C Terminus of Presenilin Is Required for Overproduction of Amyloidogenic Aβ42 through Stabilization and Endoproteolysis of Presenilin

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Mutations in presenilin (PS) genes cause early onset familial Alzheimer’s disease (FAD) by increasing production of the amyloidogenic form of amyloid β peptides (Aβ), which are proteolytically produced from β-amyloid precursor proteins (βAPP) through two sequential cleavages by as yet unidentified proteases termed the β- and γ-secretases (Selkoe, 1991, 1994). Two major forms of Aβ have distinct C termini ending at the 40th and 42nd residues (Aβ40 and Aβ42, respectively), which are differentially cleaved by γ-secretase(s) (Suzuki et al., 1994). Aβ42 aggregates much faster than Aβ40 in vitro (Jarrett and Lansbury, 1993), and Aβ42 is the initially and predominantly deposited Aβ species in the brains of patients with AD and Down’s syndrome (Iwatsubo et al., 1994, 1995). Moreover, missense mutations in βAPP genes, a rare cause of familial AD (FAD), lead to increased production of Aβ42, strongly implicating Aβ42 in the pathogenesis of AD (Suzuki et al., 1994).

Presenilin (PS) 1 (Sherrington et al., 1995) and PS2 (Levy-Lahad et al., 1995) genes were identified as the major causative genes for early onset FAD that encode homologous polytopic membrane proteins spanning the membrane eight times (Doan et al., 1996; Li and Greenwald, 1998). Although a major proportion of nascent PS is rapidly degraded (Kim et al., 1997), a small fraction of PS is stabilized and undergoes endoproteolysis, resulting in a heterodimeric complex of N- and C-terminal derivatives (NTF and CTF, respectively) (Thinakaran et al., 1996; Capell et al., 1998) with an unusually long half-life (Thinakaran et al., 1996, 1997; Ratovitski et al., 1997). Overexpression of exogenous PS results in the replacement of endogenous PS fragments, suggesting that stabilization of PS is a saturable process competing for a limiting cellular factor (Thinakaran et al., 1996, 1997).

The finding that ablation of PS1 in mice dramatically decreased γ-cleavage of βAPP indicated that PS1 physiologically serves as a coactivator of γ-secretase (De Strooper et al., 1998). Moreover, data from studies in Caenorhabditis elegans (Leviton and Greenwald, 1995; Baumeister et al., 1997), PS1 knock-out mice (Shen et al., 1997; Wong et al., 1997; De Strooper et al., 1999), and Drosophila melanogaster (Song et al., 1999; Struhl and Greenwald, 1999; Ye et al., 1999) suggest that PS1 facilitates Notch signaling by activating a γ-secretase-like protease to release Notch intracellular domain (NICD), which activates transcription in nucleus.

More than 50 missense mutations in PS1, and two in PS2, have been identified in FAD pedigrees (Hardy, 1997). Accumulating data suggest that PS mutations cause AD by promoting the secretion of Aβ42 (Borchelt et al., 1996; Duff et al., 1996; Citron et al., 1997; Tomita et al., 1997), although the mechanism whereby mutant (mt) PS leads to the increased production of Aβ42 remains unknown.

We recently reported that NTF of FAD mt PS2 alone cannot promote secretion of Aβ42 (Tomita et al., 1998), and others showed that NTF of mt PS1 also does not enhance Aβ42 production (Citron et al., 1998; Steiner et al., 1998). These data prompted us to postulate that a subdomain in the PS C terminus mediates Aβ42 overproduction and to undertake molecular dissection studies to identify this subdomain.
**MATERIALS AND METHODS**

