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Molecular Interactions of Purinoceptor Subtype (P2Y12) with its Agonist Adenosine Diphosphate - An *in silico* Approach

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The P2Y12 receptor is a member of the family of rhodopsin like G-protein coupled receptors (GPCRs) and represents an interesting new therapeutical target since it is involved in the platelet aggregation and the disorders related to thrombosis. In order to shed light on the molecular basis of the interactions of the P2Y12 with its agonists, the interactions of receptor and the ligands were examined by molecular modeling methods. The 3D models of the P2Y12 receptor were constructed by using homology-modeling method using the crystal structure of bovine rhodopsin as template. Among the modeled structures, the one with appropriate stereochemical quality was selected by using PROCHECK. The docking software HEX 4.0 was used to dock the natural ligand, adenosine diphosphate (ADP) into the model of P2Y12.The amino acid residue Thr-264 of P2Y12 model served as an important residue for nonbonded interaction with ADP.

Key Words: Molecular interaction, Purinoceptor subtype (P2Y12), Adenosine diphosphate.

INTRODUCTION

P2Y12, a G-protein coupled receptor (GPCR), is one of the three purinoceptors expressed on the platelets[1], binds to adenosine diphosphate (ADP) and signals through G-protein to induce platelet aggregation[2], having important role in heamostasis of thrombosis and thus also involved in vascular disorders like stroke, myocardial infraction, peripheral vascular thrombosis and other severe conditions[3]. Its concomitant signaling with P2Y1 (another GPCR expressed on platelets) is necessary to bring out complete platelet aggregation[4]. P2Y12 signals through Gi protein to cause inhibition of adenylyl cyclaze followed by decreased cellular cAMP level, which causes phosphorylation of vasodilator phosphoprotein (VASP), which is a cytoskeleton protein of platelet. Thus platelet shape change occurs and finally aggregation occurs[2,5].

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Currently, the marketed antiplatelet drugs like ticlopidine and clopidogrel are found to be showing their effect by binding with P2Y12[6-8].

GPCRs, membrane bound proteins, have seven transmembrane helices, are the most intriguing and challenging molecular machinery designated by nature, involved in diverse biological functions by different signal transduction mechanisms[9-11]. They share a characteristic topology and can be subdivided in to seven families based on sequence similarity. P2Y12 and bovine rhodopsin both belong to the same class[12].

The researchers used homology modeling method but based them respectively on bacteriorhodopsin and 9 Å resolution model of bovine rhodopsin (a prototypical G protein-coupled receptor). Presently, the first high-resolution (2.8 Å) 3D structure of bovine rhodopsin has been acquired *via* X-ray crystallography[13]. This structure made further refinement by offering a high resolution structure to model 3 dimensional structures of GPCRs.

In the present study, we constructed a three-dimensional model of the human P2Y12 receptor based on the structure of bovine rhodopsin having resolution of 2.8 Å to which it has sequence homology. Since the P2Y12 receptor is linked to G-protein, it is important to understand how binding of ligands to this receptor induces activation of the G-protein in a signal transduction cascade. We also provide the results of ligand binding that might be applicable for rational drug design.

EXPERIMENTAL

Entire computations and molecular modeling of P2Y12 receptor were carried out on a linux workstation (P4, 3 GHz, HT processor). Protein was modeled using Modeller 7V2[14]. The amino acid sequence of P2Y12 receptor (gi:29029605) was obtained from the NCBI (http://www.ncbi.nlm.nih.gov/entrez) Gene bank data base. BLAST was used to find homologous protein sequences for P2Y12 from NCBI protein data bank. The X-ray structure of bovine rhodopsin with the highest resolution (PDB code: 1L9H) was used as a template for P2Y12 3D structure modeling.

The secondary structure prediction was done using PHD[15] secondary structure prediction server. Multiple sequence alignment was carried out using CLUSTAL-W (http://www.ebi.ac.uk/clustalw). The dendrogram obtained from the ET analysis indicated the evolutionary relationship between P2Y12 and its homologous proteins[16-19]. The alignment between the P2Y12 and bovine rhodopsin obtained in multiple sequence alignment was used for modelling[20]. The template for modeling 3D structural model was selected from the threading results obtained from LOOPP server (http://ser-loopp.tc.cornell.edu/loopp_old.htmL). Stereochemical qualities of the models were evaluated by Ramachandran plot obtained from PROCHECK[21].

An automated docking method HEX 4.0[22] was used to dock the natural agonist ADP, into binding pocket of P2Y12 receptor. All the figures were prepared using the SPDBV molecular viewer program[23] and RASMOL[24].

