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Original article

Homology modeling and atomic level binding study of $GABA_A$ receptor with novel enaminone amides

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ABSTRACT

A series of novel enaminone amides with improved side effect were synthesized by Hogenkamp et al. To explore the action mechanisms of enaminone amides, the homology model of rat α1β2γ2 GABAR was generated using the cryo-electron microscopy structure of the nAChR of *Torpedo marmorata* and the AChBP of *Lymnaea stagnalis* as the templates. Molecular docking and pharmacophore analyses allowed us to speculate the critical residues involving to the recognition of the ligands. The docking results indicated His128, Tyr186 and Tyr236 of α subunit were essential to form H-bond interactions contacts with the ligands. Specially, the N-substituents of enaminone amides as the sterically favored areas could form the important hydrophobic interactions with the residue Tyr186.

1. Introduction

γ-Aminobutyric acid (GABA) is one of the major inhibitory neurotransmitters in the central nervous system (CNS) and exerts its physiological effect by binding to GABA receptor [1,2]. Mammalian GABARs can be classified simply as into two types: the ionotropic receptors, which are ligand-gated ion channels ($GABA_AR$, $GABA_CR$); and metabotropic receptors ($GABA_BR$) [3]. The ionotropic GABARs are the members of the superfamily of Cys-loop ligand-gated ion channels, which also includes nicotinic acetylcholine receptors (nAChR), strychnine-sensitive glycine receptors, and serotonin type 3 receptors [4].

Mammalian GABARs are distributed widely in the nervous systems. Cloning from cDNA or genomic libraries has so far revealed 19 related GABAR subunits in the mammalian nervous system. These subunits are of the classes 6α, 3β, 3γ, 1δ, 1ϵ, 1π, 1θ, and 3ρ [5–7]. The structures of all subunits contain three main domains: an N-terminal ligand-binding domain (LBD), transmembrane domains (TMDs), and a long cytoplasmic loop that links TM3 and TM4. The LBD is a hydrophilic long chain located on the extracellular side of the membrane, consisting primarily of β-strands linked by disulfide bonds between cysteine residues. Subunits in the same class share rather high similarity (70–80%), while similarities between subunits in different classes are relatively low (20–40%). One of the most common GABARs in the human brain is the α1β2γ2 subunit combination, and the proposed receptor stoichiometry is 2α2β1γ [8–10].

$GABA_ARs$ as therapeutic targets have played an important role in central transmission processes, due to involvement with several neurological and psychiatric disorders such as anxiety, sleeplessness, epilepsy, and amnesia [11]. It has been shown that $GABA_AR$ have multiple allosteric modulatory sites for barbiturates, steroid anesthetics, and benzodiazepines (BZ) that all modulate opening of the channel through different mechanisms of action [12]. Specially, a lot of non-BZ ligands have also been discovered, including imidazopyridines and pyrazolopyrimidines [13–15]. These non-BZ compounds bind to the BZ site which occurs at the interface of the α and γ subunit of the $GABA_AR$ [12]. But the use of allosteric modulators is limited because of side effects such as sedation, myorelaxation, ethanol interaction, psychical and physical dependence, tolerane, and abuse liability [16,17].

Hogenkamp et al. [18] have synthesized a series of novel enaminone amides, which bind to GABAR as the novel neurotransmitter with improved side effect. Meanwhile, they discovered that these compounds likely interact with a novel site on the $GABA_AR$ distinct from the BZ, GABA, and neuroactive steroid binding sites by in vitro binding studies. However, the detailed binding site of these compounds is still ambiguous. This work is mainly to study

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the interaction between the $GABA_AR$ and enaminone amides by computational simulations. Hence, a three-dimensional (3D) crystal structure of the GABAR will be constructed by homology modeling and the molecular docking is used to search the binding mode of the ligands with the receptor. Meanwhile, pharmacophore model built by DISCOtech will further confirm the interaction mechanism of enaminone amides. The results will be used to explain and consolidate experimental data, and help to design the highly active compounds.

2. Methods

All work containing homology modeling, Surflex-docking and DISCOtech studies was performed using the SYBYL 7.3 software package (http://www.tripos.com/) running on a Linux workstation [19].

2.1. Building homology modeling

To build the homology models of the ligand-binding domain (LBD) of rat α1β2γ2 $GABA_ARs$, the sequences of rat α1 (P62813), β2 (P63138), and γ2 (P18508) subunits were obtained from the Swiss-Prot/TrEMBL database. These three sequences were all edited to remove the TMDs and the long cytoplasmic loop linking TM3 and TM4.

Template selection is an important starting point in homology modeling because the template directly determines the main folding of the target structures, and influences their quality. Due to the technological limitations of membrane-bound protein crystallization, few well-resolved structures of membrane-bound proteins have been obtained through X-ray crystallography or NMR methods. In the study, all GABAR subunits were modeled using the cryo-electron microscopy structure of nAChR of *Torpedo marmorata* (PDB code 2BG9, 4 Å) [20] or the AChBP of *Lymnaea stagnalis* (PDB code 2ZJU, 2.58 Å) [21] as the templates.

