Inhibition of β-Amyloid Aggregation and Neurotoxicity by Complementary (Antisense) Peptides**

Jonathan R. Heal,[b] Gareth W. Roberts,[b] Gary Christie,[c] and Andrew D. Miller*[a]

Complementary peptides are coded for by the nucleotide sequence (read 5′ →3′) of the complementary strand of DNA. By reading the sequence of complementary DNA in the 3′ →5′ direction, alternative complementary peptides may be derived. We describe the derivation, testing and analysis of six complementary peptides designed against β-amyloid peptide 1-40 ($A\beta_{1-40}$). Data is presented to show that one peptide, designated 3′ →5′ βCP_{1-15}, binds specifically to $A\beta_{1-40}$, and inhibits both fibrilisation and neurotoxicity in vitro. This suggests that complementary peptides could be useful leads for drug discovery, especially where diseases of protein misfolding are concerned.

KEYWORDS:

Alzheimer's Disease · antisense agents · antisense peptides · complementary peptides · molecular recognition

Introduction

Amyloid peptides $A\beta_{1-40}$ and $A\beta_{1-42}$ are significant components of neurofibrillary tangles whose deposits are associated with the neurodegenerative disorder Alzheimer's disease (AD).[1] These Aβ peptides and fragments thereof aggregate readily into fibrils,[2] and these are neurotoxic.[3] Fragmentation of Aβ peptides may produce reactive oxygen species (ROS) that cause neuronal damage by oxidative stress.[4] Alternative mechanisms of toxicity may involve RAGE – Aβ interactions (RAGE = receptor for advanced glycation end products).[5] Amino-terminal truncations of Aβ peptides are also found in AD deposits,[6] and are associated with even more severe pathophysiological effects than the full-length parent peptides.[7] Recent evidence now suggests that the γ-secretase that generates Aβ peptides from full length amyloid precursor proteins (APP) may be presenilin-1 (PS1).[8] Mutations in the PS1 gene are the most common cause of familial early-onset AD.[9] Therefore, the pathophysiology of AD appears to be converging on one main pathway involving the generation and aggregation (fibrilisation) of excess levels of Aβ peptides, which culminates in acute neurotoxity.[10] Without doubt, blocking Aβ fibrilisation is an important therapeutic strategy to counter AD. Here we report a novel use of the complementary peptide approach to try and devise a simple peptide able to selectively bind to a full-length Aβ peptide ($A\beta_{1-40}$), thereby inhibiting both Aβ fibrilisation and the attendant neurotoxicity.

Results and Discussion

Double helical DNA is comprised of two antiparallel 2′-deoxypolynucleotide chains. Traditionally, one of these chains, the sense strand has been thought to harbor the coding information for proteins and peptides, whilst the antisense (complementary) strand provides the means of propagating that information. However, recent evidence shows that coding information may be extracted from the complementary strand as well.[11] Furthermore, peptides coded for by sense and complementary strands of DNA are actually able to interact specifically in a way that may be comparable to the specific interaction between the two strands of DNA.[12] By definition, a complementary peptide is coded for by the nucleotide sequence (read 5′ →3′) of the complementary strand of DNA (or, more precisely, by codons in complementary mRNA whose sequence contains the same coding information as the complementary strand of DNA). The codons in complementary mRNA may also be read continuously in the 3′ →5′ (Root – Bernstein direction) to give an alternative complementary peptide (Table 1).[12, 13] Our approach to devising a novel inhibitor of Aβ fibrilisation has been to exploit this concept of complementary peptides, including importantly the concept of 3′ →5′ complementary peptides.

