A STRUCTURAL MODEL OF HUMAN MT2 MELATONIN RECEPTOR AND ITS MELATONIN RECOGNITION SITE

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Abstract
Homology modeling of the hMT2 melatonin receptor is reported. The deduced amino acid sequence shows high homology with bovine rhodopsin, whose tertiary structure has been solved at 2.6 Å resolution by X-ray crystallography. Docking of melatonin into the receptor site of the protein structure was explored. The resulting structure contains seven putative transmembrane domains connected by three extracellular and three intracellular loops.

We have identified that for high-affinity melatonin binding to hMT2 receptor are essential Val 204 and Leu 272 in transmembrane domains five and six respectively as well as Tyr 298 in transmembrane domain seven. We have also demonstrated the importance of Gly 271 for high-affinity melatonin binding to the hMT2 melatonin receptor

The pineal hormone melatonin, is present in all vertebrate species including humans. Aside from being an important regulator of seasonal reproduction and circadian rhythms melatonin was reported to be potentially important immunomodulator, powerful free radical scavenger and exerts oncostatic activity. Melatonin binding to specific G protein-coupled receptors (GPCRs), designated as MT1, MT2 and Mel1c, modulates wide range of intracellular messengers mediating hormone effects. MT1 and MT2 subtypes are expressed in mammals whereas Mel1c subtype has been cloned from lower vertebrates (reviewed in [1] and [2]).

GPCRs contain seven putative transmembrane domains connected by three extracellular and three intracellular loops. It is widely accepted that TMs are involved in specific interactions with ligand. Still, very little is known about actual arrangement of TMs in majority of GPCRs, except the light receptor rhodopsin [3] structures of GPCRs at the atomic level are unknown.

Thus, the absence of detailed structure of second mammalian melatonin receptor led us to construction of three-dimensional model of the helical part of human MT2 (hMT2) receptor generated by homology to the known crystal structure of the bovine rhodopsin determined at a 2.6 Å resolution [3].

The choice of the templates was restricted to the bovine rhodopsin, whose tertiary structure has been solved at 2.6 Å resolution by X-ray crystallography and for which the PDB coordinates were available [3]. The structure (1L9H) was extracted from the Protein Data Bank (www.rcsb.org/pdb/) and loaded into SwissPdbViewer [4], where we extracted a construct containing only one monomer. The primary structures were aligned with by CLUSTALX [5].

The slow-accurate mode with a gap opening penalty of 10 and a gap extensions penalty of 0.1 for the local alignment was used as well as the Gonnet 250 protein weight matrix and hydrophobic penalties for the amino acids

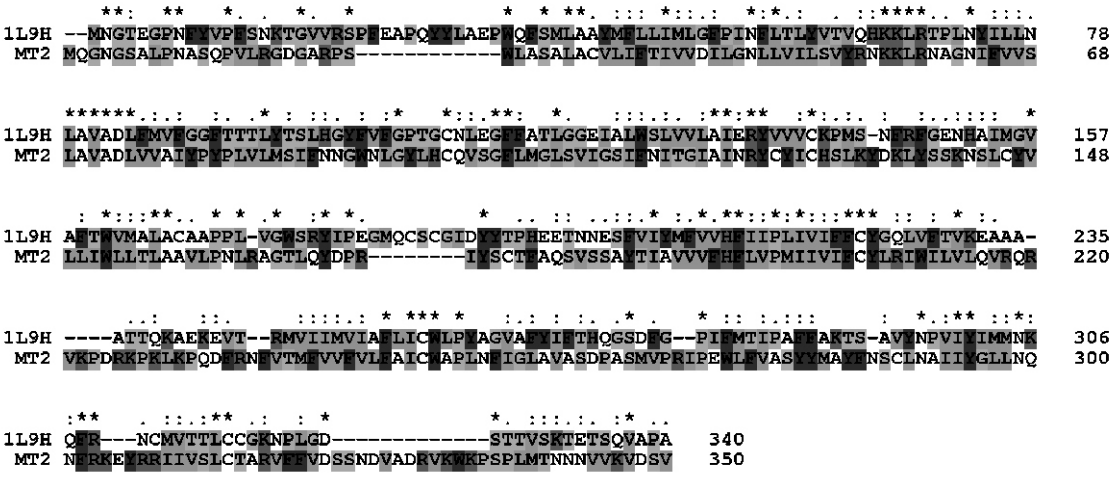

Fig. 1. Sequence alignment of the MT2 melatonin receptor and bovine rhodopsin. Identical and similar amino acids of the stronger groups are indicated with an asterisk and colon, respectively. These amino acids should conserve the structure with a probability of 95%. Dots indicate similar amino acids of the lower groups that should conserve the structure with a lower probability.

