Current Topics

D-Amino Acid Biosystem

Biological Significance of Isoaspartate and Its Repair System

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Isomerization of L-aspartate and deamidation of L-asparagine in proteins or peptides dominantly give rise to L-isoaspartate by a non-enzymatic reaction *via* succinimide as a intermediate under physiological conditions. Isoaspartates have been identified in a variety of cellular proteins *in vivo* as well as pathologically deposited proteins in neurodegenerative brain tissue. We described here that the formation of isoaspartate is enhanced in amyloid-β (Aβ) peptides in Alzheimer's disease (AD). Specific antibodies recognizing isoaspartate of Aβ revealed that isomerized Aβ peptides were deposited in senile plaques as well as amyloid-bearing vessels. Moreover, it was revealed that Aβ peptides, isomerized at position 7 or 23, were differentially deposited in senile plaques and vascular amyloids in AD brains. *In vitro* experiments showed that the modification at position 23 greatly enhanced the aggregation of Aβ. Furthermore, systematic proline substitution analyses revealed that the β-turn structure at positions 22 and 23 of Aβ42 plays a crucial role in the aggregation and neurotoxicity of Aβ peptides. It is suggested that spontaneous isomerization at position 23 induces the conformational change to form a β-turn at position 23, which plays a pathogenic role in the deposition of Aβ peptides in sporadic AD. Protein L-isoaspartyl methyltransferase (PIMT) is a putative protein repair enzyme, which converts L-isoaspartyl residues in damaged proteins to normal L-aspartyl residues. PIMT-deficient mice manifested neurodegenerative changes concomitant with the accumulation of L-isoaspartate in the brain. We discuss here the pathological implications of the formation of isoaspartate in damaged proteins during neurodegeneration in model mice and AD.

Key words isoaspartate; Alzheimer' disease; protein-L-isoaspartyl methyltransferase (PIMT); repair; aging

1. NON-ENZYMATIC FORMATION OF ISOASPARTATES IN PROTEINS

Proteins and peptides are susceptible to a variety of chemical modifications that can affect their structure and biological functions. Among these modifications, the isomerization of aspartic acid and deamidation of asparagine occur in proteins and peptides during aging.[2,3)] The reaction proceeds spontaneously under physiological conditions through a five-membered succinimide ring intermediate formed by the nucleophilic attack of the peptide bond nitrogen atom of the following residue on the side chain carbonyl group, resulting in dehydration of aspartic acid or deamidation of asparagine (Fig. 1). The L-succinimidyl intermediate then undergoes a relatively rapid hydrolysis at either the α- or β-carbonyl group to generate L-isoaspartate (L-IsoAsp) and normal L-aspartate (L-Asp) in a ratio of approximately 3 : 1 in a variety of substrates.[4)] The formation of L-succinimide can also be accompanied by enhanced racemization at the α-carbon to generate a mixture of D-succinimidyl, D-aspartyl, and D-isoaspartyl forms[4)] (Fig. 1). In the non-enzymatic reaction, the L-IsoAsp form is typically the predominant product. IsoAsp forms most easily at sequences in which the side chain of the C-flanking amino acid is relatively small and hydrophilic, and is less likely to be formed where bulky or hydrophobic residues are in this position. The most favorable C-flanking amino acids are Gly, Ser, and His.[5,6)] In general, the half-times of aspartyl and asparaginyl peptide degradation under physiological conditions (pH 7.4, 37 °C) vary between about 1 and 1000 d.[5)] L-Asn residues form L-succinimides about 10 times more rapidly than comparable L-Asp residues.[7)] A similar series of reactions would occur in glutamine and glutamate residues, generating D- and L-isoglutamate and glutamate residues, but these reactions are much slower than those of asparagines and aspartic acid residues.[8)]

