8

Inhibitors of Amyloid Aggregation: Technologies for the Discovery of Novel Lead Compounds

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Introduction

A variety of seemingly unrelated neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's diseases, CJD (the human form of 'mad cow' disease) and MND (motoneurone disease) have all been linked to a fundamental pathogenic process of protein/peptide aggregation called amyloidosis. In each case, a specific protein or peptide clumps together in a specific part of the brain to form toxic soluble oligomers and/or insoluble 'amyloid-like' fibres, which are widely believed to cause the progressive degeneration of neurones associated with these diseases.

By marked contrast with the successful application of traditional pharmaceutical technologies to other protein targets in drug discovery (see *Figure 8.1* taken from Drews, 2000), such as G-protein coupled receptors (GPCRs) and enzymes, the discovery of 'drug-like' lead compounds that can inhibit protein/peptide aggregation (amyloidosis) or any other form of protein–protein interaction *in vivo* has not yet proved to be so fruitful. Typically, the exploitation of novel therapeutic targets in the pharmaceutical industry through high-throughput screening (HTS) is a pivotal step in early stage drug discovery programmes. HTS often combines a number of technologies for the rapid screening of novel targets, but key issues to consider include assay configuration and the ability of a high-throughput screen to predict drug–target interactions accurately. This review highlights a number of issues

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Abbreviations: Aβ(1–40), 40-residue peptide of β-amyloid; Aβ(1–42), 42-residue peptide of β-amyloid; APP, amyloid precursor protein; CJD, Creutzfeldt–Jakob disease; CQ, clioquinol; CR, Congo red; ELISA, enzyme-linked immunoabsorption assay; GPCR, G-protein coupled receptor; HMP, hexadecyl-N-methylpiperidinium; HTRF, homogeneous time-resolved fluorescence; HTS, high-throughput screening; MND, motoneurone disease; NSAID, non-steroidal anti-inflammatory drug; SPA, scintillation proximity assay; TTR, tetrameric transthyretin.

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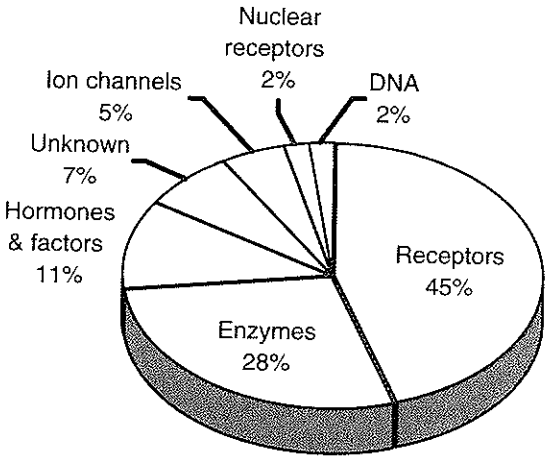

Figure 8.1. Biochemical classes of drug targets of current therapies. Data (taken and redrawn from Drews, 2000) illustrate the classes of drug targets defined by function and the relative percentages of those classes out of the 483 targets identified.

relating to the use of HTS to discover inhibitors of amyloidosis. Particular emphasis is placed on the mechanistic basis of drug–target interactions that is of prime importance in the design of HTS approaches. Furthermore, this review summarizes the known literature on a wide variety of small organic compounds that have been found by HTS and/or other serendipitous discoveries to inhibit amyloid aggregation.

An alternative, but complementary, strategy to screening large, diverse libraries of small organic molecules has been to design and test individual derivatives of the wild-type peptide that still bind to the amyloid target but prevent further aggregation. In particular, this review describes the latest research on a novel class of short synthetic N-methylated peptide derivatives ('meptides') that can inhibit and reverse this fundamental pathogenic process of protein/peptide aggregation *in vitro*. Meptides could be used in combination with HTS to design and screen entire combinatorial libraries comprising more drug-like amyloidosis inhibitors to discover novel lead compounds.

Amyloidosis as a common pathogenic mechanism

Alzheimer's disease 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 42-residue peptide called β-amyloid (Aβ(1–40) or Aβ(1–42)) aggregates into insoluble fibres that clump together. Many other neurodegenerative diseases have been associated with the aggregation of specific proteins or peptides in various parts of the brain, including the aggregation of α-synuclein in Parkinson's disease, huntingtin in Huntington's disease, prions in CJD, and superoxide dismutase in MND (Dobson, 1999; Murphy, 2002). 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 maturity-onset diabetes, for example,

a 37-residue peptide called amylin aggregates to form insoluble amyloid fibres in the islet cells of the pancreas, leading to the death of the β-cells that produce insulin (Clark *et al.*, 1987). In total, at least 20 otherwise unrelated diseases have now been associated with amyloidosis, or some other form of protein/peptide aggregation.

