

Amyloid β -Targeted Inhibitory Peptides for Alzheimer's Disease: Current State and Future Perspectives

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Abstract: Alzheimer's disease is the most common irreversible neurodegenerative disorder. To date, there is no cure for Alzheimer's disease. While multiple pathological mechanisms have been proposed for the onset and progression of Alzheimer's disease, the hypothesis that attracted much attention is the amyloid hypothesis. The senile plaques that accumulate in the brain of Alzheimer's disease

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patients are predominantly composed of beta amyloid ($A\beta$). $A\beta$ deposition in the brain is thought to occur years before the emergence of clinical symptoms. The overproduction, aggregation, and fibrillation of $A\beta$, combined with reduced clearance, eventually lead to amyloid plaque formation and subsequent neurotoxicity. Hence, inhibition of $A\beta$ aggregation and the promotion of $A\beta$ clearance have been actively explored as therapeutic strategies for Alzheimer's disease. This chapter provides an overview of the current knowledge on one such strategy, $A\beta$ -targeted inhibitory peptides.

Keywords: $A\beta$ aggregation in Alzheimer's disease; biopanning; inhibitory peptides for Alzheimer's disease; peptide–nanostructure conjugates; peptidomimetics

INTRODUCTION

Alzheimer's disease is an age-dependent disorder that is the fifth leading cause of death in people aged 65 years and older. It is estimated that over 50 million people worldwide suffer from Alzheimer's disease, and this figure is set to increase to 152 million by 2050 with a financial burden of 1.1 trillion US dollars by 2050 (1–3). Several hypotheses, including the amyloid, cholinergic (4), and Tau protein hypotheses have been proposed to explain the pathophysiology and etiology of Alzheimer's disease (5). Because of the presence of $A\beta$ in the brain tissue, cerebrospinal fluid, and plasma, the amyloid cascade hypothesis is the most widely accepted. The amyloid cascade hypothesis states that neurodegeneration in Alzheimer's disease is the result of amyloid plaque and neurofibrillary tangle formations (6, 7). The overproduction, clearance failure, aggregation, and fibrillation of $A\beta$ eventually leads to amyloid plaque formation. These factors also contribute to neuroinflammation and cell death. $A\beta$ deposition in the brain is likely to be the first pathological incident that occurs years before the emergence of clinical symptoms. $A\beta$ is produced through the proteolytic cleavage of the amyloid precursor protein (APP), a transmembrane glycoprotein, which is made up of a cytoplasmic domain with 55 amino acids and a long extracellular domain with 590–680 amino acids (8). APP cleavage by the proteases β - and γ -secretases produce $A\beta$ fragments of varying size depending on the cleavage site (9), of which $A\beta_{40}$ (about 90%) and $A\beta_{42}$ (about 5–10%) are the most prevalent (Figure 1). $A\beta_{42}$ is more toxic than $A\beta_{40}$. After production, the $A\beta$ peptides aggregate to form amyloid deposits. There are different aggregation forms such as low molecular weight oligomers, protofibrils, as well as mature fibrils that eventually come together to form amyloid deposits in the brain parenchyma and cerebrovascular spaces (10, 11).

Therefore, inhibition of $A\beta$ aggregation and the promotion of $A\beta$ clearance have been investigated as therapeutic strategies for Alzheimer's disease. Some of these strategies include the use of metal chelators (12), peptides (13), organic molecules (14), and biomolecules (15, 16). Peptides are considered a better option than small molecule-based compounds because of their high affinity for $A\beta$ and low toxicity (17). Although natural amino acid-based peptides are effective inhibitors of $A\beta$ aggregation, they are prone to faster enzymatic degradation and show a tendency for self-assembly into fibrils during administration (15). To overcome these problems, modified peptides have been generated (18) with