2. ISOASPARTATES IN DAMAGED AND AGED PROTEINS

It has been reported that isomerization and deamidation reactions occur during the isolation, purification, and storage of proteins such as recombinant calmodulin,[9)] recombinant human growth hormone,[10)] tissue plasminogen activator,[11)] synapsin I,[12)] tubulin,[13)] recombinant interleukin-11,[14)] and recombinant monoclonal antibody.[15)] Isoaspartate has also been identified in a variety of cellular proteins, including erythrocyte membrane protein 4.1,[16)] phosphacan,[17)] ribosomal protein S11,[18)] histone H10,[19)] histone H2B,[20)] Bcl-x_L,[21,22)] cAMP-dependent protein kinase (PKA),[23)] and collagen type I[24)] *in vivo*. Evidence has been accumulating that modified calmodulin,[9)] Bcl-x_L,[25)] collagen type-I[26)] and PKA[23)] show altered biochemical or biological activity when altered aspartyl residues are incorporated into their structure. Furthermore, these modifications have been detected in long-lasting proteins, including eye lens crystalline,[27,28)] and myelin basic protein.[29,30)] In addition, environmental factors such as heat shock,[31)] photochemical stress[32)] and oxidative stress,[33,34)] also induce degradation at aspartyl and asparaginyl residues in proteins. In fact, the presence of D-IsoAsp-containing peptides in elastic fibers of skin can be a marker for ultraviolet-

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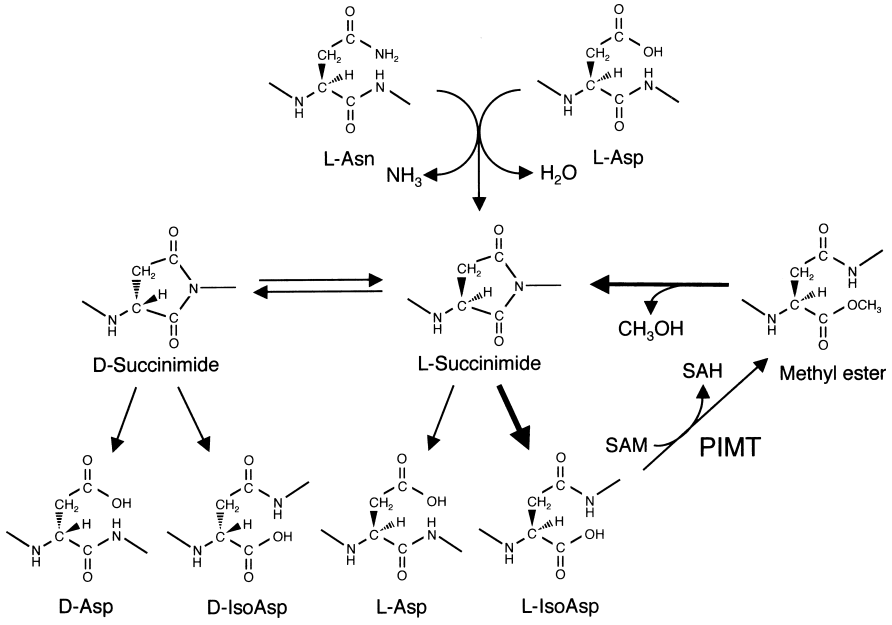

Fig. 1. Isoaspartate Formation and Repair by PIMT

Deamidation of aspargine (L-Asn) and dehydration of aspartic acid (L-Asp) spontaneously occur on nucleophilic attack of the α-amino group of the C-flanking amino acid. This reaction, which forms a succinimidyl intermediate (L-succinimide), is followed by hydrolyzation to form L-isoaspartate (L-IsoAsp) and L-Asp. L-Succinimide reversely racemizes to D-succinimide. The D-succinimide intermediate also undergoes a rapid hydrolysis at either the α- or β-carbonyl group to generate D-isoaspartate (D-IsoAsp) and D-aspartate (D-Asp). The free α-carbonyl group of L-IsoAsp is methylated by PIMT with *S*-adenosylmethionine (SAM) as the methyl donor. Enzymatic methylation followed by spontaneous ester hydrolysis leads to reformation of the L-succinimide intermediate, which is again hydrolyzed to a mixture of L-Asp and L-IsoAsp. The nonenzymatic reactions denoted by the thick arrows are faster than those denoted by the thin arrows. SAH represents *S*-adenosylhomocysteine.

