Unlocking aggregation in amyloid peptides opens up Alzheimer's research

Review Article

The effort to treat a number of diseases is presenting new challenges in peptide synthesis. The amyloid beta peptides involved in Alzheimer's disease are prone to aggregation, challenging both synthesis and purification. A group based in Auckland New Zealand therefore developed a method to reversibly introduce double linkers into the synthetic peptide that reduce aggregation and improve solubility, resulting in higher yields and purity.

The ongoing fight against Alzheimer's disease

Alzheimer's is a progressive degenerative disease of the brain that accounts for 60–80% of dementia cases, which cover a range of cognitive disabilities including memory loss (1). Alzheimer's is the sixth leading cause of death in the United States, with an expected life expectancy of 4–8 years after diagnosis. This disease is not a normal part of aging, and approximately 200,000 Americans under the age of 65 have younger-onset Alzheimer's disease. There is currently no cure, but symptoms can be treated, and great efforts are being made worldwide to improve treatment, delay onset, and ultimately prevent the disease from developing.

The critical role of amyloid plaques

One of the most characteristic features of Alzheimer's is the formation of senile plaques consisting mainly of amyloid beta (Aβ) peptide, which is involved in the 'amyloid cascade hypothesis' that postulates that the disease starts with aberrant amyloid metabolism. Aβ peptide is a product of processing Amyloid Precursor Protein that results in a number of variants, including $A\beta_{42}$, which is the most neurotoxic form with the greatest tendency to aggregate, making it a promising druggable target.

A barrier to amyloid synthesis – meeting the challenge of peptide aggregation

There is a fundamental challenge in developing effective methods for the synthesis of $A\beta_{42}$ for research into Alzheimer's – the natural propensity of the peptide to aggregate, which complicates synthesis and also chromatographic purification. The $A\beta_{42}$ molecule contains a 13 amino acid segment at its C-terminus that is highly hydrophobic and has a high propensity to aggregate into β-sheet structures (2). There are a number of other peptides that present similar problems in synthesis, including α-synuclein, and acyl carrier protein $(ACP)_{65–74}$, and some hydrophobic transmembrane segments of proteins, such as glycophorin A, epidermal growth factor (EGFR), and the M2 ion channel.

The challenge of synthesizing $A\beta_{42}$ has stimulated the development of a number of modifications to the Fmoc solid-phase peptide synthesis (SPPS) method commonly used, including DBU as deprotection agent, DMSO as a coupling cosolvent, the '*O*-acyl isopeptide' method, use of poly (ethylene glycol)-based, low-loading resins, and high temperature SPPS. Other approaches have involved reversibly incorporating specific residues or tags that modify the peptide. For example, Chemuru and coworkers added Lys residues to the C-terminus through standard peptide bonds that were then removed post-purification using immobilized carboxypeptidase B (3). The result was improved total synthetic yield, yield after purification, and final purity. Another approach involved

incorporating a short, monodisperse oligoethylene glycol-containing photocleavable lysine tag to increase solubility and decrease the propensity to aggregate (4). The solubilizing tag was then removed by UV irradiation, and this method enabled preparation and isolation of $A\beta_{42}$ in high purity and yield. It was approaches such as these that stimulated Kasim and coworkers to develop a method that included double linkers to improve hydrophilicity and reduce aggregation in order to improve synthesis yield and final purity (5).

Synthesis of $A\beta_{42}$ with double linkers

The synthesis method commenced with anchoring the Rink amide linker to ChemMatrix resin, followed by removal of the temporary Fmoc protecting group and the hexalysine tag was then assembled [Structure 1 in Figure 1]. After Fmoc deprotection, the HMBA linker was coupled to the N-terminus of the preceding lysine residue to complete the double linker system [2]. The peptide assembly was started by coupling Fmoc-Ala-OH to the free hydroxyl group to generate the base-labile ester bond of [3]. The peptide sequence was elongated with pseudoproline incorporation and continued on a Tribute® peptide synthesizer to yield the resin-bound peptide [4]. Following final Fmoc deprotection, the peptide was cleaved from the resin and lyophilized to produce crude peptide [5], which was purified by reverse phase HPLC. This peptide was treated with sodium hydroxide to hydrolyze the ester bond before removing the linker by aqueous extraction [6].

