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Modeling the protonation states of β-secretase binding pocket by molecular dynamics simulations and docking studies

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ABSTRACT

β-secretase (BACE1) is an aspartyl protease that processes the β-amyloid peptide in the human brain in patients with Alzheimer's disease. There are two catalytic aspartates (ASP32 and ASP228) in the active domain of BACE1. Although it is believed that the net charge of the Asp dyad is −1, the exact protonation state still remains a matter of debate. We carried out molecular dynamic (MD) simulations for the four protonation states of BACE1 proteins. We applied Glide docking studies to 21 BACE1 inhibitors against the MD extracted conformations. The dynamic results infer that the protein/ligand complex remains stable during the entire simulation course for HD32D228 model. The results show that the hydrogen bonds between the inhibitor and the Asp dyad are maintained in the 10,000th ps snapshot of HD32D228 model. Our results also reveal the significant loop residues in maintaining the active binding conformation in the HD32D228 model. Molecular docking results show that the HD32D228 model provided the best enrichment factor score, suggesting that this model was able to recognize the most active compounds. Our observations provide an evidence for the preference of the anionic state (HD32D228) in BACE1 binding site and are in accord with reported computational data. The protonation state study would provide significant information to assign the correct protonation state for structure-based drug design and docking studies targeting the BACE1 proteins as a tactic to develop potential AD inhibitors.

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1. Introduction

Alzheimer's disease (AD) is a progressive brain disease that is characterized by the deposition of amyloid β-peptide (Aβ) containing plaques and neurofibrillary tangles composed of abnormal *tau* protein in the brain of AD victims [1]. Proteolytic processing of the amyloid precursor protein (APP) results in the formation of Aβ [2]. β-secretase or β-APP cleaving enzyme (BACE1) cleaves APP generating a soluble fragment (β-APPs) and a C-terminal bound peptide (C99). The C99 is further processed by γ-secretase forming 40 and 42 residues Aβ peptide [3]. BACE1 has been shown as the rate-limiting enzyme in the production of Aβ and therefore its inhibition represents a promising therapeutic target for AD treatment [4,5]. In addition, studies have demonstrated that the level and the activity of BACE1 are twofold elevated in AD brain, suggesting that the increase in BACE1 level might participate in AD pathogenesis and that inhibition of the BACE1 may represent an effective approach for drug development targeting AD [6].

BACE1 shows 30% sequence homology to its pepsin family members, harbors two conserved active domains [(Asp93, Thr94, Gly95, Ser96) and (Asp289, Ser290, Gly291, Thr292)] and three pairs of disulfide linkages (216–420, 278–443, 330–380). Data showed that the disulfide bond (330–380) maintains the catalytic activity and protein conformation whereas the other disulfide bonds are significant for the orientation of the catalytic domain [7].

BACE1 activity is observed in many body tissues with highest levels in neural tissues and cell lines [8,9]. Actually, β-secretase prevails in neurons and exhibits low levels in astrocytes [10]. Additionally, BACE1 cleaves only membrane-bound substrates referring that this enzyme is a membrane-bound or associated with a membrane protein [11]. Moreover, the highest BACE1 activity existed at acidic pH, particularly in *trans*-Golgi apparatus and endosomes [12–14]. Indeed, the active site of BACE1 was identified in the acidic intracellular compartments [15]. In the cellular membrane, BACE1 performs as a dimer facilitating the binding and cleavage of protein substrates. Contrarily, the extracellular BACE1 is a soluble monomer [16,17]. Therefore, determining the underlying mechanism of interaction between BACE1 and its protein substrate is beneficial to design inhibitors targeting the dimers' site and thus in turn inhibiting the deposition of Aβ [18].

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BACE1 belongs to the family of aspartyl proteases [6,19,20]. The active site of aspartyl proteases harbors two catalytic aspartic acids with a net charge of −1 [21]. β-secretase contains ASP32 and ASP228 participating in substrate binding and enzymatic reaction. Interestingly, the exact protonation state of Asp dyad represents an ambiguous matter [22]. The precise location of protons on the two aspartic acids cannot be determined by X-ray crystallography, because of the absence of hydrogen coordinates which in turn prevents the proper identification of proton donors and acceptors. The protonation state represents a key factor in the mechanism of aspartyl proteases and plays an important role in the discovery of potent BACE1 inhibitors.

The protonation state of aspartyl protease has been a subject of intensive research, where many experimental and computational studies have shown no consensus on the protonation state and the precise location of protons. Yamazaki et al. suggested the diprotonated (neutral) state (HD32HD228, or 2H model) in the presence of an inhibitor based on the estimates of the pKa of aspartates and glutamates [23]. A monoprotonated state with ASP32 charged and ASP228 protonated (D32HD228, or DHD model) on the outer oxygen was postulated by Coates et al. based on their studies of endothiapepsin complexes using high resolution crystal structures and neutron diffraction [24,25].

Kinetic isotope studies on BACE1 confirmed the monoprotonated form in the presence of the inhibitors [26]. Hyland et al. proposed the monoprotonated state for HIV-1 based on pH rate studies and solvent kinetic isotope effects [27,28]. Piana et al. suggested the monoprotonated and deprotonated forms (D32D228, or 2D model) for HIV-1 with a low barrier hydrogen bond based on their studies on the ^{13}C NMR and *ab initio* molecular dynamic simulations [29,30]. Park et al. suggested the monoprotonated state for BACE1, particularly neutral ASP32 and ionized ASP228 (HD32D228, or HDD model) [31]. Improvement of enrichment factor was observed with the HDD model of BACE1, i.e., neutral ASP32 and ionized ASP228 [32,33].