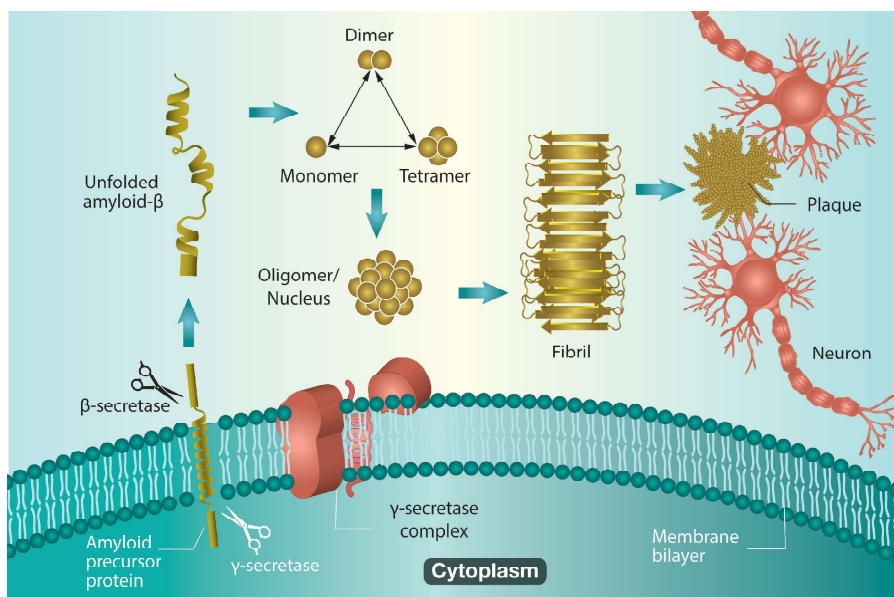


Figure 1. Amyloid- β fibrillation and neuronal damage. First, amyloid precursor protein is cleaved by β and γ secretases, respectively. A peptide fragment of 39–42 amino acid is formed depending on the site of cleavage. After cleavage, $A\beta$ monomers start to self-assemble to form soluble toxic aggregates, and finally into insoluble fibrils, which subsequently cause synaptic dysfunction and neuronal death.

D-amino acids, retro-inverso cyclization, fluorination, as well as N-methylation of the ester bond (19). With this knowledge, peptides could be potential candidates for inhibiting $A\beta$ conformational transitions, self-assembly, and toxicity against neurons, and promotion of the pathways of the nontoxic fibrillation and early diagnosis of Alzheimer's disease (20). This chapter provides an overview of the therapeutic potential peptides as $A\beta$ aggregation inhibitors.

PEPTIDIC INHIBITORS

Luhers and co-workers first experimentally described the structure of the $A\beta_{42}$ fibril (Figure 2, right) (21). At least four specific structural sites for interaction have been identified on the $A\beta$ fibril (22): (i), hydrophobic regions of Ala30–Val36, and Leu17–Ala21 residues from the C and N-terminal β -sheets respectively; (ii), hydrophilic part using electrostatic interactions between Asp23 and Lys28 residues; (iii), central cleft in the interior of the U-shaped turn; and (iv), Glu22 ladder between the side chains of the Glu22 residues of the adjacent β -strands (Figure 2, bottom right). The formation of the salt-bridge between Asp23 and Lys28 is an essential β -sheet conformation stabilizer. Moreover, it might stimulate the oligomerization of $A\beta$ via stabilizing the Val24–Asn27 turn (23). The hydrophobic residue of Met35 in the C-terminus domain could support

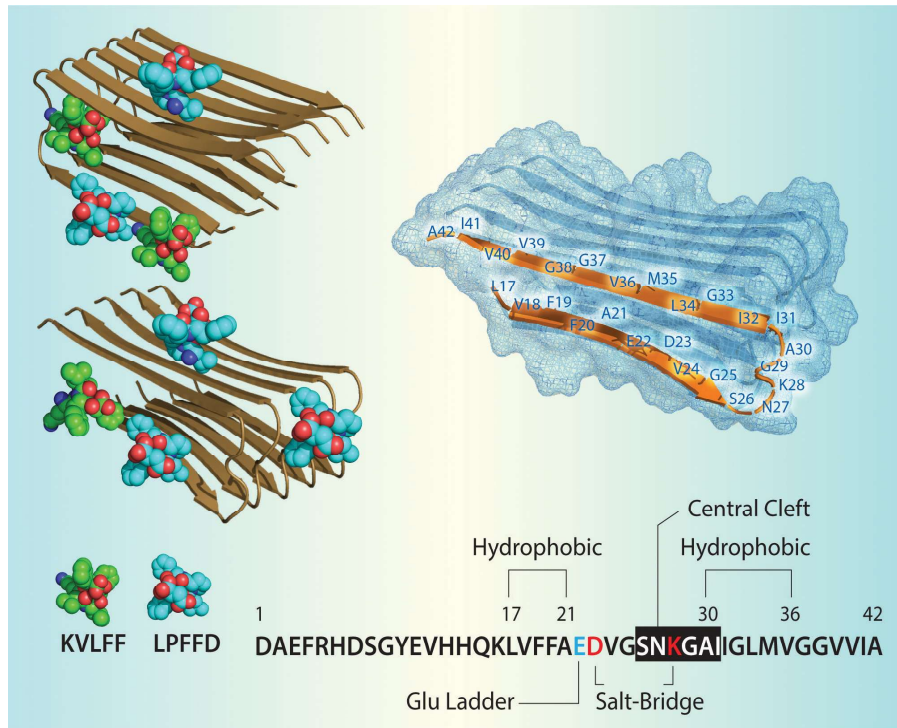


Figure 2. The structure of Aβ. Right, schematic of the Aβ₄₂ amyloid fibril. Left, binding sites of inhibitory peptides of LPFFD and KVLFF on Aβ₄₀ fibril.

