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Homology Modeling of Rdlβ5 GABA receptor

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Abstract

Three-dimensional model of Rdlβ5 GABA receptor in house Fly was generated with homology modeling, using the nACHR (1OED) as the template. To get the most reasonable and lowest energy conformation, energy minimization of molecular structure was used in our experiment. Finally, conformation of the modeled protein was evaluated with ProCheck. Results demonstrated that the percentage of reasonable amino acids in our constructed model is 98.1 %.

1. Introduction

GABA receptor, one member of the cys-loop ligand-gated ion channel, can be activated by neurotransmitter and controls the nerve conduction of the central nervous system and the accessory nervous system. GABA receptor is the major inhibitory neurotransmitter in the nervous system of mammal. About 40% of mammalian neurons use GABA receptor acid as a neurotransmitter [1]. The reinforcement of the synaptic transmission of GABA receptor is the basis of the drug therapy of a variety of neurological disease [2]. GABA receptor involved in a lot of different neurological and psychiatric disorders, such as anxiety, seizures, coma, and cognitive impairment [3]. In terms of physiology and pharmacology, it is sensitive to avermectins such as avermectin, ivermectin and

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milbemycin. According to the sensitivity with agonist and inhibitors, GABA receptors can be divided into three pharmacology subtypes: $GABA_A$, $GABA_B$ and $GABA_C$.

The three-dimensional molecular structure of protein can be used to guide biological experiment, such as site-directed mutagenesis, the drug design and the interaction of proteins and so on. Recently, the three-dimensional structure of protein was mainly determined by X-ray diffraction method and NMR spectroscopy. However, due to the technical limitation, a lot of the three-dimensional structures of proteins can not be obtained. Therefore the computer method was used to predict the protein's tertiary structure based on its sequence. In our research, the method of homology modeling is used to build the three-dimensional molecular structure of GABA receptor.

Because nACHR and GABAR belong to the super family of the ligand-gating ion channels, and they have the similarity structure and function, nACHR can be used as the template of GABAR for homology modeling. In order to achieve the ideal geometrical structure and the lowest energy state, the built model was optimized by molecular mechanics. Finally, the ProCheck was used to evaluate the three-dimensional structure of the protein. Results demonstrated that the percentage of reasonable amino acids in our constructed model is 98.1 %.

2. Methods

All work on homology modeling, molecular optimization and Surflex-docking studies was performed on the Dell 390 workstation with Redhat Linux system using Sybyl software package developed by Tripos.

2.1 Homology modeling of the single subunit Rdlβ GABA receptor

Preparing the target sequence and template: The amino acid sequence of GABA receptor β subunit of house fly was obtained from the Swiss-Prot/TrEMBL database (the accession number Q75NA5) [4]. In this study, the cryo-electron microscopy structure of nAChR of Torpedo marmorata was chosen as the template which was obtained from Protein Data Bank (accession numbers 1OED) [5].

Modeling of the single subunit Rdlβ: The Swiss-Model homology modeling server was used to model the target sequence. The steps included selecting the template, comparing with the template target sequence, recognizing structure conserved regions (SCR), searching the gaps, adding side chains and optimizing model. The step is repeated until we get a satisfactory model. In the step of choosing a template, the specified template 1OED was selected in this experiment. The sequence of Rdlβ single subunit and the template 1OED were imported into the Swiss-model, and the single subunit was built. Sybylx1.1 was used to optimize the built single subunit, which makes its structure more stable.

2.2. Subunits assembling

The online program of Symmdock server was used to assemble subunits. We input protein monomer and symmetric point group. The subunits were assembled with the docking method. The single subunit of GABA receptor protein was inputted the program. Then we input number 5 in the symmetric order.

2.3 Energy optimization and evaluation of structures

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The energy minimization of overall structure of t he built Rdlβ5 was carried out with Sybylx1.1 so ftware. ProCheck was used to assess the main chain and side chain of GABA receptors structure [6]. The ramachandran map expresses that the proportion of the amino acid residues is in reasonable range.

