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

INSIGHT THE BINDING SITE OF HUMAN MELATONIN MTR1A RECEPTOR

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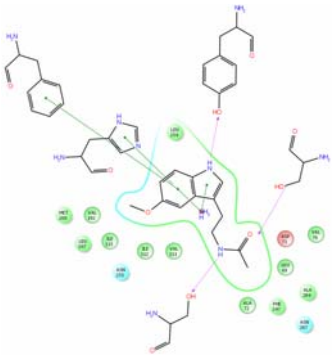
Besides the known applications of melatonin which include circadian rhythm's regulation, sleep disorders and depression, recent studies have reported the melatonin implication in cardiovascular regulation, neurodegenerative diseases or cancer treatment. In this light, the melatonin receptors have gained much attention in the last few years, and many drug discovery programs have been focused on the identification of new small molecules which selectively bind to the two melatonin receptor subtypes. In this study, we report the homology modeling of the human melatonin MTR1A based on the crystal structure of the beta1-adrenergic receptor (beta1-AR) receptor, the characterization of the binding site and the most relevant ligand–receptor interactions. Docking simulations revealed an agonist binding mode characterized by interactions with two residues from helix 3, Ser3.35 and Ser 3.39, respectively and a residue from helix 5, His5.46.



INTRODUCTION

Melatonin is a hormone primarily synthesized and secreted by pineal glad following the light-dark cycle. Besides its important role in the regulation of circadian rhythm and the treatment of sleep disorders or depression, melatonin was reported to influence several other important physiological functions like modulation of the cardiovascular response, tumor suppression or diabetes.[1–5] The most important actions of melatonin are exerted via two membrane receptors, melatonin MTR1A (MT1) and MTR1B (MT2), members of G-protein coupled receptor family (GPCRs). Currently, all marketed melatonergic drugs target on both melatonin receptor subtypes showing no significant selectivity. In order to develop new therapeutic agents with improved selectivity, it is necessary to understand and determine all the aspects regarding ligand binding and functional efficacy for each melatonin subtype. In the present work, the full characterization of the melatonin MTR1A binding site and the primary hot spots for agonist binding are provided in order to facilitate the generation of pharmacophore models or to setup the most appropriate conditions and parameters for successful virtual screening studies.

METHODS

Sequence alignment and template selection

The sequence of the human melatonin MTR1A receptor was extracted from the UniProt database[6]

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(accession code: P48039) in Fasta format and was automatically aligned using the T-coffee server[7] with the sequences of the all class A GPCRs receptors for which experimental the three-dimensional (3D) structure are available. The alignment was manually adjusted in order to preserve the highly-conserved amino acid motif specific for each transmembrane[8] and to avoid deletions or insertions in the transmembrane domain (TMD). When necessary, the deletions and insertions were pasted into a single piece per loop and placed in the most adequate point according to the 3D structure of the template. The disulfide bridge between the second extracellular loop (XL2) and the extracellular part of the third helix, conserved in all GPCRs, was taken in consideration.

Amino acid numbering

Specific amino acids from the transmembrane helices were named using the numbering system proposed by Ballesteros and Weinstein in 1995.[9] Briefly, for the most highly conserved residue in each transmembrane[8] a value of 50 was assigned, preceded by the number of the transmembrane helix. The other residues on the same helix will have a value corresponding to their position relative to the most conserved residue in transmembrane.

Model building and loop modeling

The homology modeling package Modeller[10] (version 9v11) was used to generate the three-dimensional model for melatonin MTR1A receptor based on the crystal structure of beta1-AR. The resulted model was geometrically minimized to reduce sterical clashes. The final model has over 90% of residues in the favorable regions of the Ramachandran map and all the main-chain and side-chain parameters are in the normal range.

Binding site detection

The location of the primary binding site in the MTR1A receptor was determined by using results from mutagenesis studies[11,12] and from computational predictions with SiteMap3.0[13–15] application implemented in Schrödinger software package.

