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REVIEW

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The Role of α-sheet in Amyloid Oligomer Aggregation and Toxicity

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A major barrier to developing effective treatments and diagnostics for amyloid diseases is the inability of traditional protein structure characterization methods to elucidate the structure of the toxic oligomers that form during amyloidogenesis. Some years ago, our lab "discovered" a novel protein secondary structure in molecular dynamics simulations of multiple unrelated amyloid proteins, which we call α-sheet. We hypothesize that α-sheet plays an important role in amyloid aggregation and oligomer toxicity. *De novo* monomeric α-sheet peptides designed to be complementary to the structure observed in simulations inhibit amyloid aggregation and toxicity and specifically bind to the toxic oligomeric species in a variety of unrelated mammalian and bacterial amyloid systems associated with a range of diseases. Furthermore, spectroscopic analysis of α-sheet structure, including nuclear magnetic resonance (NMR†), circular dichroism (CD), and Fourier-transform infrared spectroscopy (FTIR), correspond well to values predicted for α-sheet. These α-sheet designs are now being tested for their ability to detect and neutralize toxic oligomers in animals and in patient samples, demonstrating the potential of this nonstandard secondary structure as a target for therapeutic and diagnostic agents for amyloid diseases.

INTRODUCTION

Amyloid diseases are typically characterized by the formation and deposition of large, insoluble, extracellular protein / peptide aggregates known as fibrils or plaques. These fibrils are characterized by cross β-sheet structure [1-3]. There are over fifty such proteins that have been associated with human amyloid diseases [4], some of which affect large populations, including the β-amyloid peptide (Aβ), which is linked to Alzheimer's Disease [5]; islet amyloid polypeptide (IAPP or amylin), which is linked to Type 2 diabetes [6]; and transthyretin (TTR), which is linked to peripheral polyneuropathy, systemic amyloidosis, and heart disease [7]. These diseases afflict millions of individuals worldwide and have devastating, irreversible consequences on the body. Interestingly, amyloid fibrils also play a functional role by serving as scaffolds upon which the bacteria can rapidly form an extracellular biofilm matrix, greatly increasing their resistance to antibiotic regimens [8,9]. Recently, it was proposed that amyloidogenic Aβ plays a role in innate immunity, acting against external pathogens and parasites in the brain [10].

How do amyloid plaques and fibrils form? It is generally accepted that soluble, proteins undergo a con-

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†Abbreviations: NMR, nuclear magnetic resonance; Aβ, β-amyloid peptide; TTR, transthyretin; IAPP, islet amyloid polypeptide; CD, circular dichroism; FTIR, Fourier-transform infrared; NOE, Nuclear Overhauser Effect.

Keywords: toxic soluble oligomer, α-sheet, protein aggregation, amyloid

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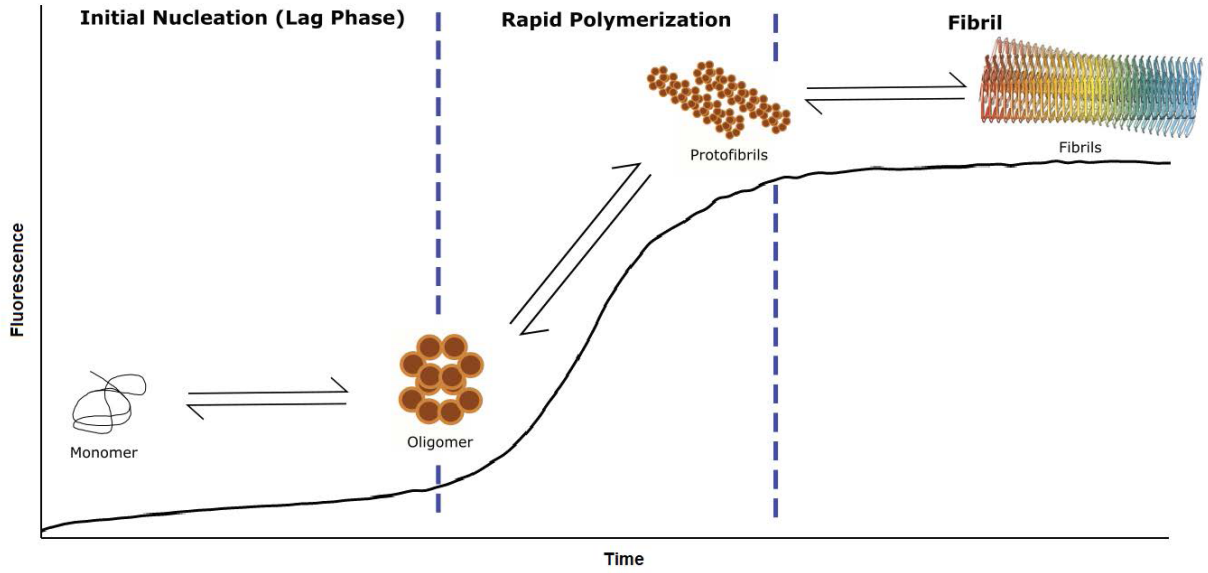

