Design strategies for anti-amyloid agents

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Numerous diseases have been linked to a common pathogenic process called amyloidosis, whereby proteins or peptides clump together in the brain or body to form toxic soluble oligomers and/or insoluble fibres. An attractive strategy to develop therapies for these diseases is therefore to inhibit or reverse protein/peptide aggregation. A diverse range of small organic ligands have been found to act as aggregation inhibitors. Alternatively, the wild-type peptide can be derivatised so that it still binds to the amyloid target, but prevents further aggregation. This can be achieved by adding a bulky group or charged amino acid to either end of the peptide, or by incorporating proline residues or *N*-methylated amide groups.

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Abbreviations

Aβ	β-amyloid peptide
AD	Alzheimer's disease
CR	Congo red
IAPP	islet amyloid polypeptide
polyQ	polyglutamine
TTR	transthyretin

Introduction

Alzheimer's disease (AD) has long been associated with the accumulation of insoluble amyloid 'plaques' in the brain. These plaques form by a process called amyloidosis, whereby a 40- to 43-residue peptide called β-amyloid (Aβ) aggregates into insoluble fibres. Many other neurodegenerative diseases have been associated with the aggregation of specific proteins or peptides in various parts of the brain, including α-synuclein in Parkinson's disease, huntingtin in Huntington's disease, prions in the spongiform encephalopathies and transthyretin (TTR) in transthyretin amyloidosis [1,2]. Furthermore, several non-neurodegenerative ageing-related diseases have been associated with the aggregation of specific proteins or peptides in other parts of the body. In type II diabetes, for example, a 37-residue peptide called islet amyloid polypeptide (IAPP, also known as amylin) aggregates to form insoluble amyloid fibres in the islet cells of the pancreas, which contain the β cells that produce insulin [3]. The role of amyloidosis in AD has been a topic of intense debate because the distribution of amyloid plaque in the brain tends to correlate rather poorly with the specific regions of the brain that are actually affected by the disease, leading some to believe that amyloid plaques might simply form as a downstream effect of the disease or may even help to protect against the real cause of the disease, rather than actually cause it [4,5]. A resolution to these anomalies may be that the soluble oligomeric form of the amyloid is the principal pathogenic form, rather than the mature plaque [6,7].

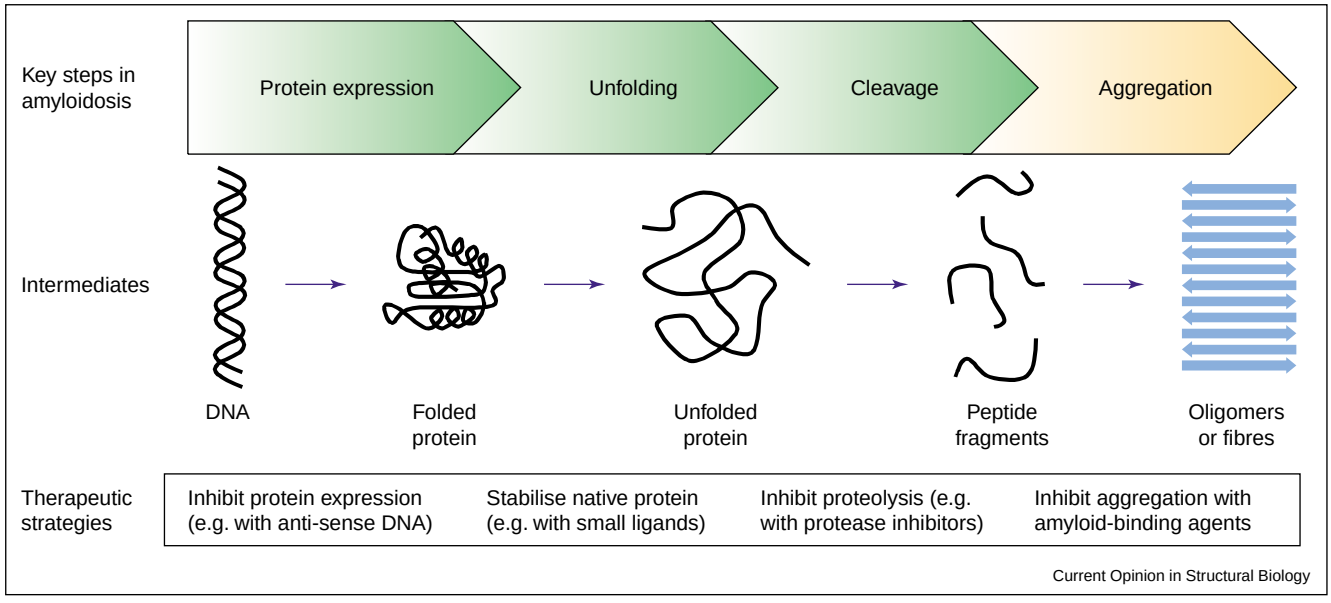
Alternative strategies to treat amyloidosis