3. Results and discussion

3.1 The construction of GABA receptors of single subunit

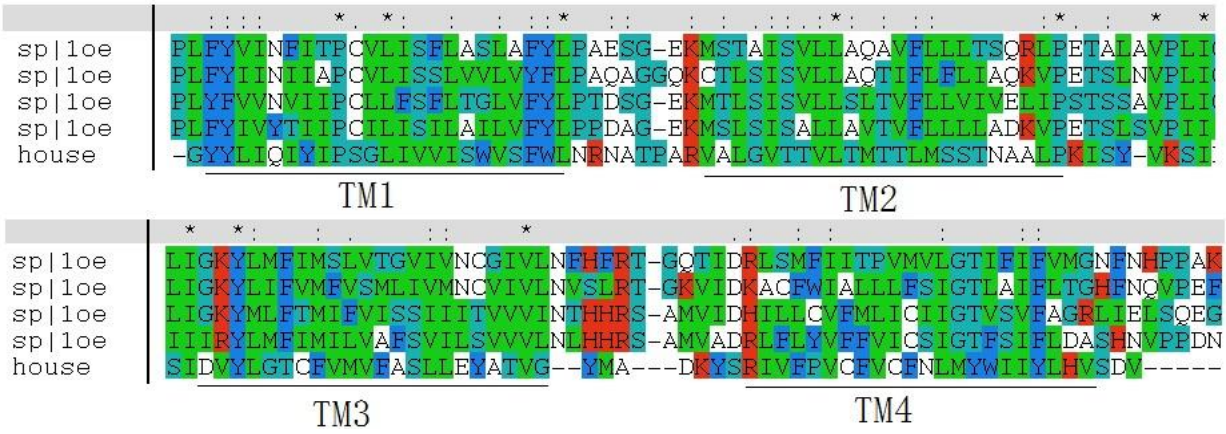

Fig. 1 The target sequence (four transmembrane domains of one subunit of Rdlβ5 GABA receptor,TM1-TM4) was aligned with all four template sequences (δ, γ, β, and α subunits of nAChR (1oed)).The amino acid sequences of TM1 toTM4 were 4G-24L, 33V-55P, 64D-85G and 96I-116V, separately

In this article, it was 18.1% that the percentage identity of the house fly sequence aligned with the chain A of the 1OED sequences, which was below the B chain. However, the subunit α of nAChR (Protein Data Bank identifier 1OED) was chosen as the template. There were two reasons about that. One was that nAChR and GABA belong to a super family of ligand gating ion channels, and their structure and function has similarity. Another was that 1OED has been repeatedly used as a template to build the GABA receptors [7]. Chain A was used as the template and can get four transmembrane domains. Then, the multiple structure-sequence alignment method was used to build all of the models. That was showed in Figure 1.

Next, the loop was added into models. Through the program search, three loop areas (Ile57-Val60, Tyr91-Ser92 and Asp116-Val117) were found. Though observing the model, we get that the part of 1-57 amino acids for two transmembrane domains (TM1 and TM2), 60-91 amino acid for TM3 and 92-117 amino acid for TM4.

3.2 Building Rdlβ5 GABA receptor

Rdlβ5 GABA receptor is pentameric. One single subunit was built by Swissmodel and assembled by Symmdock online procedures. Rdlβ5 GABA receptor is made up of five β GABA subunit. Each subunit has extracellular area, four transmembrane areas (TM1, TM2, TM3 and TM4) and the carbon domain (Figure 2).

The structure of Rdlβ5 GABA receptor was simulated in vacuum conditions. In fact, the cells live in the water environment, so the modeled receptor proteins have to be simulated. Then water box was added and the molecular dynamics optimization was carried out. The potential energy of the model of housefly GABA receptor fell

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sharply before 500 fs and then a steady state was obtained (Figure 3).

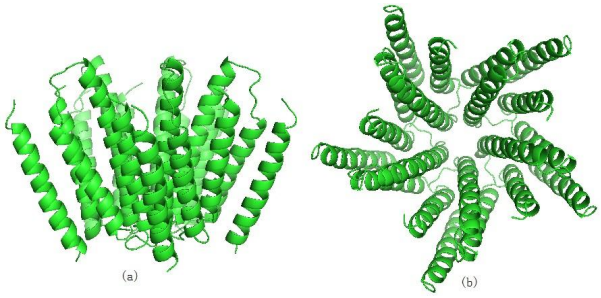

Fig. 2 This is the 3D molecular structure of the RDLβ5 GABA receptor of house fly. (a) Front view; (b) vertical view.