Figure 1. Schematic of amyloid formation from monomer, heterogeneous oligomers / protofibrils, to mature fibrils. During the lag phase, small, soluble nuclei begin to form from misfolded, aggregation-prone monomers. Once a critical concentration of nuclei is reached, the oligomers polymerize into protofibrils, which eventually mature into fibrils. A common assay for fibril formation involves the use of Thioflavin-T, which fluoresces upon binding β-sheet structure, particularly fibrils. Fibril image adapted with permission [2].

formational change that makes them aggregation-prone. Aggregation-prone monomers then interact to form soluble oligomers, which serve as nuclei upon which polymerization and aggregation take place [11,12]. Formation of the initial nucleus is generally considered the rate-determining step in amyloid aggregation. This idea is supported by the discovery that addition of fragments of amyloid fibrils or smaller soluble aggregates rapidly accelerates the conversion of monomeric amyloid proteins into fibrils [13,14]. It's been proposed that once a critical concentration of fibrils is reached, the fibrils themselves begin to catalyze the formation of more nuclei, which becomes the primary driving force for aggregation [15] (as opposed to monomeric association into nuclei). See Figure 1 schematic for an overview of amyloid aggregation kinetics and major species populated en route.

Aggregation is not a unidirectional process. The reverse, breakdown of fibrils into oligomers and oligomers into monomers is also possible, which results in further formation of nuclei. In addition, the oligomers themselves are heterogeneous, comprised of different interconverting oligomer species [16]. In short, aggregation is complex, may occur via multiple reaction mechanisms, and contains a large number of heterogeneous intermediates. Despite advances in our understanding of how this process may take place, there are still significant gaps that are of critical importance to developing effective treatments and detection agents for amyloid diseases.

TOPICS

Oligomers are the Toxic Conformer of Amyloid Diseases

It has been traditionally assumed that mature amyloid fibrils and plaques are responsible for the toxicity associated with amyloid diseases [17,18]. As a result, research efforts were (and still are) primarily focused on developing treatments that either prevent the formation or degradation of fibrils, as well as generally lowering amyloid burden. However, treatments focused on lowering amyloid deposition in Alzheimer's Disease have failed in various stages of clinical trials and are generally ineffective in treating symptomatic humans [19,20]. This, along with mounting evidence that the soluble oligomers are the primary source of toxicity not just in Alzheimer's Disease but also in a variety of other amyloid diseases [21-23], has resulted in a shift in focus to attempting to understand and elucidate oligomer toxicity. In fact, the fibrils themselves may serve as nontoxic repositories, an idea supported by the discovery of a compound that reduces cellular toxicity by promoting fibril formation [24]. Furthermore, it is the presence and amount of toxic oligomers that is correlated with symptoms in Alzheimer's Disease patients, not plaque burden [25].

Intuitively, the ability to develop effective treatments to combat oligomer-associated toxicity is closely linked to our ability to elucidate the structure of these oligomers.