Numerous groups are developing treatments designed to block various key steps in the amyloidosis process. Specific therapeutic strategies currently being pursued include (Figure 1) [8–10]:

1. Inhibiting expression of the amyloidogenic protein or stabilizing its native form using small organic ligands.
2. Inhibiting release of the amyloidogenic peptide from its parent protein using protease inhibitors.
3. Inhibiting aggregation of the protein or peptide directly using small ligands or indirectly by vaccination.
4. Inhibiting other effects of the disease that may or may not be directly associated with amyloidosis (e.g. inflammation and oxidative stress) — replacing cells that have been killed by the disease (e.g. by cell or gene therapy) and alleviating the symptoms of the disease, but without necessarily blocking the pathogenic process.

The most effective treatments may be those designed to inhibit steps that precede protein/peptide aggregation, by blocking production of the amyloidogenic protein or peptide in the first place. However, this requires blocking the expression or activity of a natural protein or peptide that has presumably evolved to perform some other, important biological function *in vivo*. For example, many large pharma companies are currently developing inhibitors of β- or γ-secretase as potential drugs for AD. These two enzymes cleave amyloid precursor protein to produce the Aβ peptide associated with the disease, but they have also been shown to perform other, important biological functions [11], so it may not be possible to identify any inhibitors of these enzymes that are safe enough for use as drugs *in vivo*. Moreover, this strategy is inapplicable to Parkinson's disease, Huntington's disease, Creutzfeldt–Jakob disease and type II diabetes, for which it is the

Figure 1



Alternative therapeutic strategies to block amyloidosis.

full-length protein or peptide that aggregates, rather than an intermediate within the protein degradation pathway.

On the other hand, treatments designed to target steps that follow protein/peptide aggregation are less likely to be effective because they would not prevent the formation of toxic soluble oligomers or insoluble fibres, which could continue to kill cells. Thus, an attractive therapeutic strategy in principle is to inhibit and preferably reverse protein/peptide aggregation itself, because this appears to be the first step in the pathogenic process of amyloidosis (Figure 2) that is not associated with some natural biological function [12]. In this review, we discuss a range of molecules recently found to inhibit aggregation. Most work in this field has focused on Aβ, although a wider range of targets have been addressed in recent years.

