Journal of Molecular Graphics and Modelling 40 (2013) 1–9

Contents lists available at SciVerse ScienceDirect

Journal of Molecular Graphics and Modelling

journal homepage: www.elsevier.com/locate/JMGM

# Elucidating the catalytic mechanism of β-secretase (BACE1): A quantum mechanics/molecular mechanics (QM/MM) approach

Arghya Barman, Rajeev Prabhakar*

*Department of Chemistry, University of Miami, 1301 Memorial Drive, Coral Gables, FL 33146, United States*

ARTICLE INFO

*Article history:*
Accepted 17 December 2012
Available online 5 January 2013

*Keywords:*
Alzheimer's disease
Aspartyl proteases
Beta-secretase
Protein hydrolysis
Acid-base mechanism

ABSTRACT

In this quantum mechanics/molecular mechanics (QM/MM) study, the mechanisms of the hydrolytic cleavage of the Met2-Asp3 and Leu2-Asp3 peptide bonds of the amyloid precursor protein (WT-substrate) and its Swedish mutant (SW) respectively catalyzed by β-secretase (BACE1) have been investigated by explicitly including the electrostatic and steric effects of the protein environment in the calculations. BACE1 catalyzes the rate-determining step in the generation of Alzheimer amyloid beta peptides and is widely acknowledged as a promising therapeutic target. The general acid-base mechanism followed by the enzyme proceeds through the following two steps: (1) formation of the gem-diol intermediate and (2) cleavage of the peptide bond. The formation of the gem-diol intermediate occurs with the barriers of 19.6 and 16.1 kcal/mol for the WT- and SW-substrate respectively. The QM/MM energetics predict that with the barriers of 21.9 and 17.2 kcal/mol for the WT- and SW-substrate respectively the cleavage of the peptide bond occurs in the rate-determining step. The computed barriers are in excellent agreement with the measured barrier of ~18.0 kcal/mol for the SW-substrate and in line with the experimental observation that the cleavage of this substrate is sixty times more efficient than the WT-substrate.

© 2013 Elsevier Inc. All rights reserved.

## 1. Introduction

Beta-secretase (BACE1) constitutes a family of aspartyl protease enzymes that is present in vertebrates, fungi, plants and retroviruses [1–4]. These enzymes are divided into the following two classes: (I) pepsin-like (renin, cathepsin D, chymosin, beta secretase, etc.) and (II) retroviral (HIV protease and malaria protease) [5,6]. BACE1 catalyzes the hydrolytic cleavage of a large number (~80) of proteins that are involved in several critical biological reactions [7,8]. One of the most notable functions of this enzyme is the catalysis of the rate-limiting step of Alzheimer amyloid beta (Aβ) peptide generation through the cleavage of the Met671-Asp672 amide bond of amyloid precursor protein (APP) (isoform 770, identifier −P05067) [9,10]. The Aβ peptides are the major components of plaques that are deposited in the brains of patients suffering from Alzheimer's disease (AD) [10–12]. A wealth of experimental data suggests that the inhibition of this enzyme is a very promising therapeutic target for the treatment of AD [9,10,13,14]. BACE1 knockout transgenic mice have been reported to be incapable of producing Aβ from either endogenous APP [15] or mutant APP transgene [16]. These animals were found to be normal in all other measures examined; giving encouragement that BACE1 inhibitors might prevent Aβ production without major side effects.

A large number of X-ray structures (ca. 170) of apo and inhibitor bound forms of this enzyme have been resolved [17–20]. These structures revealed that BACE1 consists of two main domains, namely the N-terminal domain and the C-terminal domain, and several specific sub-regions are distributed between them. The most critical regions of the enzyme known as 10s-loop (Lys9-Tyr14), flap (Val67-Glu77), insert-A (Gly158-Leu167), insert-D (Trp270-Thr274) and insert-F (Asp311-Asp317) facilitate the entry and binding of a substrate at the active site through their movements (Fig. 1) [17,21]. The active site of BACE1 contains a catalytic Asp dyad formed by two aspartate residues (Asp32 and Asp228) [17]. This dyad has been implicated in the catalytic functioning of the entire family of aspartyl proteases including pepsin, renin, cathepsin D and HIV protease [22–29]. The mutation of either one of these aspartate residues destroys the activity of BACE1 [30]. A number of theoretical calculations and recent X-ray and neutron diffraction data show that one of the Asp residues remains protonated and the second one unprotonated during the catalytic cycle [31–33]. BACE1 has also been reported to cleave a double mutant (Lys670 (P2) → Asn and Met671 (P1) → Leu) of APP known as the Swedish mutant (SW) sixty time more efficiently than the wild-type (WT)-substrate (P1, P2,. . .,consecutively denote the amino acid residues that flank the N-terminal of the substrate) (Fig. 2a) [34].