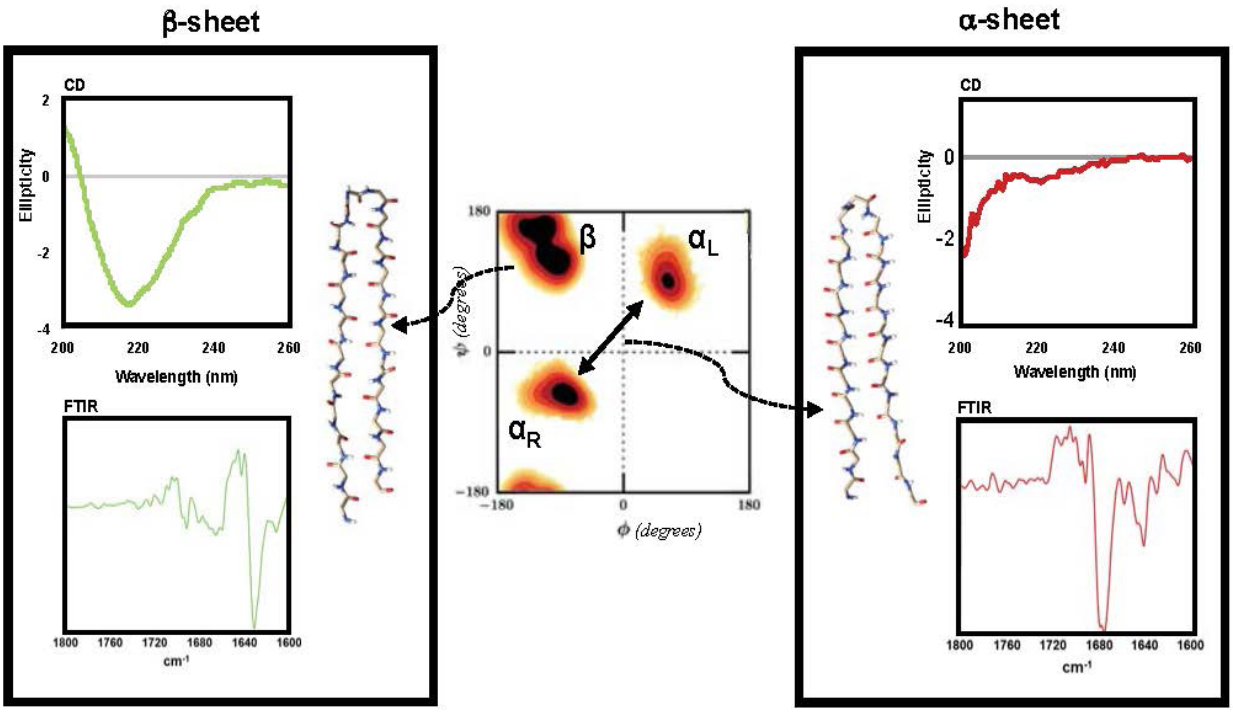

Figure 2. β-sheet and α-sheet occupy different regions of Ramachandran space and have distinct spectroscopic signatures. α-sheet CD spectra is predicted and observed to be relatively featureless due to the alternating rotation of light from the alternative α_L and α_R configurations with a dip near 200 nm from the L-amino acids in the turn. In contrast, β-sheet contains a minimum around 218 nm and positive ellipticity near 200 nm. The second derivative FTIR spectrum of α-sheet displays a strong band around 1680 cm^{-1} and a weaker band near 1640^{-1}, while the β-sheet FTIR contains a prominent band near 1620 cm^{-1}.

Unfortunately, however, this has proved impossible due to the transient, heterogeneous, and dynamic nature of these oligomers. Because the oligomers exist in dynamic equilibrium, perturbations to oligomers can cause them to easily dissociate into monomers, adopt different oligomeric states, or readily aggregate into fibrils, which makes it difficult to isolate and maintain "pure" species of oligomer. As such, methods that are traditionally used to characterize protein structure, such as x-ray crystallography or protein NMR, are of limited use. This has been a major barrier in progressing understanding of oligomeric assemblies in amyloid diseases and therefore the development of effective treatments of amyloid disease.

Yet, what we do know is that it is likely these oligomers share a common structure. Kayed and colleagues developed an antibody that binds to a variety of soluble amyloid oligomers, implicating a common structure [26]. In addition, they found that antibody binding inhibited the toxicity of these oligomers, suggesting that this structure confers a shared mechanism of toxicity across multiple unrelated amyloid species. Later, the same research group demonstrated that other antibodies bound fibrils and fibrillar oligomers specifically, but not prefibrillar oligomers, suggesting that this ubiquitous, toxic structure may be unique to soluble prefibrillar oligomers [27]. These findings correspond particularly well to data demonstrating that fibrils may serve as nontoxic repositories of amyloid oligomers.