fibril stability by hydrophobic interactions. The Met35 binding site can potentially inhibit protein-protein interactions and prevent amyloid fibril formation (24). These sites are probably critical regions in the initiation of Aβ nucleation, conformational transition promotion, and fibril formation. The residues ¹⁶KLVFFA²¹ (Figure 2) of the central hydrophobic core (CHC) region is a critical nucleation site, or self-recognition sequence. The Ile41 and Ala₄₂ residues can modulate Aβ₄₂ oligomer formation (25) by interacting with the N and C-terminus of Aβ₄₂ (26). Figure 2, left, shows the binding sites of the most common inhibitory peptides, such as LPFFD and KVLFF, on the Aβ₄₀ fibril structure.

Peptide inhibitors are generally divided into Aβ-based peptide inhibitors and non-Aβ-based peptide inhibitors. A list of select Aβ inhibitory peptides are presented in Table 1.

Aβ-based peptide inhibitors

These are based on the structure of the C-terminal fragments (CTFs) and the CHC sequences of the Aβ peptide. They bind to the Aβ peptide at specific sites and prevent its assembly into amyloid fibrils. Peptides consisting of D-enantiomeric amino acids exhibit greater stability against proteases and show a higher binding

TABLE 1 A select list of inhibitory peptides against A β peptide aggregation

No	Name/sequence of inhibitor peptide	Configuration	Type	Therapeutic findings	Ref.
1	RR (RYAAFFARR)	L-enantiomer	Rational design based on hydrophobic core and salt bridge region	<ul style="list-style-type: none"> Inhibition of Aβ_{40} fibrillation and decrease of Aβ_{40} induced toxicity (<i>in vitro</i>) 	(27)
2	D-(PGKLVYA) and D(KKLVFFARRRA)	D-enantiomer	CHC-derived sequence	<ul style="list-style-type: none"> Inhibition of Aβ aggregation Increase life time in an animal model of expressing Aβ_{42} 	(28)
3	CP-2 (ILwHsK)	Cyclic d,L- α -peptide	Screening of the randomly library members (500)	<ul style="list-style-type: none"> Nontoxic Stabilize small Aβ oligomers Disassemble formed Aβ fibrils 	(29)
4	D3D3 (D-RPRTRLHTRNRRRPRVTRLHLLHTRNR)	D-enantiomer	Not derived from A β sequence	<ul style="list-style-type: none"> More inhibitory effect against Aβ_{42} oligomerization than D3 peptide Formation of nontoxic amorphous instead of toxic oligomers (<i>In vivo</i>) 	(30)
5	RD2 (DPTLHTRNRRR-NH ₂)	D-enantiomer	<ul style="list-style-type: none"> Not derived from Aβ sequence 	<ul style="list-style-type: none"> Good binding affinity to Aβ Reduce Aβ fibrillation formation High stability in mouse plasma High bioavailability (<i>In vivo</i>) 	(31, 32)
6	AOEP2 (FDYKAEFMPWDT)	D-enantiomer	<ul style="list-style-type: none"> A mimotope of the Aβ oligomer Selected by phage display 	<ul style="list-style-type: none"> Targeting of all forms of Aβ The considerable reduction of TNF-α 	(33)
7	LPYFD-amide	L-enantiomer	CHC-derived sequence	<ul style="list-style-type: none"> Neuroprotective Decrease tau aggregation (<i>In vivo</i>) 	(34)
8	Ac-LPFFN-NH ₂	L-enantiomer	CHC-derived sequence	<ul style="list-style-type: none"> Inhibit of Aβ_{40} aggregation Stabilize the α-helical conformation of Aβ_{40} Prolong the fibril formation 	(35)
9	D-4F peptide	D-enantiomer	Not derived from A β sequence	<ul style="list-style-type: none"> Inhibit Aβ deposition Improve cognitive function 	(36)

Table continued on following page

TABLE 1 A select list of inhibitory peptides against A β peptide aggregation (Continued)