induced skin aging.[35] Recent studies have also reported the formation of isoaspartate in age-associated human neurodegenerative diseases. Isoaspartyl residues in the scrapie form of prions in the brains of mice infected with scrapie,[36] amyloid-β (Aβ) peptide deposited on senile plaques in Alzheimer's disease (AD),[37—40] and tau in paired helical filaments of AD[41] have been found. It is still unclear, however, whether the isoaspartyl residues in these deposited proteins are the cause or the result of protein insolubility and toxicity in such pathological conditions.

3. REPAIR OF ISOASPARTATE BY PROTEIN-L-ISOASPARTYL METHYLTRANSFERASE (PIMT)

Protein-L-isoaspartyl methyltransferase (PIMT, EC2.1.1.77) is a widely distributed enzyme that catalyzes the transfer of an active methyl group from *S*-adenosyl-L-methionine (SAM) to the α-carboxyl group of atypical L-isoaspartyl and D-aspartyl residues, but not normal L-aspartyl residues, in peptides or proteins[3,42] (Fig. 1). Lowenson and Clarke reported that the best substrates for PIMT, at least in synthetic peptides, contain L-IsoAsp, rather than D-Asp.[43,44] It is therefore proposed that PIMT plays a physiological role in the repair of damaged proteins containing L-IsoAsp. Two splicing isoforms, PIMT-I and PIMT-II, have been reported, although the difference in their functions is still to be defined.[45] Previous studies have shown that the PIMT-II isoform is a splice variant of PIMT-I, which carries an Arg-Asp-Glu-Leu motif at the carboxy-terminal end, suggesting that PIMT-II localizes in the ER.[46,47] It is therefore believed that PIMT-I and PIMT-II are expressed in the cytoplasm and ER, respectively. However, it has not yet been demonstrated whether PIMT-II is actually retained in the ER *in vivo*. Furthermore, exactly where the proteins are deamidated or isomerized and how they are repaired by PIMT-I and PIMT-II are not well understood.

Recently, Lanthier and Desrosiers showed that PIMT repairs abnormal aspartyl residues accumulated in type-I collagen and restores cell migration.[26] Although collagen proteins were expressed in the extracellular space where PIMT was not distributed, the damaged proteins were actually repaired by PIMT *in vitro*, indicating that PIMT could be a therapeutic tool to restore the physiological function of damaged proteins. Interestingly, overexpression of PIMT in flies can lead to a marked increase in lifespan under certain conditions.[48] Chavous *et al.* showed that a 3- to 7-fold enhancement in the activity of PIMT leads to a dramatic 32—39% increase in lifespan at 29 °C, mild heat stress conditions for these flies.[48] On the other hand, there was no change in lifespan at 25 °C indicating that PIMT may only be limiting under stressful conditions.[48] As a similar phenotype, it is reported that overexpression of PIMT in *E. coli* increases heat shock survival.[49] These results suggest that overexpression of PIMT provides protection from environmental stress in aging tissues. It is still unclear, however, whether PIMT activity is responsible for the increase in survival because even greater levels of heat tolerance were observed with the overexpression of inactive PIMT mutants in *E. coli*.[49]

4. ISOASPARTATE IN Aβ PEPTIDES IN SENILE PLAQUES AND BLOOD VESSELS IN AD

AD is a neurodegenerative disease characterized by the progressive deposition of amyloid fibrils in the brain parenchyma and cortical blood vessels.[50] The deposits mainly consist of 40- or 42-mer peptides (Aβ40 and Aβ42)