[a] *Dr. A. D. Miller*
Imperial College Genetic Therapies Centre
Department of Chemistry, Flowers Building, Armstrong Road
Imperial College of Science, Technology and Medicine
South Kensington, London, SW7 2AZ (UK)
Fax: (+ 44) 20-7594-5803
E-mail: a.miller@ic.ac.uk

[b] *Dr. J. R. Heal, Dr. G. W. Roberts*
Proteom Ltd, Babraham Hall
Babraham, Cambridge, CB2 4AT (UK)

[c] *Dr. G. Christie*
Glaxo Smith Kline Pharmaceuticals
New Frontiers Science Park (North)
Third Avenue, Harlow, Essex, CM19 5AW (UK)

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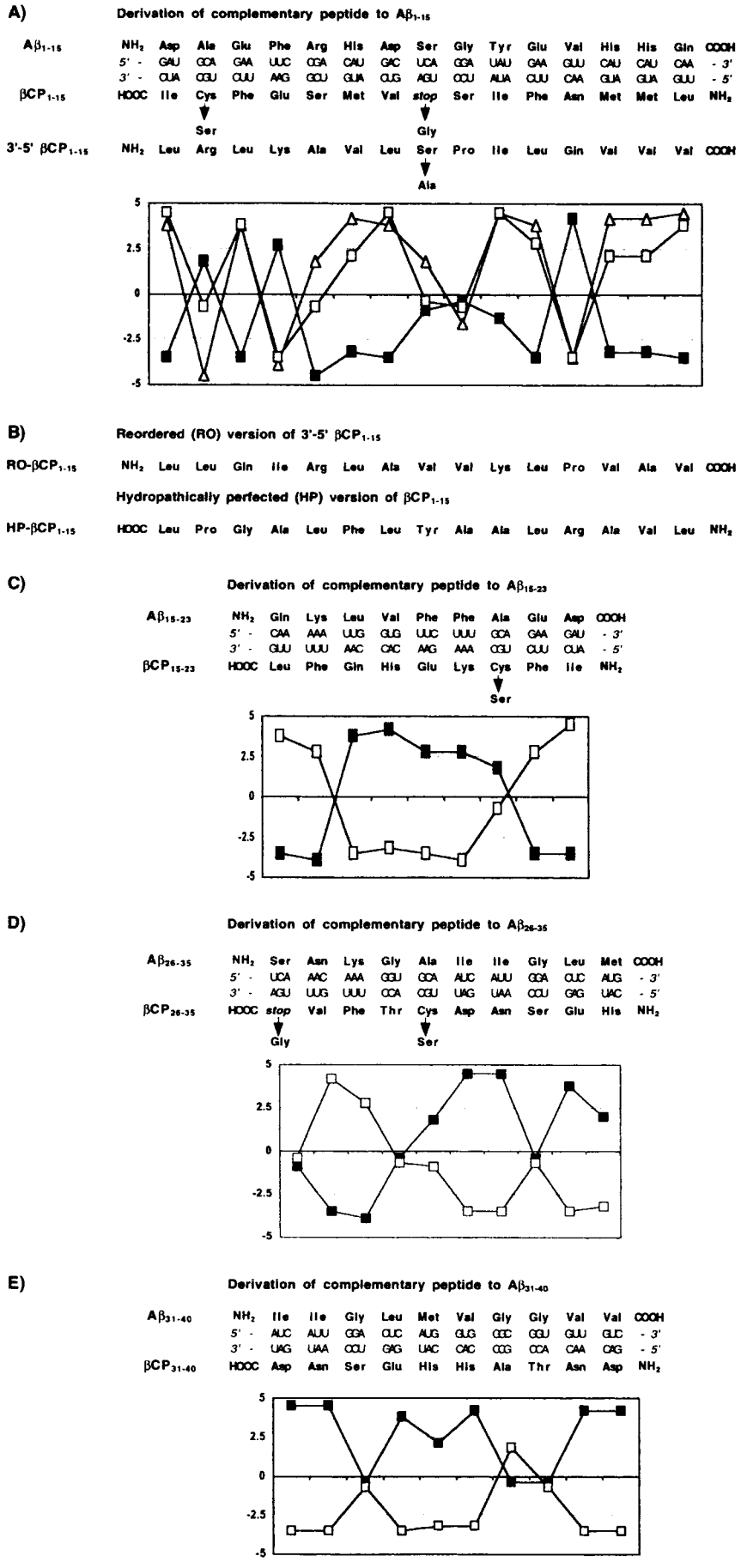
Table 1. *Table to show the Root–Bernstein derivation of 3′ →5′ complementary peptides.*[13]