No	Name/sequence of inhibitor peptide	Configuration	Type	Therapeutic findings	Ref.
10	Diazirine-equipped cyclo-KLVF(b-Ph)F	cyclic	CHC-derived sequence	<ul style="list-style-type: none"> Inhibit Aβ aggregation Prevent Aβ_{42} induced toxicity 	(37)
11	Attached flavin to an A β -binding peptide	cyclic	CHC-derived sequence	<ul style="list-style-type: none"> Inhibition of Aβ aggregation Decrease of the aggregation potency and Aβ induced neurotoxicity 	(38)
12	ZAb3 affibody	L-enantiomer	Selected by phage display technique	<ul style="list-style-type: none"> Inhibit Aβ aggregation Disassemble preformed oligomers 	(39)
13	H102 (HKQLPFEEED)	L-enantiomer	CHC-derived sequence	<ul style="list-style-type: none"> Inhibit Aβ_{42} fibrillization 	(40)
14	Th-NT, Th-CT, and Th-SC peptides, L-enantiomer	Trehalose conjugated	CHC-derived sequence	<ul style="list-style-type: none"> Inhibit fibril formation 	(41)
15	Fc-KLVFF	Ferrocene-conjugated peptide, L-enantiomer	CHC-derived sequence	<ul style="list-style-type: none"> Inhibit Aβ aggregation 	(42)
16	N-methylated A β (32–37)	L-enantiomer	C-terminal fragment	<ul style="list-style-type: none"> Inhibits Aβ aggregation Improve <i>Drosophila</i> longevity Increase locomotion 	(43)
17	D-Trp-Aib	D-enantiomer	Rational design	<ul style="list-style-type: none"> Inhibits Aβ oligomerization Decrease cerebral amyloid deposits in transgenic model Improves cognitive activity (<i>in vivo</i>) 	(44)
18	GABA-FPLIAlMA	D-enantiomer	C-terminal fragment	<ul style="list-style-type: none"> Inhibit and reduce the Aβ aggregation 	(22)
19	Tyr(Allyl-RCM)-Xaa-Gly(Allyl-RCM) and Gly(Allyl-RCM)-Xaa-Tyr(Allyl-RCM)	cyclic	CHC-derived sequence	<ul style="list-style-type: none"> Inhibit significantly the Aβ_{42} aggregation 	(45)
20	D(KLVFW)- aminobutyric acid (Aib)	D-enantiomer	CHC-derived sequence	<ul style="list-style-type: none"> High specific interaction with Aβ_{42} monomers and oligomers Remarkable inhibition of A Aβ_{42} fibril formation No cytotoxic effects 	(46)

affinity for A β compared with their L-enantiomeric counterparts. Moreover, D-peptides inhibit A β aggregation in animal models (28). Retro-inverso peptides are a special class of modified peptides that contain D-amino acids and reversed NH and CO groups in the peptide bonds. These peptides could keep the same spatial position in the side chain of the residues and preserve the desirable 3D structure compared to unchanged L-peptides (47). They also displayed advantages in terms of A β aggregation inhibition, higher proteolytic stability, lower self-assembly, and better blood-brain-barrier (BBB) permeability when compared with L-peptides in an animal model (48, 49). Fluorinated hydrophobic valine or phenylalanine in the LVFFA-based peptides can considerably delay the formation of A β aggregation. Fluorinated amino acids can also inhibit A β aggregation (50). Modification of amide functional groups with a methyl group is another strategy in the development of new inhibitors. N-methylated amide groups could enhance the peptide's solubility in aqueous solutions and decrease A β -induced toxicity. Cyclic peptides have a higher inhibitory activity than acyclic derivatives (51). Because of their high enzymatic resistance, they are degraded slowly. Residues of lysine and glutamic acid have been known to be effective stabilizing and enhancing agents of A β fibrillation due to their ability to improve surface tension. In contrast, arginine residues have been reported as aggregation inhibitors or destabilizers (chaotropes) (52).

The 16 KLVFF 20 -based peptides play a crucial role in disrupting A β aggregation by binding to full-length A β peptides and preventing fibril formation (53, 54). Ac-LVFFARK-NH₂ (LK7), designed by adding arginine and lysine to KLVFF, induced a dose-dependent inhibition on A β_{42} fibrillation; however, it was cytotoxic due to high self-assembling properties (55). When conjugated with poly (lactic-co-glycolic acid) nanoparticles (NPs), the LK7-PLGA-NPs complex resulted in the elimination of the LK7 self-assembly feature while inhibiting A β_{42} fibrillation (55). Binding β -cyclodextrin to LK7 (56) improved LK7 peptide solubility, inhibited its tendency to self-aggregate, improved its binding to A β , and inhibited A β aggregation. Head-to-tail cyclization of LK7 peptide also resulted in a decrease in self-assembly of the LK7, an increase in binding affinity to the A β_{40} peptide, and proteolytic stability in serum. This derivative also can stabilize the A β_{40} secondary structure and inhibit A β_{40} -mediated cytotoxicity. Another derivative of LK7 peptide is Ac-LVFFARKHH-NH₂ (LK7-HH), in which LK7 has been conjugated to the HH ligand as a chelator for reducing reactive oxygen species (ROS) production and capturing free and complexed ions of Cu $^{2+}$ (57). This chelator also improved the anti-aggregate effects of LK7 on A β peptide and reduced its self-aggregation properties.