RESULTS AND DISCUSSION

Primary structure: Length : 342; molecular weight : 39438 Daltons; Sequence obtained from NCBI Gene bank: >gi|29029605|ref|NP_795345.1| purinergic receptor P2Y12 [Homo sapiens]
MQAVDNLTSAPGNTSLCTRDYKITQVLFPLLYTVLFFVGLITNGLAM
RIFFQIRSKSNFIIFLKNTVISDLLMILTFPFKILSDAKLGTGPLRTFVC
QVTSVIFYFTMYISISFLGLITIDRYQKTTRPFKTSNPKNLLGAKILSVV
IWAFMFLLSLPNMILTNRQPRDKNVKKCSFLKSEFGLVWHEIVNYI
CQVIFWINFLIVIVCYTLITKELYRSYVRTRGVGKVPRKKVNVKVFII
IAVFFICFVPFHFARIPYTLSQTRDVFDCTAENTLFYVKESTLWLTSLNAC
LDPFIYFFLCKSFRNSLISMLKCPNSATSLSQDNRKKEQDGGDPNEE
TPM.

Secondary structure: Transmembrane helices were consensually predicted from various predictions obtained through various secondary predictions tools available with PHD server and they are represented in Table-1. The possible β-strands comprises of residues RQPRD (165-169) and KCSFL (174-178). Both of these β-strands were present in loop between 4th and 5th transmembrane helices.

TABLE-1
CONSENSUS SEQUENCE OF α-HELICES

α-Helix	Consensus sequence
1.	PLLYTVLFFVGLITN
2.	TVISDLLMIL
3.	SVIFYFTMYISISFLGL
4.	ILSVVIWAFMFLLSLPNMI
5.	ICQVIFWINFLIVIV
6.	FIIIAVFFICFVPFH
7.	WLTSLNACLDPFIYFFL

33 GPCRs protein sequences homologous to P2Y12 were selected from the NCBI non-redundant data base using BLAST and aligned along with the P2Y12 and bovine rhodopsin. Transmembrane (TM) helices and β-sheets predicted by secondary structure prediction methods were depicted as cylinders and arrow headed marks respectively in the multiple sequence alignment. Multiple sequence alignment gave knowledge about the conserved or invariant residues, which might be important for maintaining functional identity for particular class of receptors. Mostly

hydrophobic residues were present in all the TM helices. Most of the conserved amino residues were present in TM helices than loop regions and these regions must be involved in either maintaining functional integrity or structural integrity of GPCRs. In second TM helix three amino acids Glu45, Asp50 and Pro58 were conserved in all sequences except bacterial rhodopsin. In third TM helix Tyr89, Ser91were conserved well. In fourth TM helix Trp152 was conserved in all sequences except bacterial rhodopsin. In sixth and seventh TM helix two prolines were fully conserved. Since all these proteins were differs in their respective function and all these conserved residues might be responsible for important functional features of GPCRs. The only acidic residue was conserved in loop region is Arg122. In the first β-strand, five residues insertion was found in two PAF receptors (AAP88803, AAF01439) and hence these residues might be involved in specific function in PAF receptors.

ET Analysis: The phylogenic tree obtained from ET analysis (Fig. 1) was used to locate the evolutionary cut-off between two different GPCRs. ET analysis divideds all sequences into 10 groups and gives insights into conservation pattern. ET analysis is guided by two observations: First, protein structures descending from a common ancestor were remarkably similar, with backbone deviations remaining within 2 Å even when the sequence identity falls to 25 %. Second, the active site residues under evolutionary pressure tend to maintain their functional integrity and undergo fewer mutations than functionally less important amino acids. These observations imply that evolutionarily related sequences can be compared with each other to extract the structural and functional data.

Only two residues were found to be invariant among all GPCRs while number of residues were identified as class specific and may play role in subtype specific activity of GPCRs.

Tertiary strucutre

Homology modeling: Since no homologous protein obtained from the Blast search results was having 3d structure the template was selected from LOOPP (learning, observing and outputting protein patterns) server. It is a fold recognition program and its results were presented in Table-2.

The bacterial rhodopsin was selected as template for modeling because it was having high confidence value and P2Y12,bovin rhodopsin (1L9H) belongs to same structural class.

Ten different models of P2Y12 were built by Modeller 7v2. Models were ranked according to their energy levels and one with lowest free energy was chosen for further stereochemical analysis and that structural model was shown in Fig. 2.

