore, for all compounds docked to the hEP1 receptor.

DISCUSSION

The model constructed on the basis of ALIGN-II (Figure 1), MODEL-II, has the least PDF violations and its structural conformation, geometry, stereochemistry and loop conformation are in better position compared to other models. The stereochemistry of nearly all of the residues in MODEL-II is in accordance to preferred ϕ and ψ

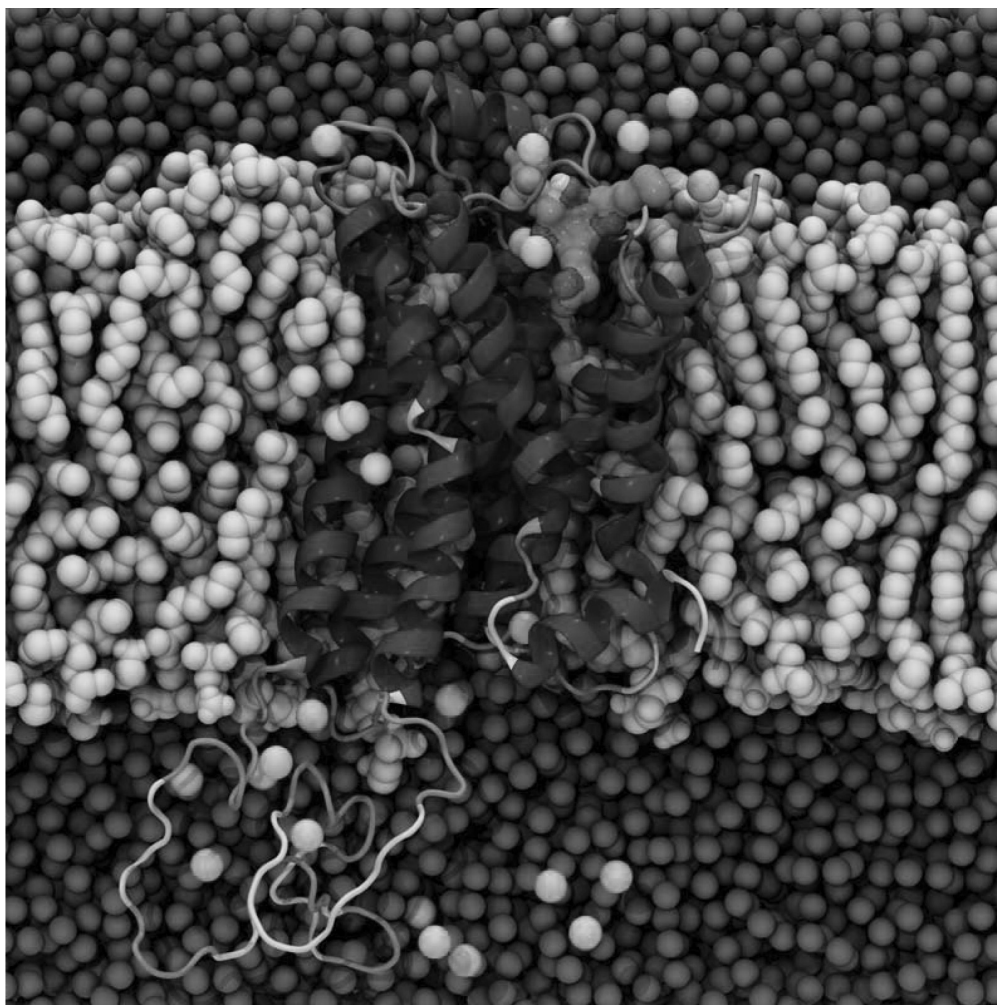


Figure 4. Overall view of the hEP1 receptor (MODEL-IV) and its ligand inserted in the hydrated lipids bilayer after 10 ns MD simulation.

values of Ramachandran plot (Figure 2). Intracellular loop 3 of MODEL-II was subjected to loop modeling to get the best conformation of this section of the protein (MODEL-IV).