Proline and aspartic acid were exchanged for valine and alanine, respectively, in KVLFFA (58, 59). The derived peptide, referred to as 5-mer iA β 5 with sequence LPFFD, inhibited A β aggregation, neurotoxicity, and reduced plaque load (58). Due to the lack of a proton on the secondary substituted nitrogen in the peptide bond of proline residue, it could inhibit the formation of the intramolecular hydrogen bonds into fibrils. Since these small peptides are prone to faster enzymatic degradation and have reduced BBB permeability *in vivo*, iA β 5p was modified by N-methylation between Pro and Phe residues to improve its stability (60). The results from *in vitro* and *in vivo* studies showed that it has the same inhibitory activity as the parental iA β 5 peptide against amyloid fibril formation and neurotoxicity but with improved protease resistance. Also, molecular dynamics

simulations show that this peptide has more durable binding and enhanced activity against A β ₄₀ aggregation in comparison to the iA β 5 peptide. In a similar study, the RIVFF sequence was produced by residue mutations of lysine16 (K) to arginine (R) and leucine17 (L) to isoleucine (I) on the KLVFF segment (61). The results indicated that this peptide could self-aggregate into β -sheet structures by reducing the surface tension of water and at higher concentrations (>250 μ M) enhanced the A β -induced cytotoxicity.

The peptide D-GRKKRRQRRR-GGGG-DVEFRH (A β ₁₋₆ A2V-TAT) was investigated *in vivo* (62, 63). It was generated by modifying the N-terminal fragment of ¹DAEFRH⁶ through mutation of alanine in position 2 to valine and conjugating with the HIV protein transduction domain GRKKRRQRRR (TAT). The resulting peptide showed strong anti-amyloidogenic effects *in vitro* and A β aggregation inhibition in mouse models of Alzheimer's disease (64). The KLVFWAK motif was designed based on the ¹⁶KLVFFAE²² sequence with mutations introduced at phenylalanine and glutamic acid residues to tryptophan and lysine respectively to enhance solubility and disrupt self-assembly via electrostatic repulsion. Results showed that the designed motif could only target the C-terminal region of A β oligomers. The designed motif exhibited a lower self-aggregation tendency in comparison to other KLVFF-related sequences. Moreover, it demonstrated a higher binding affinity to A β aggregates and fibrils than monomers (65).

RGKLVFFGR (OR1) and RGKLVFFGR-NH (OR2) are retro-inverso peptides (66), designed by the addition of arginine (R) and glycine (G) to the KLVFF sequence. They exhibit high solubility and stability against enzymes. However, only the OR2 peptide showed inhibitory effects on A β oligomer formation and cytotoxicity. OR2 was modified to HN-rGklvffGr-Ac (RI-OR2) by acetylation of the C-terminal residue (49). The result illustrated that the peptide has a high resistance to proteolysis, while maintaining the same inhibitory activity *in vivo*. In a follow-up study, the RI-OR2 peptide was attached to the TAT peptide to improve its permeability into cells and the BBB (48). The results showed the peptide was able to decrease A β aggregation, plaque levels, and oxidative damages as well as increase the number of young neurons in the brain.

³¹IIGLMVGGVVIA⁴² and ³⁹VVIA⁴² sequences were designed based on the C-terminal domain of A β ₄₂ (67). The ³⁹VVIA⁴² sequences could interact with A β ₄₂ monomers and smaller oligomers at several sites, specifically at the N-terminal domain. At micromolar concentrations, the VVIA-NH₂ peptide inhibited A β ₄₂ aggregation, exhibited less toxicity, and protected synaptic activity. However, these effects were not observed for the acetylated Ac-VVIA sequence (68). The non-acetylated VVIA-NH₂ sequence particularly interacts with the C-terminal domain while the Ac-VVIA peptide has a dispersed binding distribution (68). The Ac-³²IIGLMVG³⁷-NH₂ sequence, a hexapeptide from the C-terminal fragment, has been shown to have a moderate efficacy with less toxicity (69).

