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Molecular dynamics simulation of the $P2Y_{14}$ receptor. Ligand docking and identification of a putative binding site of the distal hexose moiety

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Abstract—A rhodopsin-based homology model of the $P2Y_{14}$ receptor was inserted into a phospholipid bilayer and refined by molecular dynamics (MD) simulation. The binding modes of several known agonists, namely UDP-glucose and its analogues, were proposed using automatic molecular docking combined with Monte Carlo Multiple Minimum calculations. Compared to other P2Y receptors, the $P2Y_{14}$ receptor has an atypical binding mode of the nucleobase, ribose, and phosphate moieties. The diphosphate moiety interacts with only one cationic residue, namely Lys171 of EL2, while in other P2Y receptor subtypes three Arg or Lys residues interact with the phosphate chain. Two other conserved cationic residues, namely Arg253 (6.55) and Lys277 (7.35) of the $P2Y_{14}$ receptor together with two anionic residues (Glu166 and Glu174, located in EL2), are likely involved in interactions with the distal hexose moiety.

Eight subtypes of P2Y receptors, namely $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$, have been cloned and characterized.[1] All of these are G protein-coupled receptors (GPCRs) belonging to the rhodopsin family. The $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ are G_q-coupled receptors and comprise the $P2Y_1$-like receptor family.[2] A second family referred to as the $P2Y_{12}$-like family consists of the $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ receptors that couple via G_i proteins to the inhibition of adenylate cyclase.[2] P2Y receptors are widely distributed throughout the body and are involved in many physiological processes.[3] In particular, the $P2Y_{14}$ receptor is likely involved in regulation of neuroimmune functions and is highly expressed in placenta, stomach, and intestine, and with moderate expression in brain, heart, lung, and spleen.[4]

In contrast to other P2Y receptor subtypes, the $P2Y_{14}$ receptor is not activated by either nucleotide di- or triphosphates, such as ATP, UTP or UDP, and dinucleotide.[5–7]

However, sugar-substituted analogues of UDP, namely UDP-glucose, UDP-galactose, and UDP-*N*-acetyl-glucosamine, are naturally occurring agonists of the $P2Y_{14}$ receptor.[4,8,9] Among these ligands, UDP-glucose is the most potent agonist.

Recently, molecular models of all known subtypes of P2Y receptors, including the $P2Y_{14}$ receptor were, published, and general configurations of the binding sites were proposed based on docking studies performed for the $P2Y_1$ and $P2Y_{12}$ receptors.[2] Several amino acid residues were suggested to be critical for ligand binding at both $P2Y_1$-like and $P2Y_{12}$-like receptor families of P2Y receptors.

Concerning the $P2Y_{12}$-like family three cationic residues, namely Arg3.29, Lys located in EL2, and Lys7.36 were proposed to be critical for coordination of the phosphate chain of a ligand. Also, Ser7.43, present in the $P2Y_1$-like family and in the $P2Y_{12}$ receptor, seems to be involved in ligand binding via H bonding with the nucleobase ring. This position is Ala7.43 in

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the $P2Y_{13}$ and $P2Y_{14}$ receptors. Tyr1.39, which was found to be involved in ligand recognition in the $P2Y_1$ receptor, is conserved among all P2Y receptors, with the exception of the $P2Y_{11}$ subtype. Tyr2.53 can interact with the native ligands at $P2Y_{1,2,4,6}$ subtypes, while in the $P2Y_{12,13,14}$ receptors the corresponding residue is Met2.53. The $P2Y_{12}$-like receptors, especially $P2Y_{13}$ and $P2Y_{14}$ receptors, likely have a different configuration of the binding site or different binding mode of its agonists in comparison to $P2Y_1$-like receptors.[2]

In this study, a molecular model of the human $P2Y_{14}$ receptor was refined by insertion into the phospholipid bilayer followed by 20 ns molecular dynamics simulation, and possible binding modes of four agonists of the $P2Y_{14}$ receptor were studied using automatic docking followed by conformational search analysis.