The prostanoid receptors for whom the greatest number of mutagenesis studies have been carried out are IP (22), EP3 (23), EP2 (24) and TP (25). While there is no mutagenesis data yet available for the EP1 receptor, by using mutagenesis data of other prostanoid receptors including phylogenetic data, some important structural features of EP1 receptor can be predicted. According to these findings the active site of EP1 receptor is the area between TM1, TM2, TM3 and TM7 which lies within the upper half of the transmembrane-spanning region and the key residue, highly conserved (100% across all prostanoid receptors), Arg338, located in the middle of TM7, which has been proved to interact with the carboxylic moiety of the ligand (23,24,26).

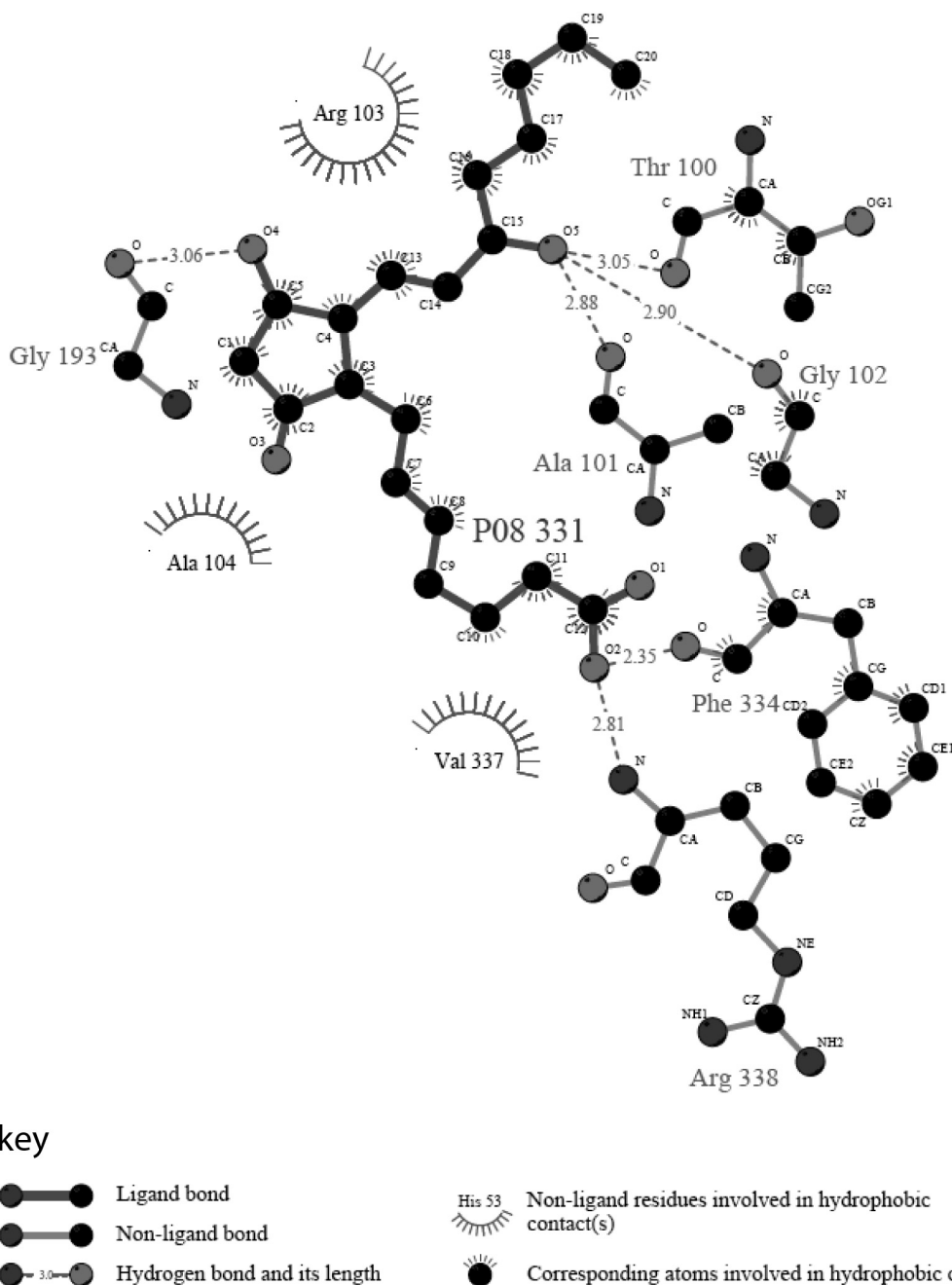
Also ECL2 (extracellular loop 2) has been revealed to have an important role in ligand binding (27,28). However the existence of a short part of the ECL2 in the crystallographic structure of h β_2 -adrenergic

