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Chemical and Physical Variability in Structural Isomers of an L/D α -Sheet Peptide Designed To Inhibit Amyloidogenesis

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Abstract

There has been much interest in synthetic peptides as inhibitors of aggregation associated with amyloid diseases. Of particular interest are compounds that target the cytotoxic soluble oligomers preceding the formation of mature, nontoxic fibrils. This study explores physical and chemical differences between two *de novo*-designed peptides that share an identical primary structure but differ in backbone chirality at six key positions. We show that the presence of alternating L/D-amino acid motifs dramatically increases aqueous solubility, enforces α -sheet secondary structure, and inhibits aggregation of the β -amyloid peptide implicated in Alzheimer's disease, in addition to neutralizing its cytotoxicity. In contrast, the all-L-amino acid isomer does not form α -sheet structure and is insoluble and inactive.

With their functional versatility and inherently modular character, synthetic peptides have long been considered a viable option for the treatment of amyloid diseases,^{1,2} a group of more than 40 conformational disorders that arise when native proteins misfold and aggregate into cytotoxic oligomers and insoluble fibrils (Figure 1).^{3,4} The ideal therapeutic peptide for inhibiting the propagation of these pathogenic species would do so by binding and sequestering a specific structural or sequential motif.⁵ With this theoretical target in mind, our peptide design efforts have focused on an atypical secondary structure termed the “ α -sheet”. This structure was observed in molecular dynamics (MD) simulations of several amyloidogenic species, and we proposed that it is a defining feature of cytotoxic soluble oligomers.^{6,7} First described by Pauling and Corey in 1951, the α -sheet backbone conformation is typified by the regular alternation of consecutive residue (ϕ and ψ) angles in the α_L and α_R helical conformations, resulting in the polar alignment of carbonyl groups on one side of the sheet and amide protons on the other (Figure 1).^{7,8} Of particular interest is

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Notes

The authors declare no competing financial interest.

Supporting Information

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the consistent appearance of α -sheet structure in low-pH MD simulations for several unrelated amyloid-forming polypeptides, a finding that supports the hypothesis that these diseases share a common etiology that is independent of sequence and structure.⁹

Our previous studies show that synthetic peptides designed to adopt an α -sheet backbone conformation complementary to the proposed α -sheet structural motif in the amyloidogenic species inhibit aggregation in three different mammalian amyloid systems,^{10,11} as well as a disease-associated bacterial system.¹² Structurally, the presence of an alternating L/D-amino acid motif heavily influences the inhibitory efficacy of these synthetic peptides by enforcing a polar α -sheet backbone configuration and encouraging complementarity between the designed peptide and its amyloidogenic target.^{7,11} In what may be the most striking example of this duality, we have observed remarkably different chemical and structural behavior between our alternating L/D-templated “benchmark” design”, AP90 (“alternating L/D peptide 90”, previously called “ α 1” by Hopping et al.¹⁰), and its all-L structural isomer, P90 [“peptide 90 (Table 1)”. Below we show that while AP90 is highly soluble in aqueous solutions, inhibits aggregation, and displays α -sheet structure as indicated by circular dichroism (CD) and Fourier transform infrared (FTIR) spectroscopy, P90 lacks substantial solubility and displays β -sheet structure in the same analyses. To characterize the conspicuous differences between these two sequentially identical designs, we conducted a series of experiments to highlight their substantial structural and chemical disparities. We confirmed that, even with identical primary structures, peptides differ significantly in their chemical and physical behavior upon introduction of α -strand motifs through swapping chirality at particular positions in the sequence.

Using the same sequence of 23 amino acids, we designed two structural isomers (Table 1). The first of these peptides, AP90, incorporates two seven-residue strands with residues of alternating chirality (i.e., “LDLDDL”) connected by a designed -NEYSG- turn and flanked by -RG to increase solubility. The aqueous solubility of the resulting AP90 peptide is >25 mg mL⁻¹. We use alternating chirality in the strands to bias the peptide to adopt the alternating $\alpha_R\alpha_L$ local structure that defines the α -sheet.^{6,7} Notably, the theoretical hydrophilicity plot for AP90 constructed using Hopp–Woods scoring values, which are based on individual amino acid transfer free energies (Figure S1),¹³ suggests that this sequence should have poor solubility. Other than five charged residues primarily clustered around the N- and C-termini, the peptide’s 18 remaining amino acids are either neutrally hydrophilic or hydrophobic. Contrary to expectations, AP90 was very soluble and its turbidity in water [evaluated by measuring the solution absorbance at 420 nm (Figure 2a)] was indistinguishable from that of neat water, suggesting that the solubility of AP90 is dependent on factors other than side chain hydrophilicity. A solution of AP90 absorbed 420 nm light at an average of 0.0442 AU, while pure water absorbed at an average of 0.0438 AU (values within instrumentation error of each other). Further microscopic analysis of AP90 in water (Figure 2b) could not discern any notable particulate matter present in the solution that would point to insoluble character. Additionally, when AP90 was dissolved to a concentration of 350 μ M and filtered through a 0.2 μ m cellulose–acetate membrane to remove large particulates, the concentration of peptide still measured $\sim$ 300 μ M as determined by the aromatic side chain absorbance at 280 nm, indicating the high solubility