Molecular Docking

Ligand setup. Ligands were prepared for docking using LigPrep 2.9 application[16] from Schrödinger. The polar and non-polar hydrogen atoms were added to ligands and all possible tautomers at physiological conditions (pH=7.4) were generated. Also, the ionization states were set and the final geometries were minimized using OPLS_2005 force field.

Receptor preparation. Protein Preparation Workflow[17,18] was used to add all the hydrogen atoms to the MTR1A receptor, to optimize the charge state of Asp, Glu, Arg, Lys and His residues and the orientation of hydroxyl and amide groups.

Docking Protocol. Glide receptor grids were generated from the prepared receptor model, with the docking grids centered on the binding site. The binding site was defined by a cubic docking box with the dimensions large enough to accommodate ligands with a length $\leq$ 20 Å, while to the ligand-midpoint box a side of 14 Å was given. The docking runs were performed with Glide 4.5[19–22] in SP mode using default settings for parameters.

RESULTS AND DISCUSSION

Template selection and model building. Selection of the most appropriate template structure represents one of the most critical step in the comparative modeling technique since it can affect significantly the models' final accuracy. The typical method for template identification consists in finding the closest neighbor or a given sequence protein, called target, by serial pairwise sequence alignments. For melatonin MTR1A receptor, the available sequence protein space is given by a number of 28 GPCRs for which structural information is available and deposited in Protein Data Bank.[23,24] Out of these, 24 sequences belong to class A GPCRs – same as melatonin MTR1A receptor – and therefore were further considered as potential templates for homology modeling. Since all the class A GPCRs share an orthosteric binding cavity, with different sizes and shapes, located in the extracellular side of the transmembrane bundle, the binding site similarity is also an important parameter to consider, when new therapeutic compounds are pursued. Consequently, the finding of the most appropriate template for melatonin MT1A receptor was conducted based on two main criteria: receptor and binding site similarity. Binding site pocket was restricted to a reference set of 44 residues previously defined by Gloriam.[25]

Classification of MTR1A and those 24 class A GPCRs was based on receptor and binding site similarity output results with minor or moderate differences regarding the number of clusters and their components (*e.g.* H1 receptor). For MTR1A receptor, the minimum distance in the class A

sequences space is given by a cluster of three receptors: beta1-adrenergic receptor (AR), beta2-AR and human dopamine D3 receptor (Fig. 1). For each applied criteria, the minimum distances between melatonin MTR1A receptor and the three potential templates are comparable, with beta1-AR being the closest relative. Moreover, when sequence identity was calculated, beta1-AR showed also the highest value with respect to MTR1A receptor.

The sequence alignment between template (beta1-AR) and target (MTR1A) was manually refined according to 3D coordinates of the beta1-AR (Fig. 2). The homology models for melatonin MTR1A receptor was generated based on the alignment shown in Fig. 2 and the resulted model, including the main backbone, was energetically minimized.

Binding site characterization. The location of the primary binding site identified by SiteMap application[13–15] is in agreement with the available experimental data.[11,12] Therefore, the following residues, Ser3.35, Ser3.39, Val5.42, His5.46 and Gly6.55– reported to be critical or having relevant involvements in the agonist binding at MTR1A receptor[11,12] – were located towards the extracellular side of helices 3, 5 and 6. The side-chains of these residues are oriented inside the transmembrane bundle. Noteworthy, compared to other class A GPCRs, binding cavity of melatonin MTR1A receptor goes deeply inside the transmembrane bundle (Fig. 3) and it has a strong hydrophobic character in several positions, well-known as being important for GPCRs agonist binding pocket. For example, positions 3.32 and 3.33 which are known as important anchor points of the biogenic amines in the GPCRs binding site, are occupied in the MTR1A receptor by a methionine and a glycine residue. The potential hot spot residues for the melatonin binding – a high lipophilic compound – are represented by polar or charged amino acids like Ser3.39, Tyr5.38, His5.46, Asn6.52, Tyr7.39 and Tyr7.43 (Fig. 4).