The role of amyloidosis in Alzheimer's disease has been a topic of intense debate. This is due, in part, to the observation that the distribution of amyloid plaques in the brain tends to correlate rather poorly with the specific regions of the brain affected by the disease. This has led some to believe that the amyloid plaques might form simply as a downstream effect of the disease, or even may help to protect against the real cause of disease, rather than actually give rise to it (Joseph *et al.*, 2001; Robinson and Bishop, 2002). Mounting evidence, however, suggests that Alzheimer's and all these other diseases really are caused by amyloidosis, or at least some other form of protein/peptide aggregation (Hardy and Higgins, 1992; Hardy and Selkoe, 2002). The evidence for this hypothesis is summarized as follows. Firstly, the fact that amyloidosis seems to be the only common link between at least 20 otherwise unrelated diseases suggests that this process does not play any kind of defensive role, since it is clearly not effective in this regard. Secondly, various hereditary forms of Alzheimer's disease have been linked to genetic mutations where the protein or peptide fragment encoded by the mutant gene is more prone to aggregation, promotes aggregation of some other protein or peptide, or is over-produced (Wisniewski *et al.*, 1991; Citron *et al.*, 1992; Cai *et al.*, 1993; Suzuki *et al.*, 1994). Thirdly, many transgenic mouse models of these diseases have been made by promoting amyloidosis *in vivo*, and these have been shown to develop some functional deficits that resemble those of the human diseases (Dodart *et al.*, 2002). Finally, the proteins and peptides associated with these diseases have also been shown to aggregate into soluble, β-sheet-rich oligomers, which are toxic to cells *in vitro* (Pike *et al.*, 1993). Moreover, compounds which can inhibit their aggregation have been shown to reduce their inherent toxicity *in vitro* and, in one or two cases, the associated neurodegeneration *in vivo* (Soto *et al.*, 1996, 1998, 2000). Hence, targeting amyloidosis could well be a viable therapeutic strategy for their effective treatment (Mason *et al.*, 2003).

Alternative strategies to treat amyloidosis

A number of companies are known to be developing treatments designed to block various key steps throughout the amyloidosis process (de Felice FG, 2002; Conway *et al.*, 2003; Lahiri *et al.*, 2003). Specific therapeutic strategies currently being pursued include: 1) inhibiting the expression of the amyloidogenic protein, or stabilizing its native form using small ligands; 2) inhibiting release of the amyloidogenic peptide from its parent protein using protease inhibitors; 3) inhibiting aggregation of the amyloidogenic protein or peptide directly (the focus of this review); 4) inhibiting other effects of the disease which may or may not be directly associated with amyloidosis (e.g. inflammation and oxidative stress); 5) replacing cells that have been killed by the disease (cell therapy); and 6) developing drugs to alleviate the symptoms of the disease but without necessarily blocking the pathogenic process itself.

The most effective treatments could be argued to 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 inhibiting 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, some companies are currently developing inhibitors of β- or γ-secretase as potential drugs for Alzheimer's disease. These two enzymes cleave a protein called APP (amyloid precursor protein) to produce the β-amyloid peptide associated with this disease, but they have also been shown to perform other important biological functions. Consequently, it may not be possible to identify any inhibitors of these enzymes with an adequate safety profile for human use (Haass and de Strooper, 1999). On the other hand, treatments designed to target steps that *follow* protein/peptide aggregation may be well tolerated, but are potentially less likely to be effective because they would not prevent formation of the toxic soluble oligomers and/or insoluble fibres, which could continue to kill cells. Thus, it follows that the most viable therapeutic strategy could be to inhibit, and preferably reverse, protein/peptide aggregation itself, since this appears to be the first step in the pathogenic process of amyloidosis which is not associated with some natural biological function (Lansbury, 1997).