Homology modeling is one of the most effective methods to study the structure and ligand–receptor interactions of GPCRs. The models obtained using this approach can provide information about residue–residue interactions, configuration of the putative ligand binding site, and possible binding modes of the ligands. However, such models have very similar configurations of the transmembrane domains (TMs) in comparison to the corresponding TMs of the template, usually rhodopsin. Homology modeling often cannot provide an accurate prediction of the configuration of the loops and terminal domains, especially if the sequences are quite long. Moreover, in most cases homology modeling does not take into account the natural environment of the membrane-bound GPCRs, although this is an important factor for the general configuration of a receptor structure and orientation of its sidechains.[10]

In this study, a previously published rhodopsin-based molecular model of the human $P2Y_{14}$ receptor[2] was refined using 20 ns molecular dynamics (MD) simulation in the phospholipid bilayer.

The MD simulation was performed using the protocol developed by Woolf and Roux[11–14] and applied to MD simulation of the $P2Y_6$ receptor by Costanzi et al.[15] This simulation was run on the Biowulf cluster at the NIH (Bethesda, MD) using CHARMM 32a2[16] (details given in Supporting data). As with the $P2Y_6$ receptor,[15] attempts to generate a MD trajectory without applying nuclear Overhauser enhancement (NOE) restraints led to a loss of the secondary structure of TM7. For this reason the first 5 ns of the MD was performed with the NOE restraints applied for the distances between the backbone carbonyl–oxygen atom of the residue n and the backbone NH-group of the residue n+4 of TM7. That constraint preserved the helical structure of TM7, which remained stable after removing the NOE restraints.

The root mean square deviation (RMSD) of all atoms of the $P2Y_{14}$ receptor was calculated from the MD trajectory. As shown in Figure 1 the first plateau of the RMSD was reached after 2.5 ns of MD simulation.

After 5 ns (when the NOE restraints were removed) the value of the RMSD was slightly increased, and after approximately 8.5 ns of MD simulation the structure of the receptor became stable. The typical structure of the last 100 ps of the trajectory was considered as a final structure of the MD simulation. The C_α-atoms of the transmembrane α-helices of this structure and an initial structure of the $P2Y_{14}$ receptor were aligned with RMSD of 3.1 Å (Fig. 2).

It was found that in general both structures have very similar configurations of the TMs. However, the angles along the axes of TM6 and TM7 changed slightly in comparison to the initial structure. The calculated values of the RMS fluctuations (RMSF) allowed us to indicate the residues with positions that were most significantly changed during the MD simulation. Not surprisingly, the highest values of the RMSF corresponded to the residues located in the extracellular (EL) and intracellular (IL) hydrophilic loops and terminal domains, while the residues located in the TM domain had the lowest values of the RMSF (Fig. 3). In particular, the greatest change occurred in EL1 and IL3. In addition, during the simulation EL2 was shifted slightly outward from its initial position. However, the configuration of this longest hydrophilic loop was not significantly altered during the simulation.

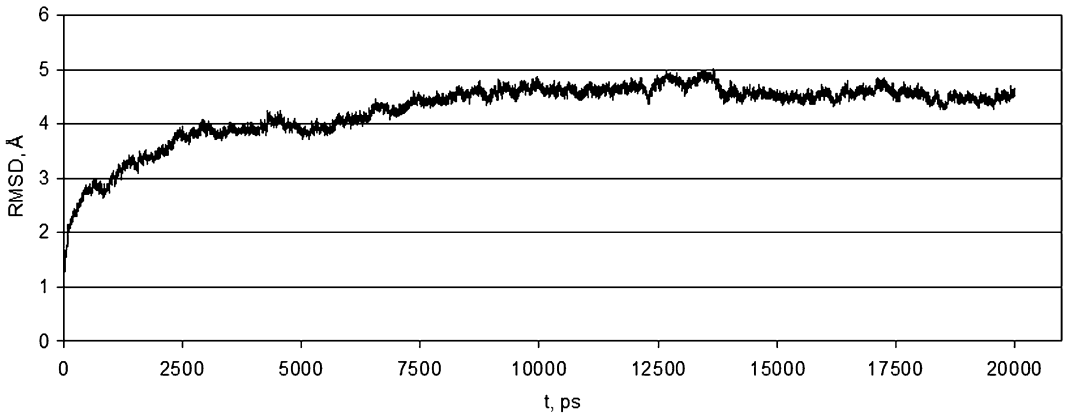

Figure 1. Changes in RMSD of atoms of the $P2Y_{14}$ receptor during the MD simulation.