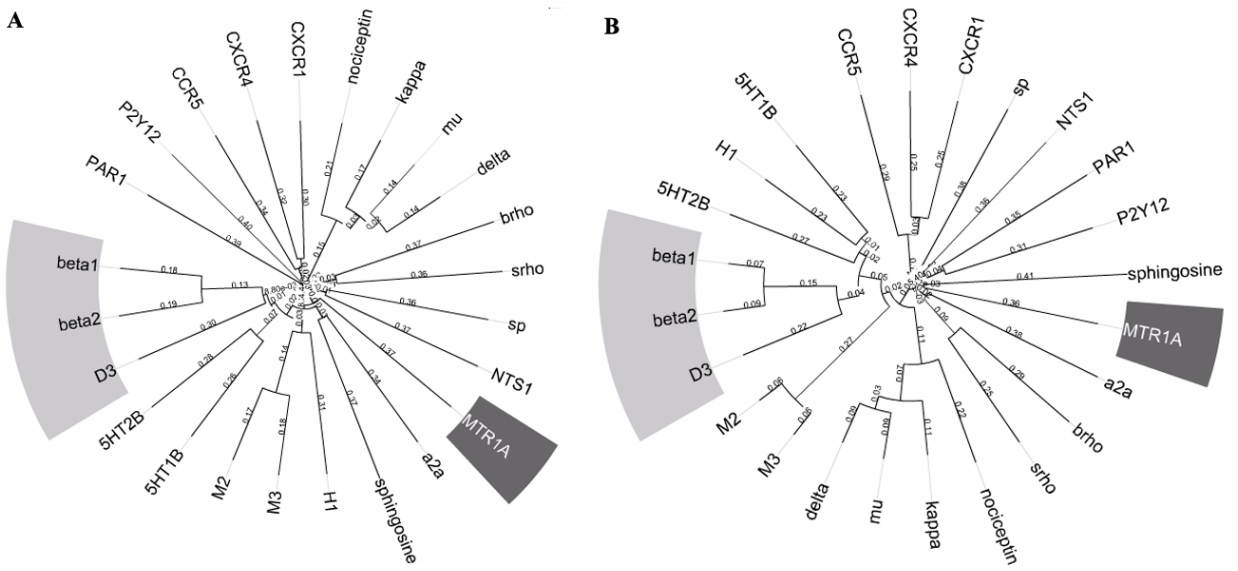

Fig. 1 – Dendrogram of melatonin MTR1A receptor and class A GPCRs with known 3D structure based on receptor (A) and binding site (B) similarity. Melatonin MTR1A receptor is highlighted in dark gray and its closest neighbors in light gray.

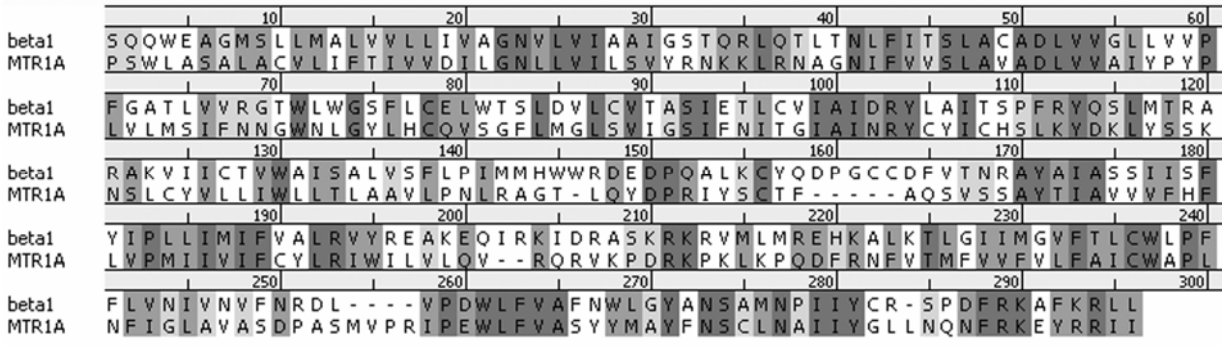

Fig. 2 – Sequence alignment of melatonin MTR1A receptor and beta1-AR.