Since then, there have been a wide variety of possible structures that have been proposed for these oligomeric intermediates. While no conclusive evidence exists for any single proposed structure, our lab "discovered" a novel secondary structure, which we call α-sheet, that we propose is linked to toxicity in the soluble oligomeric state. The remainder of this review will focus on the α-sheet hypothesis.

The Discovery of α-sheet Structure and the α-sheet Hypothesis

The α-sheet hypothesis originated from the finding that α-sheet structure formed repeatedly in molecular dynamics simulations of a variety of unrelated amyloid proteins under amyloidogenic conditions, but not under

native conditions [28,30]. α-sheet secondary structure is very similar to β-sheet secondary structure save for one major difference: the -NH and -C=O groups in α-sheet structure align on opposite faces of the strand, whereas in β-sheet structure they alternate, pointing in opposite directions (Figure 2). Notably, this alignment confers a molecular dipole to the α-sheet, which may play a role in the association of aggregation-prone monomers [28-30]. Interestingly, after "discovering" α-sheet we learned that the structure was first proposed by Pauling and Corey in 1951 as an alternative conformation to the native protein secondary structure we now know as β-sheet [31]. They eventually rejected what they called the "polar-pleated sheet" in favor of β-sheet in native proteins (and rightfully so), assuming that it was a high-energy state. This is consistent with our proposal that α-sheet structure is associated with non-native toxic states not native states.

Due to the rigidity of the peptide bond, there are two degrees of freedom that each amino acid in a peptide chain can rotate about. These are defined by two angles, φ (the angle between the C_{i-1}-N_i-$C\alpha_i$-C_i bonds) and ψ (the angle between N_i-$C\alpha_i$-C_i-N_{i+1}), which can be plotted against each other in a Ramachandran map [32]. The repetitive nature of the protein backbone in native secondary structure means that there are certain characteristic regions of Ramachandran space that successive amino acids in each secondary structure tend to populate. Ramachandran plots of α-sheet structures observed in the MD simulations illustrate that α-sheet is derived from the alternation of amino acids between local α_L and α_R helical regions (Figure 2). This alternation leads to an extended strand, as opposed to the formation of either a left-handed or right-handed α-helix.

The proposed involvement of α-sheet structure in amyloidogenic intermediate oligomers aligns nicely with the current body of knowledge surrounding oligomer aggregation and toxicity. The appearance of this structure in simulations across multiple unrelated amyloid systems supports Glabe and co-workers' assertion that toxic oligomers possess a common structural motif that is unique and distinct from standard secondary structures, such as those found in native proteins and in amyloid fibrils [26]. The proposed conversion of α-sheet to β-sheet during fibril formation aligns with Bodner *et al*. finding that promoting fibril formation lowers amyloid toxicity [24]. Though this is by no means proof that α-sheet is the definitive intermediate in amyloid disease, it makes a strong argument for further experimental investigation of the α-sheet structure and its role in amyloid aggregation and toxicity, as well as its potential as a target for amyloid therapeutics and diagnostics, as targeting α-sheet should avoid adverse reactions and false positives associated with interactions with native conformations.

Experimental Support for the Existence of α-sheet Structure

Characterization of a new secondary structure is challenging for a variety of reasons, foremost among them being the unstable, transient nature of small α-sheet structures and the lack of established reference compounds to interpret spectroscopic results. To test whether α-sheet exists outside of the computer, we set out to design and make α-sheet hairpins. To design small peptides that can stably adopt this unique secondary structure, our lab utilized amino acids of alternating L and D chirality to mimic the α_L / α_R conformation that defines α-sheet while avoiding introducing destabilizing strain to the peptide backbone. Specifically, the intrinsic propensities of each amino acid to adopt α-helical structure locally were analyzed via simulation for both L- and D- chirality amino acids [33,34]. Amino acids with high local α_L / α_R propensities were selected to make up the α-strands (Figure 2), which were linked together with a turn designed to support an α-sheet hairpin. Promising designs were identified from simulation based on the stability of the template hairpin structure and the strength of the inter-strand backbone hydrogen bonds. The top-ranked designs were then synthesized for experimental characterization and testing [35-37].