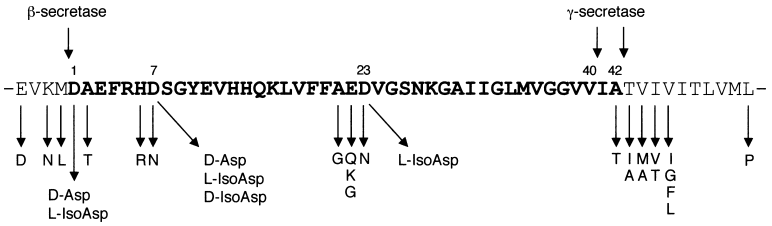

Fig. 2. Mutations of Amyloid Precursor Protein (APP) Found in Familial AD (FAD), and Isomerization and Racemization at the Asp Residue of Aβ Found in AD Brains

Aβ40 and Aβ42 peptides are generated by proteolysis of β- and γ-secretase at indicated sites. Bold letters indicate amino acid sequence in Aβ1—42. Interestingly, all FAD mutations in APP are clustered in the N-, C-terminal region, or central portion of Aβ. All Asp residues at positions 1, 7, and 23 in Aβ were isomerized or racemized.

generated by the proteolysis of amyloid precursor protein (APP).[51] Aβ42 plays a pivotal role in the pathogenesis of AD since the aggregative ability and neurotoxicity of Aβ42 are considerably greater than those of Aβ40.[52] In early onset familial cases of AD, missense mutations have been found either outside or inside the Aβ-coding region of the APP gene[53] (Fig. 2). Within this coding region, five mutations have been characterized; the Flemish mutation (Ala21 to Gly),[54] Dutch mutation (Glu22 to Gln),[55] Italian mutation (Glu22 to Lys),[56] Arctic mutation (Glu22 to Gly),[57] and Iowa mutation (Asp23 to Asn).[58] Interestingly, these mutations were exclusively found in the middle portion of the Aβ sequence from position 21 to 23, and were characteristically associated with an extensive cerebrovascular pathology of cerebral amyloid angiopathy (CAA, Fig. 2). On the other hand, the majority of patients develop AD sporadically without an apparent predisposing background. In both hereditary and sporadic cases of AD, it is considered that the aggregated Aβ peptides play a major role in the development and progression of the disease. However, it is not clear what molecular mechanisms induce the aggregation in the brain.

Biochemical studies revealed that Aβ peptides isolated from AD brains were posttranslationally modified by isomerization, racemization, pyroglutamylation, and truncation.[37,59] Roher *et al.* revealed that Asp residues of Aβ were modified to L-IsoAsp, D-IsoAsp, and D-Asp at positions 1 and 7 in AD brains[37,60] (Fig. 2). In particular, the Asp7 residue of Aβ isolated from senile plaques was dominantly (55.0%) composed of L-IsoAsp7.[37] Kuo *et al.* also suggested that the isomerization at positions 1 and 7 of Aβ1—42 contributes to the enhanced insolubility of Aβ and its resistance to enzymatic degradation.[61] As to Asp23, however, no isoaspartyl formation has yet been detected in the biochemical analysis of AD brains.

In order to characterize the pathological significance of the isomerization at positions 7 and 23 of Aβ, we investigated in detail the formation of isoaspartate in Aβ peptides in AD as well as control brains using site-specific IsoAsp-Aβ antibodies. We demonstrated that isomerized peptides were mainly deposited in senile plaques and amyloid-bearing vessels using anti-L-IsoAsp7 and anti-L-IsoAsp23 antibodies[39,40] (Fig. 3). Moreover, we revealed that Aβ peptides isomerized at either position 7 or 23, were differentially deposited on senile plaques and vascular amyloids of AD brains (Figs. 2, 3). *In vitro* experiments showed that isomerization at position 23 of Aβ40 and Aβ42, but not position 7, greatly enhanced aggregation.[40,62] Mori *et al.* have also reported that racemization at Asp23 of Aβ1—35 and Aβ1—40 accelerated peptide aggregation and fibril formation.[63,64] Furthermore, Aβ40 and