Aggregation inhibitor molecules

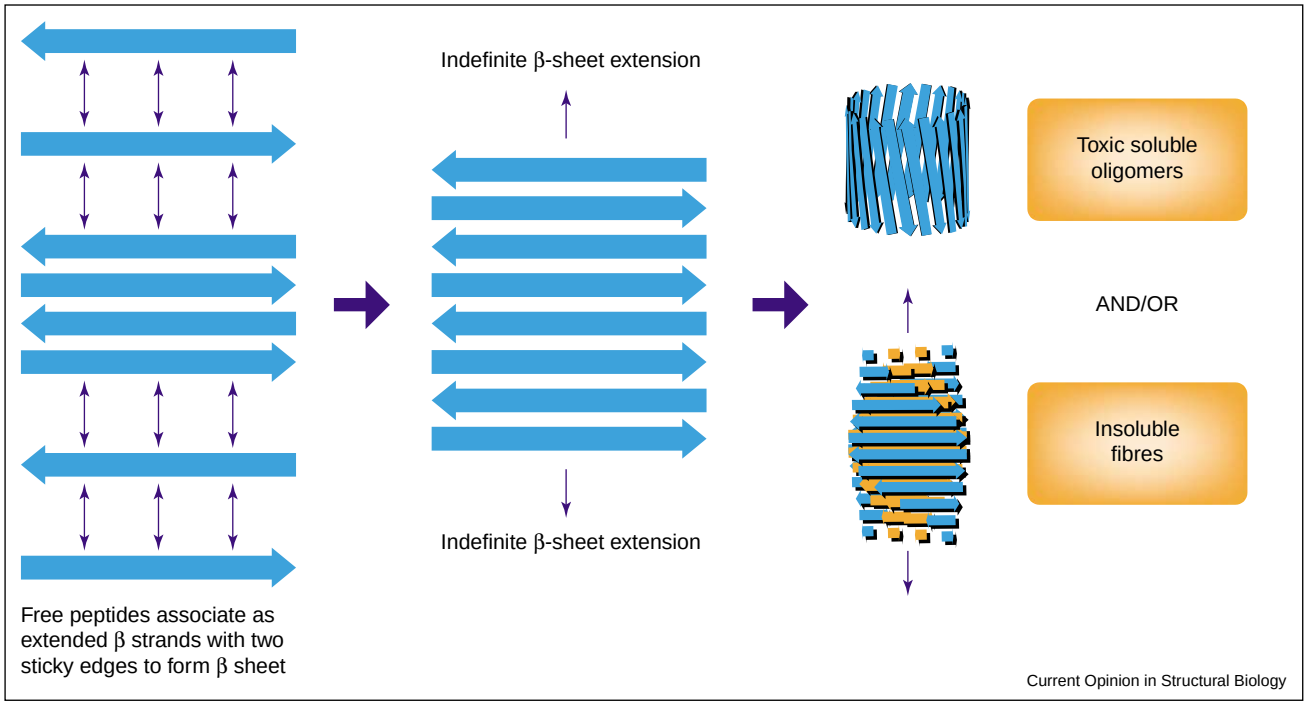
A vast range of diverse molecules have been studied as potential inhibitors of amyloid formation. Here, we discuss some of these, illustrating the types of compound found to be effective (Figure 3).

Tetrameric TTR, involved in thyroxine transport, can form amyloid fibrils, leading to TTR amyloidosis. Using mass spectrometric methods, McCammon *et al.* [13] found 18 ligands (*N*-phenyl phenoxazines and flufenamic acid derivatives) that function as inhibitors of amyloid formation through their ability to stabilize the tetrameric structure of human wild-type TTR and amyloidogenic TTR variants V30M and L55P. Another series of TTR amyloidosis inhibitors has been studied that function by stabilizing the monomeric native state, hence increasing the kinetic barrier associated with misfolding [14•].

Congo red (CR) is a hydrophilic symmetrical sulfonated azodye that binds specifically to amyloid fibrils in an as yet unidentified manner. Studies on CR binding have suggested that it can inhibit Aβ aggregation in AD [15]. Sanchez *et al.* [16••] showed that CR was also able to promote the clearance of expanded polyglutamine (polyQ)-containing aggregates (present in Huntington's disease) both *in vivo* and *in vitro*. Several derivatives of CR, as well as thioflavin S, chrysamine G and direct fast yellow, are also effective inhibitors of huntingtin protein aggregation in a dose-dependent manner [17].

A great number of diverse organic compounds have been found to inhibit or reduce the aggregation of Aβ into fibrils *in vitro*. These include nicotine (some rare good news for smokers) [18], β-cyclodextrin [19], hemin and related porphyrins [20], anthracycline 4′-iodo-4′-deoxydoxorubicin [21], hexadecyl-*N*-methylpiperidinium (HMP) bromide [22], rifampicin [23], (−)-5,8-dihydroxy-3R-methyl-2R-(dipropylamino)-1,2,3,4-tetrahydronapthalene [24] and melatonin [25]. Salvianolic acid B also reduces PC-12 cellular toxicity of aged Aβ [26]. Kiuchi *et al.* [27] tested type IV collagen, a molecule that localises in senile plaques of AD patients, as a potential inhibitor of amyloid. Thioflavin T fluorescence and electron microscopy studies demonstrated that collagen IV inhibited Aβ(1–40) fibril formation. Bartolini *et al.* [28] induced Aβ aggregation using human recombinant acetylcholinesterase and small molecules were tested for