Construction of expression plasmids. A full-length cDNA encoding wild-type (wt) or N141I FAD mt human PS2 was obtained as described (Tomita et al., 1997, 1998). cDNAs encoding C-terminally modified wt or mt PS2 were similarly generated by PCR using *Pfu* polymerase (Stratagene, La Jolla, CA), and the following oligonucleotides were used as PCR primers: 5'-CCGGATCCAGACCTCTCTGCGCCCAAG-3' as a sense primer, 5'-CCGGATCCCTACTTCTTGAAACACGCGC-3' for PS2/111stop, 5'-CTGCTCGAGCTACAGGGTGGTCTCATGAA-3' for PS2/411stop, 5'-CCGGATCTACTGATGGGAGCAGCAG-3' for PS2/441stop, 5'-GGGAGGATGCACTCTGACCTGATGGGAAGG-3' for PS2/445stop, 5'-ACACCGAGATTTTCGATGAGGAGGCTGATGGGAAGG-3' for PS2/547A, 5'-ACACCGAGATTTTCGATGAGGAGGCTGATGGGAAGG-3' for PS2/1448A, 5'-CCGGATCTACTGATGGGAGCAGCAG-3' for PS2/1448F, 5'-CCGGATCTACTGATGGGAGCAGCAG-3' for PS2/1448R, 5'-CCGGATCTACTGATGGGAGCAGCAG-3' for PS2/I448A, 5'-CCGGATCTACTGATGGGAGCAGCAG-3' for PS2/I448F, and 5'-CCGGATCTACTGATGGGAGCAGCAG-3' for PS2/I448R. A full-length cDNA encoding wt human PS1 containing VRSQ motif was obtained by PCR from a normal human brain cDNA library, and the P267S PS1 mutation was introduced by the dU-template method as described previously (Tomita et al., 1997, 1998). cDNAs encoding C-terminally modified wt or mt PS1 were generated by PCR using the following primers: 5'-CCCAAGCTTGCCACCATGTCGGCCGAGAGC-3' for PS1/460stop, and 5'-CCGGATCCATGGGAGCAGCAG-3' for PS2/460stop. cDNAs encoding N-terminally truncated wt or mt PS2 were similarly generated by PCR using the following primers: 5'-GGGCTCTGAGTTGTTAACATATACACCTGAGC-3' as a sense primer, 5'-CCGGATCCCTACTTCTTGAACACAGC-3' for PS2/2DS/24, and 5'-CCGGATCCCTACTTCTTGAACACAGC-3' for PS2/2DS/42.

Cell culture and transfection. Mouse neuro2a (N2a) cells were maintained in DMEM supplemented with 10% fetal calf serum and penicillin/streptomycin at 37°C in 5% CO2 atmosphere as described (Tomita et al., 1997, 1998). Stable N2a cells were transfected by the calcium phosphate precipitation method (Sudoh et al., 1998), with some modifications. Briefly, confluent N2a cells stably expressing PS2 or its derivatives were cultured in DMEM containing fetal bovine serum for 36 hr. Conditioned media (4.5 ml) were incubated with 2 μg of BNT77, 5 μl of goat IgG against mouse IgG, IgA, and IgM (Cappel, West Chester, PA), and 100 μl of 25% protein G agarose (Life Technologies) at 4°C for 12 hr on a rotary shaker. The immunoprecipitates were spun down, boiled in 2% SDS sample buffer, separated by SDS-PAGE on a 16.5% Tris/Tricine gel, and then blotted to a Hybond-ECL membrane filter (Amersham). After boiling in PBS, the membrane was probed with BA27 or BC05 (8 μg/ml, respectively) and then detected by the ECL system as described above.