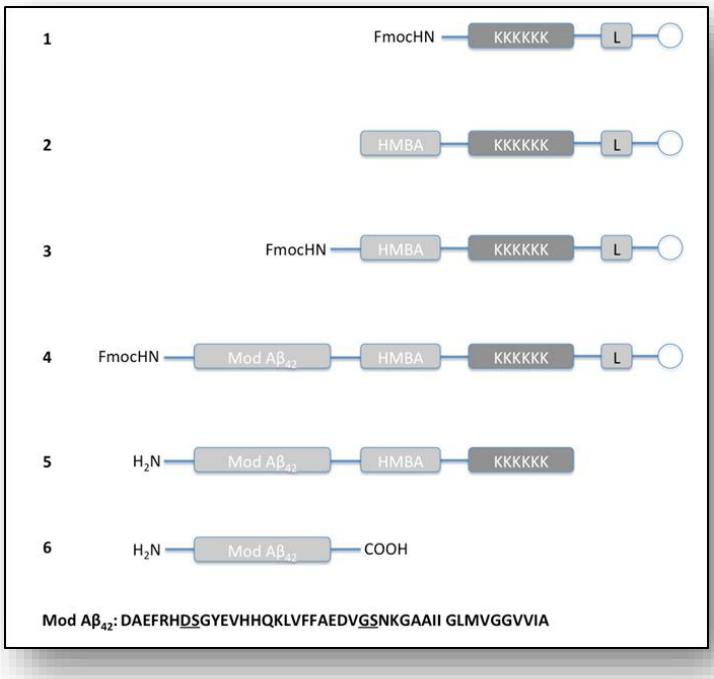

***Figure 1**. Synthesis of $A\beta_{42}$ using a double linker system, with pseudoprolines replacing underlined residues in the resin-bound peptide sequence. Based on Scheme 1, Kasim et al, 2019.*

Adding the positively charged lysine residues at the C-terminus increased the hydrophilicity, resulting in earlier elution compared with other variants (Fig. 2). The tag also increased solubility, greatly reduced the risk of aggregation, and enabled purification in acidic medium, which is not possible with unmodified $A\beta_{42}$.

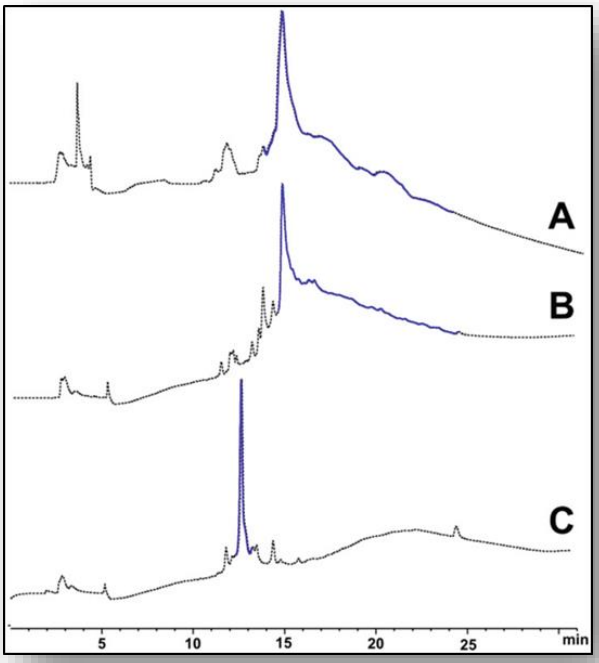

Figure 2. *Liquid chromatography profiles of peptides prepared by three synthesis strategies: A, crude Aβ$_{42}$ from standard Fmoc/tBU SPPS; B, crude Aβ$_{42}$ prepared using pseudoprolines; C, crude Aβ$_{42}$-HMBA-Lys$_6$-CONH$_2$. The regions in blue indicate the time range in which the target peptide eluted. Figure 2, Kasim et al, 2019.*

Characterization confirms correct functionality

The preparation of Aβ$_{42}$ formed by fibrils in a fibril promoting solution over time, as shown by transmission electron microscopy, while a thioflavin T assay confirmed the extensive formation of beta-sheets at high concentrations, which confirmed the concentration-dependent aggregation of Aβ$_{42}$ observed by other researchers. The circular dichroism spectrum showed the presence of both beta-sheets and random coils, which are characteristic secondary structures of amyloid fibrils.

Expanding the toolbox for peptide synthesis

The method tested here is relatively straightforward and should be accessible to most laboratories. When applied to Aβ$_{42}$, the pseudoproline dipeptides disrupted on-resin aggregation while the temporary C-terminal hexalysine tag improved water solubility and reduced aggregation. This and similar approaches promise to help significantly in the synthesis and purification of problematic peptides.

References

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