Fig. 3 – MTR1A binding site, detected by SiteMap3.0[14,15].
The hydrophobic regions are represented with black, while the hydrophilic regions are represented with gray surfaces.

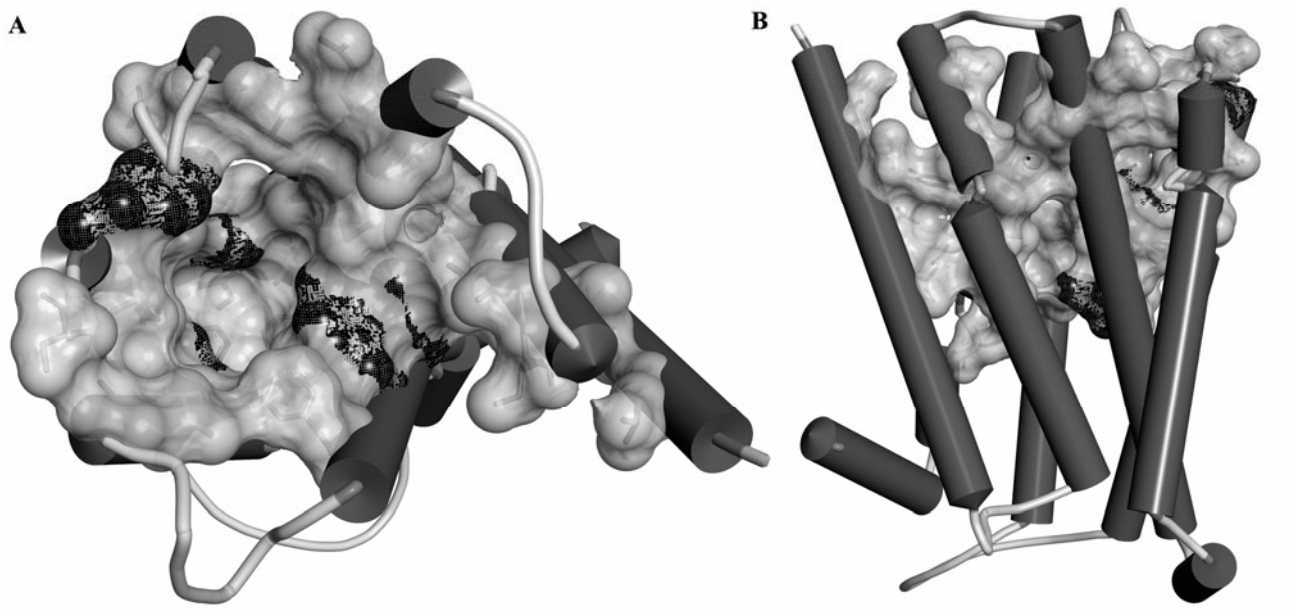

Fig. 4 – The potential hot spot residues (black surface) for melatonin interaction
at the MTR1A binding site, upper- (A) and lateral (B) view.

Molecular docking of the endogenous ligand, melatonin, revealed a binding mode characterized by two types of ligand-receptor interactions: hydrogen bonds and π-π interactions (Fig.5).

The amide moiety is positioned in the close neighborhood of Ser3.35 and Ser3.39 located on helix 3 and has a favorable orientation to establish hydrogen bonds with their side-chains. The indole

ring is stabilized in the situs by π-π interactions established with the side-chains of several aromatic amino acids like His5.46, Phe5.47 and Trp6.48. Moreover, the nitrogen indole acts as a hydrogen bond donor to the hydroxyl group of Tyr7.39. The ethyl linker is accommodated in a hydrophobic space defined by amino acids like Val3.36 and Ile3.37, from helix 3 and Ala7.42 from helix 7. The 5-methoxy substituent is faced towards aliphatic amino acids like Ile3.40 from helix 3 and Val5.42 and Leu5.48 from helix 5 (Fig. 5).