receptor (3) causes a helical ECL2 in the structures constructed in this study.

The dynamic model is particularly accurate at modeling the 7TM domains, and the ligand binding site (Figure 4). With respect to molecular dynamic properties the contact of Arg63 and Pro348 connecting TM1 and TM7 remained stable. This contact can also be observed in the crystal structural model of β_2 -adrenergic receptor between Asn51 and Ser319 (3). It is believed this connection to be an important and conserved connection motif of GPCRs. Asn51 is the most conserved residue in TM1 and may be analogous to the Arg63 residue of hEP1 receptor. Point mutation studies on the analogous α_{1B} -adrenergic receptor demonstrated that a mutation to Ala results in a constitutively active receptor, while mutation to Asp, which can exhibit the observed hydrogen bond as well, did not have an impact on the receptor function (11). Also the highly conserved part G(x3)N in the TM1 of GPCRs (Table 3), has been predicted to have an important role in conformational properties of these proteins. There is a hydrogen

Table 3. Key residues conserved in the GPCR family.

TM	Pattern	hB2-Adrenergic	hEP1
TM1	Gx ^a (3)N	Gx(3)S	+
TM2	Lx(3)Dx(7)P	Lx(3)D	Lx(3)D
TM3	Sx(3)Lx(2)Ix(2)DRY	+	Px(3)Gx(2)Mx(2)ERC
TM4	Wx(7,8)P	Wx(9)P	Ax(9)P
TM5	Fx(2)Px(7)Y	+	P
TM6	Fx(2)CWxP	+	SX(2)CWxP
TM7	Lx(5)NPx(2)Y	Lx(10)NPx(2)Y	Lx(7)DPx(2)Y

^aAny residue**Figure 5.** Schematic two-dimensional representations of the binding interactions between the PGE₂ and hEP1 receptor (MODEL-IV).

bonding between Gly46 and Asn50 in MODEL-IV which remained stable during 10 ns of molecular dynamics simulation. Arg338 in TM7, the key residue in the binding site, forms hydrogen bond with Ser341 which was maintained for all 10 ns simulation. There is another hydrogen bonding in TM7 of MODEL-IV and 100% conserved across all prostanoid receptors (22), that is related to highly conserved DP(x2)Y motif of TM7 (Table 3). In this interaction Asp347 and Tyr351 form a hydrogen bond within TM7, which has been shown to be important for proper receptor activity (22) and remained fixed during simulation.

The present hEP1 model (Model IV) was further validated by docking a series of prostanoid compounds in the binding site. Homology models based on the bovine rhodopsin as a template, have a narrow pocket for docking the ligand. The narrow binding pocket is possibly the result of the flat nature of 11-cis retinal in the binding pocket of bovine rhodopsin crystal structure, and the misplacement of side-chains of binding site residues during homology modeling (29). This problem is not the case when β_2 -adrenergic receptor are used as a template in homology modeling studies and the active site area is completely suitable for ligands as big as prostanoid analogs. As can be seen in figure 4, the ligand binding site is well predicted. In the SAR study carried out by Ungrin et al. (21) it has been revealed that the most important parts of a prostanoid analog are carboxylic moiety, 11 and 15 hydroxyl groups and also α and ω chains. Arg338 and Phe334 in TM7 formed hydrogen bonding with the carboxylic acid part of the α chain of PGE₂.