Figure 2



Mechanism of peptide aggregation in amyloidosis.

their ability to inhibit the aggregation of Aβ. Molecules such as propidium, a peripheral anionic site ligand, decamethonium, donepezil and physostigmine were found to inhibit Aβ peptide aggregation. Propidium, decamethonium and physostigmine are known as acetylcholinesterase inhibitors, whereas donepezil is a drug already used by AD patients. Nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin have also been reported to inhibit human aluminium-induced Aβ and amylin aggregation *in vitro* [29]. More recently, Kim and Lee [30] found 1,2-(dimethoxymethano)fullerene was able to bind specifically to the 16–20 region of Aβ peptides with high affinity, thus inhibiting amyloid aggregation during the early stages.

Copper and iron are present in Aβ deposits and induce the production of hydrogen peroxide, which may mediate oxidative damage to the brain in AD [31]. Bush [32] developed metal-binding compounds that inhibit the *in vitro* generation of hydrogen peroxide by Aβ. These compounds are also able to reverse the aggregation of Aβ *in vitro* and from human brain post-mortem specimens. One of the compounds, clioquinol (CQ), a copper/zinc chelator, was given orally to APP2576 transgenic mice and induced a 49% decrease in brain Aβ deposition.

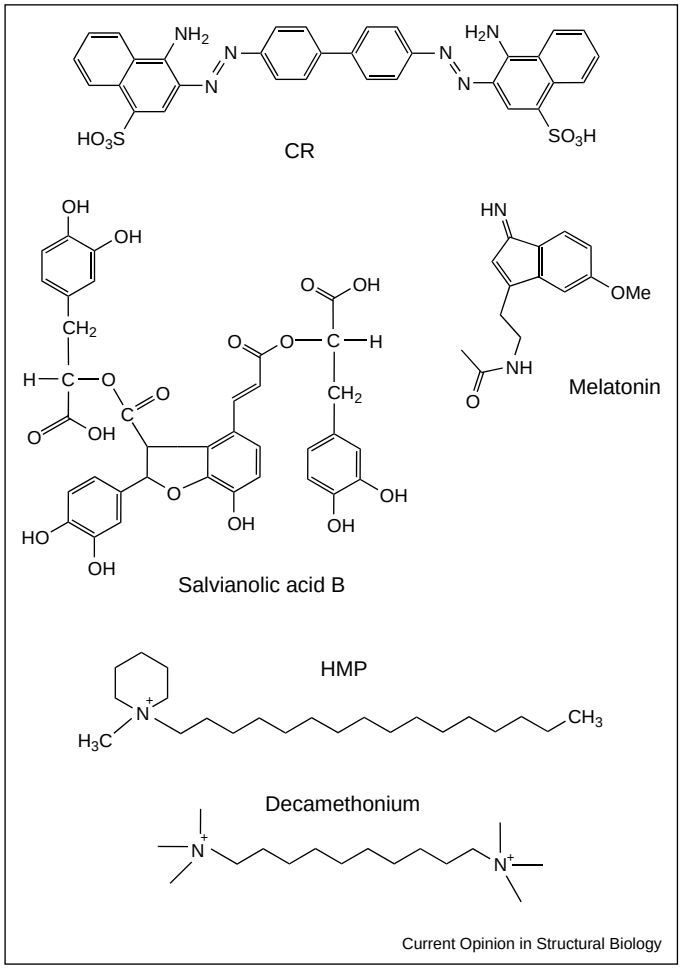
Immunisation of AD mouse models with Aβ significantly reduces both the density of cerebral amyloid plaques and the degree of cognitive impairment [33–35]. Furthermore, McLaurin *et al.* [36•] showed that immunisation with protofibrillar forms of Aβ(1–42) induced therapeutically effective IgG2b antibodies that recognize Aβ(4–10) and inhibit Aβ protofibril aggregation and toxicity. The antibody 1C2, which recognizes elongated polyQ chains, was also effective in inhibiting huntingtin protein aggregation [17].

