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Computer modelling of the CB1 receptor by Molecular Operating Environment

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Thus far two classes of G-protein-coupled receptors (GPCRs) have been discovered and validated as the main therapeutic targets of this system: the cannabinoid receptor type 1 (CB1), which is most widely expressed in the brain and the cannabinoid receptor type 2 (CB2), predominantly found in the immune system. These receptors have been intensively studied for drug development and for their role in the signalling pathway. The computer modelling and homology modelling approaches can be used in the design and discovery of cannabinoid analogues, because the computational structure prediction methods provide a cost-effective alternative in the absence of experimental structures. This study aims to present an attempt to construct a homology model of the cannabinoid receptor using Molecular Operating Environment. The present investigation provides a consistent framework for further investigation of the ligand-receptor interactions.

Keywords: Cannabinoid receptors, CB1, Molecular Operating Environment, G protein–coupled receptors, Homology modelling, Ligand-receptor interactions

INTRODUCTION

Homology modelling, also known as comparative modelling of protein is a computational technique, within structural biology, to determine the 3D structure of proteins [1,2]. More detailed information about homology modelling is available in recently published reviews [3-8].

Gaoni and Mechoulam identified Δ-9-tetrahydrocannabinol (THC) as the principal psychoactive molecule present in cannabis [9]. The pharmacological effects of cannabinoids are mediated through at least two cannabinoid receptors-CB1 and CB2. Regarding their distribution and functionality, CB1 receptors are located in the central nervous system, and they are responsible for most of the pharmacological effects of cannabinoids [10-13]. The CB2 receptor is found in peripheral tissues [14]. The CB1 and CB2 are seven transmembrane receptors that belong to the rhodopsin-like family Class A of G protein coupled receptors (GPCRs).

When the X-ray structure of a ligand-bound receptor is not available, homology models of the target protein can be used to obtain the ligand-receptor interactions. A knowledge of the three-dimensional structure of CB1 receptors could be helpful in the task of understanding their function and in the rational design of specific ligands. For this reason, many biochemical, pharmacological, and computational studies have been carried out on CB1 receptors. Different theoretical models were proposed in the literature but they are not available for the investigators in that field, because theoretical models have not been published in the data base (Table 1).

The objective of this research is to construct and refine a three-dimensional model of the human CB1 receptors in their activated forms by Molecular Operating Environment (MOE).

MATERIALS AND METHODS

Receptor

The protein sequence for the human cannabinoid receptor 1 was obtained from the Swiss Prot database (accession number P21554).

Table 1. Number of protein and protein/nucleic acid complex structures obtained by various experimental methods, available in the PDB as of 15 June 2017 (modified from www.rcsb.org/pdb/statistics/holdings.do).

Experimental method	Proteins	Nucleic Acids	Protein/NA complexes	Other	Total
X-RAY	110584	1866	5630	4	118084
NMR	10426	1217	244	8	11895
El. microscopy	1159	30	412	0	1601
HYBRID	102	3	2	1	108
Other	194	4	6	13	217
Total	122465	3120	6294	26	131905

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For homology models based on multiple templates, the template search was done with sequence search option of RCSB [15].

Computational tools

In order to perform computational studies a different software was used in the present work. The protein sequence of CB1 receptor was obtained from UNIPROT (http://www.uniprot.org/).

Homology modelling studies and ligands preparation were carried out using MOE (http://www.chemcomp.com/). The software MOE is a chemical computing and molecular modelling tool, which is a very widely used program in scientific applications. In the present investigation the MOE's Protein Modelling applications: MOE-SearchPDB, MOE-Align, Homology Model, and Protein Geometry were used. As the search progresses, pre-aligned families appear in the software with their calculated E-values. All docking calculations were performed with the software GOLD (Genetic Optimisation for Ligand Docking) 5.2 using the scoring functions available in the tool: ChemPLP, GoldScore, ChemScore and ASP (Astex Statistical Potential) scoring functions, [16-19]. Molegro Molecular Viewer was used for generating the figures (http://molegro.com/ index.php).

The correlation between the affinity of cannabinoid ligands from the literature [20] and the docking results for the obtained model by homology modelling was carried out in software GraphPad Prism 3.0 (http://www.graphpad.com/ scientific-software/prism). The Pearson's correlation coefficient was used, which is a measure of the correlation between normally distributed variables.

