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Inhibition of A β 42 aggregation using peptides selected from combinatorial libraries

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Abstract

Increasing evidence suggests that the aggregation of the small peptide A β 42 plays an important role in the development of Alzheimer's disease. Inhibiting the initial aggregation of A β 42 may be an effective treatment for preventing, or slowing, the onset of the disease. Using an *in vivo* screen based on the enzyme EGFP, we have searched through two combinatorially diverse peptide libraries to identify peptides capable of inhibiting A β 42 aggregation. From this initial screen, three candidate peptides were selected and characterized. ThT studies indicated that the selected peptides were capable of inhibiting amyloid aggregation. Additional ThT studies showed that one of the selected peptides was capable of disaggregating preformed A β 42 fibers.

Keywords

peptide libraries; A β 42; Thioflavin T; Alzheimer's disease; amyloid inhibition

Introduction

Many proteins are known to adopt alternative misfolded structures that can be linked to a variety of diseases [1–3]. These alternative structures no longer retain the function associated with the native structure of the protein, and thereby become functionally useless or toxic to the cell. Misfolded proteins are capable of aggregating together to yield a variety of oligomeric states, ultimately forming fibers and amyloid plaque. The formation of fibers and amyloid in human tissue appears to be a nearly irreversible reaction.

While resolubilizing amyloid appears to be very difficult to achieve, there has been some success made in slowing the rate of aggregation, if not preventing aggregation altogether. Several chaperon-like proteins, such as clusterin and HSP20, as well as several antibodies have been shown to be capable of preventing amyloid formation [4–10]. Likewise, several small molecules and peptides have been shown to slow the formation of amyloid [11–26]. Recently, Sato *et al.* synthesized several peptides found to inhibit the aggregation of A β 42 [27]. These

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Supporting information

Supporting information may be found in the online version of this article.

peptides synthesized by Sato and coworkers were rationally designed to bind to, and inhibit the aggregation of, A β 42.

The use of small peptides as aggregation-inhibiting agents could prove therapeutically useful for a variety of diseases, including Alzheimer's disease. Slowing the progress of drug discovery for Alzheimer's disease is the lack of an inexpensive and easily accessible screen. Most of the screens that have been reported make use of synthetic A β 42 [28,29]. The synthesis of A β 42 is time-intensive and prohibitively expensive for use in screens of peptide libraries. Additionally, synthetic A β 42 has an extremely high propensity to form homo-oligomeric complexes in solution. These initial oligomers act as seeds to promote the formation of amyloid. Because of the difficulty in removing the seed oligomers, screens aimed at finding compounds that prevent the earliest stages of amyloid formation are likely to fail.

Here, we describe an EGFP-based screen, developed by Hecht and coworkers [30–32], to select for small peptides capable of inhibiting the aggregation of A β 42. Two peptide libraries were constructed to associate with, and prevent the aggregation of, A β 42. In this screen, the peptide A β 42 was genetically fused to EGFP. When expressed in *Escherichia coli*, the A β 42-EGFP fusion protein produces virtually no green color or fluorescence due to the amyloidogenic nature of A β 42. Aggregation of A β 42 precludes folding, and hence fluorescence, of the EGFP reporter. However, when A β 42 is prevented from aggregating, the fused EGFP is capable of folding and fluorescing brightly. In this screen, combinatorially randomized peptides were co-expressed in *E. coli* with the A β 42-EGFP fusion protein. Peptides that resisted cellular degradation and prevented the aggregation of A β 42 permitted EGFP to fold and fluoresce. Therefore, individual *E. coli* colonies expressing both a library peptide and A β 42-EGFP were screened to select for those colonies that showed the greatest fluorescence.

Materials and Methods

Materials

Synthetic peptides were prepared by GenScript Corporation. DNA purification kits were from Qiagen Inc. Klenow Fragment DNA polymerase and restriction enzymes were from New England Biolabs. Expand High Fidelity DNA Polymerase was from Roche. pET 28a and pCDF-1b plasmids were from Stratagene. DNA sequencing was performed by Davis Sequencing.

Construction of the A β 42 Gene

The A β 42 gene was constructed using polymerase chain reaction (PCR)-based gene assembly [33]. Ten single-stranded DNA oligonucleotides (Table S1, Supporting information) were designed to base-pair with their upstream and downstream pairing partners. Codons were optimized for expression in *E. coli*. An oligo master mix was prepared by adding 2 μ l of a 100 μ M solution of each oligonucleotide to a single tube. To assemble the full-length A β 42 gene, 1 μ l of the oligo master mix was PCR amplified using Expand High Fidelity Polymerase (Roche).