Strong evidence in support of this interaction is that the mutation of this Arg in EP2 (24) and EP3 (26) to Gln and Leu, leads to significant loss of binding. Also Thr100, Ala101 and Gly102 of TM2 interact with the 15-hydroxyl group of PGE₂ molecule with different hydrogen bond distances. Among them the length of the hydrogen bond between Ala101 and 15-hydroxyl moiety of PGE₂ was the shortest one. Some residues participated also in hydrophobic interaction such as Arg103, Ala104 and Val337. The role of ECL2 in receptor activity has been proved in some mutagenesis studies. Figure 5 shows a hydrogen bond between Gly193 in the ECL2 of MODEL-IV and 11-hydroxyl group of PGE₂. Therefore the predicted active site covers some important interactions between prostanoid ligands and residues in this area. A correlation value of $r^2=0.75$ was obtained and it appears to be quite reasonable for the structure which is constructed by homology modeling technique.

CONCLUSION

In conclusion the model which was constructed in this study has a reasonable conformation and active site properties and remained stable after 10 ns molecular dynamics simulation in membrane. The crystallographic structure of h β_2 adrenergic receptor is somehow a suitable and useful template for performing homology modeling of GPCRs.

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REFERENCES

1. Breyer R.M., Bagdassarian C.K., Myers S.A., Breyer M.D. Prostanoid receptors: Subtypes and signaling. *Annual Rev Pharmacol.* 2001; 41:661-690.
2. Narumiya S., Sugimoto Y., Ushikubi F. Prostanoid receptors: Structures, properties, and functions. *Physiol Rev.* 1999; 79:1193-1226.
3. Cherezov V., Rosenbaum D.M., Hanson M.A., Rasmussen S.G., Thian F.S., Kobilka T.S., Choi H.J., Kuhn P., Weis W.I., Kobilka B.K., Stevens R.C. High-Resolution Crystal Structure of an Engineered Human β_2 -Adrenergic G Protein-Coupled Receptor. *Science.* 2007; 318:1258-1265.
4. Warne T., Serrano-Vega M.J., Baker J.G., et al. Structure of a beta(1)-adrenergic G-protein-coupled receptor. *Nature.* 2008; 454:486-U482.
5. Jaakola V.P., Griffith M.T., Hanson M.A., Cherezov V., Chien E.Y., Lane J.R., Ijzerman A.P., Stevens R.C. Adenosine Receptor Bound to an Antagonist. *Science.* 2008; 322:1211-1217.
6. Flower D.R. Modelling G-protein-coupled receptors for drug design. *Biochim Biophys Acta.* 1999; 1422:207-234.
7. Findlay J.B.C., Eliopoulos E.E. Three-dimensional modelling of G protein-linked receptors. *Trends Pharmacol Sci.* 1991; 11:492-499.
8. Piserchio A., Zelesky V., Yu J., Taylor L., Polgar P., Mierke D.F. Bradykinin B2 Receptor Signaling: Structural and Functional Characterization of the C-Terminus. *Biopolymers.* 2005; 80:367-373.
9. Soderhall J.A., Polymeropoulos E.E., Paulini K., Gunther E., Kuhne R. Antagonist and agonist binding models of the human gonadotropin-releasing hormone receptor. *Biochem Biophys Res Commun.* 2005; 333:568-582.
10. Montero C., Campillo N.E., Goya P., Páez J.A. Homology models of the cannabinoid CB1 and CB2 receptors. A docking analysis study. *Eur J Med Chem.* 2005; 40:75-83.
11. Wolf S., Bockmann M., Howeler U., Schlitter J., Gerwert K. Simulations of a G protein-coupled receptor homology model predict dynamic features and a ligand binding site. *FEBS Lett.* 2008; 582:3335-3342.