In a similar manner, other MTR1A agonists ramelteon, 2-iodo-melatonin and agomelatine have a similar binding mode in which the amide function is interacting with the two serine residues from helix 3 and the aromatic ring is involved in a stacking interaction with His5.46 (Fig. 6).

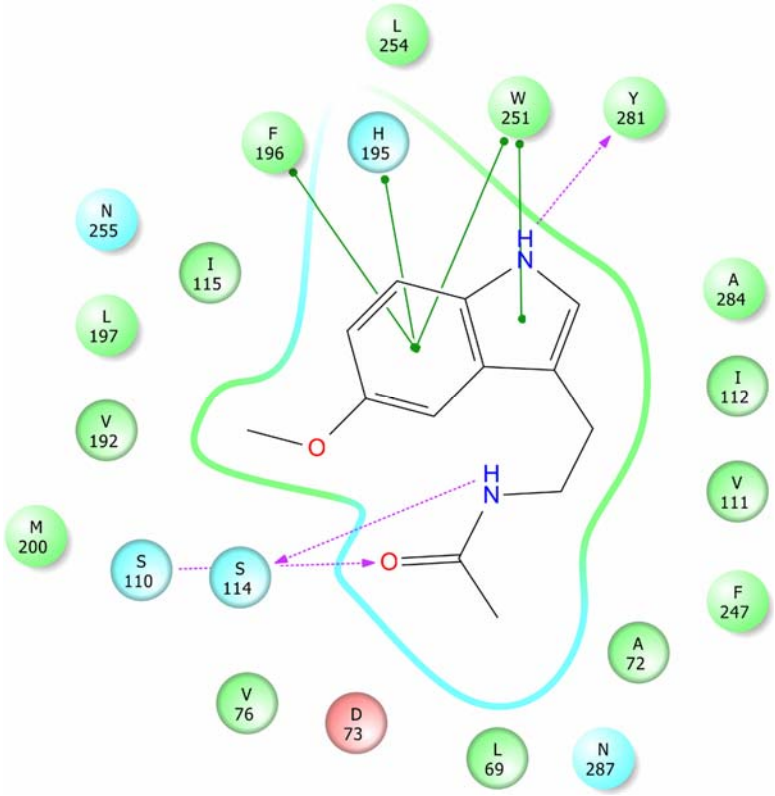

Fig. 5 – Binding mode of melatonin in human MTR1A binding site.

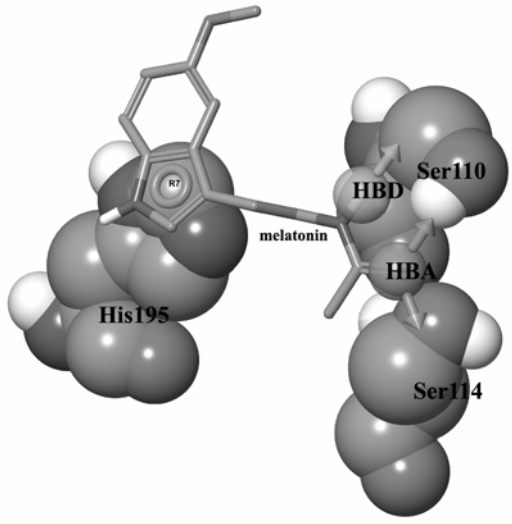

Fig. 6 – Agonist binding mode at MTR1A receptor, illustrated on melatonin.

CONCLUSIONS

The homology model of the MTR1A receptor based on the crystal structure of the beta1-adrenergic receptor was generated using homology modeling. The final model has all steric and topologic parameters within the normal range and it has allowed the recapitulation of experimental data by docking simulations using the endogenous ligand and several known agonists. The agonist binding site was mapped and the most relevant ligand–receptor interactions were described. The agonist binding mode is characterized by the formation of hydrogen bonds with two polar residues from helix 3, Ser3.35 and Ser 3.36 and a stacking interaction with His5.46 from helix 5.