Construction of p A β 42-EGFP

The synthetic A β 42 gene was doubly digested with HindIII and EcoRI and ligated into an analogously digested p28EGFP plasmid that contains the gene for EGFP. [The EGFP gene, and other similar fluorescent proteins, is commercially available from Clontech Laboratories, Inc. The EGFP gene was PCR amplified using the primers 5'-GAA CTG GAC CAT ATG GTG AGC AAG GGC GAG GAG-3' and 5'-GTT ACG CTG GAA TTC TTA CTT GTA CAG CTC GTC CAT GCC-3' which produce an NdeI restriction site at the 5' end of the gene and an EcoRI site at the 3' end of the gene. The PCR product was doubly digested with NdeI and EcoRI restriction endonucleases (New England Biolabs) and ligated into an analogously

digested pET28a plasmid (Novagen)]. The final construct, p28A β 42-EGFP was verified with DNA sequencing. In this construct, the A β 42 peptide is genetically linked to EGFP through a short flexible linker sequence. The A β 42-EGFP gene was removed from the pET28a plasmid using NcoI and HindIII restriction endonucleases and ligated into an analogously digested pCDF-1b plasmid yielding pA β 42-EGFP.

Construction of the Peptide Libraries

Two peptide libraries were designed to match the suspected aggregation-prone regions of A β 42. Combinatorial variation was introduced to the libraries using degenerate codons (Table S2, Supporting information). Library 1 was designed to associate with A β 42 amino acids 29–42 and Library 2 was designed to associate with A β 42 amino acids 17–21 (Figure 1). The two libraries were constructed using three synthetic DNA oligonucleotides (Table S2, Supporting information), as described in the supplementary materials.

Screening Peptide Libraries

The pA β 42-EGFP plasmid (Strep^r) was cotransformed with either the Library 1 or the Library 2 plasmid library (Amp^r) into electrocompetent BL21 (DE3) *E. coli* cells (Stratagene) using a BTX ECM388 electroporator. The transformed colonies were plated on sterile nitrocellulose discs on LB media plates that contained both ampicillin and streptomycin. The plates were incubated for 15 h at 37 °C. The nitrocellulose discs, covered in individual colonies, were transferred to LB media plates containing ampicillin, streptomycin, and 2 mM IPTG. These plates were incubated at 37 °C for 3–6 h. Plates were scanned both visually and under 490 nm wavelength light (Figure 2) to select green-colored fluorescent colonies.

Quantification of Cell Culture Fluorescence

Selected colonies were grown to an O.D.₆₀₀ of 0.7 before protein induction with 1 mM IPTG. The induced *E. coli* were incubated at 37 °C with shaking for 4 h. After 4 h, O.D.₆₀₀ and fluorescence emission (Ex_{490 nm} and Em_{516 nm}) of each culture was recorded. Only colonies showing an increase in fluorescence compared with cultures expressing A β 42-EGFP alone were selected for further testing.

Preparing Disaggregated A β 42

In 4.0 ml of HFIP, 0.5 mg synthetic A β 42 (GenScript Corp) was dissolved and placed in a sonicating water bath for 20 min. The solution was divided into 400 μ l aliquots and stored at –80 °C.

ThT Binding of A β 42 in the Presence of Selected Peptide Inhibitors

ThT binding studies were performed as described by LeVine [34]. Disaggregated A β 42 (as described above) was thawed and the HFIP removed over a stream of nitrogen gas. The resulting solid A β 42 was dissolved in PBS buffer to yield a 0.2 mg/ml stock solution. This stock solution was divided evenly among tubes containing the selected peptides dissolved in PBS buffer. The in-solution concentration of A β 42 peptide was 40 μ M for each sample and the concentrations of selected peptides ranged from 1.2 mM to 20 μ M. The samples containing A β 42 and selected peptides were incubated at 37 °C with shaking (120 rpm). At various time points, 15 μ l aliquots were removed and mixed with 485 μ l of 3 μ M ThT in 50 mM glycine buffer pH8.5. The ThT mixture was incubated at room temperature in the dark for 15 min before recording the ThT fluorescence spectrum (Ex_{450 nm}) using a Hitachi F-7000 fluorescence spectrophotometer. ThT fluorescence (Em_{488 nm}) in the presence of each peptide inhibitor was taken as a percentage of the ThT fluorescence of A β 42 alone.