**RESULTS**

A small deletion at the C terminus of mt PS2 abrogates increased secretion of Aβ42

We previously showed that C-terminally truncated PS2 harboring the N141I FAD mutation (mt PS2) corresponding to endoproteolytic NTF (terminating at amino acid residue 303; 303stop), or retaining the entire sixth loop but truncated at the putative seventh transmembrane (TM) domain (388stop), lost the capacity to increase secretion of Aβ42 by N2a cells stably overexpressing these proteins (Tomita et al., 1998). To define the minimal PS2 C-terminal region required for the overproduction of Aβ42, we constructed cDNAs encoding mt PS2 (full-length, 448 residues) with the following C-terminal deletions ending at residues 411 (PS2/411stop), 441 (PS2/441stop), or 445 (PS2/445stop) as shown in Figure 1. Stably transfected N2a cells expressing these constructs were used to measure the secretion of Aβ42 by Aβ C-terminal-specific ELISAs. The percentage Aβ42 (%Aβ42) as a
fraction of total \( \beta \) \((= \alpha x-40 + \alpha x-42) \) secreted by cells stably expressing mt PS2/411stop, PS2/441stop or PS2/445stop was \(-10\%\), and this was similar to the \%A\beta2 secreted by cells expressing full-length (FL), wt PS2 or wt PS2/411stop, PS2/441stop, or PS2/445stop, whereas the \%A\beta42 secreted from cells expressing FL mt PS2 was constantly elevated to \(\sim 35-55\%\) as previously documented (Tomita et al., 1997, 1998) (Fig. 2). The total amounts of secreted \( \alpha \beta \) from transfected with these C-terminally truncated PS2 as determined by ELISA were comparable to those with FL PS2 (data not shown).

**Effects of substitution of the C-terminal residues of mt PS2 on A\(\beta\)42 production**

Because truncation of three amino acid residues at the C terminus of mt PS2 [i.e., Leu (L) 446, Tyr (Y) 447, and Ile (I) 448] completely inhibited the increase in secretion of A\(\beta\)42, we next replaced each of these single residues with Ala and examined their effects on A\(\beta\)42 secreted by N2a cells, to determine whether one or more of these three residues critically affect the production of A\(\beta\)42. mt PS2/L446A and PS2/Y447A increased the \%A\beta42 to comparable levels seen in N2a cells with FL mt PS2 (\(\sim 45-55\%\)), whereas \%A\beta42 from cells expressing mt PS2/I448A was \(-25\%\), which was at an intermediate level between those detected in the N2a cells with FL wt and mt PS2 (Fig. 3A).

We then focused on the role of residue I448, which is unusually hydrophobic for a C-terminal residue oriented at the cytoplasmic side, on A\(\beta\)42 secretion and examined the effects that resulted from replacing this residue with amino acids having different properties, i.e., Val (V), which is similarly hydrophobic but has a slightly shorter carbon chain, Phe (F), which also is hydrophobic but harbors an aromatic ring, or Arg (R), which is hydrophilic with positive charges. mt PS2/I448V and PS2/I448F enhanced secretion of A\(\beta\)42 from N2a cells at comparable levels to those in N2a cells expressing FL mt PS2. In sharp contrast, \%A\beta42 secreted from cells expressing mt PS2/I448R was \(-10\%\), which was similar to levels in cells with wt PS2 (Fig. 3B).

We further examined the secretion of A\(\beta\)40 and A\(\beta\)42 from cells transfected with these C-terminally substituted PS2 by immunoprecipitation with BNT77 and immunoblotting with BA27 and BC05, respectively (Fig. 3C). In N2a cells expressing FL wt PS2, robust 4 kDa (Fig. 3C, A\(\beta\)40, and A\(\beta\)42 (arrows) as well as the 3.7 kDa p3.740 and p3.742 (arrows) from conditioned media of N2a cells stably expressing wt or mt FL PS2 (\(\beta\)), PS2/L446A, or PS2/I448R by BNT77. Immunoprecipitates were visualized by Western blotting with BA27 or BC05.
positive bands were weaker compared with those in cells expressing wt PS2, whereas stronger BC05-positive 4 kDa (Fig. 3C, Aβx22) and 3.7 kDa (Fig. 3C, p3.752) bands were observed. The amounts of the BC05-positive bands were similarly increased in N2a cells expressing mt PS2/L446A, whereas no increase in the amount of BC05-positive bands was observed in cells expressing mt PS2/I448R. These data were in agreement with those obtained by ELISA, suggesting that the levels as well as ratios of Aβ40 and Aβ42 as determined by ELISA correctly represent those of bona fide Aβ peptides (the 4 kDa peptides may correspond to full-length Aβ and the 3.7 kDa peptides to N-terminally truncated Aβ, respectively). The total amounts of secreted Aβ from cells transfected with these C-terminally substituted PS2 as determined by ELISA also were at levels similar to those with wt PS2 (data not shown).