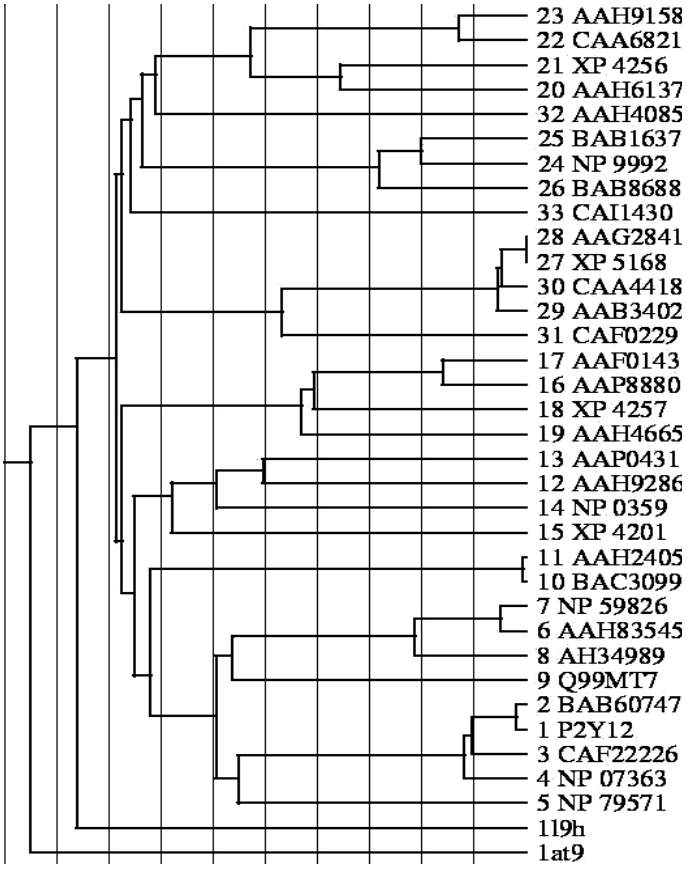

Fig. 1. Sequence similiarity based dendogram for P2Y12 and its homologous sequences. The vertical lines show the partitions considered for evolutionary analysis. Each partition indicates 10% percentage identity decreasing for right to left

TABLE-2
LOOPP THREADING RESULTS

No.	PDB code of the protein	Parameters		
		Confidence	Sequence identity (%)	Length (%)
1.	1L9H_A	High	15.43	94.74
2.	1GZM_B	High	14.38	91.23
3.	1F88_B	High	14.47	84.50
4.	1RH5_A	Good	11.43	92.11
5.	1LN6_A	Low	17.16	86.84
6.	1LD8_B	Low	10.78	92.11
7.	1PW4_A	Low	11.54	91.23
8.	1MXR_A	Low	8.00	85.38
9.	1IOM_A	Low	9.15	85.96
10.	1AJ8_A	Low	7.94	99.42

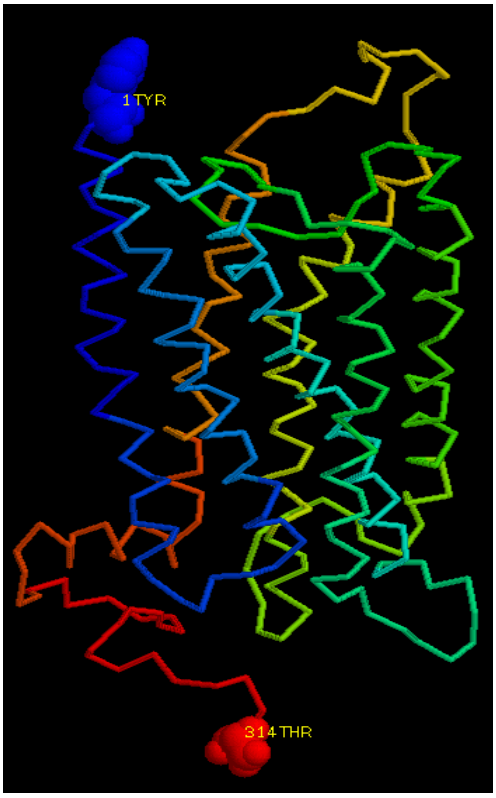

Fig. 2. Homology model of P2Y12 with lowest free energy visualized by Ras Mol. and depicted as backbone view. First amino acid (Tyr 1) and last amino acid (Thr 314) are shown in spacefill view

Validation by Procheck: Procheck automatically checked the stereochemical quality of P2Y12 and provided Ramachandran plot (Fig. 3). The distribution of the phi/psi angles of the model were within the allowed regions in Ramachandran plot. Only two out of the 314 residues had disallowed conformations. A model of a protein structure of high quality is achieved when > 90 % of the residues are found within the most favoured region of the resulting Ramachandran plot. In case of P2Y12 model structure, 88.5 % found to be in most favoured regions. However, it has to be noted that the amino acids occupying disallowed regions (Cyc292 and Lys150) are located in regions that are remote from the binding packet, mainly in the loop regions.