Discovery of amyloid 'β-sheet breakers'

The soluble oligomers and insoluble fibres of amyloid are comprised of β-sheets, which form by the association of peptides as extended β-strands. The peptide β-strands associate with each other by forming hydrogen bonds between their peptide backbones and specific interactions between their amino acid side chains. Each β-strand has two free edges that can associate with either free edge of a second β-strand in this way, so many β-strands can aggregate together to form β-sheets, ultimately leading to the formation of soluble oligomers or insoluble fibres (*Figure 8.2*). That all these diverse proteins and peptides fold to form a common structure offers the exciting possibility that a single class of molecule may be developed as therapies for all these diseases. However, identifying potent and selective inhibitors of such

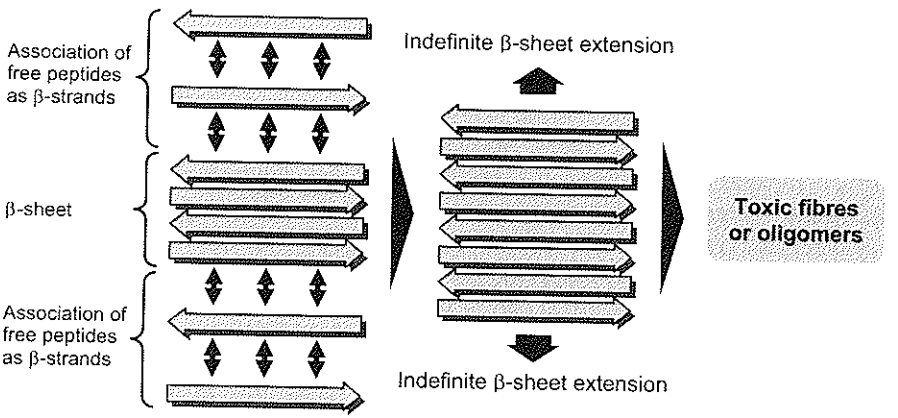

Figure 8.2. Mechanism of protein/peptide aggregation in amyloidosis.

protein–protein interactions is a challenging goal, and is seen by some as the 'holy grail of drug discovery'. The quest that all 'β-sheet breakers' face is how to disrupt a relatively flat surface interaction in a β-sheet of considerably greater than 140 Å^2 (Veselovsky *et al.*, 2002), rather than bind within a well-defined cleft, such as the active site or ligand-binding pocket of a specific enzyme or receptor. So far as we are currently aware from the literature, no 'designed', natural product or mass-screened potent small molecule inhibitor of protein–protein interactions between β-strands has yet been discovered that is sufficiently 'drug-like' for serious clinical development. Therefore, there is a clear need, and an exciting opportunity, to identify more drug-like lead compounds that work by this mechanism.

Drug discovery using HTS

One strategy to discover a novel lead compound is to use HTS to screen a large and diverse chemical library (Kenny *et al.*, 1998). Developments in instrumentation technology have led to significant changes in the way HTS is performed. As recently as 10 years ago, screening was still very much a manual process, and limited to the use of a number of bench-top instruments for all reagent dispensing, plate mixing, incubation, and plate reading, with the scientist transferring microtitre plates to each instrument by hand. This equipment was usually only able to handle a limited number of 96-well microtitre plates at a time. As a consequence, screening was both repetitive and labour-intensive. The introduction in the early nineties of robotics and automation to the screening laboratory has led to significant changes, both in the equipment used and, more importantly, in the way that screening is now approached. It is now possible to produce fully integrated, 'turn-key' screening systems combining the technologies of both industry and basic research (e.g. Banks *et al.*, 1997). The core of many screening systems is an industrial articulated robotic arm, mounted on a linear track. This links all instrumentation and other system hardware, enabling the transfer of microtitre plates to any location. The configuration of a typical HTS system of this type is illustrated in *Figure 8.3*. Such systems are capable of performing multiple assay types that could be suitable for screening for 'β-sheet breakers', including homogeneous (non-separation), ELISA, and filtration-based assays.

The majority of modern HTS systems are now capable of handling multiple readout formats, including radioisotopic, luminescence, colourimetric, fluorescence, time-resolved fluorescence, and fluorescence polarization. Data quality assurance issues often arise as a result of screen automation: automated systems are not compatible with poorly developed assays. Often, the key to success is a rigorous development process for each screen, which will typically include careful sourcing and batch testing of all reagents. Assay performance over a number of experiments also needs to be assessed to ensure that the assay performs reliably over a number of days. Full characterization of assay conditions should also include generation of data on assay kinetics where appropriate, and inclusion of controls and standard compound inhibitors. Tolerance to solvents should also be assessed, as organic solvents are frequently used to dissolve test compounds, most frequently dimethyl sulfoxide (e.g. Mellor *et al.*, 1997).