**Addition of amino acids to the PS2 C terminus abolishes increased Aβ42 secretion**

We next examined the effects of the addition of amino acid residues to the C terminus of mt PS2 on Aβ42 secretion. To this end, we used two different types of six amino acid long sequences: one contained six His residues (designated CHis), which is often used as an epitope tag, and the other was Ser-His-Gln-Leu-Tyr-Ile (designated CDup), which was identical to the last six amino acid residues of PS2. The latter was designed to determine whether the effect of mt PS2 on Aβ42 secretion is dependent on the integrity of the C-terminal amino acid sequences, regardless of the length of the C terminus. When stably transfected into N2a cells, neither mt PS2/CHis nor mt PS2/CDup retained the capacity to increase secretion of Aβ42, and the %Aβ42 was ~10%, which was similar to cells with wt PS2 (Fig. 4). The total amounts of secreted Aβ from cells transfected with PS2 with additional residues at the C terminus again were comparable to those with FL PS2 (data not shown).

**Relationship between overproduction of Aβ42 and stabilization and endoproteolysis of PS2**

It has not been definitively proven whether nascent holoproteins or stable complexes of endoproteolytic fragments (composed of NTF and CTF) of PS are the biologically active forms of these proteins. To investigate the relationship between stabilization and endoproteolysis of the C-terminally modified forms of PS2 studied here and their pathologial overproduction of Aβ42, we analyzed the expression and metabolism of these proteins in N2a stable cell lines by Western blots combined with cycloheximide treatment. All of the C-terminally truncated (i.e., PS2/411stop, PS2/411stop, or PS2/445stop) as well as tagged (i.e., PS2/CHis or PS2/CDup) PS2 that lacked the capacity to promote endoproteolysis did not undergo endoproteolysis to give rise to a ~35 kDa NTF and a ~23 kDa CTF normally produced from FL PS2 (Fig. 5A, arrowheads), although abundant holoproteins of corresponding sizes were expressed (Fig. 5A, arrows). With respect to mt PS2 with C-terminal single amino acid substitution, mt PS2/L446A, PS2/Y447A, PS2/I448V, or PS2/I448F, which promoted Aβ42 secretion, were cleaved to form endoproteolytic...
N-terminally truncated PS2 in stably transfected N2a cells. Cell lysates (20 μg protein) from N2a cells stably transfected with N-terminally truncated PS2 genes (wt) or without (mt) N141I FAD mutation (mt) quantitated by two-site ELISAs. FL, Full-length PS2; dAS, PS2 lacking the N-terminal acidic stretch corresponding to residues 1–20; dN, PS2 lacking the entire N-terminal cytoplasmic domain corresponding to residues 1–75. Mean values ± SE in four independent experiments are shown. Transfected PS2 cDNAs are indicated below the columns. A, Western blot analysis of N-terminally truncated PS2 in stably transfected N2a cells. Cell lysates (20 μg protein) from N2a cells transfected with cDNAs encoding full-length (FL) or N-terminally truncated PS2 were fractionated by SDS-PAGE and analyzed by immunoblotting with anti-G2N2 antibodies for dAS (left panel) and with anti-G2L for dN, which lacks the epitopes for anti-G2N2 (right panel). Note that correspondingly smaller holoproteins (arrows in both panels) and NTFs (arrowhead in the left panel) compared with those in cells with FL PS2 were detected and that transfection of dN gave rise to increased levels of CTF (arrowhead in dN; right panel) compared with those of endogenous CTF (arrowhead in Vector, right panel), indicating an effective generation of endoproteolytic fragments. Vector, Cells transfected with an empty pcDNA3 vector. C, Analysis of the half-lives of N-terminally truncated PS2. Cells were grown in the presence of cycloheximide (CHX) for 0, 4, or 10 hr and then harvested and analyzed as in fragments, i.e., NTF (Fig. 5B, arrowheads) as well as CTF (data not shown), whereas mt PS2/I448R lacking this property was not cleaved (Fig. 5B, arrowheads). mt PS2/I448A, which showed intermediate levels of Aβ42 overproduction, yielded moderate levels of endoproteolytic fragments (Fig. 5B, left, arrowhead). Western blots after cycloheximide pretreatment revealed that holoproteins of C-terminally modified PS2 were short-lived with half-lives of <12 hr (Fig. 5C, arrow), whether endoproteolysis occurs or not. In contrast, the endoproteolytic fragments, if any, derived from transfected PS2 [e.g., NTF indicated by arrowhead in L446A of Fig. 5C, as well as corresponding CTF (data not shown)] acquired extraordinarily long half-lives of >24 hr, as observed with fragments produced from FL PS2 (Fig. 5C). These results strongly suggest that the stable NTF/CTF complexes of mt PS are the pathologically active forms of PS that induce overproduction of Aβ42, and that the integrity of the C-terminal structure of PS is critical for the stabilization of these complexes and the endoproteolysis of PS.