O-acyl isopeptide and NMe-b-Ala26 (70) were derived from the full-length A β sequence with modification of an ester bond at the Gly25-Ser26 moiety and an N-methyl amide- β -Ala26, respectively. O-acyl isopeptide inhibited A β ₄₂ fibrillation at equimolar concentrations through an inhibitory mechanism distinct from any other peptidic inhibitors reported previously. Also, this derivative was more soluble than A β ₄₂ peptides and rapidly decomposed to A β ₄₂ monomers under physiological conditions through an O-to-N acyl rearrangement reaction whereas NMe-b-Ala26 showed higher chemical stability at physiological conditions.

Non-A β -based peptide inhibitors

Carnosine, a natural imidazole-containing dipeptide is a metal ion chelator (71). It inhibits the fibrillation and toxicity of amyloidogenic species such as glycosylated α -Crystallin, A β peptide, and prions. This peptide also inhibits the intramolecular salt bridge formation, which is vital to the stability and elongation of fibrils (71). Peptide D1, QSHYRHISPAQV (72), is another non-A β peptide that reduces A β aggregation and A β -associated cytotoxicity at high concentrations. N-methylated proprietary peptides such as D-NH₂ (SEN304) and SEN1576 can inhibit A β -associated toxicity *in vivo* (73). Furthermore, SEN304 is a more potent inhibitor than customized versions of the KLVFF peptide. These peptides could interfere with the nucleation of A β , convert them into non-toxic forms, and eliminate toxic oligomers.

PEPTIDE LIBRARY SCREENING

There are many screening approaches to identify target-specific ligands (74, 75). Phage display is one such efficient high-throughput screening method that allows the screening of a wide variety of peptide libraries to identify specific peptide sequences against the desired target (76, 77). Wang et al. synthesized a linear peptide with sequence PYRWQLWVHNWS selected based on the screening of a randomized 12-mer peptide library against the target A β ₁₋₁₀ sequence (78). After screening, specific phages were selected and their binding affinity to A β ₁₋₁₀ was evaluated by real-time biomolecular interaction analysis. This peptide could specifically bind to A β ₁₋₁₀, inhibit the aggregation of A β into plaques, and reduce A β ₁₋₄₂ induced-apoptosis. Furthermore, it illustrated a protective effect against A β ₁₋₄₂-induced memory and learning impairments in animal models (59).

Larbanoix et al. utilized the phage display method to discover a linear hexapeptide against A β ₁₋₄₂ aggregation (79). Two of the selected clones, Pep1: LIAIMA and Pep2: IFALMG, corresponding to fragment ³¹IIGLMV³⁶ from A β ₁₋₄₂ peptide, demonstrated the highest binding affinities to A β ₁₋₄₂ with K_d values in the micromolar range. Their specific interactions with A β ₁₋₄₂ plaques were identified by immunohistochemistry on harvested brain tissue from an animal model of Alzheimer's disease. The peptides did not induce any toxicity in neurons *in vitro*. Moreover, the thioflavin T aggregation assay indicated that the designed peptides could suppress the amyloid fibril formation.

In 2010, a random heptapeptide library (XX-P-XXXX) on T7 phage was reported by Kawasaki et al. (80). The library was designed based on the LPFFD sequence XX-P-XXXX, where P is proline, and X is any amino acid. After the fifth-round of biopanning against A β ₁₋₄₂ soluble oligomers, eight new peptides containing arginine residues were obtained. The peptide with the strongest affinity to A β (RGPRGRV) suppressed the formation of 37–48 kDa oligomers and maintained the monomeric form of A β ₁₋₄₂ for up to 24 h. In follow-up studies, to assess the effect of the peptide length on the inhibition of soluble oligomers formation, random libraries containing 3-residue and 4-residue peptides were prepared by phage display and evaluated. The results demonstrated that the 3-residue peptides could not significantly inhibit oligomers formation because of their