The second key hardware development in HTS has been miniaturization, driven

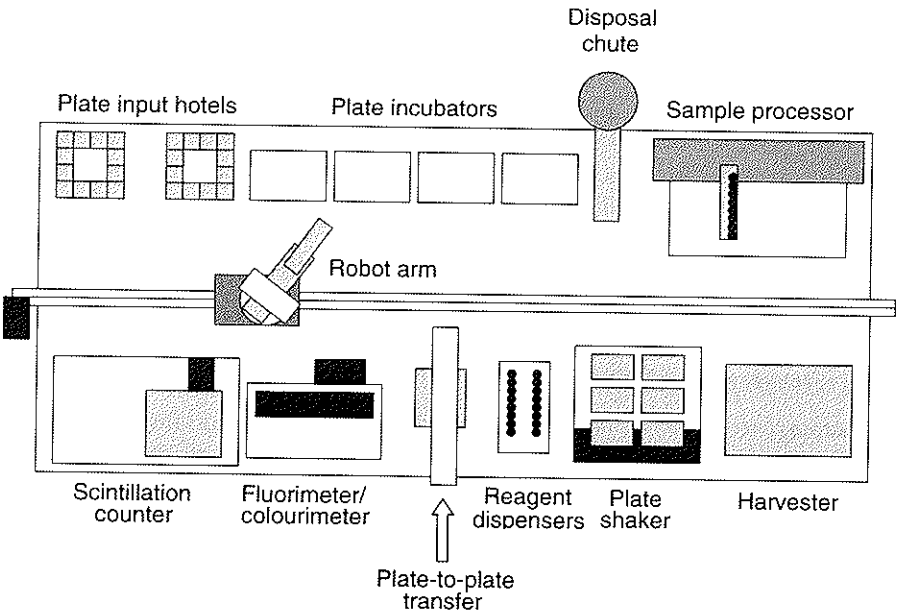

Figure 8.3. Schematic layout of a typical robotic screening system.

almost exclusively by cost and excessive compound depletion from screening libraries. This has led to the development of plates with the same footprint as the 96-well microtitre plate, but having increasingly higher density arrays (e.g. 384, 864, 1536 and 3456). Miniaturization with such plates can offer benefits in terms of reduction in the quantity of compound and reagent required for an assay, time taken to complete a screen, and reduced disposal costs. The technology to process these plates is also expanding rapidly, with instrumentation for the handling of 384-well plates now almost level with that of 96-well microtitre plates in terms of both availability and reliability. This enables screening scientists to run 384-well-based assays with the same reliability as that achieved with 96-well microtitre plates.

The development of 384-well plate capability has been very much an evolutionary process from 96-well technology. In many cases, the same equipment is used to process both plate types. A more revolutionary approach is required to enable higher density formats (1536 and 3456-well microtitre plates) to be used in routine screening. Liquid handling and plate reading instrumentation for high-density (sub-microlitre) formats relies on the development of very different technologies. For example, fluorescence polarization and homogeneous time-resolved fluorescence techniques will play a much more prominent role in miniaturized assays. These are sensitive, non-radioactive, homogeneous assay formats that lend themselves to very low volumes. The following sub-sections outline some of the key assay methodologies currently in general use that could potentially be adapted to screen for 'β-sheet breakers'.

SCINTILLATION PROXIMITY ASSAYS (SPA)

Scintillation Proximity Assays (SPA) utilize a homogeneous assay technique ideally suited for high-throughput screening. SPA relies on the observation that a β-particle emitted from a radioisotope will travel only a short distance in an aqueous buffer. On this basis, the close proximity of the emitted β-particle to a scintillant molecule leads to transfer of energy and light emission. In SPA, microspheres comprising a solid scintillant, usually a core of hydrophobic polymer polyvinyltoluene encased in a surface coating of polyhydroxy film, are coated with the target molecule or receptor of interest. When an isotope or labelled molecule binds to the target (i.e. is in close proximity), energy transfer from the β-particle will take place, resulting in light emission. When binding is inhibited and the isotopically labelled ligand remains free in solution, the energy of the β-particle dissipates in aqueous medium and no light is emitted.

The simplicity of SPA has meant that it is an ideal technique for HTS, especially as a means to automate screening (Kenny *et al.*, 1998). There are many advantages to SPA that can be incorporated into assay design for a screening programme: the need for separation of bound and free ligand through glass fibre membrane is eliminated, as is the need for addition of liquid scintillant. The assay protocol can be simplified, often to a three-step addition: 1) compound and/or unlabelled ligand control; 2) radiolabelled ligand; and 3) target-bead complex. The main disadvantage to SPA, as with all radioisotope-based techniques, are counting time and disposal issues. For SPA, the speed of counting is often rate-limiting.