Figure 3 shows examples of some of these Aβ inhibitors. Their lack of structural similarity is striking, suggesting that they bind to different sites within amyloid, a situation in contrast to most drugs, which bind to a single active site. This makes inferring conclusions from structure/activity relationships, and hence rational drug design, difficult.

Rational design of peptide-based inhibitors of amyloid aggregation

An attractive strategy to develop amyloid aggregation inhibitors is to start with the wild-type peptide as a lead, as it is already known to bind to itself (Figure 4). The first group to make use of a core section of Aβ as a potential drug lead was Tjernberg and co-workers [37], who showed that Aβ(16–20) is able to bind full-length Aβ and thus prevent its assembly into fibrils. Using molecular graphics simulations, they hypothesized that it bound stereospecifically and in an antiparallel conformation to Aβ [38]. Despite being shown to form fibrils in isolation, Aβ(16–20) was proposed to be a key region from which

Figure 3



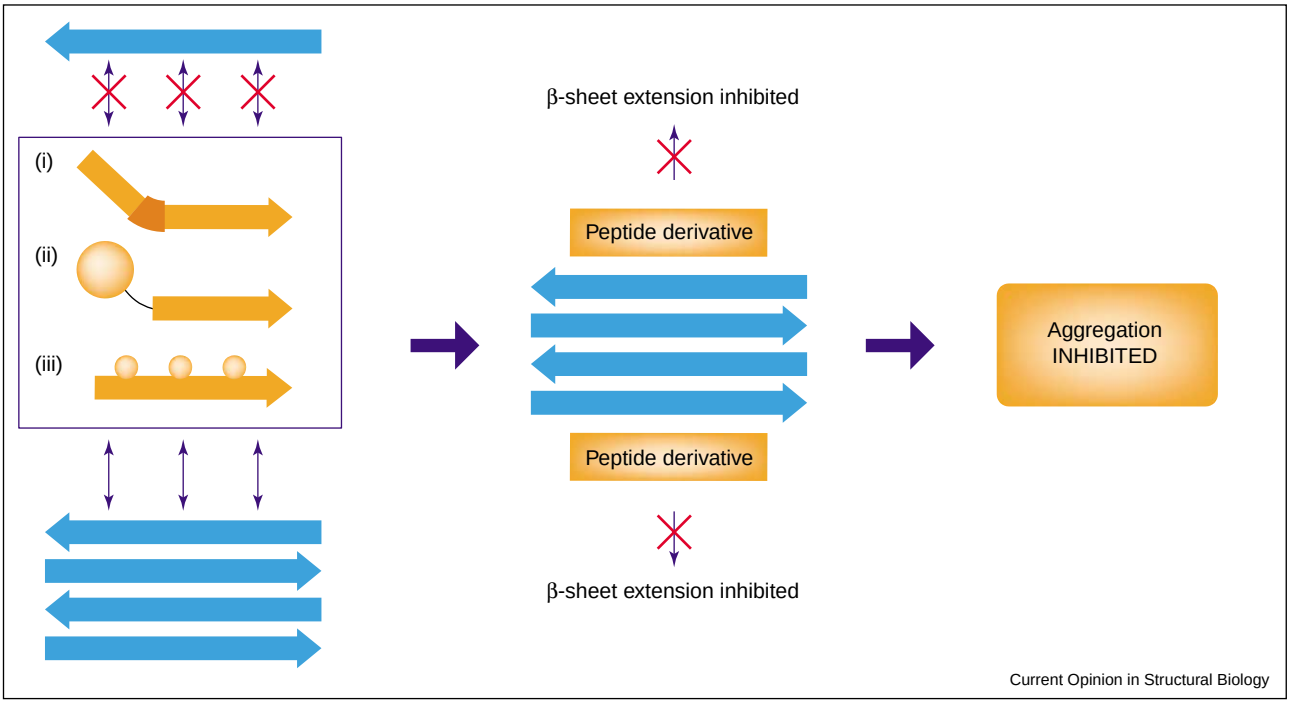
Examples of 'small-molecule' peptide aggregation inhibitors. HMP, hexadecyl-*N*-methylpiperidinium.

a lead compound could be created against amyloid. Ligands based on Aβ(16–20) and composed entirely of D-amino acids were comparable to all-L ligands in their ability to prevent fibril formation, with the additional benefit of protease resistance.