The structural similarity between the obtained model by homology modelling and the real structure of the human CB1 receptor from the PDB was assessed from the root mean square deviation (RMSD) values [21]. In general, a RMSD value, which is less than 3Å, implies a fairly good similarity between the structures.

RESULTS AND DISCUSSION

In our case, the search identifies members of rhodopsin as homologues of our target sequence. The displayed code represents the PDB structure from within the family that scored the highest against the target sequence.

Homology modelling starts with template identification that defines appropriate homologue(s) of known protein structure, called template(s), which are sufficiently similar to the target sequence to be modelled. Using MOE a simple search was performed by submitting the sequence of the human cannabinoid receptor 1 (CB1) obtained from Swiss Prot database (accession number P21554, Fig. 1). It was found that the conformation of the crystal structure of squid rhodopsin (PDBid:2z73) [22], the structure of bovine rhodopsin (dark adapted) (PDBid:1jfp) [23] and the structure of bovine rhodopsin (Metarhodopsin II) (PDBid:1ln6) [24] have high similarity with CB1 sequence.

In order to build a homology model, we aligned the target sequence to the protein family and after that decided which chain is to be the template for our model, and then built the model. This process goes through the following steps: Performing a Homology Search, Building a Homology Model and Evaluating the Homology Model.

The second step of the homology modelling procedure in MOE involves creating an alignment of the target sequence of CB1 receptor with some similar structures (Fig. 2). It was found in sequence alignment that there was a sequence similarity greater than 50% in almost all transmembrane regions. Thus, it could be expected that homology models built with this alignment would be accurate.

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>sp|P21554|CNR1_HUMAN Cannabinoid receptor 1 OS=Homo sapiens GN=CNR1 PE=1 SV=1
MKSILDGLADTTFRTITTDLLYVGSNDIQYEDIKGDMASKLGYFPQKFPLTSFRGSPFQE
KMTAGDNPQLVPADQVNITEFYNKSLSSFKENEENIQCGENFMDIECFMVLNPSQQLAIA
VLSLTLGTFTVLENLLVLCVILHSRSLRCRPSYHFIGSLAVADLLGSVIFVYSFIDFHVF
HRKDSRNVFLFKLGGVTASFTASVGSLFLTAIDRYISIHRPLAYKRIVTRPKAVVAFCLM
WTIAIVIAVLPLLGWNCEKLQSVCSDIFPHIDETYLMFWIGVTSVLLLFIVYAYMYILWK
AHSHAVRMIQRGTQKSIIIHTSEDGKVQVTRPDQARMDIRLAKTLVLILVVLIICWGPLL
AIMVYDVFGKMNKLIKTVFAFCSMLCLLNSTVNPIIYALRSKDLRHAFRSMFPSCEGTAQ
PLDNSMGDSDCLHKHANNAASVHRAAESCIKSTVKIAKVTMSVSTDTSAEAL
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Fig. 1. Sequence alignment of the human CB1 obtained from Uniprot database (P21554).