**N terminus of mt PS2 is dispensable for production of Aβ42 as well as stabilization and endoproteolysis of PS2**

To gain insights into the role of the N terminus of PS2 in Aβ42 production as well as on the stabilization and endoproteolysis of PS2, we constructed cDNAs encoding two types of N-terminally truncated PS2, i.e., dAS lacking the N-terminal 20 residues encompassing the acidic stretch, and dN lacking the entire N-terminal cytoplasmic domain corresponding to residues 1–75. When stably expressed in N2a cells, PS2/dAS as well as PS2/dN with N141I FAD mutation both increased the %Aβ42 at levels similar to that of cells with FL mt PS2 (Fig. 6A), although the total amounts of secreted Aβ were not altered (data not shown). Western blot analysis showed that both PS2/dAS and PS2/dN undergo endoproteolysis, yielding smaller NTFs and a ~23 kDa CTF of the same size as that derived from cells with FL PS2 (Fig. 6B). Cycloheximide treatment demonstrated that these endoproteolytic fragments have long half-lives (>10 hr) (Fig. 6C, arrowheads), whereas their corresponding holoproteins are short-lived (Fig. 6C, arrows). Taken together, we conclude that the N terminus of PS2 is dispensable for overproduction of Aβ42 as well as for stabilization and endoproteolysis of PS2.

**Replacement of endogenous PS1 by PS2 derivatives overexpressed in N2a cells**

To determine whether various types of PS2 derivatives overexpressed in N2a cells replace endogenous PS, we selected single clonal cell lines stably expressing C- or N-terminally modified PS2 and examined the levels of endogenous PS1 in representative clones (Fig. 7). The amounts of ~30 kDa NTF and ~23 kDa CTF of endogenous mouse PS1 were decreased in cells expressing FL wt or mt PS2, as well as in cells expressing PS2/I446A or PS2/dN, whereas they were maintained at levels similar to those in mock transfectants in cells expressing wt or mt PS2/445stop, PS2/I448R, or PS2/CHis. These results clearly showed that the pathologically active forms of mt PS2 that promote overproduction of Aβ42 can

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**B.** The positions of holoproteins of PS2/dAS or dN are marked by arrows (both panels), and endoproteolytic NTF (left panel) and CTF (right panel) are marked by arrowheads, respectively. The names of the transfected cDNA constructs are indicated at the top of each lane. Molecular mass standards are shown in kilodaltons.
replace endogenous PS, whereas replacement does not occur with C-terminally modified PS2 lacking the Aβ42-promoting effects.