SPA could potentially be adapted to screen for amyloid inhibitors by attaching the target amyloid peptide directly to the scintillation beads. Radiolabelled ligands that bind to the amyloid would stimulate the scintillant to emit light, thus indicating their potential activity as amyloid inhibitors. Alternatively, a radiolabelled form of the wild-type amyloid could be added to induce emission of light so that non-radiolabelled compounds can be screened directly for their ability to *reverse* the self-association of amyloid, or *vice versa* to screen for their ability to *inhibit* amyloid aggregation on the surface of the bead.

HOMOGENEOUS TIME-RESOLVED FLUORESCENCE

Homogeneous time-resolved fluorescence (HTRF) is a screening technique based on fluorescence resonance energy transfer (FRET, Hemmila and Webb, 1997). In principle, an excited donor fluorophore will transfer its energy to an acceptor fluorophore when the two are in close proximity. In the case of Packard's HTRF system, the donor fluorophore, europium cryptate [(Eu)K], is excited at 337 nm, and the energy is transferred to the acceptor fluorophore, XL665 (a stabilized allophycocyanine), with subsequent light emission at 665 nm (Kolb *et al.*, 1997). The emission of light from XL665 has a slow decay time and is measured after a delay of 50 ms, eliminating background interference. Free [(Eu)K] emits light at 620 nm, with a slow decay time, and can be used as an internal reference. Therefore, the 665/620 ratio can be used as an assay end-point. This allows compensation for variations in absorbance of emitted light, excitation energy and turbidity in different assay mixtures. This technique, therefore, provides a highly sensitive, homogeneous assay, and is ideal for dense format assays.

A key limitation of HTRF is the need to label the relevant assay components with fluorophores that display appropriate excitation, emission and quenching properties. Indeed, considerable advances have been made in HTRF on the basis of Nobel prize-winning chemistry by Jean Marie Lehn. This identified a method of protecting Eu^{3+} ions by means of a crypate cage that greatly increases assay sensitivity (Dietrich and Sauvage, 1988). The technique allows europium to be protected from fluorescence quenching in aqueous medium. The cryptate cage effectively acts as an antenna to increase energy transfer, and allows conjugation of a range of peptides and proteins to the fluorophore. Highly sensitive, dense format assays have been developed using commercial instrumentation.

HTRF could potentially be adapted to screen for amyloid inhibitors by labelling the target amyloid peptide with both donor and acceptor fluorophores at appropriate positions, so that only the aggregated or oligomeric form of the peptide generates a signal. Compounds can then be screened directly for their ability to inhibit or reverse the self-association of amyloid, depending on whether they are added before or after aggregation has taken place.

DATA ANALYSIS

HTS generates substantial quantities of data. With a typical library of 500 000 compounds, and depending on the type of assay, about 5 million data-points could realistically be collected. Larger libraries and more complex assays ultimately yield numbers closer to 10 million data-points. On a daily basis, a fully functioning screening laboratory aims to collect up to 100 000 data-points per day. Managing the flow of information from the assay environment into the analysis environment requires a robust information management strategy, but the resulting datasets can be used to analyse structure–activity relationships and identify key pharmacophores of interest.

As a result of HTS and other serendipitous discoveries, a vast range of diverse molecules have been reported in the literature to act as inhibitors of amyloid formation, which are summarized in the next section.

The diversity of known amyloidosis inhibitors

Figure 8.4 illustrates some examples of the vast range of compounds that have been found to be effective as inhibitors of amyloid aggregation.

Tetrameric transthyretin (TTR), involved in thyroxine transport, can form amyloid fibrils leading to transthyretin amyloidosis. McCammon *et al.* found 18 ligands (N-phenyl phenoxazines and flufenamic acid derivatives) that function as inhibitors of amyloid formation by their ability to stabilize the tetrameric structure of human wild-type TTR and of amyloidogenic TTR variants, V30M and L55P (McCammon *et al.*, 2002). Another series of transthyretin amyloidosis inhibitors function by stabilizing the monomeric native state, and hence increasing the kinetic barrier associated with misfolding (Hammarstrom *et al.*, 2003).