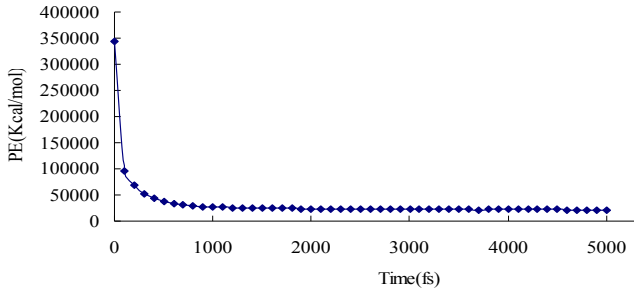

Fig. 3 The molecular dynamics simulation curve of housefly GABA receptor

3.3 Evaluating the rationality of the model protein

After potential energy of the housefly GABA receptor was optimized to the stable structure, PROCHECK program was used to evaluate the rationality of the protein in this experiment. In the process, the ramachandran map expresses proportion of the amino acid residues in reasonable range and the main chain structure and the side chain structure of housefly GABA receptor protein were evaluated by PROCHECK program.

The ramachandran map of house fly GABA receptor protein was shown in Figure 4. Proportion of the amino acid residues in reasonable range reached 98.1%, which indicates that the modeled housefly GABA receptor structure is reasonable.

Figure 5 is the evaluation of the main chain structure of protein. Compared the built protein with the similar resolution of protein, this evaluation method instructs the rationality of the built protein structure. The purple strip in figure is a better structure to identify. The small black square in figure represents the evaluation results of constructed protein structure in this test. The results of the main chain showed that the proportion of amino acids in the best areas is a little higher than the average proportion of the same types. The collision of the main chain and the bond energy of hydrogen bonding are in a reasonable scope. The deflection of peptide plane and the torsion of α carbon tetrahedron deviate from the mean variation. To consider comprehensively, the main chain of protein structure is reasonable.

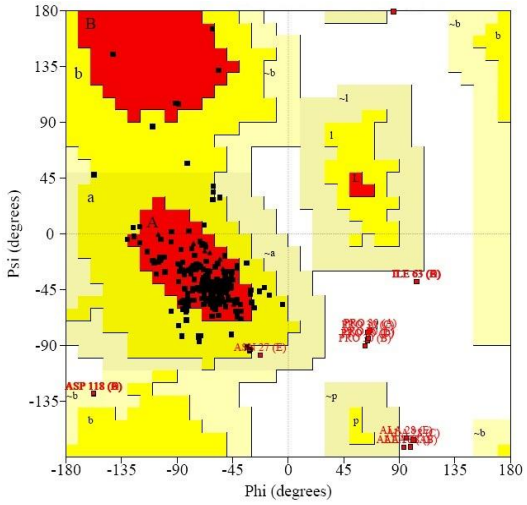

Fig. 4 The ramachandran map of housefly GABA receptor, red: the best regions, brown: allowed regions, others are disallowed.

Figure 6 is the evaluation of the side-chain properties of the house fly receptor protein structure. Similar to the evaluation of main chain structure, the purple strip in figure is also a better structure to identify. The results can illustrate that the negative deflection, reversal, positive deflection

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and dihedral angle of side-chain are in the reasonable scope.