Docking: Knowledge about the ligand-binding region of X-ray crystal structure of the bovine rhodopsin was directly extrapolated to the P2Y12 receptor to determine the putative ligand-binding pocket[25,26]. ADP was placed inside the binding pocket manually and docked automatically using

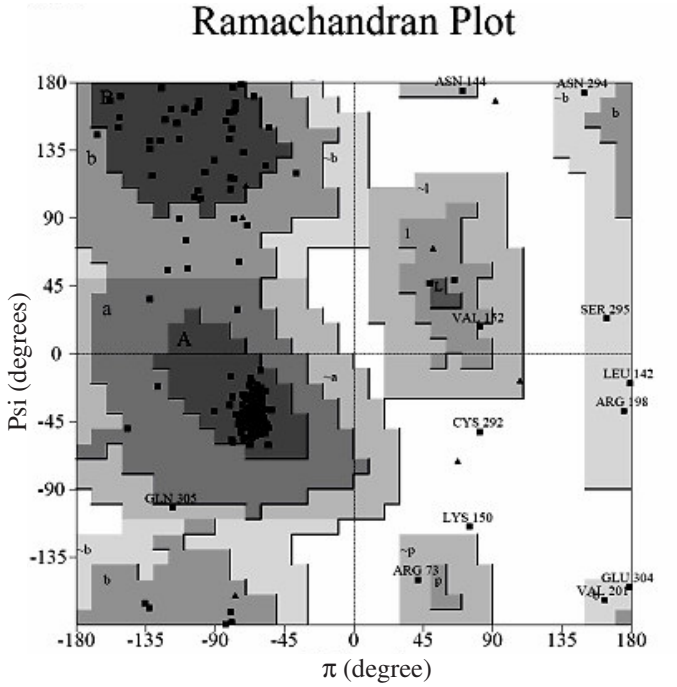

Fig. 3. Ramachandran plot of best 3D structural model of P2Y12

HEX-4.0. After docking, it automatically performed a short molecular dynamic simulation to rearrange the conformation of the ligand-receptor complex to the minimum energy level and it was represented in Fig. 4. Docked ATP in P2Y12 gave molecular level information of the interaction of ADP with different amino acids of P2Y12. One hydrogen bond interaction between oxygen of β-phosphate and hydrogen of hydroxyl group of Thr264 reveals that thr264 present in the seventh TM helix might be the key residue for the agonistic activity. As shown in Fig. 4 other atoms of ADP were found to be likely interacting with cys225, Phe226, leu261and glu258 (present in 6th and 7th TM helix) thr80, phe84 (present in second TM helix). Thus above all residues might forma ligand-binding pocket of ADP in P2Y12.

Conclusion

The present study carried out to explore the 3D structure of the P2Y12 receptor and interaction with its agonist ADP. This study provides information about some crucial interactions between receptor and agonist. A major finding of this study was the H-bond formed between oxegen of β-phosphate of ADP and hydrogen of hydroxyl group of Thr264 P2Y12 which might be crucial interaction for agonistic activity. Furthermore, lipophillic residues delineate the binding pocket for the ligand (ADP).

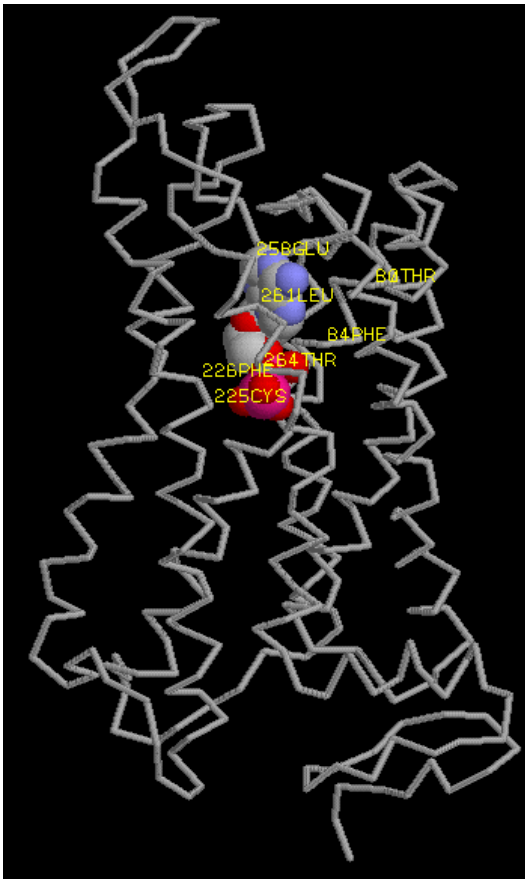

Fig. 4. The possible interaction of ADP with different amino acids present in binding pocket of P2Y12 visualized by Ras Mol. ADP is shown in spacefill view while P2Y12 is shown in backbone view

In short, these findings may be helpful for the pharmacophore designing to develop potenet and selective P2Y12 receptor agonists.

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