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 β-amyloid aggregation in Alzheimer's

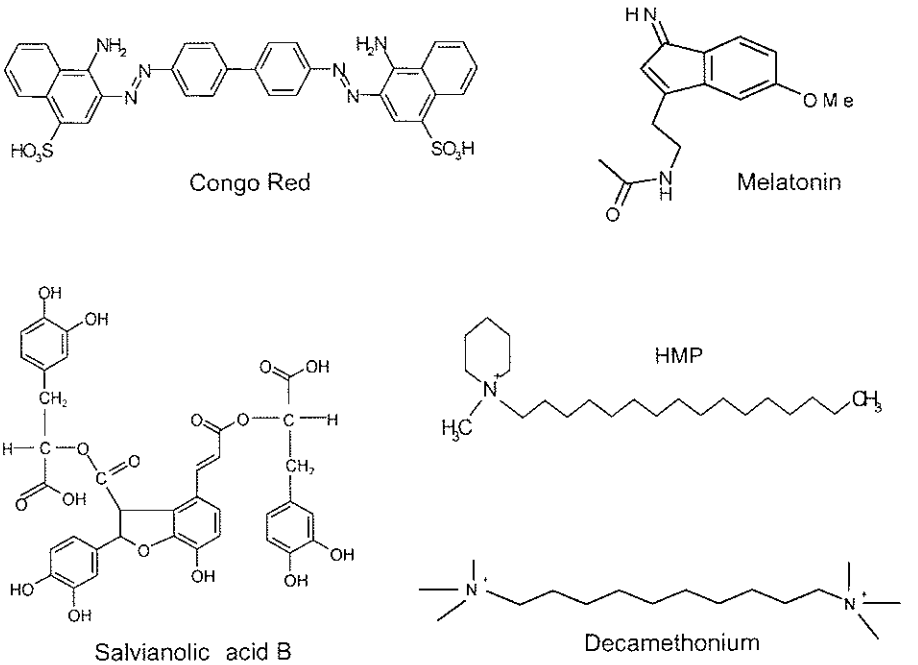

Figure 8.4. Examples of small molecule amyloid aggregation inhibitors.

disease (Burgevin *et al.*, 1994). Sanchez *et al.* 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* (Sanchez *et al.*, 2003). 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 (Heiser *et al.*, 2002).

A great number of diverse organic compounds has been found to inhibit or reduce the aggregation of β-amyloid into fibrils *in vitro*. These include nicotine (Salomon *et al.*, 1996), β-cyclodextrin (Camilleri *et al.*, 1994), hemin and related porphyrins (Howlett *et al.*, 1997), anthracycline 4'-iodo-4'-deoxydoxorubicin (Merlini *et al.*, 1995), hexadecyl-N-methylpiperidinium (HMP) bromide (Wood *et al.*, 1996), rifampicin (Tomiyama *et al.*, 1996), (-)-5,8-dihydroxy-3R-methyl-2R-(dipropyl-amino)-1,2,3,4-tetrahydronapthalene (Parker *et al.*, 2002), and melatonin (Pappola *et al.*, 1998). Salvianolic acid B also reduces PC12 cellular toxicity of aged β-amlyoid (Tang and Zhang, 2001). Kiuchi *et al.* tested Type IV collagen, a molecule that localizes in senile plaques of Alzheimer's patients, as a potential inhibitor of amyloid. Thioflavin T fluorescence and electron microscopy studies demonstrated that collagen IV inhibited Aβ(1–40) fibril formation (Kiuchi *et al.*, 2002). Bartolini *et al.* induced β-amyloid aggregation by human recombinant acetylcholinesterase, and small molecules were tested for their ability to inhibit the aggregation of β-amyloid. Molecules, such as propidium, a peripheral anionic site ligand, decamethonium, donepezil, and physostigmine, were found to inhibit β-amyloid aggregation (Bartolini *et al.*, 2003). Propidium, decamethonium, and physostigmine are known as acetylcholinisterase inhibitors, whereas donepezil is a

drug already used for Alzheimer's patients. Non-steroidal anti-inflammatory drugs (NSAIDs) and aspirin have also been reported to inhibit human aluminium-induced β-amyloid and amylin aggregation *in vitro* (Thomas *et al.*, 2003). Even fullerene, a football-like sphere of 60 carbon atoms, has recently been reported to inhibit the aggregation of β-amyloid (Kim and Lee, 2003).

Copper and iron are present in β-amyloid deposits and induce the production of hydrogen peroxide that may mediate oxidative damage to the brain in Alzheimer's disease (Varadarajan *et al.*, 2000). Bush developed metal-binding compounds that inhibit the *in vitro* generation of hydrogen peroxide by β-amyloid (Bush, 2002). They are also able to reverse the aggregation of β-amyloid *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.

Immunization of Alzheimer's mouse models with β-amyloid peptide significantly reduces both the density of cerebral amyloid plaques and the degree of cognitive impairment (Schenk *et al.*, 1999; Janus *et al.*, 2000; Morgan *et al.*, 2000). Furthermore, McLaurin *et al.* showed that immunization with protofibrillar forms of Aβ(1–42) induced therapeutically effective IgG2b antibodies that recognize Aβ(4–10) and inhibit β-amyloid protofibril aggregation and toxicity (McLaurin *et al.*, 2002). The antibody 1C2, which recognizes elongated polyQ chains, was also effective in inhibiting huntingtin protein aggregation (Heiser *et al.*, 2002).

