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Alzheimer's disease (AD) is the most common form of dementia-the 7th leading cause of death worldwide. At the tissue level, AD is characterized by the presence of extracellular amyloid plaques that are comprised primarily of the amyloid-β peptide (Aβ), and by intraneuronal neurofibrillary tangles composed of tau. Molecular genetics of early-onset AD and longitudinal brain-imaging studies of late-onset AD indicate that extracellular Aβ deposition, in general, precedes neurofibrillary tangle formation in neurons (Hampel et al., 2021; Young-Pearse et al., 2023).

Aβ is produced by proteolytic processing of the Aβ precursor protein (AβPP) via the amyloidogenic pathway, that is, AβPP is cleaved consecutively by β- and γ-secretase to generate Aβ peptides of varying lengths, of which the two predominant forms are $Aβ_{1–40}$ and $Aβ_{1–42}$. Early-onset AD missense mutations in AβPP and presenilin – the catalytic subunit of γ-secretase – affect AβPP processing by increasing the production of $Aβ_{1–42}$ relative to $Aβ_{1–40}$. Recently, a polygenic risk score for late-onset AD has also been associated with increased production of $Aβ_{1–42}$ (Lagomarsino et al., 2021). $Aβ_{1–42}$ is more neurotoxic than shorter Aβ variants, as revealed by many studies using *in vitro* and *in vivo* systems. Moreover, $Aβ_{1–42}$, under conditions that are not well understood, undergoes sequential post-translational modifications by dipeptidyl peptidase 4 and glutaminyl cyclase (QC) to produce $Aβ_{pyroE3–42}$ (**Figure 1**). Compared to $Aβ_{1–42}$, $Aβ_{pyroE3–42}$ is more hydrophobic, which in turn, makes it more insoluble and amyloidogenic (Bayer, 2022). $Aβ_{pyroE3–42}$ is a major component of diffuse and compacted amyloid plaques in AD. Importantly, $Aβ_{pyroE3–42}$ has been shown to cause template-induced misfolding of $Aβ_{1–42}$ into oligomers that spread in a prion-like fashion. *In vitro* and *in vivo* studies have shown that the $Aβ_{pyroE3–42}$-seeded oligomers are highly neurotoxic, synaptotoxic, and proinflammatory, suggesting that $Aβ_{pyroE3–42}$ even when present in small amounts contributes significantly to early neuronal cell death in AD.

Nature has provided quality control mechanisms for clearing Aβ from the brain. These include enzymatic degradation, phagocytosis by glial cells, transport across the blood-brain barrier, and clearance mediated by the bulk flow of cerebrospinal and interstitial fluids (Tarasoff-Conway et al., 2015). The relative importance of these mechanisms has not yet been established. Nonetheless, increasing evidence suggests that enzymatic degradation of Aβ monomer is an important and efficient mechanism for Aβ clearance.

The insulin-degrading enzyme (IDE; EC 3.4.24.56) is a conserved and ubiquitous zinc metalloprotease that could be the most important enzyme for degrading Aβ monomer in the brain (Kurochkin et al., 2018). IDE hypofunction and hyperfunction increase and decrease brain levels of Aβ *in vivo*, respectively. As its name suggests, IDE degrades α-helical insulin; however, IDE's natural substrates are predominantly unstructured molecules that have a high propensity to form β-sheet, including Aβ and the islet amyloid polypeptide. IDE's catalytic activity is allosterically regulated by small molecules such as ATP (Im et al., 2007), carnosine (Distefano et al., 2022), and short peptides (Song et al., 2003).

Recently, we found that $Aβ_{pyroE3–42}$ inhibits IDE's activity towards $Aβ_{1–40}$ (Kemeh and Lazo, 2023). A plausible explanation for this inhibition is the co-aggregation of $Aβ_{1–40}$ with $Aβ_{pyroE3–42}$, secluding

