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Why does β-secretase zymogen possess catalytic activity? Molecular modeling and molecular dynamics simulation studies

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Abstract

β-secretase is a potential target for inhibitory drugs against Alzheimer's disease as it cleaves amyloid precursor protein (APP) to form insoluble amyloid plaques and vascular deposits in the brain. β-secretase is matured from its precursor protein, called β-secretase zymogen, which, different from most of other zymogens, is also partially active in cleaving APP. Hence, it is important to study on the mechanism of the zymogen's activation process. This study was to model the 3-D structure of the zymogen, followed by intensive molecular dynamics (MD) simulations to identify the most probable 3-D model and to study the dynamic structural behavior of the zymogen for understanding the effects of pro-segment on the function of the enzyme. The results revealed that the dropping in catalytic activity of the β-secretase zymogen could be attributed to the occupation of the entrance of the catalytic site of the zymogen by its pro-segment. On the other hand, the partial catalytic activity of the zymogen could be explained by high fluctuation of the pro-segment in comparison with that of other zymogens, resulting in the occasionally exposure of the catalytic site for access its substrate APP. Indeed, steered MD (SMD) simulation revealed a weak pulling force at quasi-equilibrium state for the pro-segment of the zymogen leaving from the entrance, indicating that this swinging process could take place spontaneously. Furthermore, MM-PBSA calculation revealed a small change of free energy of 10.56 kal/mol between the initial and final states of the process of pro-segment swung outside the binding pocket of β-secretase zymogen. These results not only account for the partial catalytic activity of β-secretase zymogen, but also provide useful clues for discovering new potent ligands, as new type of drug leads for curing Alzheimer's disease, to prevent the pro-segment of the zymogen from leaving its catalytic site.

Keywords: Homolgy modeling; Steered MD; MM-PBSA; Free energy; Drug design

1. Introduction

Alzheimer's disease (AD) is the major cause of dementia in many developed countries. AD is characterized by the progressive formation of insoluble amyloid plaques and vascular deposits consisting of the 4 KD amyloid β-peptide (Aβ) (Selkoe, 2000) in the brain. Aβ occurs in two predominant forms with different C-termini, $A\beta_{40}$ and $A\beta_{42}$. Overproduction of $A\beta_{42}$ has been suggested to be the cause of familial early-onset Alzheimer's disease. Researchers have revealed that $A\beta_{42}$ is one of the proteolytic products of amyloid precursor protein (APP) (Lahiri et al., 2002; Selkoe, 1999). To initiate Aβ formation, β-secretase cleaves APP at the N-terminus to release APPsβ, a ∼100 KD soluble N-terminal fragment containing 22X11 epitope, and CTF99, a ∼12 KD C-terminal fragment which remains membrane bound (Phimister, 2000) containing 6E10 and C8 epitopes (Fig. 1). Then, γ-secretase goes on to cleave CTF99 and release Aβ and fragment P6 containing C8 epitope. In an alternate pathway of the hydrolization of APP, α-secretase cleaves it within the Aβ sequence, thus precluding the formation of Aβ (Fig. 1). Cleavage by α-secretase produces a large soluble N-terminal fragment containing 22X11 and 6E10 epitopes, APPsα, and a ∼10 KD membrane-bound C-terminus, CTF83 (Parvathy et al., 1999). CTF83 is further cleaved by γ-secretase to produce fragments P3 and P6, respectively. Accordingly, the inhibition of β-secretase may lead to cessation of Aβ formation, hence, making β-secretase a potential therapeutic target for Alzheimer's

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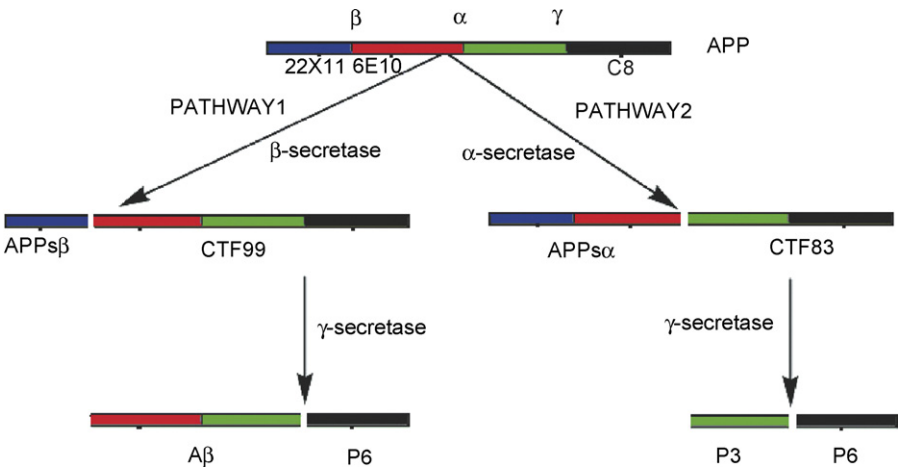