* Corresponding author. Tel.: +1 305 284 9372; fax: +1 305 284 4571.
*E-mail address:* rpr@miami.edu (R. Prabhakar).

1093-3263/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jmgm.2012.12.010

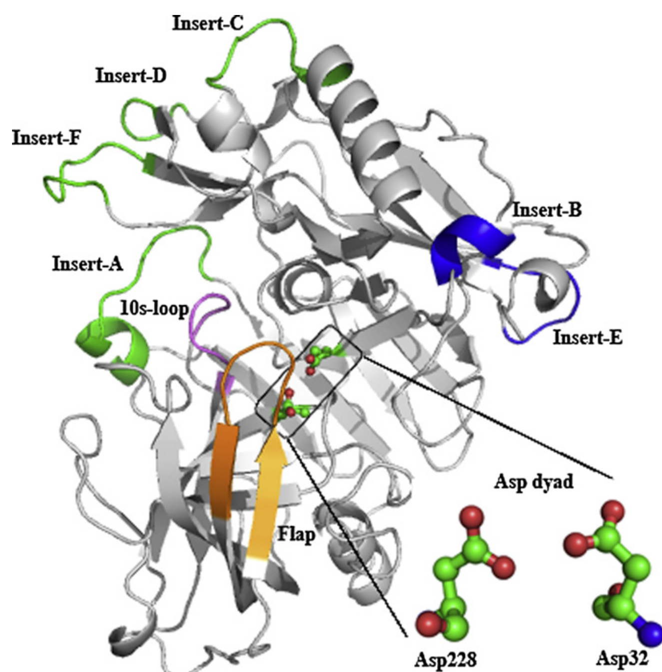


**Fig. 1.** Key regions of BACE1 from the X-ray structure (PDB ID: 1FKN).

BACE1 utilizes the general acid-base mechanism (discussed below) to cleave the peptide bond during its catalytic cycle [22]. This mechanism has previously been theoretically investigated for the other aspartyl proteases such as HIV protease [23–27] and presenilin (PS1) [28]. In addition, it has been studied by applying *ab initio* methods on simple models [35–37]. The most plausible mechanism of peptide hydrolysis has been proposed to proceed through the following two steps (Fig. 2b) [26,38]. In the first step, the unprotonated Asp228 acts as a base and the protonated Asp32 acts as an acid. From reactant (I), the Asp228 residue abstracts a proton from the catalytic water molecule (W1) and generates a hydroxyl ion ($OH^-$). The generated hydroxyl ion subsequently makes a nucleophilic attack on the carbonyl carbon of the scissile peptide bond and concomitantly the Asp32 donates its proton to the carbonyl oxygen of the peptide bond to produce the tetrahedral gem-diol intermediate (II). From (II), the two aspartates reverse their functions and Asp32 now acts as a base and Asp228 acts as an acid. The protonated Asp228 donates its proton to the amide nitrogen (—NH) of the scissile peptide bond and in a synchronous manner Asp32 abstracts a proton from one of the hydroxyl groups (—OH) of the gem-diol intermediate. This process leads to the cleavage of the peptide bond that generates the amino ($—NH_2$) and carboxy (—COOH) terminals (III). Experimentally measured kinetic data ($k_{cat} = 2.45\ s^{-1}$) for the SW-substrate shows that the reaction proceeds through a barrier of ca. 18.0 kcal/mol [34]. In a QM/MM study, Carloni et al. also showed that the formation of the gem-diol species (first step) for the Leu-Ala substrate peptide occurs through a barrier of ca. 20.0 kcal/mol [25]. In this study, only the first step of the mechanism was investigated and the barrier was estimated by keeping the distance between the oxygen atom of the catalytic water and the carbon atom of the peptide bond of the substrate fixed at increasingly shorter distances. In a previous QM only (DFT) study using a pruned model of the active site, with the barrier of 22.4 and 19.1 kcal/mol for the WT- and SW-substrate respectively, this step was proposed to be the rate-determining step for both substrates [29]. However, a Car-Parrinello MD simulation study on another member of the aspartyl protease family, HIV protease, suggested that the peptide bond cleavage in the second step (barrier = 21.0 kcal/mol) occurs in the rate-determining step [26]. Here, the barriers for both steps were estimated by constraining the reaction coordinates. The main conclusion of this study was also supported by a recent crystallographic study on HIV protease [39].