Figure 8.4 shows examples of the structures of some of the diverse range of β-amyloid inhibitors. There is no obvious structural similarity between these compounds, suggesting that they may bind to different sites within the amyloid and/or to different intermediates in the aggregation pathway, which would make identifying any possible structure–activity relationships difficult.

Meptides as potential therapeutic lead compounds

In addition to the discoveries described above, a further strategy to develop amyloid inhibitors is to start with the wild-type peptide itself as a lead, since it is known to bind to the target. This can then be modified so that it still binds, but further addition of wild-type peptide is blocked. This strategy has been shown to be effective by adding poly(Lys) to the core hydrophobic LVFFA peptide fragment of β-amyloid (Ghanta *et al.*, 1996), replacing internal amino acids with proline (Soto *et al.*, 1996, 1998), or by adding a bulky blocking group to its N-terminus (Findeis *et al.*, 1999). In addition, unmodified amyloid peptide fragments can also act as inhibitors (Tjernberg *et al.*, 1996).

Recently, a new class of short synthetic N-methylated peptide derivatives called meptides have been found to inhibit and reverse the aggregation of amyloidogenic proteins and peptides, as well as their associated toxicity to cells *in vitro*. Meptides are virtually identical to natural peptides, except they have methyl groups attached to the backbone nitrogen atoms of alternate amino acid residues. The methyl groups force the meptide molecule into the active β-strand conformation (Manavalan and Momany, 1980; Gordon *et al.*, 2002) but occupy only one edge of the β-strand (*Figure 8.5*). The methyl groups block this edge of the molecule from associating with another peptide β-strand, while the other edge remains free to associate with the

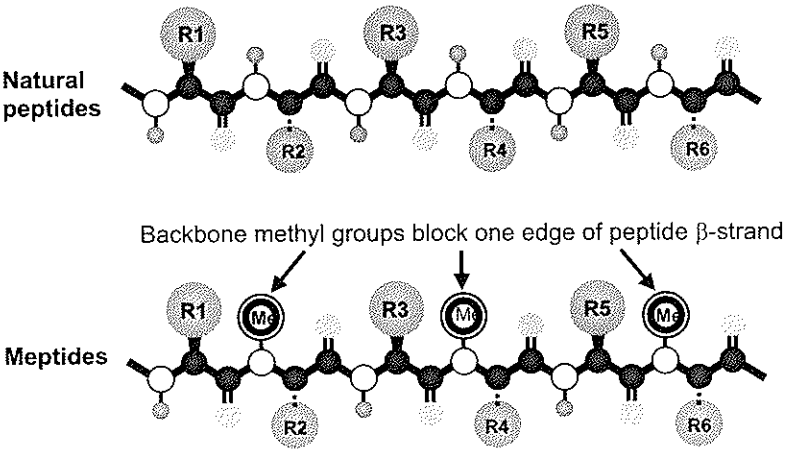

Figure 8.5. β-strand structure of natural peptides compared with meptides.

target β-strand in the same way as the wild-type peptide. Consequently, the meptides are able to bind specifically to the peripheral strands of a growing β-sheet and prevent its extension by blocking the association of further β-strands (*Figure 8.6*).

Whereas small synthetic organic molecules typically found in large screening libraries are best suited for binding to the active sites and ligand-binding pockets of enzymes and GPCRs (see *Figure 8.1*), meptides are more suited for binding tightly and specifically to the surfaces of extended peptide β-strands because they retain the same structural features that allow natural peptides to associate with each other as extended β-strands. The amino acid sequence of the meptides can be derived directly, simply by methylating the core hydrophobic section of the target peptide (*Figure 8.5*), since this is already known to associate tightly and specifically with itself as it aggregates. This means that potent inhibitors can be rationally designed for any amyloidogenic protein or peptide without the need for extensive screening of chemical libraries, or even knowledge of the target peptide structure beyond its simple amino acid sequence. A number of academic groups have reported that N-methylated fragments of β-amyloid, amylin, and α-synuclein can effectively inhibit and reverse the aggregation and associated toxicity of the full-length peptides *in vitro* (Hughes *et al.*, 2000; Gordon *et al.*, 2001, 2002; Irvine *et al.*, 2002; Kapurniotu *et al.*, 2002; Adessi *et al.*, 2003). Furthermore, meptides have several other advantages in common with natural peptides, not least their chemical versatility and compatibility with solid-phase synthesis of combinatorial libraries for rapid optimization by HTS. The use of substituents larger than a methyl group to block hydrogen bonding is also effective (Rijkers *et al.*, 2002).

