

## Structure of a presenilin family intramembrane aspartate protease

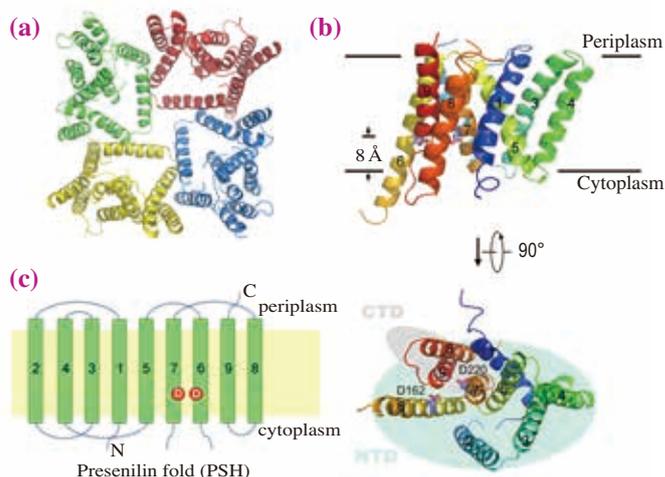
Alzheimer's disease (AD), a fatal neurodegenerative disease, mostly occurs in elderly people over 65 years of age. However, a rare early-onset AD is diagnosed earlier. With the aging of the population, the incidence of AD is increasing yearly. In 2006, there were approximately twenty-six million AD patients worldwide, and it is estimated that 1 in 85 people will be suffering from this disease by 2050. One of the clinical manifestations of AD is an increasing severity of cognitive impairment, which seriously impacts patients and their families. AD is one of the most expensive diseases in the society of developed countries. However, there is still no effective treatment for this disease.

Recent studies showed that AD is due to the aberrant accumulation of a 42-residue amyloid- $\beta$  peptide (A $\beta$ 42) over a 40-residue product (A $\beta$ 40) from an altered cleavage of the amyloid precursor protein (APP), which is caused by mutated presenilin, the catalytic component of  $\gamma$ -secretase. In addition to presenilin, three other components, PEN-2, APH-1 and nicastrin, are also essential for the activity of  $\gamma$ -secretase. Research on the atomic structures of presenilin and  $\gamma$ -secretase will help us to understand the mechanism behind Alzheimer's disease and provide some hints on therapeutic intervention. However, up to now, there has been very limited structural information on presenilin or  $\gamma$ -secretase. This has obviously hampered our understanding of the mechanism of presenilin and  $\gamma$ -secretase, and their relevance to AD.

For protein engineering and the crystallization of PSH, we cloned human presenilin 1 (hPS1) and its eukaryotic homologs from other organisms, such as zebrafish and arabidopsis. For a long time, we have struggled with the low expression level of these targets. We then sought their archaeal homologues. Of 13

archaeal presenilin homologues, only one, from the archaeon *Methanoculleus marisnigri* JR1 (PSH, also known as MCMJR1), could be expressed well and purified successfully. However, its behavior in solution is too poor for crystallization, even after all nonionic detergents are tested. In order to improve the behavior of PSH in solution, we set up a protein engineering system based on the sequence alignment between PSH and other presenilin homologues from archaeal species. First, we replaced the residue of PSH with a residue that is highly conserved between other archaeal homologues at the corresponding positions through sequence alignment. Then, we selected mutations that not only affect its activity but also improve its behavior in solution evaluated by gel filtration, and combined them in the same PSH variant for another cycle selection and possible crystallization trials. Finally, we used V8 protease to delete the flexible surface region for easy crystallization. The PSH used for the final crystallization contained five mutants, D40N, E42S, A147E, V148P, and A229V, and the loop between residues 182 and 209 was also removed. We adopted more than 160,000 crystallization conditions for generating suitable crystals for X-ray data collection.

The high-resolution native data was collected at Shanghai Synchrotron Radiation Facility (SSRF) beamline BL17U. All other diffraction data were collected at SPRING-8 beamline **BL41XU**. In order to solve the phase problem, we soaked crystals in a mother liquor containing 2 mM K<sub>2</sub>PtCl<sub>4</sub>. The platinum-derived crystal was sensitive to radiation damage even at 100 K. We fine-tuned the size of the collimated X-ray beam to maximize the number of exposures on a single crystal to avoid this problem and collected a complete high-redundancy, three-wavelength MAD data set. After more than 100 crystal trials, we finally collected the



**Fig. 1.** Overall structure of PSH. (a) PSH protein forms a tetrameric complex in C222 crystals. (b) Overall structure of PSH. Nine TMs are divided into NTD (TM1-6) and CTD (TM7-9). Putative catalytic residues Asp 162 and Asp 220 are located on TM6 and TM7, respectively. The structure is rainbow colored, with the N terminus in blue and the C terminus in red. The top and bottom panels represent two perpendicular views. Figures (a) and (b) were prepared using PyMol. (c) Membrane topology diagram of PSH. The two putative catalytic residues, Asp 162 and Asp 220, are indicated.

three anomalous data sets from the same crystal with different wavelengths at the peak, inflection, and high-energy remote around the absorption edge of platinum. The PSH variant was crystallized in three space groups, *C222*, *C222<sub>1</sub>*, and *P2*. The structure was determined by platinum-based multiwavelength anomalous dispersion (MAD) and refined at 3.3 Å resolution in the *C222* space group with the help of selenium anomalous peak data [1,2].