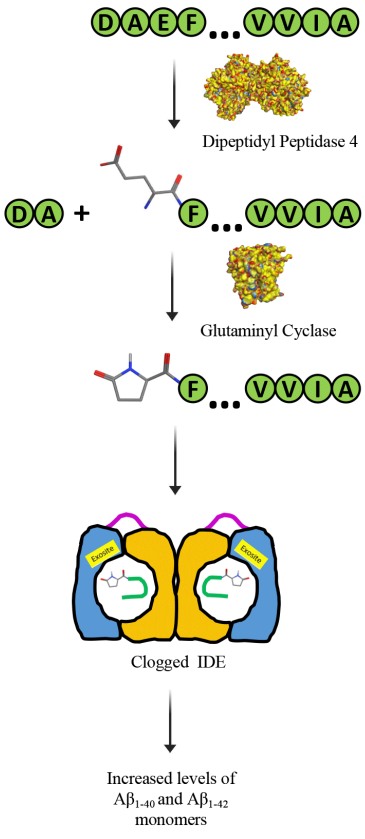

Figure 1 | Highly toxic $Aβ_{pyroE3–42}$ begets more Aβ. $Aβ_{1–42}$ is modified to $Aβ_{pyroE3–42}$ by the sequential action of dipeptidyl peptidase 4 (PDB ID: 1NU6) and glutaminyl cyclase (PDB ID: 2AFM). $Aβ_{pyroE3–42}$, arguably the most toxic form of Aβ, deactivates the insulin-degrading enzyme by clogging it up. In turn, the loss of IDE activity leads to increased brain levels of $Aβ_{1–40}$ and $Aβ_{1–42}$. Created using Microsoft PowerPoint. Aβ: Amyloid-β; IDE: insulin-degrading enzyme.

the former from IDE. Given that $Aβ_{pyroE3–42}$ also inhibits the IDE-dependent degradation of insulin (Kemeh and Lazo, 2023), a more reasonable mechanism is that the modified Aβ peptide causes IDE to become inactive. X-ray-crystallographic and cryogenic-electron microscopic studies have revealed key structural and enzymological features of the enzyme (Tang, 2016; Zhang et al., 2018). IDE's functional form is a homodimer, with each monomer composed of two bowl-shaped halves held together by a flexible linker. Furthermore, each monomer can exist in two dominant conformations: an open conformation for the capture of substrate and release of products; and, a closed conformation to form a catalytic chamber (aka crypt) for proteolysis to occur. Because the substrate must fit within IDE's crypt, substrates are limited to peptides that contain less than 80 residues. IDE contains a highly conserved exosite that presumably interacts with the N-terminus of its substrate through electrostatic interactions (Tang, 2016; Ivancic et al., 2018), facilitating the substrate's unfolding prior to its degradation. We posit that because $Aβ_{pyroE3–42}$'s N-terminus is uncharged, its exosite-assisted unfolding does not occur. As a result, $Aβ_{pyroE3–42}$ clogs up each "clamshell" of IDE, and by doing so, the enzyme becomes deactivated (Kemeh and Lazo, 2023; **Figure 1**). Our finding implicates $Aβ_{pyroE3–42}$ in the reduction of IDE activity which has been associated with late-onset AD.

Could the clogging up of IDE by $Aβ_{pyroE3–42}$ be prevented by small molecules? Maybe. Small molecules such as ATP (Patel et al., 2017) and polyphenols (Zheng et al., 2019) could modulate the interactions between $Aβ_{pyroE3–42}$ and IDE in such a way that the Aβ peptide does not clog up IDE's crypt. ATP has properties of biological hydrotrope in that it has the ability to inhibit the formation of protein aggregates and keep proteins in monomeric form. Polyphenols possess the ability to modulate protein-protein interactions found in amyloidogenic assemblies. Interestingly, resveratrol a polyphenol found in red wine also sustains IDE activity towards $Aβ_{1–42}$ (Krasinski et al., 2018). Could the formation of $Aβ_{pyroE3–42}$ be inhibited *in vivo* by small molecules? Of the two enzymes implicated in the conversion of $Aβ_{1–42}$ to $Aβ_{pyroE3–42}$ (**Figure 1**), QC appears to be the more attractive target for inhibitor development. We speculate that as long as the N-terminus of Aβ is negatively charged, as would be in the case in $Aβ_{3–42}$ that is produced by N-terminal truncation of $Aβ_{1–42}$ by dipeptidyl peptidase 4, the peptide would be susceptible to degradation by IDE. Furthermore, pyroglutamate formation in truncated proteins such as α-synuclein and TDP-43 could contribute to co-occurring pathologies in AD, providing an additional rationale for inhibiting QC. Of the QC inhibitors under development, varoglutamstat has advanced to Phase 2a/b trials in the US. The hope is that this small molecule could be an alternative to monoclonal antibodies targeting $Aβ_{pyroE3–42}$ or $Aβ_{1–42}$, which have been shown to cause amyloid-related imaging abnormalities in some patients.

Finally, our finding that a highly toxic Aβ begets more Aβ by deactivating IDE also brings to the forefront the development of small molecules that could increase IDE's activity towards a particular substrate. Because IDE is an allosteric enzyme, and significant progress has been made in the elucidation of its structural biology, we believe that the stage is now set for the development of IDE activators that specifically target the enzyme's inactivity towards the most toxic form of Aβ.

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