GPSNDQEKR. The alignment used for further modeling is shown in figure 1.

Three-dimensional models comprising all non-hydrogen atoms were generated by the MODELLER6 package. [6] This is based on a distance restraint algorithm, satisfying spatial constraints extracted from the alignment of the known protein, which is the template structure, with the target sequence and from the CHARMM-22 force-field [7]. A bundle of five models from random generation of the starting structure was calculated. The resulting models showed MODELLER target function values of 2403, 2446, 2530, 2673, 2451, respectively. All models obtained were subjected to a short simulated annealing refinement protocol available in MODELLER. The tertiary structure models were checked with PROCHECK [8]. It produces a Ramachandran diagram and allows examination of various structural features such as bond lengths and angles, secondary structures and exposure of residues to the solvent.

The structure of the melatonin was built with the sketcher module of of InSight II, v2000.1, (Accelrys Inc., San Diego, CA, USA) and geometry-optimized using the DISCOVER force field cvff. To obtain the receptor-ligand complex the melatonin was manually fitted into the binding site of the receptor. The starting point for ligand docking was the orientation proposed by Grol and Jansen [15]. The ligand was positioned by avoiding severe steric overlap with the receptor, trying to keep the aromatic part of the melatonin close to the hydrophobic side chains. The resulting complex was minimized in vacuo using SANDER with the ff99 forcefield included in AMBER 7.0 [9].

The quality of the alignment can be seen as the most important step in homology modeling. Therefore, the degree of similarity between the target sequence and the template and the reliability of the alignment are the most critical problems. These two problems are of course partially interconnected, since the degree of similarity of two structures decreases with the degree of sequence identity [10]. In our case the pair wise identity with bovine rhodopsin was quite low, just about 21 %. However, the similarity between both sequences of about 48 % was relatively high and makes homology modeling possible. Similarity in this case included not only identical amino acids, but also indicated that amino acids of the stronger groups were conserved. Stronger groups are: CSTA, NEQK, NHQK, NDEQ, QHRK, MILF, HY, FYW. These amino acids should conserve the structure and are marked in the alignment with two stars. For such a degree of similarity, alignment errors were possible [11]. One basis of homology modeling is the assumption that it is possible to define a unique optimal sequence-based alignment that coincides with a structure-based alignment. This is not true in general because every alignment program tries to maximize the number of alignable residues, although these residues might not be spatially superposable. This limitation and source of error is intrinsic and should always be taken into account when estimating the degree of confidence of a certain model.

The final model of the MT2 melatonin receptor calculated with PROCHECK, shown in Fig.2, revealed a good quality stereochemistry, as indicated by the torsion angles

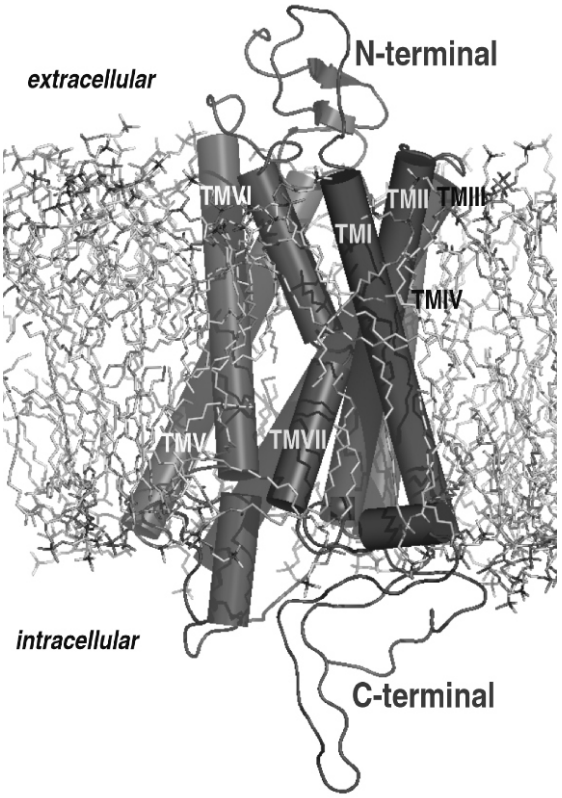

Fig. 2. The final model of the MT2 melatonin receptor. The protein structure is placed into a lipid bilayer system with 128 POPC lipids that was preequilibrated.