With these synthetic α-sheet designs in hand we began to evaluate their structural properties. This is difficult with a non-standard structure. While we can test whether a presumed new structure is consistent with known conventional structures, the assignment and deconvolution of spectra, for example, is contingent upon knowing the behavior of model compounds displaying that structure. In effect we must also use our designs to determine the spectroscopic signatures for α-sheet structure.

Circular dichroism (CD) is the most common method for obtaining secondary structure information. This method is based on the rotation of circularly polarized light, with each secondary structure displaying different characteristic spectra [38,39]. Due to the alternating chirality of the α-strand designs and the fact that mirror-images display inverted spectra [40], it was predicted that the α-sheet spectrum would be flat and featureless. CD spectra of our designed α-sheet compounds appear as predicted with the exception of a slight dip around 195-200 nm, emanating from the L-amino acids in the turn (Figure 2). This spectrum is distinct from conventional α-helix, β-sheet, or random coil spectra [35,36].

The unique interactions and alignment of atoms and functional groups within the α-sheet conformation give rise to a number of unique features by other spectroscopic techniques, as well. For instance, the alignment of the amide functional groups results in a molecular dipole, which was computationally estimated to generate a strong peak around 1675-1680 nm^{-1} by Fourier-transform

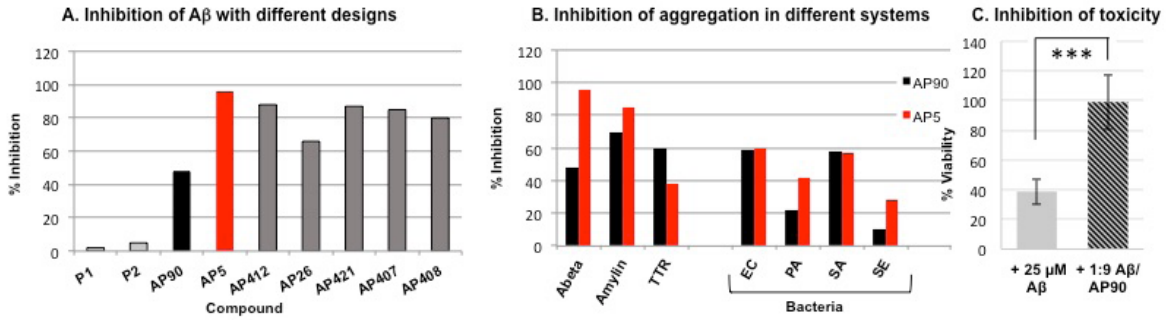

Figure 3. Multiple designed α-sheet peptides inhibit Aβ aggregation and are active in numerous human and bacterial amyloid systems by specifically binding to toxic oligomer species. A) Aβ aggregation is inhibited by multiple α-sheet designs but not by random coil peptide controls (P1 and P2). **B)** α-sheet designs broadly inhibit unrelated amyloid systems. AP90 and AP5 inhibit fibril formation across a variety of human [Aβ(1-42), Alzheimer's Disease; Amylin, Type 2 diabetes); TTR, transthyretin, systemic amyloid disease] and four different biofilm-forming bacterial systems [*E. coli* (EC)*, P. aeruginosa* (PA), *S. aureus* (SA), *S. epidermidis* (SE)]. **C)** Tests of the ability of α-sheet to neutralize Aβ toxicity and ability to preferentially bind toxic species. MTT viability assay (as a surrogate for toxicity) of SH-SY5Y neuroblastoma cells exposed to preincubated Ab (100 μM, 3h 37ºC) in the presence and absence of a molar excess of AP90. AP90 significantly decreased the cytotoxicity of oligomeric Aβ ($p = 0.0002$) [37].

infrared (FTIR) spectroscopy and a weaker band around 1640^{-1} [41]. This, in fact, was what we later observed in FTIR spectra of film samples of our α-sheet designs (Figure 2). It bears noting that these peaks are distinct from those found in conventional β-sheet and α-helical structures, which have bands at ~1620 nm^{-1} and ~1670 nm^{-1}, respectively [42].