Monitoring the Disaggregation of A β 42 with Selected Peptides

For 24 h, 0.2 mg/ml stock A β 42 (described above) was incubated at 37 °C with shaking (120 rpm) to promote formation of A β 42 fibrils. This resulting solution was evenly divided among tubes containing the peptide inhibitors at concentrations ranging from 10 μ M to 1.2 mM. The preformed A β 42 fibrils with the peptide inhibitors were incubated at 37 °C with shaking (120 rpm). At 1, 3, 5 and 24 h, 15 μ l aliquots were removed and added to 485 μ l of 3 μ M ThT in 50 mM glycine buffer pH 8.5. The ThT mixture was incubated at room temperature in the dark for 15 min before recording the ThT fluorescence spectrum (Ex_{450 nm}) using a Hitachi F-7000 fluorescence spectrophotometer.

Results and Discussion

The 42-amino acid peptide, A β 42, is highly amyloidogenic. The exact amino acids responsible for the self-aggregation of A β 42 are not known, but it is believed that the two hydrophobic patches of A β 42 may play a role. With that in mind, we constructed two peptide libraries designed to anneal to either of the two hydrophobic patches of A β 42 (Figure 1). The degenerate peptide libraries were designed to maintain much of the hydrophobic character of the A β 42 sequence. However, negatively charged aspartic acid residues were introduced into the combinatorial mix to act as potential aggregation breakers. The goal was to produce a peptide (or series of peptides) having a nonpolar face capable of annealing tightly to A β 42, while displaying a highly charged and polar aspartic acid residue that could sterically block additional A β 42 peptides from binding. The theoretical size of peptide Library 1 was 16 384 possible sequence variants while that of Library 2 was 1600. The actual number of clones generated was 55 000 for Library 1 and 35 000 for Library 2.

E. coli colonies co-expressing A β 42-EGFP with library peptides were incubated on LB media plates containing IPTG. Colonies were irradiated with 490 nm light to visually identify those with the greatest amount of fluorescence emission at 516 nm (Figure 2). Six colonies were initially selected based on their fluorescence emission intensity. Of the six colonies selected, only three showed a substantially increased amount of fluorescence when grown in solution (Figure S1, Supporting information). Two of these colonies were selected from Library 1 (named peptides 1A and 1B) and the third colony was selected from Library 2 (named peptide 2). The plasmid DNA from these clones was purified and sequenced to identify the selected peptides (Table 1).

With any screen, it is important to verify the activity of the selected candidates using additional experimental tests. Candidates selected with this screen should be capable of inhibiting A β 42 aggregation. However, this screen could unintentionally select for peptides that help EGFP to fold and fluoresce without necessarily preventing A β 42 aggregation. To directly test the ability of the selected peptides to inhibit A β 42 aggregation, the ThT fluorescence assay was used. ThT fluorescence has been shown to be a useful indicator for detecting and quantifying amyloid fibril formation [35,36]. ThT binds to the cross β -structure of amyloid proteins. The fluorescent properties of ThT change when the dye moves from an aqueous environment, to the aggregated amyloid protein. These fluorescent changes can be quantitated. As the concentration of amyloid increases, so too does the ThT fluorescence. The three selected peptides were commercially synthesized and dissolved in PBS buffer, pH 7.0. The aggregation time course of A β 42 alone and in the presence of each of the selected peptides (at 185 and 249 μ M) is shown in Figure 3. At 185 μ M, peptide 1A shows modest ability to inhibit A β 42 aggregation, while peptides 1B and 2 show strong inhibitory potential. At 249 μ M, all three selected peptides show considerable inhibitory potential. Selected peptides incubated with ThT (in the absence of A β 42) had the same fluorescence properties as ThT alone in buffer (data not shown).

While many substances have been shown to inhibit A β 42 aggregation, few are known to disaggregate preformed A β 42 fibers. Peptides 1A, 1B, and 2 were tested using ThT for their ability to disaggregate preformed A β 42 fibers. Figure 4 shows the time course of A β 42 disaggregation in the presence of peptide 2. While peptides 1A and 1B were found to inhibit A β 42 aggregation, they were not found capable of disaggregating preformed A β 42 fibers. However, peptide 2 was shown to inhibit A β 42 aggregation as well as disaggregate preformed A β 42 fibers. Concentrations of peptide 2 as low as 400 μ M were found to disaggregate preformed A β 42 (Figure 5) within 24 h. Peptide 2 is one of the few peptides we are aware of, along with those of Sato *et al.* and Soto *et al.*, shown to disaggregate preformed A β 42 fibers [26,27].