of the dissolved peptide. Analysis by atomic force microscopy (AFM) revealed no significant buildup of solid material on the imaging surface (Figure 2c).

Conversely, P90 displayed very different physical characteristics compared to those of its L/D-templated counterpart, despite its sole modifications being the chiral reversal of the six D-amino acids. As a result of this alteration, P90 was completely insoluble in aqueous solutions, more accurately reflecting the character predicted by the aforementioned Hopp–Woods hydrophilicity plot. Additionally, its average turbidity value of 0.0618 AU was 41% higher than that of AP90 and pure water (Figure 2a). This fact was reflected in images taken at 200 $\times$ magnification of P90 in solution, which show glassy, insoluble fragments of a peptide film unevenly scattered throughout the sample (Figure 2b). Unlike AP90, once the P90 solution was filtered through the same type of 0.2 μ m filter, no signal could be detected by the 280 nm absorbance, suggesting near-total insolubility and complete removal of peptide solids by the filter. The properties of P90 were examined further by dissolving it in dimethyl sulfoxide and then adding phosphate-buffered saline (PBS), which induced the formation of an insoluble gel. As determined by AFM, large, nonspecific peptide aggregates were distinctly visible (Figure 2c). We note that the turbidity and other properties of P90 are independent of whether the N- and C-termini were capped (Figure S2).

As the primary structures of AP90 and P90 are identical, we hypothesize that their starkly dissimilar physical profiles result from differences in secondary structure. We analyzed each by CD and FTIR spectroscopies. Our predictions were confirmed (Figure 3). As expected, AP90 produced a nearly flat CD spectrum, caused by cancellation of the positive and negative signals in alternating L/D strands with a residual negative signal from the L-amino acids in the turn.^{10,14} Meanwhile, P90, which had to be evaluated as a suspension because of its insoluble character, adopted β -sheet structure with negative absorbance at 220 nm and positive absorbance at 200 nm. These CD results for AP90 and P90 in phosphate buffer are consistent with their spectra obtained earlier in 50% acetonitrile.¹¹ FTIR spectra were likewise indicative of significant secondary structural differences. As shown in previous studies, AP90 exhibits strong absorbance at 1675 and 1640 cm^{-1} , corresponding to predictions for α -sheet from density functional calculations for amide I vibrations.^{10,11,15} In contrast, P90 has a strong band at 1620 cm^{-1} consistent with β -sheet structure and a weak broad band at 1660 cm^{-1} .¹⁶ Additionally, in previous studies, AP90 displayed sequential main chain $\text{NH}_i\text{--H}_{i+1}$ nuclear Overhauser effect crosspeaks (NOEs) expected for the alignment of NH groups in α -sheet structure and did not give rise to characteristic α -helix or β -sheet NOEs.¹⁰

To further explore differences between these designs, we constructed *in silico* peptide models of AP90¹⁰ and P90 (see Figure 1) and calculated their *in vacuo* dipole moments (computer code provided in the Supporting Information). AP90s alternating L/D α -sheet hairpin yielded a computed dipole moment of 85 D; meanwhile, P90 gave a value of 11 D as a β -hairpin and 15 D as an extended strand. For reference, we obtained a value of 2.4 D for water, which is in excellent agreement with experiment. The large difference in the calculated dipole moments for the α -sheet and β -sheet structures for these identical sequences supports the idea that the polar α -sheet structure is responsible for the enhanced solubility of AP90.