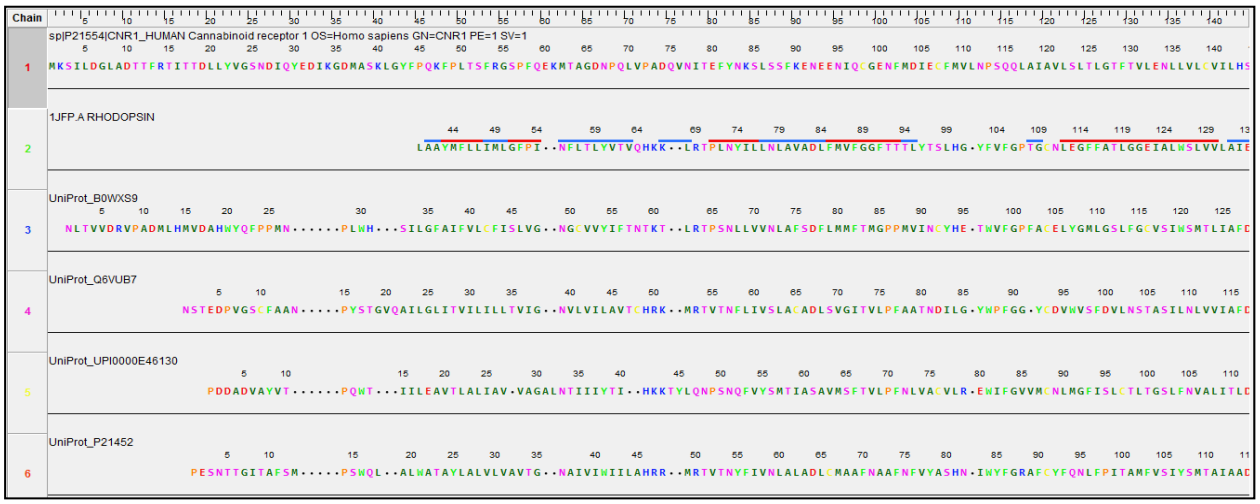

Fig. 2. Sequence alignment of CB1 receptor with some similar structures obtained from MOE.

Next step is building of homology models in MOE. The standard molecular mechanics AMBER 99 forcefield was used. Of the forcefields supported in MOE, Amber99 or Charmm27 are specifically designed to support protein simulations. The Amber99 forcefield for protein homology applications has been recommended [25].

The backbone fragments from a high-resolution structural database were collected and alternative side chain conformations for non-identical residues were assembled from an extensive rotamer library during data collection. During model building in software MOE, 10 independent models were created based upon loop and side chain placements scored by a contact energy function (Fig. 3). The final, refined model was loaded in MOE after the calculation was finished, (Fig. 4). After that the stereochemistry of homology models for unusual or geometrically unreasonable features was examined. Our model has RMSD 0.6230 < 3Å, which means that the generated model is built correctly.

The model with the best contact energy was chosen (-175.4267) and it was validated directly in MOE. The stereochemical quality of the modelled proteins was assessed from Ramachandran validation score for favoured regions and allowed regions (Fig. 5). In general, a score close to 100% implies good stereochemical quality of the models.

The molecular docking experiments with the obtained model of CB1 receptor by homology modelling in MOE and the ligands from literature [20] were carried out with software GOLD 5.2 and all four scoring functions embedded in the program: GoldScore, ChemScore, ASP and ChemPLP. According to Shim *et al*. [26] there exists a hydrophobic binding pocket that interacts with the alkyl chain of the cannabinoids.

	mol	name	RMSD to Mean	CA RMSD to Mean	Contact Energy	Packing Score	GB/VI	U	E sol	E ele	E vdw	E bond
1		Model #1(1)	0.5321	0.4335	-170.5224	2.5773	-11979.084	44.0562	-5489.7065	-3670.9124	-1578.8199	4192.5435
2		Model #2(1)	0.5999	0.4840	-171.1597	2.5411	-11946.157	55.1725	-5077.1636	-3709.1528	-1350.6154	4050.9197
3		Model #3(1)	0.6230	0.4680	-175.4267	2.4970	-12384.922	-465.5206	-4022.0649	-4885.0430	-1470.7916	4672.6147
4		Model #4(1)	0.5001	0.4120	-170.9495	2.5883	-12226.305	-642.7585	-4680.9507	-4376.6196	-1501.2986	4089.6438
5		Model #5(1)	0.5971	0.5120	-167.9658	2.4401	-12483.975	-689.5169	-3515.0066	-5215.2861	-1420.5818	4648.6338
6		Model #6(1)	0.5601	0.4499	-166.6386	2.5825	-11415.081	341.8376	-5764.7217	-2798.6863	-1600.3688	3804.0024
7		Model #7(1)	0.6687	0.5838	-161.2423	2.4580	-12526.668	-1077.5608	-3243.3823	-5334.0039	-1420.6730	4385.4292
8		Model #8(1)	0.6448	0.5364	-172.5911	2.4157	-12503.752	-760.5984	-3513.8774	-5252.4111	-1415.8101	4643.9072
9		Model #9(1)	0.5660	0.4784	-168.1365	2.4765	-12431.845	-901.6127	-4003.0608	-4989.3452	-1459.3658	4308.2290
10		Model #10(1)	0.5322	0.4337	-168.3179	2.5163	-11409.416	519.5591	-5675.1157	-2803.8230	-1589.4479	3975.6216

Fig. 3. Screenshot of database obtain after modelling by MOE.

The docking is effective when the polar residue from the receptor sequence was chosen - Asp366 and the investigated ligands bind near to it.