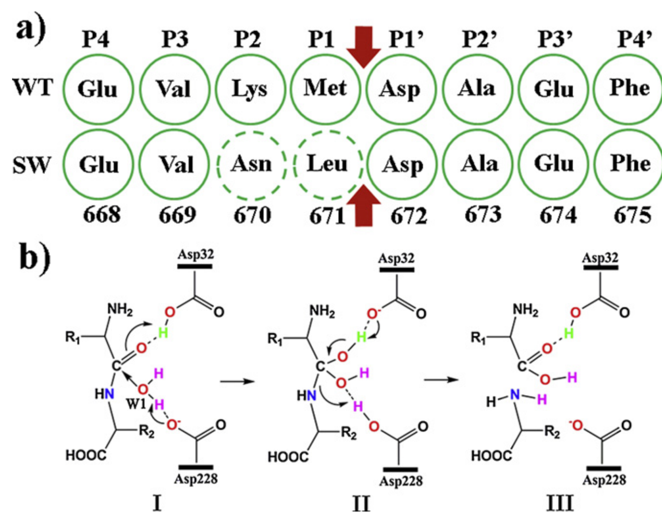


**Fig. 2.** (a) Amino acid sequences of the WT- and SW substrates. The mutated residues in the SW substrate are marked as dotted circles and the arrow signs show the cleavage site of the substrates. (b) General acid/base mechanism utilized by BACE1.

In almost all the previous studies, either the pruned models of the active sites have been utilized or key reaction coordinates were constrained to investigate catalytic mechanisms. In calculations using these models the electrostatic and steric effects of the protein environment surrounding the active site were either completely ignored or only the latter was partially included by constraining some atoms from the X-ray structures. Therefore, these studies cannot incorporate the influence of these effects on the computed energetics and elucidate the reorganization of the key regions (the 10s-loop, flap, insert-A, insert-D and insert-F) of the enzyme in each step of the reaction.

In order to address these outstanding issues, we have employed a hybrid two-layer quantum mechanics/molecular mechanics (QM/MM) method, ONIOM (B3LYP/Amber), to investigate the mechanisms of the hydrolysis of WT- and SW-substrate by including the entire enzyme in the models. The fully optimized structures derived from these calculations will provide the quantitative contribution of the electrostatic and steric effects of the protein on energetics and the structural changes in the key regions of the enzymes during the mechanism. The structural and mechanistic information revealed by these calculations will also help to understand the cleavage mechanisms of different biological substrates (~80) of this critical enzyme and other members of the aspartyl protease family.

## 2. Computational models

The starting structures of the enzyme-substrate (BACE1-WT and BACE-SW) complexes are obtained from our previous MD simulations [29]. The models used in this study include all 393 amino acid residues of the enzyme and octa-peptide substrate. The BACE-WT and BACE-SW complexes contain 6101 and 6095 atoms respectively in total and the whole enzyme-substrate complex is called the “real” system. The “model (QM)” part of the “real” system includes six residues (Asp32, Ser35, Tyr71, Asp228, Thr231 and Arg235) from the enzyme and four from the substrate (Lys1, Met2, Asp3 and Ala4 of the WT-substrate and Asn1, Leu2, Asp3 and Ala4 residues of the SW-substrate) and two water molecules (W1 and

Download English Version:

https://daneshyari.com/en/article/443394

Download Persian Version:

https://daneshyari.com/article/443394

Daneshyari.com