Soto *et al.* [39] also began work on inhibitors based on the core region of Aβ, in this case residues 17–21. The strategy is based upon substituting key residues for prolines in a bid to reduce the β-propensity of the peptide while retaining its hydrophobicity. In this way, the proline-containing Aβ(17–21) region may bind to aggregated Aβ and prevent further fibril growth (Figure 4). A lead eleven amino acid inhibitor was reduced to five amino acids with greater ability to prevent fibril formation. All-D analogues were again found to be as effective as all-L, but with increased protease resistance. These so-called β-sheet breaker peptides were shown not only to be stable *in vivo*, but also to be small and hydrophobic enough to have blood-brain barrier permeability [40,41]. Using prion protein 114–122 as a template, Soto [42] has also produced proline-containing β-sheet breaker peptides with the ability to prevent the conformational change of the prion protein to its toxic form.

Based initially on the 15–25 region of Aβ, Murphy and coworkers [43–45] designed a peptide with a 'recognition element' homologous to Aβ, but with a 'disrupting element', tagged to the C terminus, designed to interfere with Aβ aggregation (Figure 4). Having shown that at least three lysines are required as an appropriate disrupting element, the compound (KLVFFKKKK) showed considerable promise, as it accelerated Aβ aggregation kinetics, altered fibril morphology and reduced toxicity in MTT assays using PC-12 cells. The anionic disrupting compound KLVFFEEEE had similar effects, whereas the neutral disrupting compound KLVFFSSSS was ineffective, suggesting that the charged nature of the disrupting element is critical. These results were interesting because they implied that Aβ aggregation need not be

Figure 4



Inhibition of amyloidosis by synthetic peptide derivatives. **(i)** Proline introduced as β-sheet breaker. **(ii)** Terminal blocking group [e.g. cholyl or poly(lys)]. **(iii)** *N*-methylated amides to block one edge of β strand.

blocked to prevent toxicity and that the compounds perhaps work by accelerating aggregation to remove toxic soluble oligomers, or 'protofibrils'.

Findeis *et al.* [46] analysed many truncated variants of Aβ with a variety of different N-terminal modifications to establish a small yet effective inhibitor of Aβ polymerisation. Their strategy was to retain a peptide that could bind to Aβ and had a bulky group, such as a steroid, at its terminus to hinder further Aβ association (Figure 4). The all-D-amino acid peptide cholyl-LVFFA-OH was a potent inhibitor of Aβ polymerisation, but was all but cleared upon hepatic first pass, possibly because the cholyl group was recognised as an endogenous bile component [46].

Several groups are studying the incorporation of *N*-methyl amino acids into peptides as inhibitors of amyloidosis. These peptides again correspond to a region that is key to the amyloid protein. One side presents a hydrogen-bonding 'complementary' face to the protein, with the other having *N*-methyl groups in place of backbone NH groups, thus presenting a 'blocking' face (Figure 4). Hughes *et al.* [47•] have shown that *N*-methyl derivatives of Aβ(25–35) are able to prevent aggregation and inhibit toxicity in PC-12 cells. Meredith and co-workers [48,49] investigated *N*-methylated peptides of a region corresponding to residues 16–22 and subsequently 16–20 of the amyloid 'core domain' region. These peptides can prevent Aβ fibrils from forming and break down preformed fibrils. They have the added advantages of high proteolytic resistance, solubility, blood-brain barrier permeability [49,50] and propensity to form β-structure at the *N*-methylated site. Kapurniotu *et al.* [51] employed the same strategy against a region within residues 20–27 of IAPP. The use of larger substituents than a methyl to block hydrogen bonding is also effective [52].

Conclusions

Although it is not yet certain whether preventing amyloid proteins from aggregating will be therapeutically beneficial, numerous anti-amyloidogenic compounds have been developed. Such compounds can be discovered either by screening large libraries, derivatising a lead known to bind to amyloid, or by modifying core regions of the amyloidogenic peptide or protein. The common fibrillar structure adopted by diverse peptides and the success of general methods to produce inhibitors (Figure 4) offer the exciting possibility that a family of compounds may be produced to act as therapeutic molecules for a range of amyloidogenic diseases.

Acknowledgements

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