12. Labarga A., Valentin F., Anderson M., Lopez R. Web Services at the European Bioinformatics Institute. *Nucleic Acids Res.* 2007;1-6.
13. Sali A., Blundell T.L. Comparative Protein Modeling by Satisfaction of Spatial Restraints. *J Mol Biol.* 1993; 234:779-815.
14. Laskowski R.A., Macarthur M.W., Moss D.S., Thornton J.M. Procheck - a Program to Check the Stereochemical Quality of Protein Structures. *J Appl Crystallogr.* 1993; 26:283-291.
15. Van Der Spoel D, Lindahl E, Hess B, Groenhof G, E. Mark A, J. C. Berendsen H. GROMACS: Fast, flexible, and free. *Journal of Computational Chemistry* 2005; 26:1701-1718.
16. Schuettelkopf A.W., Van Aalten D.M. PRODRG - a tool for high-throughput crystallography of protein-ligand complexes. *Acta Crystallogr D* 2004; 60:1355-1363.
17. Rivail L., Chipot C., Maigret B., Bestel I., Sicsic S., Tarek M.. Large-scale molecular dynamics of a G protein-coupled receptor, the human 5-HT₄ serotonin receptor, in a lipid bilayer. *Journal of Molecular Structure: THEOCHEM.* 2007; 817:19-26.
18. Jones G., Willett P., Glen R.C., Leach A.R., Taylor R. Development and validation of a genetic algorithm for flexible docking. *J Mol Biol.* 1997; 267:727-748.
19. Humphrey W., Dalke A., Schulten K. VMD: visual molecular dynamics. *J Mol Graphics.* 1996; 14(1): 33-38, 27-28.
20. Wallace A.C., Laskowski R.A., Thornton J.M. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein Eng.* 1995; 8(2):127-134.
21. Ungrin M.D., Carrière M.C., Denis D., Lamontagne S., Sawyer N., Stocco R., Tremblay N., Metters K.M., Abramovitz M. Key structural features of prostaglandin E₂ and prostanoid analogs involved in binding and activation of the human EP₁ prostanoid receptor. *Mol Pharmacol.* 2001; 59:1446-1456.
22. Stitham J., Arehart E.J., Gleim S.R., Douville K.L., Hwa J. Human prostacyclin receptor structure and function from naturally-occurring and synthetic mutations. *Prostag Oth Lipid M* 2007; 82:95-108.
23. Audoly L., Breyer R.M. Substitution of charged amino acid residues in transmembrane regions 6 and 7 affect ligand binding and signal transduction of the prostaglandin EP₃ receptor. *Mol Pharm* 1997; 51:61-68.
24. Kedzie K.M., Donello J.E., Krauss H.A., Regan J.W., Gil D.W. A single amino-acid substitution in the EP₂ prostaglandin receptor confers responsiveness to prostacyclin analogs. *Mol Pharmacol.* 1998; 54:584-590.
25. Chiang N., Kan W.M., Tai H.H. Site-directed mutagenesis of cysteinyl and serine residues of human thromboxane A₂ receptor in insect cells. *Arch Biochem Biophys.* 1996; 334:9-17.
26. Chang C.S., Negishi M., Nishigaki N., Ichikawa A. Functional interaction of the carboxylic acid group of agonists and the arginine residue of the seventh transmembrane domain of prostaglandin E receptor EP₃ subtype. *Biochem J* 1997; 322: 597-601.
27. Audoly L., Breyer R.M. The second extracellular loop of the prostaglandin EP₃ receptor is an essential determinant of ligand selectivity. *J Biol Chem* 1997; 272:13475-13478.
28. Stillman B.A., Audoly L., Breyer R.M. A conserved threonine in the second extracellular loop of the human EP₂ and EP₄ receptors is required for ligand binding. *Eur J Pharmacol.* 1998; 357:73-82.
29. Kimura S.R., Tebben A.J., Langley D.R. Expanding GPCR homology model binding sites via a balloon potential: A molecular dynamics refinement approach. *Proteins-Structure Function and Bioinformatics* 2008; 71:1919-1929.