**Integrity of the C terminus of mt PS1 is required for overproduction of Aβ42**

To determine whether our conclusions regarding the role of PS C terminus are applicable to PS1, we constructed cDNAs encoding wt and Pro267Ser (P267S) mt PS1 lacking the last seven amino acid residues (PS1/460stop), and FL PS1 with the C-terminal residue Ile467 replaced by Arg (PS1/I467R), and stably expressed them in N2a cells. Consistent with the results obtained with PS2, FL P267S mt PS1 increased the %Aβ42 by ~1.5-fold compared with that of wt PS1, whereas the %Aβ2 was not elevated in cells with mt PS1/460stop nor with mt PS1/I467R (Fig. 8A, and the total amounts of secreted Aβ were not altered (data not shown). These C-terminally modified PS1 proteins were expressed as holoproteins (Fig. 8B, FL) but they were cleaved to produce only trace amounts of endoproteolytic fragments (Fig. 8B, huNTF). These results confirmed that the integrity of the C-terminal structure of PS1 also is important for the overproduction of Aβ42 as well as for the stabilization and endoproteolysis of this protein similar to PS2.

**DISCUSSION**

In this study, we have clearly shown that (1) the integrity of the C-terminal structure of PS is required for the ability of FAD mt PS to increase secretion of amyloidogenic Aβ42; (2) subtle modifications of the C terminus of PS, especially those eliminating the hydrophobicity of the C-terminal Ile residue, abrogate the endoproteolysis of PS; (3) the pathological activity of FAD mt PS to increase Aβ42 is most likely mediated by stabilized complexes of endoproteolytic fragments of PS; and (4) the N terminus of PS, in contrast to the C terminus, is dispensable for the overproduction of Aβ42, as well as for the stabilization or endoproteolysis of PS.

The mechanisms whereby PS proteins mediate their physiological as well as pathological functions remain elusive. Here we showed a strict parallel between the overproduction of Aβ42 and the stabilization and endoproteolysis of PS in a series of PS proteins harboring subtle modifications at the C terminus. Taken together with recent observations on PS1 (Steiner et al., 1998) or chimeric molecules of PS1NTF–PS2CTF (Saura et al., 1999), our data support the notion that stabilized fragments of mt PS are responsible for the pathological augmentation of Aβ42 production, although the precise role of endoproteolytic cleavage in PS function remains unknown. FAD-associated mt PS1 lacking exon 9, which escapes cleavage within the sixth loop domain, is stabilized (Ratovitski et al., 1997), incorporated into high molecular weight stable complexes (Capell et al., 1998), and acquires the abnormal ability to promote overproduction of Aβ42 caused by an amino acid substitution at the splice junction site (Steiner et al., 1999), suggesting that endoproteolysis merely represents a molecular signature that indicates the occurrence of stabilization but is not mandatory for the function of PS.

Very recently, it was reported that mutating either of the two conserved Asp residues in the sixth and seventh TM domains of PS1 substantially reduces γ-cleavage of βAPP as well as PS endoproteolysis, whereas these Asp-mutated PS1 species can...
replace endogenous PS1 fragments, thereby eliminating the activity of PS1 in cells (Wolfe et al., 1999). It is conceivable that the two Asp residues are essential for the ability of PS1 to activate (or alternatively, to work as a) γ-secretase that is mediated by the stabilized form of PS, whereas the C terminus of PS plays a critical role in the formation of the stabilized complexes of PS, which in turn leads to increased secretion and deposition of Aβ42 in the FAD brains and cells. In this regard, it is particularly interesting that some of the loss-of-function SEL-12 mutants in C. elegans (Levitan and Greenwald, 1995) or Drosophila PS mutants (Struhl and Greenwald, 1999), which are incapable of facilitating Notch signaling, are truncated at the C terminus (e.g., within the putative seventh loop domain in the arl133 mutant of SEL-12) (Levitan and Greenwald, 1995). Further studies will be needed to determine whether these C-terminally truncated PS homologs are stabilized, and whether stabilized complex of PS is required for γ-(like) cleavage of Notch or βAPP to release NICD or Aβ, respectively.