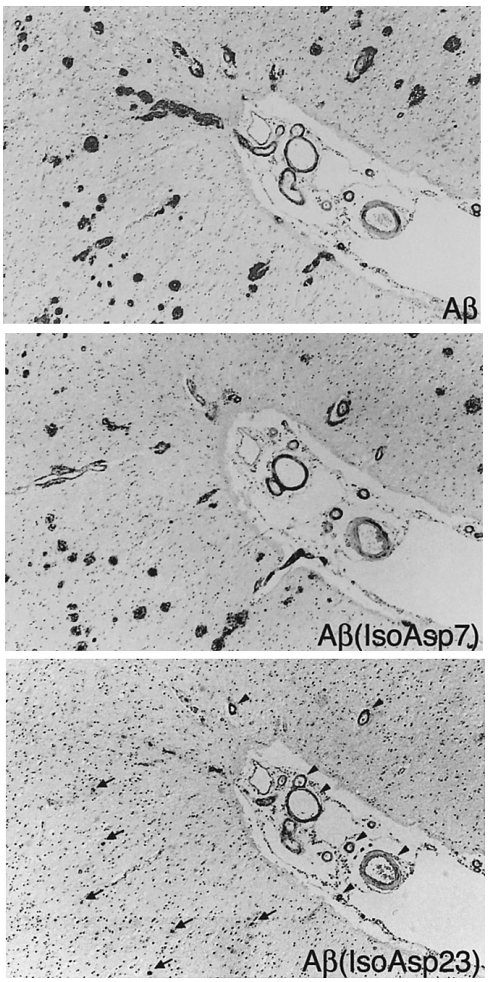

Fig. 3. Isoaspartate Formation of Aβ in Senile Plaques and Amylid-Bearing Vessels of an AD Patient

Temporal cortex sections of an AD patient were stained with anti-Aβ1—42 (upper), anti-IsoAsp7 (middle), and anti-IsoAsp23 antibodies (lower). Arrows indicate the core of mature plaques. Arrowheads indicate the amyloid-bearing vessels. Magnification: ×10.

Aβ42 with genetic mutations at positions 22 and 23 also showed greatly enhanced aggregation and neurotoxicity.[40,65,66] These results suggest that mutations or modifications at Glu22 and Asp23 have a pathogenic role in the deposition of Aβ peptides followed by the progression of AD. However, the genetic mutation at position 21, the Flemish mutation, needs an alternative explanation since it failed to result in enhanced aggregation and neurotoxicity. Recently, Tsubuki *et al.* reported that A21G-Aβ42 and other CAA-related Aβ mutants altered at position 22 are little affected by

the degradation enzyme of Aβ peptides.[67] This catabolic characterization of A21G-Aβ42 might indicate an alternative pathogenesis of Flemish-type CAA.

To clarify how the structures in Aβ42 peptides affect the ability to aggregate and neurotoxicity, Morimoto *et al.* investigated the aggregative ability and neurotoxicity of a series of proline-substituted Aβ42 mutants. When an amino acid residue of the peptides was substituted with a proline residue, the mutants easily formed β-turns but failed to form β-sheets. The results implied that the β-turn at positions 22 and 23 plays a crucial role in the aggregation and neurotoxicity of Aβ42,[68,69] suggesting that mutations or modifications at these positions alter the conformation to form a β-turn structure. In that paper, we proposed a new model of pathological conformation for aggregated Aβ42 peptides.[69]

In an analysis of FAD pedigrees, Shin *et al.* showed that Aβ species in Iowa-type familial CAA (Asp23 to Asn) presented with isoaspartyl residues at position 23, which was specifically detected by our anti-IsoAsp23 antibody.[70] This result suggested that the alteration of Asn23 to IsoAsp23 in Aβ peptides may be an important determinant in the deposition of Aβ in cerebral blood vessels. Recently, a similar FAD mutation replacing Asp7 with Asn7 has been reported in a Japanese pedigree.[71] This mutation may result in the formation of isoaspartate at position 7 of Aβ, enhancing the deposition of Aβ peptides in the FAD brain because Asn residues form succinimide intermediates more rapidly than comparable Asp residues.