Finally, H^1 nuclear magnetic resonance (NMR) spectroscopy can be used to determine the proximity of hydrogens; in particular, the backbone hydrogens can be useful for assessing secondary structure. In this regard, nuclear Overhauser effect crosspeaks (NOEs), reflecting transfer of magnetization between close protons, can be used as distance restraints to determine secondary structure and calculate 3D structures. β-sheet structure results in successive amide hydrogens pointing in opposite directions, whereas in an α-sheet they point in the same direction. In this α-sheet orientation, successive NH groups along the sheet are expected to give rise to strong NOEs. Also important are the NOEs that are not observed, *i.e*., those expected for α-helix or β-sheet structure. The experimentally derived NOEs of our designs correspond well with expectations for α-sheet structure [35]. Furthermore, we have recently determined a high-resolution structure for one of our designs, confirming the α-sheet hairpin structure of our design [43]. Moreover, the structural information obtained for our growing library of α-sheet compounds relative to other structural controls is being used to shed light on the structure of toxic soluble oligomers of Aβ and other amyloid proteins and peptides [43].

Loss of the alternating L/D templating greatly affects the properties of our α-sheet designs, even if the sequence identity is 100 percent conserved. In a study of structural isomers of a designed α-sheet peptide known as AP90, removal of the alternating L/D templating in the all-L amino acid isoform (called P90) caused its solubility to drop from > 25 mg/mL to virtually zero in water [37]. AP90 is calculated to have a dipole of 85 Debyes (D) *in vacuo*, while that of P90 is 11 D. As had been discussed above, the α-sheet structure *per se* gives rise to a molecular dipole in an otherwise relatively nonpolar peptide, which in turn vastly increases its solubility. The loss of the L/D templating that locks in the α-sheet structure also results in a loss of inhibition of both amyloid toxicity and aggregation [37], further demonstrating that this L/D templating (and thereby α-sheet structure, as confirmed by CD and FTIR) is critical to the inhibition of amyloid aggregation and toxicity, as well as the binding specificity of our designs to toxic oligomers.

Inhibition and Detection of Amyloidogenesis by α-sheet Compounds via Preferentially Binding Toxic Oligomers

Returning to our α-sheet hypothesis, we first identified α-sheet structure in molecular dynamics simulations of amyloid proteins under amyloidogenic conditions (typically low pH or in the presence of human disease-associated mutations). We proposed that the structure observed by MD is formed in the toxic oligomer species and associated with toxicity in these soluble aggregates. To test this idea, we reasoned that synthetic, monomer-

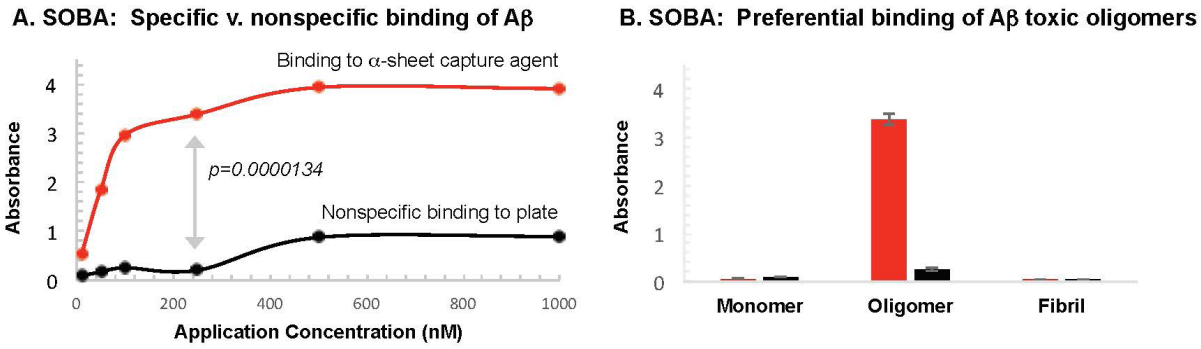

Figure 4. An ELISA-like assay for specific binding of toxic oligomers. Binding of toxic Aβ species probed in Soluble Oligomer Binding Assay (SOBA) with an α-sheet covalently immobilized in wells of 96-well plate. **A)** Application of different concentrations of toxic Aβ(1-42) (75 mM Aβ pre-incubated 24 hrs, PBS) to α-sheet peptide attached to plate (red curve). A structure-independent Aβ antibody was used to detect binding, a secondary reporter antibody, application of TMB and absorbance read at 450 nm. Nonspecific binding (black curve) is measured in paired wells lacking covalently attached peptide [43]. **B)** Application of 250 nM Aβ monomer, oligomer, and fibril samples to SOBA plate. Toxic oligomer is preferentially bound to the plate (note that this is the same sample as in panel A), while fresh nontoxic monomer and mature nontoxic fibrils are not.