The structure of PSH protein forms a tetrameric complex in the *C222* crystals (Fig. 1(a)). There are nine transmembrane  $\alpha$ -helices in each PSH molecule, which is consistent with previous studies. The amino terminal domain (NTD), comprising TM1–6, forms a horseshoe-shaped structure that partially surrounds TM7–9 of the carboxy terminal domain (CTD). The catalytic residues Asp 162 and Asp 220 in PSH are located at TM6 and TM7, respectively, and approximately 8 Å below the lipid membrane surface from the cytoplasmic side (Fig. 1(b)).

The cleavage of a target peptide within the lipid bilayer by intramembrane protease such as PSH needs the participation of water. Through structural analysis, we find that there is a cavity that links the cytoplasmic side and the two active site aspartates. Water molecules can access catalytic sites without any restriction via this cavity. This finding is also supported by a previous biochemical observation and by the electron microscopic structure study of the  $\gamma$ -secretase complex. The distance between two catalytic aspartates is about 6.7 Å, suggesting the conformation we captured in the PSH crystal structure probably presents a non-active state. After substrate binding, PSH will be active through conformation

change. This induced-fit mechanism might protect PSH from non-specific cleavage. Through the analysis of the TM organization of PSH, we find that there are two potential pathways for substrate entry: through the open space between TM6 and TM9 or that between TM2 and TM6. However, there is steric hindrance in the second possibility and the first possibility is supported by previous biochemical evidence.

The topology of PSH is quite different from those of all other membrane proteins of known structures, even rhomboid and S2P one, which are the other two families of intramembrane protease. No entry in the protein data bank is similar to the overall nine transmembranes of PSH, as searched using the DALI program. This suggests that PSH shows a previously unreported protein fold, which we call the presenilin fold (Fig. 1(c)).

The primary sequences of PSH and human presenilin PS1 share more than 50% similarity, especially between the signature motifs for catalysis (Fig. 2). Therefore, the structure features we observed in PSH should be applied to human PS1. Owing to the low expression level and other difficulties in the crystallization process, there is still no atomic structure information on human PS1 or other eukaryotic presenilin homologues. The structure of PSH, an archaeal presenilin homolog, could help us to understand the mechanism of human presenilin and  $\gamma$ -secretase. Furthermore, it will lead to the elucidation of why some mutations on presenilin can cause Alzheimer's disease, enabling us to design small molecules for treating this disease safely and effectively. Future structural studies on other conformations of PSH, especially the activity state, and human presenilin and whole  $\gamma$ -secretase complex will be highly illuminating for this field.

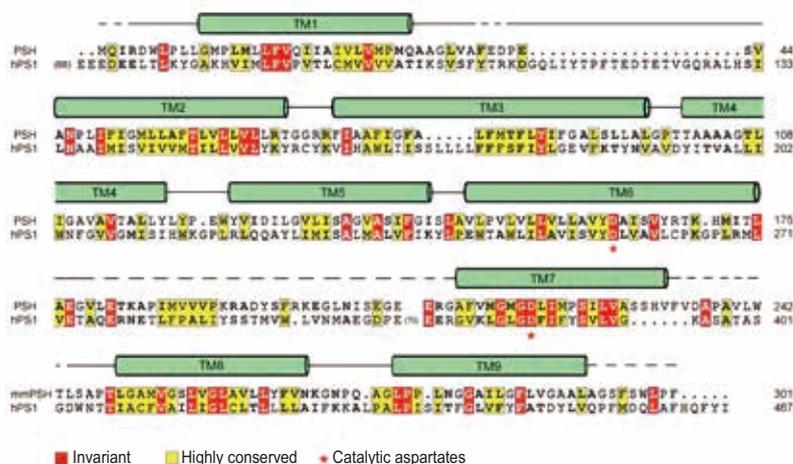


Fig. 2. Sequence alignment of PSH with human presenilin 1 (hPS1). Secondary structural elements of PSH are indicated above the sequences. Invariant and highly conserved amino acids are highlighted in red and yellow, respectively. The two putative catalytic aspartate residues are indicated by red stars. The aligned sequences are PSH (*Methanococcus marisnigri*, GI: YP\_001047832); and hPS1 (*Homo sapiens*, GI: NP\_000012). Sequence alignment was carried out using ClustalW.

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## References

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