Conclusion

An A β 42-EGFP construct was successfully used to select for peptides capable of inhibiting the aggregation of A β 42. This screen selected for peptides that could resist *in vivo* degradation and inhibit A β 42 aggregation. Two different peptide libraries, with a theoretical diversity of nearly 18 000 different sequences, were screened. From this screen, three peptides were ultimately selected for further investigation. ThT assays indicated that the selected peptides were strong inhibitors of A β 42 aggregation. Subsequent testing demonstrated that peptide 2 is one of the few peptides known to disaggregate preformed A β 42 fibers [26,27].

Supplementary Material

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Abbreviations used

EGFP	enhanced green fluorescent protein
ThT	Thioflavin T
HFIP	hexafluoroisopropanol
O.D. ₆₀₀	optical density at 600 nm
IPTG	Isopropyl β -D-1-thiogalactopyranoside
PBS	phosphate buffered saline, pH 7.0.
HSP20	Heat Shock Protein 20

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References

1. Bucciantini M, Giannoni E, Chiti F, Baroni F, Formigli L, Zurdo JS, Taddei N, Ramponi G, Dobson CM, Stefani M. Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature* 2002;416:507–511. [PubMed: 11932737]
2. Chiti F, Dobson CM. Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem* 2006;75:333–366. [PubMed: 16756495]
3. Murphy RM, Kendrick BS. Protein misfolding and aggregation. *Biotechnol. Prog* 2007;23:548–552. [PubMed: 17425329]
4. Calero M, Rostagno A, Frangione B, Ghiso J. Clusterin and Alzheimer's disease. *Subcell. Biochem* 2005;38:273–298. [PubMed: 15709484]