Natural peptides usually make very poor lead compounds due to a number of factors: their high molecular weight, which significantly reduces their potential for effective delivery through the gut wall and the blood–brain barrier, and which contributes to their rapid clearance *in vivo*; their poor solubility in both water and organic solvents, which further reduces their effective delivery through biological membranes; and their susceptibility to proteolytic degradation, which reduces their stability *in vivo* and precludes their oral bioavailability altogether. By contrast,

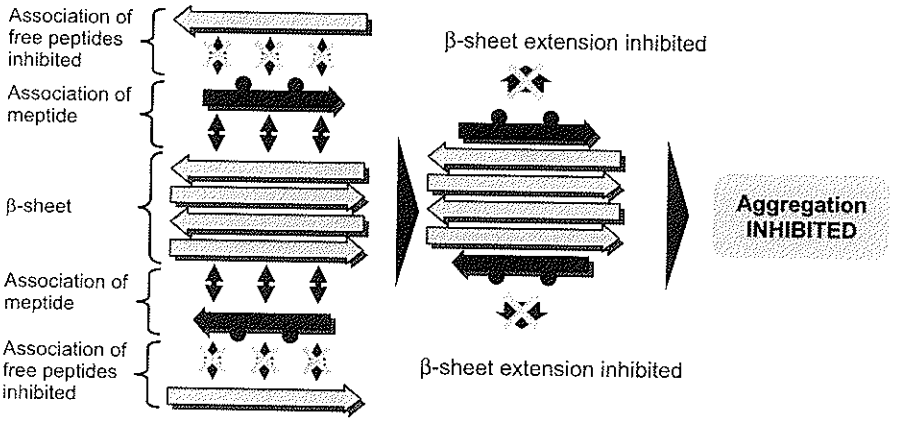

Figure 8.6. Inhibition of protein/peptide aggregation by meptides.

meptides could yield more promising lead compounds because their structure appears to overcome many of these bioavailibility-related issues typically associated with natural peptides. Firstly, meptides may be as short as 4–6 residues in length without significantly limiting their potency as inhibitors of protein or peptide aggregation, so they can fall within the desired upper molecular weight limit of 600 Da; and their small size also makes them less likely to be immunogenic. Secondly, the meptides are constrained into their active β-strand conformation, so they can bind more tightly to a natural target peptide than a natural peptide may bind to itself. Thirdly, meptides are highly soluble in both water and organic solvents, because the methyl groups stop the meptide molecules from aggregating (thus raising their solubility in water), while also introducing a non-polar group (thus raising their solubility in organic solvents). Finally, meptides are completely resistant to proteolytic degradation because their methylated backbones are not recognized by the active sites of proteolytic enzymes (Gordon *et al.*, 2001). Together, these features suggest that meptides could make very promising lead compounds, unlike natural peptides. Moreover, their unique solubility, stability, and activity compared with natural peptides, combined with their simple peptide-based chemistry, means that they can be used to construct large combinatorial libraries comprising more drug-like amyloidosis inhibitors by standard methods of automated solid-phase peptide synthesis. These libraries can then be screened by HTS to identify more potent lead compounds, but also to explore key structure–activity relationships which might indicate how the lead compounds can be developed into viable drug candidates that are worthy of serious clinical development.

Conclusion

At present, there are no cures or effective long-term treatments for any of these amyloid-related diseases. Alzheimer's disease and diabetes have *each* been estimated to cost the US economy *alone* around $100 billion per year in nursing home care and lost productivity of care workers. Moreover, the cost of all these diseases to the economy of the entire developed world could be as much as US$500 billion per

year, and this economic burden is expected to increase sharply with the ageing population unless effective treatments are discovered soon.

This review has highlighted the great diversity of compounds ('β-sheet breakers') that have been found to inhibit the fundamental pathogenic process of protein/peptide aggregation (amyloidosis) associated with these diseases. Currently, none of the amyloid inhibitors that have been identified by HTS or serendipitous discoveries have proven to be sufficiently potent, selective, and drug-like to be considered as a viable lead compound suitable for serious development. However, meptides provide a simple and reliable way to identify suitable lead compounds, either directly by rational design, or by incorporating them into large combinatorial libraries that can be screened using the most recent advances in HTS.

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