Crystal Structure of an Active Form of BACE1, an Enzyme Responsible for Amyloid β Protein Production

Alzheimer's disease (AD) is the most common neurodegenerative disorder and characterized by the extracellular deposition of insoluble amyloid plaques. The major component of amyloid plaques is the amyloid β-peptide (Aβ), which is derived from the amyloid precursor protein (APP) by sequential cleavage at the N-termini and C-termini of the Aβ domain by BACE1 and γ-secretase (Fig. 1(a)). Therefore, BACE1 is considered to be critical in the pathogenesis of Alzheimer's disease and one of the most promising pharmaceutical targets for treating AD.

During cellular trafficking, BACE1 is reinternalized from the cell surface to early endosomes and can recycle back to the cell surface via the trans-Golgi network (Fig. 1(b)). At the plasma membrane, both BACE1 and APP are located in lipid rafts on the cell surface where, at neutral pH (~pH 7), BACE1 is expected to show poor activity. BACE1 and APP follow similar trafficking routes and meet within endosomes, where BACE1 is activated due to an acidic pH environment (pH 4.5-6). When BACE1 is recycled to the cell surface, it becomes inactive again. Considering the long half-life of BACE1 and the recycling rate, BACE1 moves between the cell surface and the endosomal system many times throughout the course of its lifespan, showing an activation/inactivation transition during cellular trafficking [1]. The conformational transition of active and inactive states in BACE1 is interconvertible under physiological conditions and plays an important role in the localization-dependent activity of BACE1. However, never before has a structural view of active BACE1 been available. Here, we show the crystal structures of the catalytically active and inactive forms of BACE1 using beamlines **BL26B1**, **BL26B2** and **BL44B2** [2].

The active BACE1 has novel structural features involving the conformation of a flexible antiparallel β-hairpin, called a flap, and subsites that promote APP binding. The flap, which is believed to control substrate access to the active site and set the substrate into the correct geometry for the catalytic process, has adopted a more open conformation (Fig. 2(a)). In addition, the subsites show a new conformation, resulting in the formation of new spaces for APP binding (Fig. 2(b)). Inhibitor binding experiment suggests that these conformational

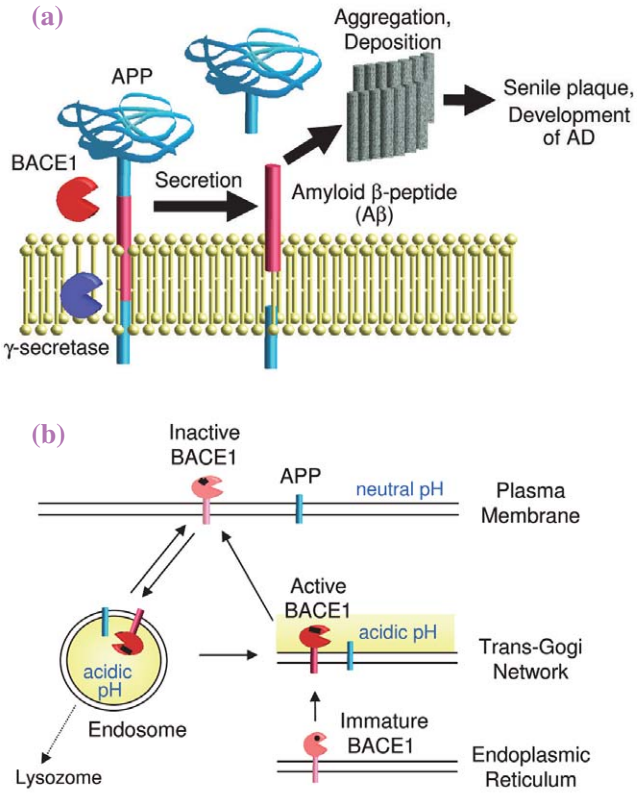

Fig. 1. **(a)** Schematic depicting generation of Aβ from APP. γ-secretase is another enzyme involved in the process. **(b)** Subcellular trafficking of BACE1.

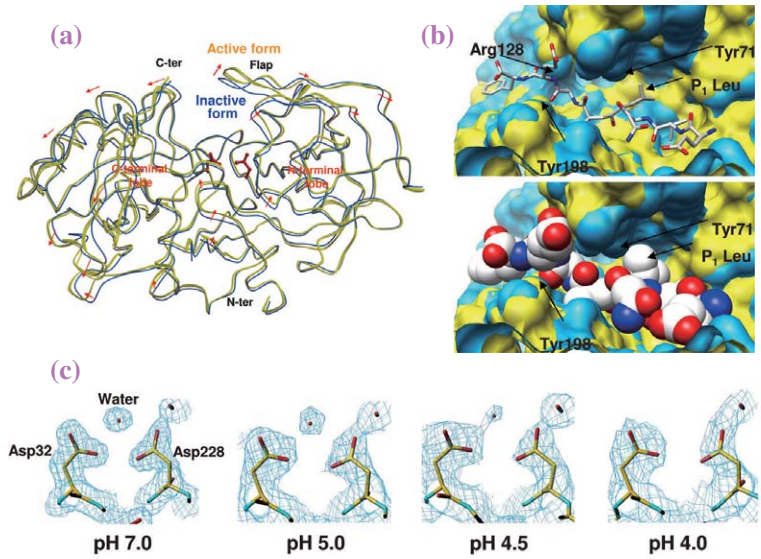

Fig. 2. Shape changes associated with activation of BACE1. **(a)** Overall structure of inactive BACE1 (dark blue) and active BACE1 (yellow). The displacement from inactive to active BACE1 is indicated by red arrows. **(b)** Composite surface representation in the APP binding site cleft with superimposed inhibitor OM99-2. Active BACE1 (yellow), inactive BACE1 (blue) and bound OM99-2 (white). **(c)** Electron densities of water molecule in the vicinity of Asp32 and Asp228.

changes are important in allowing the APP to bind. Thus, the active BACE1 could accommodate the substrate, leading to activation, whereas inactive BACE1 has a self-inhibition mode accounting for less APP binding and the loss of enzymatic activity.

Furthermore, we found that the active site water molecule –considered to be important in the chemical reaction through which BACE1 cleaves APP– became weaker as the pH was lowered, leading to the lack of activity at pH less than 4.0 (Fig. 2(c)). On the basis of these results, we propose that BACE1 has a dual regulatory mechanism of enzymatic activity at different pH values (Fig. 3).

As a template for novel inhibitor designs, the most suitable structure of BACE1 should be selected depending on the condition of the cellular compartment where the inhibitor reaches and binds to BACE1 [3]. Accordingly, information on the structure of active BACE1 would be of value in designing further selective drugs.

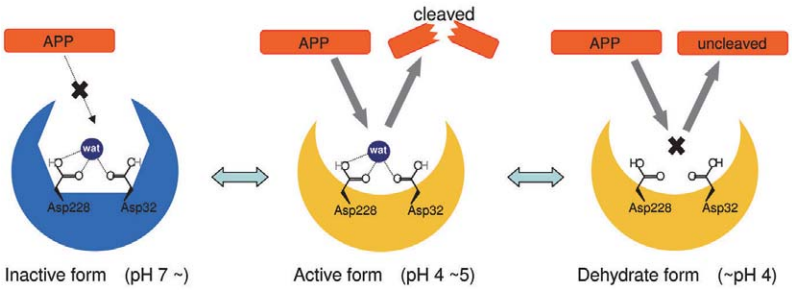

Fig. 3. Schematic representation of the regulatory mechanism of enzymatic activity in BACE1.

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