In conclusion, it is suggested that isomerization or racemization of the aspartyl residues, especially at position 23, in Aβ peptides plays an important role in the formation of fibrils, neurotoxicity, and progression of amyloid deposition in AD brains. Alternatively, it is also suggested that chemical modifications accumulated during the progression of amyloid deposition further accelerate the pathological process by enhancing fibrillogenesis and neurotoxicity in the neurodegeneration of AD. We therefore postulated that spontaneous modifications of Aβ peptides such as isomerization and racemization play a pivotal role in the development of sporadic AD.[40] Furthermore, Watanabe *et al.* have reported that hyperphosphorylated tau, a major component of neurofibrilar tangles (NFTs) from AD brains, was also modified with protein isomerization at positions 193, 381, and 387.[41] This together with the isomerization and racemization of Aβ peptides, indicated that tau proteins were selectively isomerized at specific aspartyl residues, suggesting that spontaneous isomerization and racemization accelerate the abnormal protein deposition in sporadic AD.

5. IMPLICATIONS OF ISOASPARTATE FORMATION IN AD BRAINS

Two abnormally accumulated proteins, Aβ and tau, are more isomerized in AD brains than in normal brains, indicating that the protein isomerization may be enhanced in AD, or alternatively, the altered cellular or extracellular conditions may favor protein isomerization in AD, although the molecular basis of this posttranslational modification has yet to be clarified. The latter factor led to the hypothesis that the function of PIMT *in vivo* fails to minimize the accumulation of L-isoaspartyl residues in Aβ and tau in human brains. Kondo *et al.* have reported that the expression of PIMT is transcriptionally up-regulated in AD brains compared to normal brains.[72] To clarify the distribution of the up-regulated PIMT, we investigated the expression of PIMT by immunohistochemistry in AD brains. We have showed that PIMT is up-regulated in neurodegenerative neurons and colocalizes in NFTs, but not senile plaques, in AD brains.[39] The result indicated that PIMT is associated with NFTs but not detected in senile plaques for the simple reason that NFTs localize intracellularly while amyloid deposits accumulate in the extracellular space where PIMT does not exist. Taken together with the evidence that protein isomerization is enhanced in AD brains, the results suggest that the up-regulated PIMT is associated with the increased amount of protein isomerization in AD. The mechanism by which the isomerization of tau or Aβ is involved in or linked to other modifications such as the abnormal phosphorylation of tau[73,74] or pathological aggregation of Aβ, peptides should be examined further to elucidate the complicated mechanisms of disease progression in AD.

6. ACCUMULATION OF ISOASPARTYL PROTEINS IN BRAINS OF PIMT-DEFICIENT MICE

To clarify the biological and pathological role of PIMT in higher organisms, another group and we have generated PIMT-deficient mice, which provide a relevant model system for biological and pathological studies of isoaspartate *in vivo*.[75,76] Interestingly, PIMT-deficient mice expressed epileptic seizures that eventually limited their survival to 12 weeks of age (Fig. 4A).[75,76] In addition, the PIMT$-/-$ mice, but not PIMT$+/-$ and PIMT$+/+$ mice, accumulated IsoAsp in the brain as well as brain slices (Figs. 4B, C). The accumulation of isomerized proteins progressed markedly from 2 weeks of age and continued to increase for as long as we could observe it until 9 weeks of age (Fig. 4B). These findings indicated that protein isomerization is not restricted to long-living proteins of aged brains, but is observed as early as the embryonic stage in PIMT-deficient mice. The result also suggested that the amount of isoaspartate was kept low with PIMT in the wild type brain, with an equilibrium between the repair activity of PIMT and the spontaneous generation of protein isomerization. Interestingly, PIMT-deficient mice start to show refractory seizures at 4 weeks, which can initially be controlled with an anti-epileptic agent, but become resistant to medical treatment at 9 weeks.[76] The amount of isomerized protein increased markedly in the brains of PIMT-deficient mice at 4 weeks of age when the animals show the clinical symptoms of seizures. The development of resistance to anti-epileptic agents at 7—9 weeks would be well explained by the fact that medical treatment cannot repair or degrade the isomerized proteins accumulated in the brain of PIMT-deficient mice (Fig. 4B).