ic α-sheet compounds would be complementary to the proposed structure in the toxic species and preferentially bind that species and in so doing inhibit aggregation and amyloid formation. We have now tested this experimentally for our anti-α-sheet designs, which are themselves α-sheet, in a variety of amyloid systems.

We began with studies of the inhibition of Aβ(1-42) [35]. Figure 3A shows inhibition of Aβ aggregation for two random coil control peptides (P1 and P2) as well as seven different α-sheet designs (the AP notation is used for our alternating L/D peptides). The random coil controls show no appreciable inhibition while all of the α-sheet designs inhibit aggregation (Figure 3A). This is noteworthy as these designs have different sequences, some very different, some are linear hairpins and one is a cyclic design (AP26). AP90 is our benchmark sequence and its properties relative to P90 its all L-amino acid isomer were introduced above. Consistent with our hypothesis stressing that the main chain structure is critical to inhibition, we see inhibition by a variety of different sequences. The side chains can modulate the stability of the sheet and specifics of the interactions with the amyloid species [36], but all of the α-sheet compounds we have made thus far inhibit amyloid formation.

Another expectation from our α-sheet hypothesis is that, as with inhibition being independent of sequence of the inhibitor, our compounds should cross-react with different unrelated amyloid species. Figure 3B shows the effect of adding two of these designs (AP90 and AP5) to three different amyloid systems unrelated by sequence and structure: Aβ (Alzheimer's Disease); transthyretin (TTR), systemic amyloid disease, and amylin (IAPP), type 2 diabetes. Our α-sheet designs inhibit amyloid formation in all three systems [35,36]. Furthermore, these same designs (and others) inhibit amyloid formation and the ensuing biofilm formation in live bacteria associated with a number of severe infections in which the amyloid fibrils act as scaffolds for the biofilm matrix to help evade antibiotics and the host immune system: *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA), *Staphylococcus aureus* (SA), and *Staphylococcus epidermidis* (SE) (Figure 3B). It is noteworthy and unprecedented that these α-sheet inhibitors inhibit aggregation in many different unrelated amyloid systems, spanning peptides and proteins associated with different human amyloid diseases to both gram (+) and gram (-) bacteria.

S. aureus in particular has been studied in some detail both in cells and by *in vitro* biophysical studies like those performed for the three mammalian systems [8]. The main component of *SA* amyloid fibrils is a helical peptide called PSMα1. Our α-sheet designs also inhibit the aggregation of pure PSMα1, which by CD undergoes a transformation from α-helix → α-sheet → β-sheet during aggregation. Furthermore, these compounds preferentially bind the α-sheet species to inhibit amyloid formation. The formation of α-sheet during aggregation by Aβ, for example, by CD is a bit more difficult as it begins from a disordered ensemble. Nonetheless, we see the random coil spectrum of Aβ lift and flatten out to an α-sheet-like spectrum during aggregation [43] and the toxic oligomer in particular displays an α-sheet spectrum without any evidence of β-sheet structure [37]. This same Aβ oligomer

is toxic to neuroblastoma cells and the cell viability is recovered upon administration of our AP90 design (Figure 3C). Preferential binding of our α-sheet designs to toxic soluble oligomers has also been explored through affinity columns containing the designs and random coil and β-sheet controls [35-36]. This was accomplished by immobilizing the peptides onto agarose beads in microfuge spin columns, followed by application of native species, toxic oligomers, and fibril samples to the columns. The immobilized α-sheet designs preferentially bind the toxic oligomers over the other species while the β-sheet and random-coil control peptides do not. This is noteworthy as the native structures begin in α-helix (PSMα1) [8], random coil (Aβ) [35], and β-sheet (TTR) [36], yet all three bind to our α-sheet compounds in their soluble oligomer states but not in their native or fibrillar forms.