Fig. 1. A schematic illustration of two pathways for the cleavage of APP with proteolytic processing sites and cleavage products of APP. α, β and γ are α-, β-, and γ-secretases, respectively. 22C11, 6E10, and C8 are epitopes.

disease. Indeed, some inhibitors have been developed based on the X-ray crystallographic structure of β-secretase (Ghosh et al., 2001, 2002; Hom et al., 2003, 2004; Lefranc-Jullien et al., 2005; Stachel et al., 2004; Tung et al., 2002; Turner et al., 2001).

On the other hand, the β-secretase itself is produced from its proprotein (pro-BACE or zymogen) by zymogen processing (Benjannet et al., 2001; Hussain et al., 1999). Some experiments have observed that the zymogen is partially active in cleaving APP. Ermolieff et al. discovered that the zymogen is catalytically active only below pH 5.5 and is weaker by three times in the activity than that of β-secretase (Ermolieff et al., 2000). Shi et al. showed the K_{cat}/K_m of the zymogen is about 2.3-fold less than that of β-secretase (Shi et al., 2001). Therefore, notwithstanding the complete inhibition of the β-secretase, Aβ would be still produced if the zymogen is working. So the zymogen must be taken into account in developing drugs against Alzheimer's disease based on the inhibition of Aβ formation. In other words, inhibiting zymogen could reduce not only the amount of Aβ, but also the amount of β-secretase itself. In order to better understand the role and function of the zymogen, the information on its three-dimensional (3-D) structure is essential. But no experimental 3-D structure of zymogen is available to date. In sequence, the zymogen has 24 residues more than mature β-secretase at N-terminal, called pro-segment. In 2002, Chou and Howe modeled a tertiary structure of β-secretase zymogen (Chou and Howe, 2002). A residues (proline) of pro-segment was found to be right above the binding pocket, but do not interact directly at all with the most important catalytic residues of aspartic acids in active site. It was suggested that the indirect interaction is the cause that the zymogen retains, to some extent, catalytic activity. Certainly, the model provides some information about the geometry of the zymogen, but no dynamic feature is available based on the static model.

In this study, we constructed and validated a 3-D model of β-secretase zymogen, followed by conventional molecular dynamics (MD) and steered molecular dynamics (SMD) simulations, to study the dynamic features of the zymogen. SMD simulation, which is capable of mimicking the principle of atomic force microscopy (AFM) (Binning et al., 1986; Evans et al., 1995; Svoboda and Block, 1994) has been widely used to explore the binding and unbinding properties of biomolecules and their responses to external mechanical manipulations at the atomic level. Thus, the accelerated dissociation process of the pro-segment by using SMD could reveal information about the flexibility of the zymogen structure and its response to the dissociation of pro-segment. To further assess the feasibility of the spontaneous detachment process of the pro-segment from the binding site, molecular mechanics-Poisson–Boltzmann surface area (MM-PBSA)(Chong et al., 1999; Massova and Kollman, 1999; Srinivasan et al., 1998) calculation was performed to estimate the free energy change between the initial and final conformations of the opening process identified through the SMD simulation using AMBER9.0 program (Case et al., 2006). The aim of this study is to study the activation mechanism of the zymogen via MD simulations, SMD simulations, and MM-PBSA calculations, for providing useful information to develop new mechanism-based drugs against Alzheimer's disease.

2. Materials and methods

2.1. Modeling the structure of β-secretase zymogen

The sequence of β-secretase zymogen was retrieved from the Swiss-Prot database (accession number: P56817). Templates for homology modeling are identified as 1HTR and 1FKN in PDB through NCBI-BLAST server (http://www.ncbi.nlm.nih.gov/BLAST/) (Ghosh et al., 2002; Moore et al., 1995). 1FKN is the crystal structure of mature β-secretase. 1HTR is the crystal structure of progastricsin that is a pro-enzyme in the family of aspartyl protease including β-secretase. The identity in whole sequence between β-secretase zymogen and progastricsin is about 24%, and is 23% between their pro-segments (refer to Supporting Information, Fig. S1 for details). Although the sequence identity is low than 30%, the 3-D structure of progastricsin is very similar to that of β-secretase, including β sheet and α helices. The shape of both binding pocket and the position of the most important residues (aspartic acid) have high consistency (refer to Supporting Information,