Rajamani et al. suggested that the DHD model (i.e., monoprotonated ionized ASP32 and protonated ASP228) of BACE1 was preferred in the presence of ligand [34]. They identified the dideprotonated state (2D model) if the ligands were not present, based on the full linear scaling quantum mechanical (QM) calculation [34]. Applying the QM/MM X-ray refinement for BACE1 suggested that the HDD model (neutral ASP32 and ionized ASP228) as the favored state [35].

Liu et al. investigated the entire process of BACE1 enzymatic hydrolysis using quantum chemistry calculations and showed that at the beginning of the catalytic reaction, DHD model (ionized ASP32 and neutral ASP228) was preferred and identified the reverse model HDD (neutral ASP32 and ionized ASP228) at the transition and product generation states of BACE1 [36]. Dominguez et al. found that that inhibitors with hydroxyethylene isostere scaffold preferred the diprotonated state (2H model) at low pH (4.5) while the probability of monoprotonated state increased at higher pH (7.4). This study also identified functional group dependency in that an amino group induced a HDD model (neutral ASP32 and ionized ASP228) at both high and low pH values [37]. Ellis and Shen identified three protonation models based on their observations on the constant-pH molecular dynamics (CpHMD) method: (HD32 HD228) at pH $\leq$ 2, (HD32 D228) at pH range of 2.5–4.5, and (D32D228) model at pH value $\geq$5 [38].

Therefore, the aforementioned studies suggest that controversy exist regarding which protonation states should be assigned to ASP32 and ASP228. Given the important role of ligand binding for these two residues, it is crucial to determine the exact protonation state of ASP32 and ASP228 for drug design guidance targeting BACE1. In order to assign the protonation states of ASP32 and ASP228 in BACE1, we employed molecular dynamic (MD) simulations, and docking studies against the structures extracted from the last snapshot of the MD trajectories.

There are two models for the BACE1: open and closed. The open or closed conformation is defined by a β-hairpin conformation consisted of residues 68–74 of BACE1. This β-hairpin conformation is also called the flap, capping the active site and thus controlling the ligand access to BACE1 binding domain (Fig. 1) [39]. The open conformation displays an open flap conformation; a conformation that considers the lack of stabilizing interactions with the inhibitors. Fig. 1S (Supporting information) showed that in the closed conformation (2FDP) the ligand not only interacts with binding pocket residues ASP32, ASP228 and Thr231, but also forms hydrogen bond interactions with loop residues Gln73, Tyr71, and Thr72. These hydrogen bond interactions with the loop was absent in the open conformation (2OHP, Fig. 1S). Upon ligand binding, BACE1 adopted a closed conformation which stabilizes the interaction. Since the closed conformation is the preferred conformation for ligand binding, in our study, we only selected the closed conformation (PDB id: 2FDP) as the model system for our study. We prepared four different models based on different protonation states on residues ASP32 and ASP228: (i) the dideprotonated model (D32D228, 2D model), (ii) the diprotonated model (HD32HD228, 2H model), (iii) the deprotonated D32, protonated D228 (D32HD228, DHD model), and (iv) the protonated D32, deprotonated D228 (HD32D228, HDD model).

Our results show the preference of the HD32D228 (HDD) model as this protonation state identified the most potent ligands. Additionally, MD simulations provide another clue for this preference and confirm that stable interactions occur between ASP228 and ligand positively charged amino group.

2. Computational methods

2.1. Preparation of protein structures

Our study started with comparing the crystal structures of 110 BACE1 proteins. All 110 BACE1 structures were structurally aligned using the DaliLite program and classified as open and closed conformations based on the β-hairpin conformation consisted of residues 68–74 of BACE1. We used 2OHP [40] and 2VA6 [41] as representatives for the open conformation whereas 2FDP [42] and 1YM4 [43] for the closed conformation for comparison. Superposition of 110 BACE1 proteins to 2FDP led to the classification of the open and closed conformation of BACE1. The model system for the closed conformation was further used for MD simulations. The X-ray crystal structure of 2FDP was retrieved from the RCSB Protein Data Bank. Missing residues of 2FDP (Gly158-Ala168 and Asp311-Ala313) were fixed by structural alignment to a fully resolved β-secretase (PDB id: 2G94) [44] using the DaliLite program [45] and the structures of the missing residues were adopted from 2G94 after structural alignment. Each inserted section and its surrounding 4.5 Å region were energetically minimized to reduce the steric clash. The overall protein structures were subsequently prepared using the Protein Preparation Wizard in the Schrödinger software suite [46] to maximize H-bond interactions to allow structural flips of Gln, Asn, and His side chains. The resulting four proteins were subjected to 500 iterations of energy minimization while restraining the backbone using the OPLS force field in the MacroModel module in the Schrödinger software suite. Four different protonation states were prepared: the dideprotonated model (D32D228, 2D), the diprotonated model (HD32HD228, 2H); the deprotonated D32, protonated D228 (D32HD228, DHD); and the protonated D32, deprotonated D228 (HD32D228, HDD).

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