Taking advantage of the complementarity between the α-sheet structure in both the designs and in the toxic oligomeric intermediates as well as the lack of binding to the native and fibrillar species, we are developing an ELISA-like plate-based assay, which we call a Soluble Oligomeric Binding Assay (SOBA) (Figure 4). The assay has been optimized such that it can reliably distinguish between specific (red line) and nonspecific (black line) binding of oligomer samples to the plate (Figure 4A). Statistically significant binding of oligomer is obtained at concentrations of less than 2.5 nM. (Figure 4A). For reference, while not directly applicable, the critical aggregation concentration for Aβ *in vitro* is approximately 90 nM, and aggregation is negligible below concentrations of 20 nM [44]. Nevertheless, SOBA effectively distinguishes between monomer, toxic oligomer (as confirmed through cell-based assay shown in Figure 3C), and fibrillar Aβ, with the monomer and fibril signals indistinguishable from the control for nonspecific binding. However, the concentration of the toxic oligomers *in vivo* will remain unknown until a diagnostic agent specific for the oligomers is implemented.

Though sequence-specific antibodies can be used to quantify concentrations of amyloidogenic and amyloid species [45,46], they typically cannot differentiate between monomeric, oligomeric, and fibrillar species, which often necessitates the use of multiple specific antibodies that may also bind to fragments of the amyloid precursor protein [47]. Generic polyclonal anti-oligomer antibodies like A11 have been developed [26], but immobilization of antibodies can affect yield and specificity adversely due to conformational changes, particularly in the binding site [48], and A11 is a polyclonal antibody that can also bind native β-sheet proteins [49]. The same holds true for other assays that involve amyloid immobilization via antibody capture, such as in surface-based fluorescence intensity distribution analysis assays [50]. A number of interesting approaches are being explored to circumvent these issues, including other ELISAs using peptides as capture agents, which have demonstrated selectivity of oligomers over monomeric and fibrillar aggregates in clinical CSF samples for specific Aβ sequences [51,52].

Non-ELISA-based approaches are also being considered. Some utilize the increased local concentration of binding epitopes found in oligomers to distinguish between monomeric and oligomeric species. For instance, one approach involves tagging oligomers with two antibodies targeting different Aβ epitopes, with fluorophores conjugated to the antibodies that only fluoresce when both antibodies are present [53]. Due to lower local concentrations of epitopes, fluorescent signals for monomeric Aβ are negligible, allowing for differentiation between monomers and oligomers. Other methods for distinguishing oligomers from monomers and fibrils include detecting oligomer size distributions via MALDI-MS [54] or capturing oligomers via hydrogel biosensors functionalized with peptide capture agents [55].

New, orthogonal approaches like SOBA also offer promise of an oligomer-specific diagnostic marker. SOBA has demonstrated ability to selectively bind amyloid oligomers independent of sequence. As such, it can be the basis for generic screening against amyloid diseases, which can then be discriminated further to a specific disease by employing different primary antibodies, as illustrated for Aβ(1-42) in Figure 4B. Thus, by targeting the fundamental oligomer-specific α-sheet structure, SOBA can be utilized as both a universal amyloid diagnostic and a disease-targeting tool.

CONCLUSIONS AND OUTLOOK

Though the idea that oligomers are the toxic intermediate in amyloid diseases has been discussed for over a decade, the heterogeneous and dynamic nature of these oligomeric species makes them notoriously difficult to characterize structurally, which has been a significant barrier to therapeutic and diagnostic development. There is increasing structural and experimental evidence to support the involvement of α-sheet structure in aggregation and toxicity in amyloid diseases. Peptides designed with this structure inhibit aggregation and toxicity in a variety of unrelated amyloid systems and preferentially bind toxic oligomers. They also contain spectroscopic features that differ from those found in β-sheet, α-helical, and random-coil secondary structures. Much remains to be done, but α-sheet peptides are unique in their ability to bind specifically to toxic oligomers, suggesting that this structure may be useful as both a therapeutic and diagnostic agent for a variety of amyloid diseases.

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