5. Lee S, Carson K, Rice-Ficht A, Good T. Hsp20, a novel alpha-crystallin, prevents Abeta fibril formation and toxicity. *Protein Sci* 2005;14:593–601. [PubMed: 15722443]
6. Santhoshkumar P, Sharma KK. Inhibition of amyloid fibrillogenesis and toxicity by a peptide chaperone. *Mol. Cell. Biochem* 2004;267:147–155. [PubMed: 15663196]
7. Yerbury JJ, Poon S, Meehan S, Thompson B, Kumita JR, Dobson CM, Wilson MR. The extracellular chaperone clusterin influences amyloid formation and toxicity by interacting with prefibrillar structures. *FASEB J* 2007;21:2312–2322. [PubMed: 17412999]
8. Wilhelmus MMM, Boelens WC, Otte-Holler I, Kamps B, de Waal RMW, Verbeek MM. Small heat shock proteins inhibit amyloid-beta protein aggregation and cerebrovascular amyloid-beta protein toxicity. *Brain Res* 2006;1089:67–78. [PubMed: 16635482]
9. Smith TJ, Stains CI, Meyer SC, Ghosh I. Inhibition of beta-amyloid fibrillization by directed evolution of a beta-sheet presenting miniature protein. *J. Am. Chem. Soc* 2006;128:14456–14457. [PubMed: 17090018]
10. Robert R, Dolezal O, Waddington L, Hattarki MK, Cappai R, Masters CL, Hudson PJ, Wark KL. Engineered antibody intervention strategies for Alzheimer's disease and related dementias by targeting amyloid and toxic oligomers. *Protein Eng. Des. Sel* 2009;22:199–208. [PubMed: 18927231]
11. Yang F, Lim GP, Begum AN, Ubada OJ, Simmons MR, Ambegaokar SS, Chen PP, Kaye R, Glabe CG, Frautsch SA, Cole GM. Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo. *J. Biol. Chem* 2005;280:5892–5901. [PubMed: 15590663]
12. Ritchie CW, Bush AI, Mackinnon A, Macfarlane S, Mastwyk M, Mac-Gregor L, Kiers L, Cherny R, Li QX, Tammer A, Carrington D, Mavros C, Volitakis I, Xilinas M, Ames D, Davis S, Beyreuther K, Tanzi RE, Masters CL. Metal-protein attenuation with iodocholehydroxyquin (clioquinol) targeting Abeta amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial. *Arch. Neurol* 2003;60:1685–1691. [PubMed: 14676042]
13. Ritchie CW, Bush AI, Masters CL. Metal-protein attenuating compounds and Alzheimer's disease. *Expert Opin. Investig. Drugs* 2004;13:1585–1592.
14. Wood SJ, MacKenzie L, Maleeff B, Hurle MR, Wetzel R. Selective inhibition of Abeta fibril formation. *J. Biol. Chem* 1996;271:4086–4092. [PubMed: 8626745]
15. Lashuel HA, Hartley DM, Balakhaneh D, Aggarwal A, Teichberg S, Callaway DJ. New class of inhibitors of amyloid-beta fibril formation. Implications for the mechanism of pathogenesis in Alzheimer's disease. *J. Biol. Chem* 2002;277:42881–42890. [PubMed: 12167652]
16. Talaga P. Beta-amyloid aggregation inhibitors for the treatment of Alzheimer's disease: dream or reality? *Mini Rev. Med. Chem* 2001;1:175–186. [PubMed: 12369982]
17. Cohen T, Frydman-Marom A, Rechter M, Gazit E. Inhibition of amyloid fibril formation and cytotoxicity by hydroxyindole derivatives. *Biochemistry* 2006;45:4727–4735. [PubMed: 16605241]
18. Porat Y, Abramowitz A, Gazit E. Inhibition of amyloid fibril formation by polyphenols: structural similarity and aromatic interactions as a common inhibition mechanism. *Chem. Biol. Drug Des* 2006;67:27–37. [PubMed: 16492146]
19. Porat Y, Mazor Y, Efrat S, Gazit E. Inhibition of islet amyloid polypeptide fibril formation: a potential role for heteroaromatic interactions. *Biochemistry* 2004;43:14454–14462. [PubMed: 15533050]
20. Saengkhae C, Salerno M, Ades D, Siove A, Moyec L, Migonney V, Garnier-Suillerot A. Ability of carbazole salts, inhibitors of Alzheimer beta-amyloid fibril formation, to cross cellular membranes. *Eur. J. Pharmacol* 2007;559:124–131. [PubMed: 17291491]
21. Riviere C, Richard T, Quentin L, Krisa S, Merillon JM, Monti JP. Inhibitory activity of stilbenes on Alzheimer's beta-amyloid fibrils in vitro. *Bioorg. Med. Chem* 2007;15:1160–1167. [PubMed: 17049256]
22. Hull RL, Zraika S, Udaya-Sankar J, Kisilevsky R, Szarek WA, Kahn SE. Inhibition of islet amyloid formation in vitro by a small molecule inhibitor that reduces heparan sulfate proteoglycan (HSPG) synthesis. *Diabetes* 2006;55:A372–a372.
23. Hamaguchi T, Ono K, Yamada M. Anti-amyloidogenic therapies: strategies for prevention and treatment of Alzheimer's disease. *Cell. Mol. Life Sci* 2006;63:1538–1552. [PubMed: 16804637]
24. Cherny RA, Atwood CS, Xilinas ME, Gray DN, Jones WD, McLean CA, Barnham KJ, Volitakis I, Fraser FW, Kim Y, Huang X, Goldstein LE, Moir RD, Lim JT, Beyreuther K, Zheng H, Tanzi RE,