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REFERENCES

1. A. Cavallo, S. R. Daniels, L. M. Dolan, J. A. Bean and J. C. Khoury, *J. Pineal Res.*, **2004**, *36*, 262-266.
2. S. R. Pandi-Perumal, V. Srinivasan, G. J. M. Maestroni, D. P. Cardinali, B. Poeggeler and R. Hardeland, *FEBS J.*, **2006**, *273*, 2813-2838.
3. A. Carrillo-Vico, J. M. Guerrero, P. J. Lardone and R. J. Reiter, *Endocrine*, **2005**, *27*, 189-200.
4. A. Dominguez-Rodriguez, *Cardiovasc. Pharmacol.*, **2013**, *2*, e109.
5. C. Ekmekcioglu, *Biomed. Pharmacother.*, **2006**, *60*, 97-108.
6. T. U. Consortium, *Nucleic Acids Res.*, **2012**, *40*, D71-5.
7. C. Notredame, D. G. Higgins and J. Heringa, *J. Mol. Biol.*, **2000**, *302*, 205-217.
8. J. M. Baldwin, G. F. Schertler and V. M. Unger, *J. Mol. Biol.*, **1997**, *272*, 144-64.
9. J. A. Ballesteros and H. Weinstein, in "Methods Neurosci" (Ed.: S.C.S.B.T.-M. in Neurosciences), Academic Press, 1995, pp. 366-428.
10. A. Sali and T. L. Blundell, *J. Mol. Biol.*, **1993**, *234*, 779-815.
11. T. Kokkola, M. A. Watson, J. White, S. Dowell, S. M. Foord and J. T. Laitinen, *Biochem. Biophys. Res. Commun.*, **1998**, *249*, 531-536.
12. S. Conway, E. S. Mowat, J. E. Drew, P. Barrett, P. Delagrange and P. J. Morgan, *Biochem. Biophys. Res. Commun.*, **2001**, *282*, 1229-36.
13. SiteMap, version 3.0, Schrödinger, LLC, New York, NY, 2014.
14. T. A. Halgren, *Chem. Biol. Drug Des.*, **2007**, *69*, 146-148.
15. T. A. Halgren, *J. Chem. Inf. Model.*, **2009**, *49*, 377-389.
16. Schrödinger Release 2014-1: LigPrep, version 2.9, Schrödinger, LLC, New York, NY, 2014.
17. G. M. Sastry, M. Adzhigirey, T. Day, R. Annabhimoju and W. Sherman, *J. Comput. Aided. Mol. Des.*, **2013**, *27*, 221-234.
18. Schrödinger Suite 2014-1 Protein Preparation Wizard; Epik version 2.7, Schrödinger, LLC, New York, NY, 2013; Impact version 6.2, Schrödinger, LLC, New York, NY, 2014; Prime version 3.4, Schrödinger, LLC, New York, NY, 2014.
19. R. A. Friesner, R. B. Murphy, M. P. Repasky, L. L. Frye, J. R. Greenwood, T. A. Halgren, P. C. Sanschagrin and D. T. Mainz, *J. Med. Chem.*, **2006**, *49*, 6177-6196.
20. R. A. Friesner, J. L. Banks, R. B. Murphy, T. A. Halgren, J. J. Klicic, D. T. Mainz, M. P. Repasky, E. H. Knoll, M. Shelley, J. K. Perry, et al., *J. Med. Chem.*, **2004**, *47*, 1739-1749.
21. T. A. Halgren, R. B. Murphy, R. A. Friesner, H. S. Beard, L. L. Frye, W. T. Pollard and J. L. Banks, *J. Med. Chem.*, **2004**, *47*, 1750-1759.
22. Small-Molecule Drug Discovery Suite 2014-1: Glide, version 6.2, Schrödinger, LLC, New York, NY, 2014
23. H. Berman, K. Henrick and H. Nakamura, *Nat Struct Mol Biol*, **2003**, *10*, 980.
24. http://www.rcsb.org/pdb/.
25. D. E. Gloriam, S. M. Foord, F. E. Blaney and S. L. Garland, *J. Med. Chem.*, **2009**, *52*, 4429-4442.