Models were assembled using the following method. According to the schematic presentation of subunit correspondence between nAChR and α1β2γ2 GABAR shown in Fig. 1a, the integral GABA receptor was obtained by aligning subunits of rat GABAR to the corresponding subunits of the template. That is to say, the GABAR α subunit was aligned to nAChR subunit γ and δ, the GABAR γ subunit to nAChR subunit β, and the GABAR β subunit to nAChR subunit α. This method of model construction is based mainly on the fact that the agonist binding sites of both the GABA and nACh receptors are spatially similar. The nAChR α subunits form the principal part of the acetylcholine (ACh) binding pocket, while the GABAR β subunit forms the principal side of the GABA binding pocket.

The initial models were optimized energetically using the AMBER7 FF99 force field by performing a conjugate gradient minimization to reach a root-mean-square (RMS) gradient energy of 0.5 kcal mol^{-1} $Å^{-1}$. Subsequently, a dynamics simulation was performed to find the steady-state conformation of initial rat α1β2γ2 GABARs over the 600 ps with a step size of 1 fs at a constant temperature 300 K [19].

2.2. Ligand docking

To more fully probe the interactions of this novel enaminone amides with $GABA_AR$, 12 ligands were docked into the putative binding pocket of rat α1β2γ2 GABARs. These ligands from Hogenkamp's Lab have the same skeleton structure, which is 2-(2-chlorobenzoyl)-3-(4-chlorophenylamino)acrylamide [18]. The different substitutional groups conferred these compounds to the diverse activity. Their activity data [IC_{50} (mol L^{-1})] which will be used in subsequent study have been converted to the logarithemic scale [$-lg_{10}$ IC_{50}] (Table 1).

Before performing the ligand docking, it is critical to search for the binding pocket of the prepared protein. There are three kinds of modes to determine the binding site: automatic, ligand and residues mode. In this study, Residues mode was adopted to generate the protomol in the program Surflex [22]. This mode defines the active site by considering a reasonable distance around chosen residues. In addition, two parameters that can significantly affect the size and extent of the protomol generated are the threshold and the bloat values.

The Surflex scoring function, which is based on the binding affinities of protein–ligand complexes, takes into account several terms, including hydrophobic, polar, repulsive, entropic and solvation [23]. The docking scores are expressed in $-lg_{10}$ K_d units to evaluate the docking results, where K_d represents a dissociation constant of a ligand [19]. In the study, the binding free energies (kcal mol^{-1}) of protein–ligand complexes would be obtained according to the calculation as follows, where RT = 0.59 kcal mol^{-1}:

$$\text{Free energy of binding} = RTlg_e K_d \quad (1)$$

2.3. Identification of the pharmacophore

Given these enaminone amides that bind to a common binding site, the DISCOtech program was used to identify possible pharmacophore features in compounds **e, f, g, h** and **i**. DISCOtech first assigns pharmacophore elements such as hydrogen bond donor atoms, hydrogen bond acceptor atoms, charged centers, hydrophobic groups, centers of mass of hydrophobic rings and the most likely location of binding sites in the receptor macromolecule to the molecules [19]. Then 3D alignments of the pharmacophore features in different molecules are found by automatically iterating

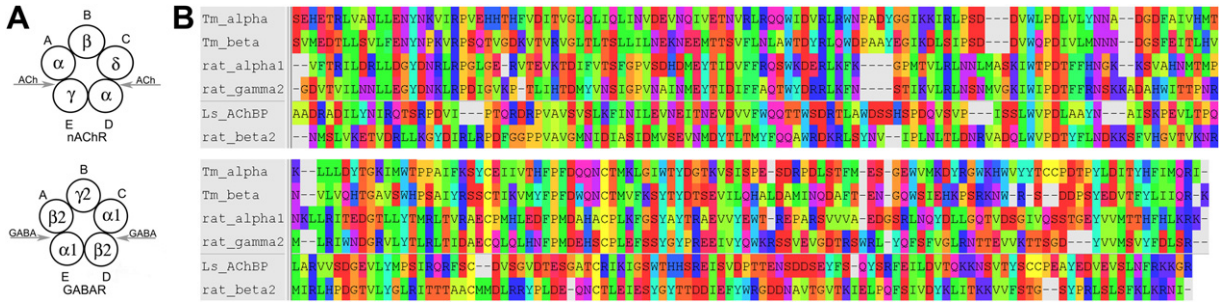

Fig. 1. (a) Subunits correspondence between nAChR and $GABA_AR$ is schematically presented. (b) Every target sequence (the LBD domain of rat GABAR α1, β2 and γ2 subunits) was aligned with the template sequences (α and β subunits of nAChR 2BG9 and the AChBP 2ZJU). Different types of amino acids are painted in different colors.