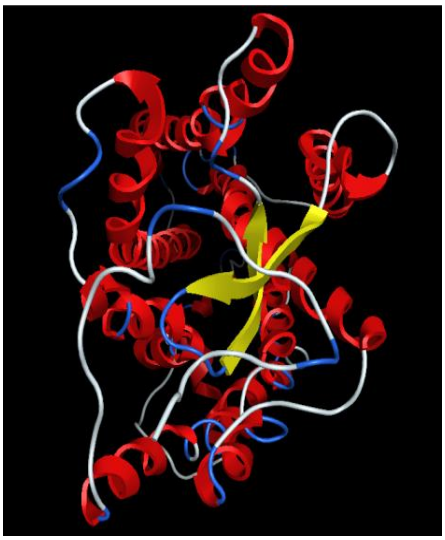

Figure 4. Refined model after the calculation was finished.

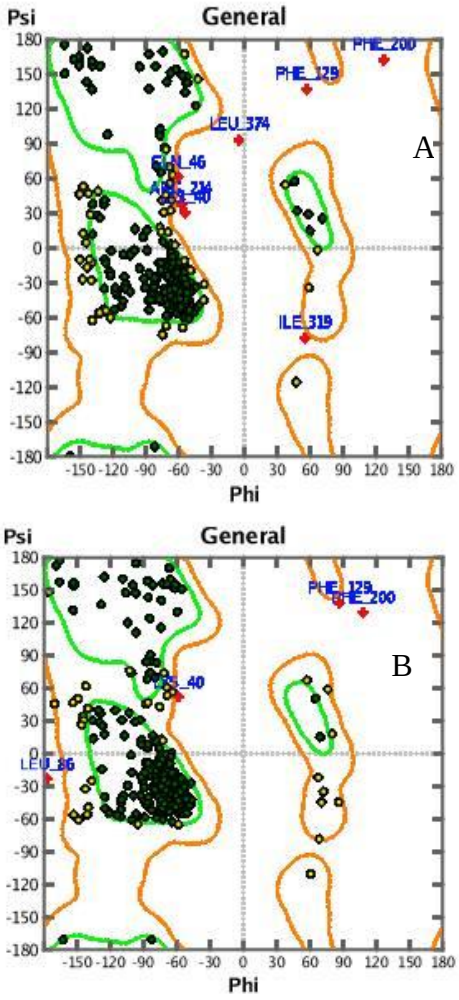

Figure 5. Ramachandran plot generated by MOE: A - before optimization of the structure; B - after optimization of the structure.

Correlations between the data of molecular docking and the affinity of ligands were performed with GraphPad Prism 3.0. The correlation between the data was assessed by the Pearson's correlation coefficient.

Significant correlation is established between the values of ASP scoring function and the values of affinity of cannabinoid ligands [20], (Pearson R=0.9594) for obtained model by homology modelling of human CB1 receptor [27-35].

The established correlation between these parameters shows that the model of CB1 receptor developed by homology modelling in MOE allows to optimally determining the binding affinity by ASP scoring function. For some work along these lines, see [36-42].

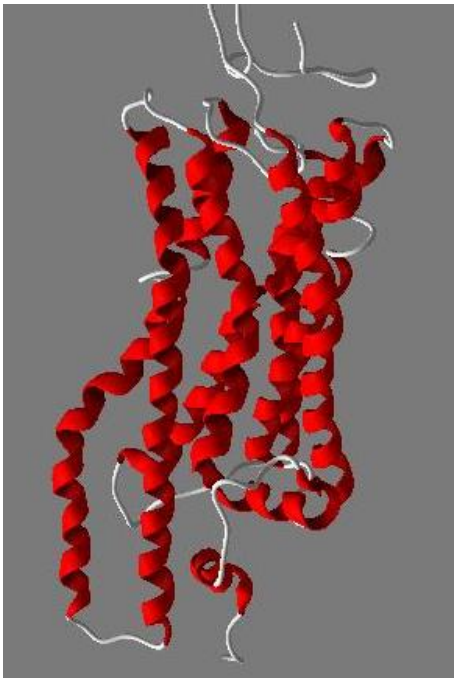

Figure 6. Graphical representation of the obtained model of homology modelling of CB1 receptor by the software MOE. The diagram was generated with the MMV.

The generated model of the human CB1 receptor obtained by homology modelling in MOE could be used in further investigations (Fig. 6). It could serve as a target in docking studies for design of new selective and effective cannabinoid ligands.

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