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Insights into the suanzaoren mechanism—From constructing the 3D structure of GABA-A receptor to its binding interaction analysis

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Abstract

Gamma aminobutyric acid type A (GABA-A) receptors are an important therapeutic target in insomnia treatment. The GABA-A protein structure is still not available. In this study, a reliable structure of GABA-A receptor was built and validated by several criteria. Zolpidem was predicted to gain the highest binding affinity at the BZ-binding site and be surrounded by α_1-His129, α_1-Tyr187, α_1-Gly228, α_1-Thr234, α_1-Tyr237, γ_2-Met96, γ_2-Phe116, and γ_2-Met169. In addition, GABA formed five hydrogen bonds with α_1-Arg159, β_2-Glu179, and β_2-Tyr181 and was surrounded by the residues α_1-Phe92, α_1-Arg147, β_2-Tyr181, β_2-Thr184, β_2-Thr226, and β_2-Tyr229 at the GABA-binding site. The two simulation results were consistent with the experimental assay, which suggested that the simulated GABA-A receptor was reliable. Jujuboside A, which was considered the effective suanzaoren constituent, had difficulty penetrating the blood–brain barrier. Besides, jujuboside A was unable to bind at both binding sites due to its large structural volume. However, jujubogenin that was hydrolyzed from jujuboside A showed the most compatible binding pose and formed five hydrogen bonds with the key residues, β_2-Thr226 and β_2-Tyr229, at the GABA-binding site. In addition, according to a docking study, jujubogenin gained higher scoring values, which indicated a higher binding affinity. Moreover, the adsorption, distribution, metabolism, excretion, and toxicity (ADMET) descriptors predicted that jujubogenin had high blood–brain barrier penetration. Conclusively, jujubogenin was suggested to be the effective suanzaoren constituent for exerting the sedative function via GABA-A receptor.

Keywords: Gamma aminobutyric acid; Suanzaoren; Jujubogenin; Molecular modeling; Docking

1. Introduction

Nowadays, a population of elderly people suffers from insomnia. The main target proteins for insomnia treatment are Gamma aminobutyric acid type A (GABA-A) receptors, which mediate the neurotransmission of the central nervous system in mammals. GABA-A receptors belonging to the Cys-loop superfamily serve as ligand-gated chloride ion channels which are built by pentameric membrane protein (Hirouchi *et al.*, 1987). GABA, an inhibitory neurotransmitter, induces rapidly increasing chlorine ion influx through GABA-A ion channel to depress excitatory depolarization (Kash *et al.*, 2004). The subunits show a variety of structural heterogeneity including several different subunits (α_1–α_6, β_1–β_3, γ_1–γ_3, δ, ε, π, θ, and ρ_1–ρ_3). The GABA-A receptor has been created by $2\alpha_1$, $2\beta_2$ and $1\gamma_2$ for molecular modeling because β_2 is the most abundant subunit in the brain, γ_2 is necessary for benzodiazepine drug binding, and α_1 is involved in mediating the sedative effect (Ci *et al.*, 2007; Kash *et al.*, 2004; Rowlett *et al.*, 2005; Stephenson, 1995). Unfortunately, the structural analysis of membrane-bound proteins through X-ray crystallography or a spectroscopic technique is not easy because of the rapid protein denaturation at the time of extraction from the physiological membrane environment. As a result of lacking a valid GABA-A receptor structure, acetylcholine binding-protein (AChBP, Protein Data Bank ID: 1I9B) was employed as the template to simulate the GABA-A receptor structure (Brejc *et al.*, 2001; Chen, 2007; Chen and Chen, 2007a; Ci *et al.*, 2007; Ernst *et al.*, 2003; Sancar *et al.*, 2007). The three-dimensional structure obtained via structural bioinformatics (Chou, 2004a) for a targeted protein can provide us a footing or starting point for drug design in a timely manner; hence, significantly expedite its development pace.

Zolpidem, a benzodiazepine-like drug that is a GABA-A receptor agonist, is used for the clinical treatment of insomnia and anxiety disorder (Falco *et al.*, 2006). The two GABA-A

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receptor agonists, GABA and zolpidem, have different binding sites. The benzodiazepine-binding site is located at the α/γ interface, whereas the GABA-binding site is located at the α/β interface (Jones *et al.*, 2006; Sigel and Buhr, 1997).

Suanzaoren decoction, a traditional prescription, has been used for insomnia treatment (Chung and Lee, 2002; Liao *et al.*, 1995; Zhao *et al.*, 2006). The decoction is composed of five herbs, Suanzaoren (*Semen ziziphi spinosae*), Chuanxiong (*Rhizoma chuanxiong*), Fuling (*Poria*), Zhimu (*Rhizoma anemarrhenae*), and Gancao (*Radixglycyrrhizae*). The main medicine of the suanzaoren decoction is suanzaoren, and the saponins, jujuboside A and jujuboside B, are considered the major effective constituents for insomnia treatment (Shou *et al.*, 2001). In addition, a study indicates that suanzaoren exerts sedative effects via GABA-A receptors (Yi *et al.*, 2007). Jujubogenin is the hydrolyzed product of jujuboside A and jujuboside B and can be further hydrolyzed and cleaved to form *cis*/*trans*-ebelin lactone isomers under the acid condition as following (Lee *et al.*, 2006):

$$\text{jujuboside A} \xrightarrow{\text{hydrolysis}} \text{jujugogenin} \xrightarrow{\text{acidic hydrolysis}} \textit{cis}/\textit{trans}\text{-ebelin lactone}$$