Histochemical analyses showed that dentate granule cells in the hippocampi of PIMT$-/-$ mice exhibited neurodegenerative changes with an extensive gliosis.[77,78] In an ultrastructural analysis, Ikegaya *et al.* observed vacuolar degeneration and cytoplasmic swelling in dentate granular cells in PIMT-deficient mice.[77] Furthermore, we also showed aberrant synaptic transmission in the hippocampal CA3 region, and cognitive deterioration in mutant mice.[77] It therefore ap-

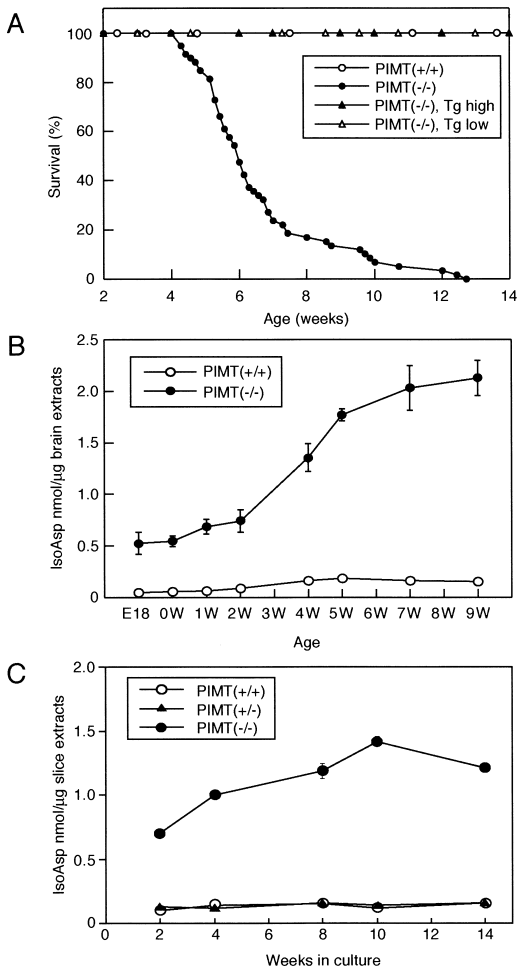

Fig. 4. Phenotype of PIMT-Deficient Mice

A: Survival of PIMT−/− and PIMT transgenic (tg) mice with a PIMT−/− background. Modified from Shimizu *et al.*[78] B: Accumulation of IsoAsp in the brain of PIMT−/− mice. The brain extracts were prepared from PIMT-deficient mice from embryonic day 18 (E18) to 9 weeks (9W) of age or littermate control mice. Modified from Shimizu *et al.*[39] C: Accumulation of IsoAsp in the brain slices of PIMT−/− mice. The brain slices were prepared from wild-type, heterozygous, and homozygous PIMT-deficient mice at 11 d of age as described previously.[86] They were cultured for 14 weeks. The IsoAsp contained in the extracts was measured at the time point indicated.

pears that the primary neurodegeneration in dentate granule cells in hippocampi results in the fatal epileptic seizures in PIMT-deficient mice. The result implied that protein isomerization may play a pathological role in neurodegeneration in an animal model as well as in AD brains. Although seizure is not a common symptom of AD patients, the rapid accumulation of isomerized proteins may cause an epileptic seizure while chronic accumulation may cause neurodegeneration. Alternatively, it is speculated that some proteins specifically isomerized during the early phase of development are responsible for the epileptic seizures while other proteins isomerized in matured or aged brains are associated with progression of the neurodegeneration. In humans, it has been reported that PIMT down-regulated in mesial temporal epilepsy and damaged proteins including tubulin were accumulated.[79] This together with our data, indicates that the accumulation of damaged protein may be a causative factor in epilepsy. The identification of substrates for PIMT should clarify this issue in PIMT-deficient mice.