Amino acid	Codon 5′ → 3′[a]	Complementary codon 3′ → 5′[a]	Complementary amino acid	Amino acid	Codon 5′ → 3′[a]	Complementary codon 3′ → 5′[a]	Complementary amino acid
Ala (A)	GCA	CGU	Arg (R)	Ser (S)	UCA	AGU	Ser (S)
	GCG	CGC	Arg (R)		UCC	AGG	Arg (R)
	GCC	CGG	Arg (R)		UCG	AGC	Ser (S)
	GCU	CGA	Arg (R)		UCU	AGA	Arg (R)
					AGC	UCG	Ser (S)
					AGU	UCA	Ser (S)
Arg (R)	CGG	GCC	Ala (A)	Gln (Q)	CAA	GUU	Val (V)
	CGA	GCU	Ala (A)		CAG	GUC	Val (V)
	CGC	GCG	Ala (A)				
	CGU	GCA	Ala (A)				
	AGG	UCC	Ser (S)				
	AGA	UCU	Ser (S)				
Asp (D)	GAC	CUG	Leu (L)	Gly (G)	GGA	CCU	Pro (P)
	GAU	CUA	Leu (L)		GGC	CCG	Pro (P)
					GGU	CCA	Pro (P)
					GGG	CCC	Pro (P)
Asn (N)	AAC	UUG	Leu (L)	His (H)	CAC	GUG	Val (V)
	AAU	UUA	Leu (L)		CAU	GUA	Val (V)
Cys (C)	UGU	ACA	Thr (T)	Ile (I)	AUA	UAU	Tyr (Y)
	UGC	ACG	Thr (T)		AUC	UAG	Stop
					AUU	UAA	Stop
Glu (E)	GAA	CUU	Leu (L)	Leu (L)	CUG	GAC	Asp (D)
	GAG	CUC	Leu (L)		CUC	GAG	Glu (E)
					CUU	GAA	Glu (E)
					UUA	AAU	Asn (N)
					CUA	GAU	Asp (D)
					UUG	AAC	Asn (N)
Lys (K)	AAA	UUU	Phe (F)	Thr (T)	ACA	UGU	Cys (C)
	AAG	UUC	Phe (F)		ACG	UGC	Cys (C)
					ACC	UGG	Trp (W)
					ACU	UGA	Stop
Met (M)	AUG	UAC	Tyr (Y)	Trp (W)	UGG	ACC	Thr (T)
Phe (F)	UUU	AAA	Lys (K)	Tyr (Y)	UAC	AUG	Met (M)
	UUC	AAG	Lys (K)		UAU	AUA	Ile (I)
Pro (P)	CCA	GGU	Gly (G)	Val (V)	GUA	CAU	His (H)
	CCC	GGG	Gly (G)		GUG	CAC	His (H)
	CCU	GGA	Gly (G)		GUC	CAG	Gln (Q)
	CCG	GGC	Gly (G)		GUU	CAA	Gln (Q)

[a] All possible complementary amino acid residues in a complementary peptide are identified in this case by reading these complementary codons in the 3′ →5′ direction.

The shortest region of Aβ able to aggregate and display neurotoxic properties corresponds to the C-terminal amino acid residues.[10, 14, 15] Therefore, we surmised that a complementary peptide to this region could interact specifically with full-length Aβ peptides and prevent both fibrilisation and neurotoxicity. With this thought in mind, the sense mRNA sequence of Aβ was obtained and the sequence of a 5′ →3′ complementary peptide βCP_{31-40} (complementary to $A\beta_{31-40}$) was deduced from the corresponding complementary mRNA sequence (Figure 1). 5′ →3′ Complementary peptides βCP_{1-15}, βCP_{15-23} and βCP_{26-35} were also designed to complement the regions $A\beta_{1-15}$, $A\beta_{15-23}$ and $A\beta_{26-35}$ respectively. The original complementary mRNA derived sequences of βCP_{1-15} and βCP_{26-35} were complicated by the appearance of "stop" codons. In both cases, glycine was substituted for the appearance of these "stop" signals. Furthermore, in the cases of βCP_{1-15}, βCP_{15-23} and βCP_{26-35}, serine was used as a substitute for cysteine to avoid any added complications from thiol group oxidation.