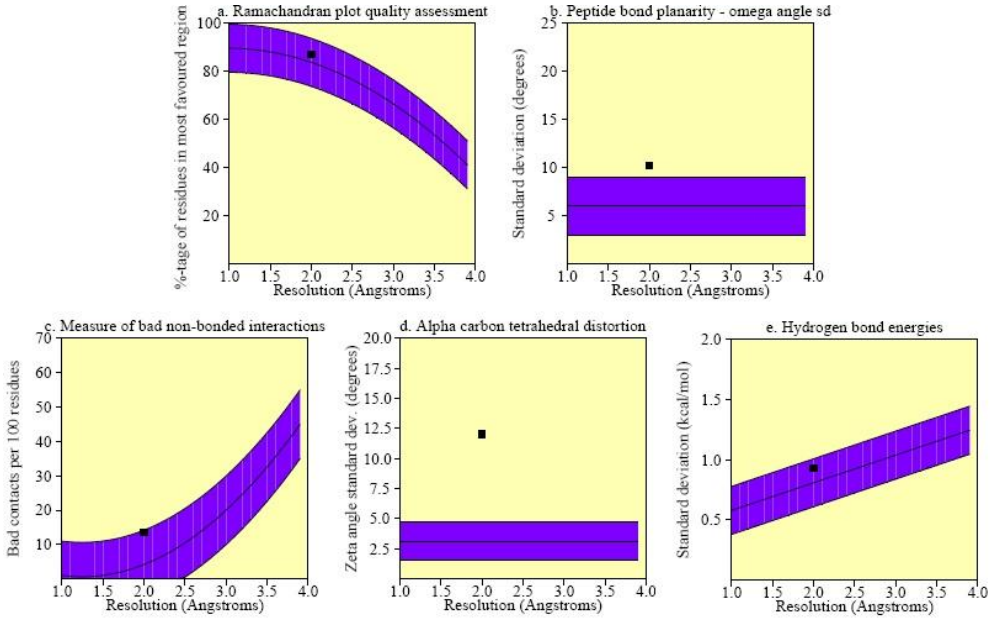

Fig. 5 The main-chain properties of the house fly receptor structure (values marked by solid squares) and how these properties compare with well refined structure at a similar resolution. (a) shows the ramachandran-plot quality; (b) shows the planarity of the peptide bond as measured by the standard deviation of the w torsion angles; (c) shows the number of bad contacts per 100 residues; (d) shows the C a tetrahedral distortion; (e) shows the standard deviation of the hydrogen-bond energies for main-chain hydrogen bonds.

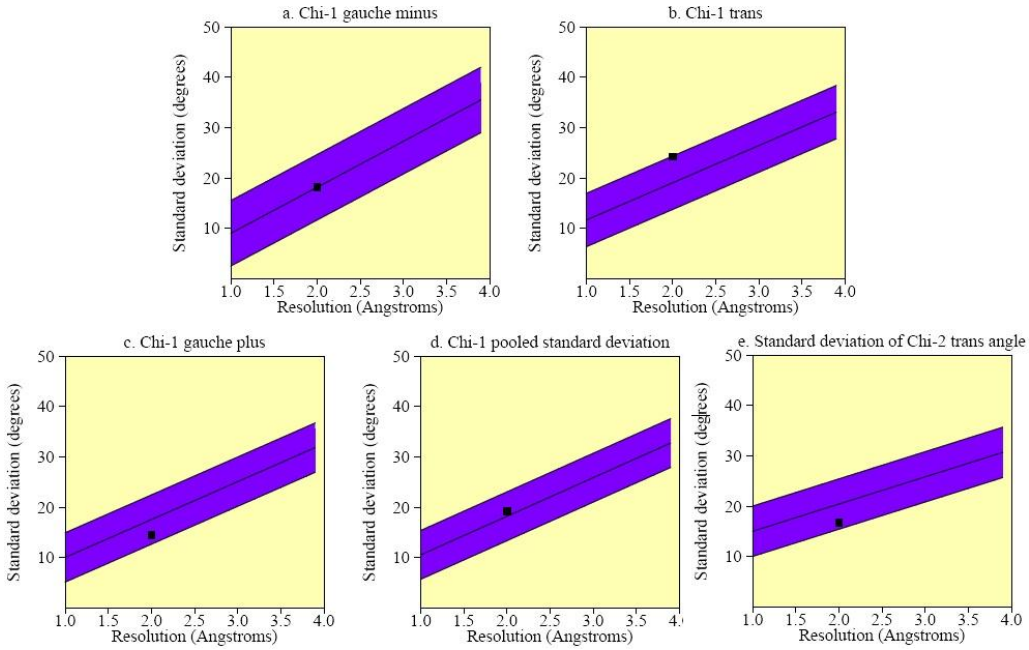

Fig. 6 The side-chain properties of the house fly receptor structure (values marked by solid squares) and howthese properties compare with well refined structure at a similar resolution. The graphs show the standard deviations of the side-chain torsion angles: (a) X1gauche minus; (b) X1 reversal; (c) X1 gauche plus; (d) pooled X1 torsion angles; (e) X2

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reversals.

4. Conclusions

1OED was chosen as a template in this test. The Swiss-model, Symmdock and Sybyl softwares were used to build Rdlβ5 GABA receptor. Compared with the template and the target protein, the sequence recognition degree is 18.1%, which is lower than the requirement of homologous simulation. The online program of Symmdock server was used to assemble subunits. The Procheck was used to evaluate the main chain and side chain structure of the target protein. The percentage of amino acid residues in the reasonable range is 98.1%, which indicated that the model is reasonable. It may provide important knowledge for the deep study of GABA receptor to some extent.

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