Biochemical studies indicated that synapsin I was accumulated with abnormal aspartyl residues in brains of PIMT-deficient mice.[78,80] Synapsin I-deficient mice exhibited late onset epileptic seizures as well as enhanced stimulation-evoked epileptic seizures.[81,82] Since PIMT-deficient mice also exhibited an epileptic phenotype, modification of synapsin I may lead to the dysregulation of neurotransmitter release and synapse formation, followed by fatal epileptic seizures. Although the two mutant strains had a common phenotype, synapsin I-deficient mice all survived whereas PIMT-deficient mice did not. It is therefore speculated that damaged synapsin I with isoaspartyl residues is a factor for the progression to fatal epileptic seizures in PIMT−/− mice. To investigate whether transgenic expression of PIMT in the brain would rescue the mice from the fatal epilepsy and accumulation of isoaspartate in proteins, especially syanpsin I, we prepared two transgenic lines. The results indicated that higher expression levels of the PIMT-I or PIMT-II transgene independently stopped the lethal effect (Fig. 4A) and accumulation of damaged proteins in PIMT-deficient mice, suggesting that PIMT-1 and PIMT-II share common biological functions and/or intracellular substrates.[78] Although a lower level of expression caused an accumulation of damaged proteins in a partially-rescued line, the mice survived (Fig. 4A). Interestingly, synapsin I was specifically repaired in a partially-rescued, but symptom-improved transgenic line.[78] Moreover, we generated recombinant adenoviral vectors that contained PIMT-I or PIMT-II in order to investigate the specific biological roles of PIMT-I and PIMT-II. When the adeno-PIMT vectors were administered to PIMT-deficient neurons, either of PIMT-I or PIMT-II effectively repaired the damaged proteins.[83] In addition, we introduced a recombinant adenovirus expressing PIMT-I into the brain of PIMT-deficient mice using an exo-utero method. The recombinant adeno-PIMT improved the symptoms of PIMT-deficient mice *in vivo*, but only partially repaired IsoAsp in damaged proteins.[83] These results suggest that an overall accumulation of damaged proteins does not necessarily lead to a fatal epileptic seizure, while certain modifications of specific molecules such as synapsin I may play a pivotal role in epilepsy. Further structural analysis of synapsin I from PIMT−/− brain should shed light on the epileptogenesis of PIMT-deficient mice. On the other hand, Farrar and Clarke reported an alternative mechanism of epilepsy, that where a deficiency of PIMT in the brain can lead to alterations in the concentrations of SAM and *S*-adenosyl homocysteine (SAH).[84] SAH appears to have anti-epileptic activity and a lower level of SAH in PIMT−/− mice may thus contribute directly to the epilepsy phenotype.[84]

7. CONCLUSIONS AND PERSPECTIVES

In this review, we described that modified Aβ peptides with isoaspartate accumulated in AD brains and a deficiency of PIMT in the mouse brain leads to progressive neurodegenerative changes concomitant with the accumulation of isoaspartyl residues, suggesting that PIMT plays a protective role in the pathological changes associated with aging, especially in the brain. The repair of damaged proteins containing isoaspartate by PIMT may be one of the molecular mechanisms delaying aging. In a genomic analysis of the human PIMT gene, Devry and Clarke found that a human polymorphism results in the production of an enzyme that contains an

isoleucine or a valine residue at position 119.[85] Interestingly, the distribution of genotypes revealed that the heterozygous genotype in the healthy elderly population presented at an unusually high frequency of 65%, given the frequency of 46% in ethically-matched younger controls.[85] This suggests possible selection for both alleles of the repair enzyme in successful aging. Further clarification of the physiological and pathological functions of PIMT and formation of isoaspartate in mammals should shed light on the significance of protein isomerization in the aging tissues of humans and mice.

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