What then is the mechanistic role of the PS C terminus in the stabilization and function of PS proteins? Holoproteins of C-terminally modified PS polypeptides studied here were robustly expressed, and as we have previously confirmed with NTFs of PS2, they were inserted into membranes and localized to endoplasmic reticulum [Tomita et al. (1998) and our unpublished observations], despite their relatively short half-lives. One possible mechanism would be that the highly hydrophobic cytoplasmic tails of PS (i.e., -Leu-Tyr-Ile for PS2 and -Phe-Tyr-Ile for PS1) serve as the binding domain of some interacting proteins that are required for the stabilization of PS. Notably, PDZ domain-containing proteins are known to bind to the C terminus of transmembrane proteins with specific motifs such as Ser/Thr-X-Val/Ile (for group I PDZ domains) or hydrophobic amino acids at positions -2 and 0 (for group II) (Songyang et al., 1997), the latter being very similar to those of the PS C terminus noted above. In fact, deletion, mutation, or addition of C-terminal amino acid residues abolishes their binding to PDZ domains (Saras et al., 1997). Taken together with the fact that the stabilization of PS is regulated by competition for limiting cellular factor(s), it is tempting to speculate that interacting proteins that bind to the C terminus of PS are the determinants for the stabilization and replacement of PS that are vital to the function of PS. Another possibility would be that the C terminus of PS per se plays an important role in the proper folding or conformation of PS required for the stabilization and/or function of PS proteins. Although little is known about the roles of different domains of polytopic membrane proteins in the stabilization of polypeptides, data from deletion studies in lac permease of Escherichia coli may have interesting implications for our findings. Lac permease is a polytopic membrane protein that spans the membrane 12 times, with the N and C termini oriented to the cytoplasmic side. Kaback and colleagues (Roepe et al., 1989) have shown that a total ablation of the 17 amino acid C-terminal cytoplasmic tail of lac permease does not have any decremental effects on the stabilization and function of this protein, whereas additional deletion of five more amino acids constituting the C-terminal portion of the 12th TM domain drastically destabilized the protein after insertion into the membrane. It was also reported that removal of the C-terminal tails of two other polytopic membrane proteins, bacteriorhodopsin (Huang et al., 1981) or melibiose permease (Botfield and Wilson, 1989), did not affect their functions. Thus, it is highly likely that the C terminus of PS, especially the hydrophobic C-terminal residues, plays a unique role in the stabilization of PS as a polytopic membrane protein. The precise mechanism whereby the C terminus stabilizes PS is unknown at present; it may bind to some important domain within the TM or loop structures of PS to maintain a structure required for stabilization or function, or alternatively, the hydrophobic C terminus may interact with, or is anchored within, membranes ensuring the proper conformation of PS. It is also possible that the conformation of the whole protein maintained by the C terminus allows the binding of a limiting factor (protein) with some portion(s) of PS outside the C terminus.

The hydrophobic C terminus of PS could be a therapeutic target for the treatment of FAD because the development of small compounds that bind to the C terminus of PS and mimic these modifications may destabilize and reduce the total amount of "functional" mt PS proteins, which promote overproduction of Aβ42, in the brains of patients with FAD linked to PS mutations. Future studies of the roles of the C terminus of PS will pave the way for understanding the pathomechanisms as well as for the development of novel therapeutic strategies for FAD and possibly sporadic AD.

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