Recently, chemical engineering and computer technology have been penetrating all fields of modern biotechnology, especially bioinformatics, pharmacoinformatics, and computer-aided drug design (CADD). Molecular modeling and CADD, a rapid raising technique including comprehensive physical chemistry and computer science, are important methods in modern high-throughput drug design. Docking studies have been used for investigating ligand–receptor interaction and revealing the binding mechanism (Chen, 2007, 2008a,b,c; Chen and Chen, 2007a,b,c; Chen *et al.*, 2008a,b; Chou, 2004a,b; Chou *et al.*, 2003, 2006; Du *et al.*, 2005a,b, 2007; Gao *et al.*, 2007; Guo *et al.*, 2008; Li *et al.*, 2007a,b; Wang *et al.*, 2007a,b,c, 2008; Wei *et al.*, 2005, 2006a,b, 2007; Zhang *et al.*, 2006; Zheng *et al.*, 2007; Zhou and Troy, 2005a,b). Several scoring functions including piecewise linear potential (PLP), potential of mean force (PMF), and dockscore are employed to estimate ligand binding affinity (Gehlhaar *et al.*, 1995; Muegge and Martin, 1999; Venkatachalam *et al.*, 2003). Structural analysis is used to identify the important ligand–receptor interaction. Therefore, we investigate the potent effective constituent of suanzaoren via molecular simulation and docking study.

2. Materials and methods

2.1. Materials

Discovery Studio (Accelrys, San Diego, USA) was used to perform the molecular simulation, docking study, and scoring function calculations. AChBP (Protein Data Bank ID: 1I9B) served as the protein template to simulate the extramembrane domain of GABA-A receptor (Chou, 2004b). The sequences of human GABA-A receptor subunits, α_1 precursor (Swiss Prot ID: P14867), β_2 precursor (Swiss Prot ID: P47870), and γ_2 precursor (Swiss Prot ID: P22300), were obtained from the Swiss Prot web site. The structures of jujuboside A, jujuboside B, betulin, betulic acid, jujubogenin, *cis*/*trans*-ebelin lactone isomers, zolpidem, and GABA were acquired from the National Center for Biotechnology Information web site (Fig. 1).

2.2. Multiple sequence alignment and modeling

Only the intracellular domain sequences were removed because GABA and benzodiazepine binding sites are located in the extramembrane domain. As a result, the sequences of α_1 subunit from the residue 28–251, β_2 subunit from the residue 25–244, and γ_2 subunit from the residue 40–273 were employed for sequence alignment. Multiple sequence alignment between each subunit and AChBP monomer was performed by CLUSTAL W program. Each alignment result was linked together to form the combination (α_1, β_2, α_1, γ_2, β_2). Based on the final sequence alignment and AChBP structure, the GABA-A receptor structure was simulated by MODELLER. Molecular dynamics (Chen *et al.*, 2005; Liu and Hsieh, 2005) were employed to minimize the violation of the steric restraint. Dynamics cascade included 500 steps of Steepest Descent, 500 steps of Adopted Basis NR, 2000 steps of heating, 1000 steps of equilibration, and 1000 steps of production.

2.3. Docking and scoring function prediction

The two-dimensional structures of compounds were built using Chemdraw. Then the three-dimensional structures were constructed using Chem3D and energy minimized by MM2 force field with the default setting. Several compound properties were predicted by adsorption, distribution, metabolism, excretion, and toxicity (ADMET) descriptors. This protocol used the quantitative structure–activity relationship models to estimate a range of properties for compounds. The related properties included aqueous solubility, blood–brain barrier penetration, cytochrome P450 2D6 inhibition, hepatotoxicity, human intestinal absorption, and plasma protein binding.

The GABA and benzodiazepine-binding site was identified based on the important residues that were indicated by mutational studies. The docking study was performed using LigandFit. According to Chou *et al.* (1999) the constituents of the binding pocket of a protein receptor to a ligand are defined by those residues that have at least one heavy atom (i.e., an atom other than hydrogen) with a distance Å from a heavy atom of the ligand. Such a criterion was originally used to define the binding pocket of ATP in the Cdk5–Nck5a* complex (Chou *et al.*, 1999) that has later proved quite useful in identifying functional domains and stimulating the relevant truncation experiments (Zhang *et al.*, 2002). A similar approach has also been used to define the binding pockets of other receptor–ligand interactions (Chou, 2004a,b; Chou *et al.*, 2003, 2006; Du *et al.*, 2007; Wang *et al.*, 2007a,c; Wei *et al.*, 2006a). All experimental compounds were docked into the GABA and benzodiazepine-binding site, respectively. The partition