As AP90 was initially designed to be complementary to putative α -sheet regions in amyloidogenic proteins, we investigated the ability of the peptides to bind to the Alzheimer's disease-associated peptide amyloid- β (residues 1–42) ($A\beta$) and inhibit its aggregation and cytotoxicity. We measured the equilibrium dissociation constant, K_D , for binding of each peptide to preincubated oligomeric $A\beta$ (3 h at 37 °C) using biolayer interferometry, a technique similar to surface plasmon resonance. After each peptide had been adhered to an aminopropylsilane-coated tip, the tip was dipped into a preaggregated oligomeric solution of $A\beta$. AP90 bound $A\beta$ 10^4 times tighter than did P90 (Table S1). The difference in affinity is consistent with previous studies showing preferential binding of preincubated toxic samples of $A\beta$ and transthyretin by AP90 over fresh nontoxic samples and the lack of binding by P90.^{10,11}

We confirmed that the $A\beta$ sample used for the binding assessments was toxic by applying 25 μ M preincubated oligomeric $A\beta$ to SH-SY5Y neuroblastoma cells (Figure 4a). In the presence of excess AP90 at a 9:1 molar ratio, which translates to 1.46 ng/cell, the cell viability recovered to that of the solvent/medium control. These findings indicate that AP90 binds to toxic soluble oligomer species and neutralizes the cytotoxic effects. Unfortunately, P90 was not soluble under these conditions; however, it was evaluated under slightly different conditions and did not block toxicity while AP90 did (Figure S3, $p = 0.009$ comparing P90 and AP90).

Next, we evaluated the ability of the peptides to inhibit the aggregation of $A\beta$, as well as their propensity for self-aggregation, using a standard thioflavin T (ThT) binding assay in which fluorescence detection of ThT binding is taken to reflect β -sheet fibril formation (Figure 4b). While P90 bound ThT, confirming the β -sheet spectroscopic results, it did not form fibrils. AP90 did not bind ThT and, despite being heated to 60 °C, inhibited the aggregation of $A\beta$ by ~50%.

Further studies were conducted to explore the binding of AP90 to $A\beta$ oligomers utilizing fresh, non-heat-treated AP90. We preincubated $A\beta$ to obtain soluble oligomer samples and separated the resulting species by size exclusion chromatography. Two species were observed, monomer and hexamer (Figure S4). Addition of excess AP90 to the $A\beta$ sample resulted in a shift of the hexamer peak to a higher molecular weight, reflecting binding of AP90 to the oligomer. To investigate the effect of AP90 binding on the structure of the oligomer, CD was performed in the presence and absence of the inhibitor. The resulting spectra are very similar and consistent with α -sheet structure (Figure S5). In addition, we tested the ability of AP90 to prevent fibrillization of a toxic oligomer sample prepared using the protocol of Ahmed et al.¹⁷ Their incubation was performed at low salt and low temperature (4 °C) in contrast to our incubations in PBS at 25 or 37 °C. AP90 effectively prevented fibril formation upon being added to the preincubated oligomers, while that same oligomer sample proceeded to form β -structure in the absence of an α -sheet inhibitor (Figure S6).

Here we confirm that the chirality of individual amino acids can dramatically affect behavior. Indeed, from the example of AP90 and P90, the alternation of chirality at six key locations results in very different physical and chemical behavior. The incorporation of

strands with alternating L/D-amino acids enforces an α -sheet backbone conformation with a molecular dipole that provides heightened solubility in an otherwise hydrophobic molecule. In turn, the α -sheet structure per se inhibits amyloidogenesis of A β *in vitro*, in agreement with earlier studies.¹¹ Furthermore, our α -sheet design inhibits A β -induced cytotoxicity in neuroblastoma cells while P90 does not. Overall, the stark differences between AP90 and P90 show that even with identical primary structures, altering the chirality of individual amino acids has a profound impact on chemical character and functional properties, defying the idea of the composition of matter based on sequence alone as a defining property.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Tjernberg LO, Naslund J, Lindqvist F, Johansson J, Karlstrom AR, Thyberg J, Terenius L, Nordstedt C. J Biol Chem. 1996; 271(15):8545–8548. [PubMed: 8621479]
2. Yamin G, Ruchala P, Teplow DB. Biochemistry. 2009; 48(48):11329–11331. [PubMed: 19877710]
3. Chiti F, Dobson CM. Annu Rev Biochem. 2006; 75(1):333–366. [PubMed: 16756495]
4. Sipe JD, Benson MD, Buxbaum JN, Ikeda S, Merlini G, Saraiva MJM, Westermark P. Amyloid. 2016; 23:209–213. [PubMed: 27884064]
5. Findeis MA. Biochim Biophys Acta, Mol Basis Dis. 2000; 1502(1):76–84.
6. Armen RS, DeMarco ML, Alonso DOV, Daggett V. Proc Natl Acad Sci U S A. 2004; 101:11622–11627. [PubMed: 15280548]
7. Daggett V. Acc Chem Res. 2006; 39(9):594–602. [PubMed: 16981675]
8. Pauling L, Corey RB. Proc Natl Acad Sci U S A. 1951; 37(5):251–256. [PubMed: 14834147]
9. Kaye R, Head E, Thompson JL, McIntire TM, Milton SC, Cotman CW, Glabe CG. Science. 2003; 300(5618):486–489. [PubMed: 12702875]
10. Hopping G, Kellock J, Barnwal RP, Law P, Bryers J, Varani G, Caughey B, Daggett V. eLife. 2014; 3:e01681. [PubMed: 25027691]
11. Kellock J, Hopping G, Caughey B, Daggett V. J Mol Biol. 2016; 428(11):2317–2328. [PubMed: 27012425]
12. Bleem A, Francisco R, Bryers J, Daggett V. npj Biofilms and Microbiomes. 2017; 3:16. [PubMed: 28685098]
13. Hopp TP, Woods KR. Proc Natl Acad Sci U S A. 1981; 78(6):3824–3828. [PubMed: 6167991]
14. Wade D, Boman A, Wählin B, Drain CM, Andreu D, Boman HG, Merrifield RB. Proc Natl Acad Sci U S A. 1990; 87(12):4761–4765. [PubMed: 1693777]
15. Torii H. J Phys Chem B. 2008; 112(29):8737–8743. [PubMed: 18582018]
16. Barth A, Zscherp C. Q Rev Biophys. 2002; 35(4):369–430. [PubMed: 12621861]