- Masters CL, Bush AI. Treatment with a copper-zinc chelator markedly and rapidly inhibits beta-amyloid accumulation in Alzheimer's disease transgenic mice. *Neuron* 2001;30:665–676. [PubMed: 11430801]
25. Orner BP, Liu L, Murphy RM, Kiessling LL. Phage display affords peptides that modulate beta-amyloid aggregation. *J. Am. Chem. Soc* 2006;128:11882–11889. [PubMed: 16953628]
26. Soto C, Sigurdsson EM, Morelli L, Kumar RA, Castano EM, Frangione B. Beta-sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: implications for Alzheimer's therapy. *Nat. Med* 1998;4:822–826. [PubMed: 9662374]
27. Sato T, Kienlen-Campard P, Ahmed M, Liu W, Li H, Elliott JI, Aimoto S, Constantinescu SN, Octave JN, Smith SO. Inhibitors of amyloid toxicity based on beta-sheet packing of Abeta40 and Abeta42. *Biochemistry* 2006;45:5503–5516. [PubMed: 16634632]
28. Esler WP, Stimson ER, Ghilardi JR, Felix AM, Lu YA, Vinters HV, Mantyh PW, Maggio JE. A beta deposition inhibitor screen using synthetic amyloid. *Nat. Biotechnol* 1997;15:258–263. [PubMed: 9062926]
29. Blanchard BJ, Chen A, Rozeboom LM, Stafford KA, Weigele P, Ingram VM. Efficient reversal of Alzheimer's disease fibril formation and elimination of neurotoxicity by a small molecule. *Proc. Natl. Acad. Sci. U.S.A* 2004;101:14326–14332. [PubMed: 15388848]
30. Wurth C, Guimard NK, Hecht MH. Mutations that reduce aggregation of the Alzheimer's Abeta42 peptide: an unbiased search for the sequence determinants of Abeta amyloidogenesis. *J. Mol. Biol* 2002;319:1279–1290. [PubMed: 12079364]
31. Wurth C, Kim W, Hecht MH. Combinatorial approaches to probe the sequence determinants of protein aggregation and amyloidogenicity. *Protein Pept. Lett* 2006;13:279–286. [PubMed: 16515456]
32. Kim W, Kim Y, Min J, Kim DJ, Chang YT, Hecht MH. A high-throughput screen for compounds that inhibit aggregation of the Alzheimer's peptide. *ACS Chem. Biol* 2006;1:461–469. [PubMed: 17168524]
33. Stemmer WPC, Cramer A, Ha KD, Brennan TM, Heyneker HL. Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides. *Gene* 1995;164:49–53. [PubMed: 7590320]
34. LeVine H 3rd. Thioflavine T interaction with synthetic Alzheimer's disease beta-amyloid peptides: detection of amyloid aggregation in solution. *Protein Sci* 1993;2:404–410. [PubMed: 8453378]
35. Rogers DR. Screening for amyloid with the thioflavin-T fluorescent method. *Am. J. Clin. Pathol* 1965;44:59–61. [PubMed: 14314221]
36. Saeed SM, Fine G. Thioflavin-T for amyloid detection. *Am. J. Clin. Pathol* 1967;47:588–593. [PubMed: 4164576]

Aβ42:	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGGVIA
Library 1:	MSNKGAI <u>IIVLMVVVVV</u> ADSHS
	TTA AAAAA
	NND DDDDD
	SSG GGGGG
Aβ42:	DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGGVIA
Library 2:	MDKL <u>VFFAEVV</u> GSNK
	ALL AA
	DII DD
	GMM GG
	VV

Figure 1.

Amino acid sequence of Aβ42 compared to the degenerate amino acid sequences of Library 1 and Library 2 peptides. Combinatorial mixtures are indicated with amino acids shown in bold. Underlined residues were conserved sequences designed to match Aβ42.

Figure 2.

Colonies expressing both A β 42-EGFP and library peptides are visualized under UV-light using a Bio-Rad Molecular Imager VersaDoc MP Imaging System. Colonies with the greatest level of fluorescence were selected for DNA sequencing and further *in vitro* tests. Note: The CCD camera detector does not show data in color. Data are shown as fluorescence emission intensity.

Figure 3.

Time course of A β 42 aggregation as monitored with ThT binding in the presence of selected peptides at (A) 185 and (B) 249 μ M. A β 42 was 40 μ M for all samples. ThT fluorescence in the presence of each peptide is shown as a percentage of the ThT fluorescence of A β 42 alone at each time point. The ThT fluorescence for A β 42 alone increased for each time point. This figure is available in colour online at www.interscience.wiley.com/journal/jpepsci.

Figure 4.

Disaggregation of preformed A β 42 aggregates as monitored with ThT binding in the presence of Peptide 2. Peptide 2 (1.2 mM) showed substantial ability to disaggregate preformed fibrils (initial A β 42 concentration was 40 μ M). Data are the average of four separate trials conducted on separate days. This figure is available in colour online at www.interscience.wiley.com/journal/jpepsci.

Figure 5.

ThT fluorescence of preformed A β 42 fibrils (40 μ M) with varying concentrations of peptide 2. To promote aggregation, A β 42 was incubated in PBS buffer for 24 h at 37 °C with vigorous shaking. Peptide 2 was added, at varying concentrations, to the preformed fibers and incubated for 24 h at 37 °C with vigorous shaking. After 24 h, the samples were tested with the ThT binding assay. The raw data are shown. This figure is available in colour online at www.interscience.wiley.com/journal/jpepsci.

Table 1

Selected peptide sequences

Peptide	Sequence
Peptide 1A	MSNKGASIGLM AGD VDIADSHS
Peptide 1B	MSNKGASNALM AGDGD IADSHS
Peptide 2	MQKLDV VVAEDAGSNK

Peptides 1A, 1B, and 2 were selected for their ability to prevent A β 42 aggregation as detected by an increase in A β 42-EGFP fluorescence. Amino acids that differ from wild-type A β 42 are shown in bold.