smaller size. In contrast, the 4-residue peptide with the RFRK sequence inhibited the soluble oligomer formation like the heptapeptide (RGPRGRV). It also showed a slight decrease on A β fibrillation (81), similar to the inhibitory activity of the N-Methylated Peptide (SEN304), against A β_{42} aggregation (25, 82, 83). Tsuji-Ueno et al. utilized the all-steps-all-combinations (ASAC) method to explore A β_{42} -binding peptide aptamers. The identified peptides from the primary and secondary libraries showed a weak binding affinity to A β_{42} (Kd values in the μ m range) (84). To further improve the peptide aptamers, Gautam et al. applied the mRNA display technique and paired-peptide library method. The library was assembled by a random shuffling method on selected peptide blocks taken from the formed primary and secondary peptide libraries by Tsuji-Ueno et al. (84). They reported two peptides with high binding affinity to A β_{42} (Kd in the nM range) which significantly inhibited the A β_{42} aggregation (85). The improved peptide aptamers, P84 (CGILDPIPWGGSGGSCGILDPIPW) and P131 (GCPCIGIIGSGGSDCSSDLTPS), where GGSGGS is the linker sequence, demonstrated a higher binding affinity for the A β_{42} peptide (Kd values in the nanomolar range) compared to the primary and secondary A β_{42} -binding peptides (86). The results showed that both peptides could inhibit the A β_{42} aggregation and result in the reduction of the cytotoxic effects of A β_{42} fibrils and A β_{42} oligomers in PC12 cells; P84 showed better efficacy than P131 on the cell line.

Groen and co-workers employed mirror-image phage display to identify selective and high-affinity D-peptide ligands for A β_{1-42} . The D-enantiomer A β_{1-42} was used as a target for selection from a randomized 12- amino acid peptide library with more than 1 billion different peptides. After six rounds of biopanning, they identified a specific D-enantiomeric peptide, RPRTRLHTHRNR, called D3 (73). The D3 ligand inhibited A β aggregation, and dissolved pre-formed A β fibrils. Additionally, D3 ligand could disaggregate pre-existing amyloid plaques in the brain and result in an increase in the amount of A β monomeric form, which has high clearance from the brain (87). FITC fluorescence data demonstrated that A β -D3 clearance might have been associated with pericytes, which have a major role in the clearance of different A $\beta_{40/42}$ species (88, 89). Glial fibrillary acidic protein (GFAP) staining of astrocytes and CD11b staining of microglia in brain sections revealed that the D3 significantly decreased the amount of plaque-related inflammation markers (active astrocytes and microglia) around the A β plaques in comparison to the untreated animals. In addition to the anti-inflammatory properties, this peptide ligand could drastically reduce the A β plaque load in brain tissue of transgenic APP-PSD mice after a 30-day treatment with administration of 9 mg D3 per day per mouse. Computational simulation studies demonstrated strong electrostatic interactions between the arginine-rich D3 and negatively charged groups of A β nonamer; D3 binding to A β nonamer could change the topology of the A β oligomers by inducing a twist in them and consequently promote the formation of A β nonfibrillar aggregations (73, 90, 91).

Luo et al. applied peptoid chemistry, N-substituted glycine oligomers as a class of peptidomimetics, to develop and improve selective high-affinity ligands for A β_{42} (92). They constructed an on-bead peptoid library of 38,416 unique peptoids. After screening for A β_{42} -selective peptoid ligands, the IAM1 ligand and its dimeric form were selected and further evaluated. IAM1 peptide showed about 10-fold more affinity for A β_{42} -binding than for A β_{40} , and inhibited A β_{42} aggregation *in vitro*. The dimeric derivative (IAM1)₂ demonstrated a 7.4-fold higher

affinity for $A\beta_{42}$ (60 nM) than the monomeric form. Moreover, (IAM1)₂ demonstrated neuroprotective effects on primary hippocampal neurons against $A\beta_{42}$ -induced toxicity.

Due to the considerable similarities between the self-assembly of cyclic d,l- α -peptides and amyloid structures, it is possible such peptides can bind to $A\beta$ non-toxic forms and stabilize them (29). Richman et al. described the cyclic peptide CP-2, cyclo-[l-J-w- H-s-K]s (J denotes l-norleucine), by screening a 6-residue library of head-to-tail cyclic d,l- α -peptides consisting of residues Lys, Glu, Ser, Leu, Trp, and His using a one-bead-one-peptide combinatorial approach (29, 93). The selected peptide strongly interacted with $A\beta_{40}/A\beta_{42}$ and prevented their assembly, entirely disassembled $A\beta_{40}$ fibrils, and protected PC12 cells against $A\beta_{40}/A\beta_{42}$ -induced toxicity, without having any toxic effects of its own. NMR spectroscopy revealed that the CP-2 peptide, in a self-assembled form, interacted with monomeric and low-oligomeric structures of $A\beta_{40}$ and induced weak α -helix structures during the initial stage of $A\beta_{40}$ aggregation and subsequently promoted the conformational transition shift from a more toxic antiparallel β -sheet conformation to the less toxic parallel β -sheet.