17. Ahmed M, Davis J, Aucoin D, Sato T, Ahuja S, Aimoto S, Elliott JI, Van Nostrand WE, Smith SO. Nat Struct Mol Biol. 2010; 17(10):561–567. [PubMed: 20383142]

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Figure 1. Simplified schematic progression of amyloidogenesis of $A\beta$. (1) Monomeric aggregation-competent $A\beta$. Aggregation proceeds, yielding (2) cytotoxic oligomers [with an atomic force microscopy image (AFM)] proposed to contain α -sheet structure, as illustrated in an AP90 model hairpin. (3) Formation of β -sheet fibrils follows, as illustrated by the P90 β -hairpin model and the AFM image of $A\beta$. AFM images are $2\ \mu\text{m} \times 2\ \mu\text{m}$.

Figure 2.

Analysis of AP90 and P90 in aqueous solutions. (a) Each peptide was dissolved in filtered water to a concentration of 1 mg mL^{-1} ; the resulting solutions and/or slurries were sonicated to break up any remaining solids and analyzed for the absorbance of 420 nm light. Though raw absorbance was somewhat low because of the lack of color in all solutions, the P90 solution had an absorbance significantly higher than that of either AP90 or pure water. (b) The same solutions from panel a were imaged by light microscopy. Neither AP90 nor water had any insoluble solids other than some small dust particles, while the P90 solution contained large pieces of insoluble peptide film. (c) P90 formed a viscous gel upon being solubilized in dimethyl sulfoxide and diluted with phosphate-buffered saline. AFM analysis of this gel revealed large, nonspecific aggregates of insoluble peptide material. AP90, in contrast, produced no discernible aggregates (pieces of dust located and shown for reference). The scale bar is 200 nm.

Figure 3.

Spectroscopic analysis of AP90 and P90. Spectra for AP90 (green) suggested α -sheet structure by their relatively flat CD signal and strong FTIR amide I absorbance at 1675 and 1640 cm^{-1} . P90 (red), in contrast, displayed β -sheet character by its negative CD absorbance around 220 nm and strong amide I vibration at 1620 cm^{-1} . FTIR spectra are annotated with α -sheet (α_s) and β -sheet (β_s) bands, indicated by dashed lines.

Figure 4.

Aggregation and amyloid inhibitory properties of AP90 and P90. (a) MTT viability assay of SH-SY5Y neuroblastoma cells exposed to preincubated A β (100 μ M, 3 h, 37 $^{\circ}$ C) in the presence and absence of excess AP90 in a 9:1 molar ratio relative to the final concentration of A β in cells of 25 μ M. AP90 significantly decreased the cytotoxicity of oligomeric A β (p = 0.0002). Because of its insolubility, P90 could not be evaluated under these conditions. Values were normalized to medium supplemented with NaOH to account for residual NaOH from the A β stock. (b) ThT binding assay of 10 μ M A β , a 1:10 A β /AP90 mixture, 100 μ M AP90 alone, and 100 μ M P90 alone. Heat-treated AP90 retained the ability to inhibit aggregation while showing no sign of self-aggregation. In contrast, 100 μ M P90 self-aggregated into β -rich deposits. The ThT concentration was 22 μ M in all cases.

Table 1

Sequence Comparison of AP90 and P90

Peptide	Sequence ^a
AP90	RGE <u>m</u> N <u>l</u> S <u>w</u> MNEYSGW <u>t</u> M <u>n</u> L <u>k</u> MGR
P90	RGEMNLSWMNEYSGWTMNLKMGR

^aUnderlined, lowercase letters refer to D-amino acids.