F and Y. The F, Y torsion angles of 85.1 % of the residues had values within the most favored areas and 10.8 % of the residues had values within additionally allowed regions of the Ramachandran plot. Six residues (1.9 %) were found in disallowed regions. This is acceptable for a structure based on a template of 2.6 Å that has 3.1 % of its residues either in generously allowed or even disallowed regions. The overall g factor of the best structure obtained showed a value of -0.18. The g factor should be above -0.5 and values below -1.0 may need investigation. To summarize we can say that the final model gave the best results in all three categories. It has the lowest MODELLER objective function, the highest percentage of residues in the most favored regions and the highest g-factor. The g-factor of our structure is only slightly lower than of the template structure (0.06) which is an ideal result and shows that our MT2 structure fulfills all criteria of a good quality model. It should be added that all five calculated models differ only slightly in all performed checks. It demonstrates the consistence of the method that should ideally give identical results for every run. The model structure placed into the membrane is shown in figure 2.

The modeled structure of the melatonin receptor consists of seven membrane helices and 6 loops (Three intracellular and three extracellular). Some of the helices are interrupted as in the template structure 1L9H, which might point to regions of low resolution in the template. The interrupted helixes are: TM V (between Phe194 and Phe196), TM VI (Arg235 to Thr239) and TM VII (Ala284 to Leu290). Whereas the intracellular loops play a crucial role in the receptor function, the role of the extracellular

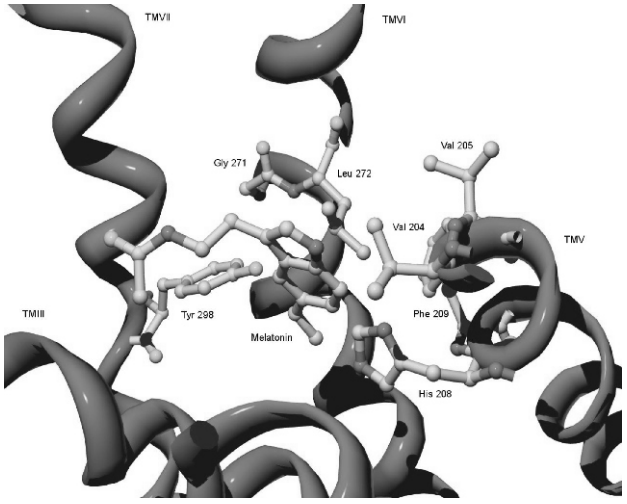

Fig. 3. The melatonin binding site of melatonin receptor. The residues important for melatonin binding (reported above) and melatonin docked into the binding site are shown.

loops seems to be marginal. Two loops seem to play the key role in the receptor function: the small intracellular loop between helices 3 and 4 (Cys143 to Ser153) and the longer loop between helices 5 and 6 (Leu226 to Pro240). Four helices form the receptor site for melatonin, these are TM III (Tyr97 to Ile 129), TM V (Ser185 to Ile212), TM VI (Lys 228 to Ser263) and TM VII (Trp275 to Cys298).

The various membrane-bound receptors the C-terminal plays an important role in regulation the receptor function [12]. Also in our case the C-terminal (Asn301-Val350) seems to have a rigid self supporting structure and therefore a functional importance might be predicted.

According to our model, Val 204 in TM V and Leu 272 in TM VI both occupy the area surrounding the indole ring of the melatonin molecule (Fig. 3). Their physical properties and proposed location indicate that they play a role in hydrophobic interactions with the indole core of the ligand. This could be crucial for adopting the correct orientation and/or stabilizing the melatonin molecule in its binding pocket. The importance of Gly 271 to high-affinity melatonin binding was previously reported for the MT I melatonin receptor [13]. We assume that the effect of this mutation introducing bulkier and slightly polar Thr instead of small and conformationally flexible Gly can be realized through the affection of proper orientation of adjacent Leu 272 in the binding pocket. Val 205 is relatively aside from a docked ligand and thus it should not affect binding parameters of the receptor. His 208 in TMV is proposed to participate in a specific interaction with the 5-methoxy group of melatonin. The role of His 208 in specific binding to the both subtypes of both mammalian melatonin receptors was subsequently confirmed in experiments based on site-directed mutagenesis showing the substantial increase of Kd value of the mutant receptor [14]. According to our model Phe 209 does not have any specific interaction with the melatonin molecule. In concord with our model we propose that the hydroxy group of Tyr 298 through hydrogen bonding specifically interacts with the 5-methoxy group in the melatonin molecule providing.

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