In another study, Acerra et al. utilized an intracellular protein-fragment complementation assay (PCA) methodology for the screening of selective high-affinity peptides to $A\beta$ (94). The $A\beta_{25-35}$ sequence, known to self-assemble into toxic fibrils (95), was inserted into one half of the murine dihydrofolate reductase enzyme as a target, and the $A\beta_{29-35}$ sequence-based peptide was inserted on the other half of the enzyme (96). After the screening of primary and secondary libraries, two new targeting peptides L2P1, FSKATSN, and L2P2, PVKATTA were selected. These peptides shared no homology with the starting template $A\beta_{29-35}$. The results showed that all selected peptides could bind $A\beta_{42}$, inhibit fibril formation, and disaggregate pre-formed fibrils. To further improve the metabolic stability of selected peptides from primary and secondary libraries, their retro-inverso (RI) analogs were evaluated (86). All RI peptide ligands, such as KAR-RI, L2P1a-RI, L2P1b-RI, L2P2a-RI, and L2P2b-RI, inhibited $A\beta$ fibrillation and disaggregated pre-formed fibrils, and reduced $A\beta_{42}$ -induced toxicity in PC-12 cells.

THE CURRENT STATE AND FUTURE DIRECTIONS OF $A\beta$ INHIBITORY PEPTIDES IN ALZHEIMER'S DISEASE

A wide range of peptide-based inhibitors has been evaluated in cellular and animal models as new therapeutic compounds for inhibition of $A\beta$ aggregation. While experimental studies generated promising results, only a few of these inhibitory peptides have been successful enough to enter clinical trials. NAP or Davunetide peptide with NAPVSIPQ sequence, derived from the activity-dependent neuroprotective protein (ADNP), was reported in 2003 by Gozes et al. (97). NAP was able to inhibit $A\beta$ aggregation, disassemble pre-formed fibrils, and protect the neuronal cells from $A\beta$ induced toxicity. Though NAP demonstrated benefits in phase II clinical trials for mild cognitive impairment, it failed in a phase III trial (98–100). PPI-1019 peptide (APAN), with a sequence of D-(H-[(Me-L)-VFFL]NH₂), is an N-methylated peptide which is derived from the D-enantiomeric

Cholyl-LVFFA-NH₂ that could inhibit A β aggregation and the induce toxicity in experimental studies (101). The phases I and II clinical trials of APAN was completed in patients with mild-moderate Alzheimer's disease in 2005 ([NCT00100282](https://clinicaltrials.gov/ct2/show/study/NCT00100282), [NCT00100334](https://clinicaltrials.gov/ct2/show/study/NCT00100334)), but the outcome of this study is still unknown (<https://clinicaltrials.gov/>, last assessed 28 October 2020). Other reported inhibitory peptides including, D3 (102), D-Trp-Aib-OH (44), D-4F (36), TAT-R1-OR2 (48), NL-R1-OR2-TAT90 (103), and R1-OR2 (49) have shown considerable efficacy in pre-clinical trials, but they have not yet entered clinical trials.

The reality is that, to date, there is no cure for Alzheimer's disease. Only optimism remains. Therefore, it is necessary to discover potential peptides for testing in clinical trials. The current inhibitory peptides have certain limitations such as poor BBB permeability and high cytotoxicity. To overcome these problems and further improve the inhibitory activity, a number of studies have focused on peptide-nanostructure conjugates (PNCs) approach that provides an opportunity to increase the capabilities of both these classes of materials (55, 104, 105). Nanostructures could be considered as a potential vehicle to overcome poor BBB permeability and bring hope for neurodegenerative diseases therapy due to their size and various surface modifications (106). As an example, multivalent inhibitors can be developed against A β aggregation by decorating gold nanoparticles with VVIA and LPFFD (107). The PNCs approach gives a fascinating insight into the fields of diagnosis and treatment, and provides new opportunities for the design of high-performance peptides (108, 109).

CONCLUSION

Despite a better understanding of the pathogenic mechanisms of Alzheimer's disease, finding efficient therapeutic compounds to prevent or halt the progression of Alzheimer's disease continues to be a challenge. A β aggregation inhibition-based approaches are being developed with the aim to stop disease progression. While the reported inhibitory peptides have considerable advantages over other compounds, and experimental evidence has been encouraging, bench-to-bedside has not yet become a reality. Therefore, adequate knowledge of binding interactions of these peptides with their biological targets, the ligand-target complex, is required to design more accurate therapeutic biomolecules. Peptide inhibitors have unique properties, particularly, high selectivity, low accumulation in tissues, low side-effects and toxicity, and different chemical and biological synthesis routes when compared with other compounds. As researchers continue to focus on rational design, characterization, optimization, and interaction between the inhibitor and the A β peptide complex, more peptide inhibitors are expected to succeed in clinical trials.

Conflict of Interest: The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this chapter.

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