Two alternatives of βCP_{1-15} were also designed, namely a "hydropathically perfected" version, HP-βCP_{1-15} and a 3′ →5′ complementary peptide, 3′ →5′ βCP_{1-15} (Figure 1). In the past, much has been made of the fact that sense and complementary peptides are mutually complementary with respect to their hydropathic profiles (according to the Kyte–Doolittle scale)[16] and are, therefore, able to interact specifically on account of their "mutually complementary shapes" (secondary and tertiary structures).[17] The HP-βCP_{1-15} peptide was designed for this reason.[18] These same considerations resulted in the use of alanine as a substitute for a serine residue in the sequence of 3′ →5′ βCP_{1-15}, so as to maximise the mutual complementarity of the hydropathic profiles of 3′ →5′ βCP_{1-15} and $A\beta_{1-15}$ (see Figure 1).



Interactions between complementary peptides and Aβ were investigated by means of resonant mirror biosensor and enzyme-linked immunosorbent assays (ELISA) assays. Resonant mirror biosensor assays rely on being able to immobilise a "receptor" in a hydrogel layer mounted over a prism block. Interaction of a "ligand" with an immobilised "receptor" provokes refractive index changes in the hydrogel layer that lead to changes in the resonant angle at which evanescent waves are able to penetrate (tunnel) into the layer. Hence binding events may be monitored in real time by observing changes in the resonant angle (in arcs) as a function of time. A typical set of experimental association data is shown (Figure 2a) for the interaction of the peptide 3′ $\rightarrow$5′ βCP$_{1-15}$ and Aβ_{1-40} immobilised on a biosensor carboxymethyl dextran (CMD) cuvette. Unfortunately, this binding data set was too far from saturation ($\ll$70%) to use the software supplied by Affinity Sensors (FASTfit) in order to derive meaningful kinetic and binding constants for this interaction. Peptide aggregation in the cuvette at higher concentrations proved to be the main problem. Therefore, no constants were derived. The 3′ $\rightarrow$5′ βCP$_{1-15}$ was found not to associate with either immobilised bovine serum albumin (BSA) or a blank CMD cuvette (results not shown). In addition, all the other complementary peptides showed no detectable affinity for immobilised Aβ_{1-40}, BSA or blank CMD cuvette.

The sequence specificity of the interaction between 3′ $\rightarrow$5′ βCP$_{1-15}$ and Aβ_{1-40} was shown in two main ways. Firstly, reordered (RO) βCP$_{1-15}$ was prepared as a control (Figure 1). This peptide was designed to have the same amino acid residue composition as 3′ $\rightarrow$5′ βCP$_{1-15}$ but with the sequence reordered so as to completely alter the hydropathic profile. As expected, this did not associate significantly with immobilised Aβ_{1-40} (results not shown). Second, the ability of 3′ $\rightarrow$5′ βCP$_{1-15}$ to block the interaction between Aβ-specific polyclonal antibody (2FI2; anti-Aβ_{4-17}) and immobilised

Figure 1. *Derivation of complementary and control peptides for Aβ. Kyte – Doolittle hydropathy plots are also shown to illustrate mutual hydropathic complementarity between Aβ sense peptide fragments and the complementary peptides as follows: A) Aβ$_{1-15}$ (■), βCP$_{1-15}$ (□), 3′ $\rightarrow$5′ βCP$_{1-15}$ (△); C) Aβ$_{15-23}$ (■), βCP$_{15-23}$ (□); D) Aβ$_{26-35}$ (■), βCP$_{26-35}$ (□) and E) Aβ$_{31-40}$ (■), βCP$_{31-40}$ (□), respectively. In (B), profiles of HP βCP$_{1-15}$ and RO βCP$_{1-15}$ are not shown but may be inferred from their respective sequences and from the explanations in the text concerning their derivation.*

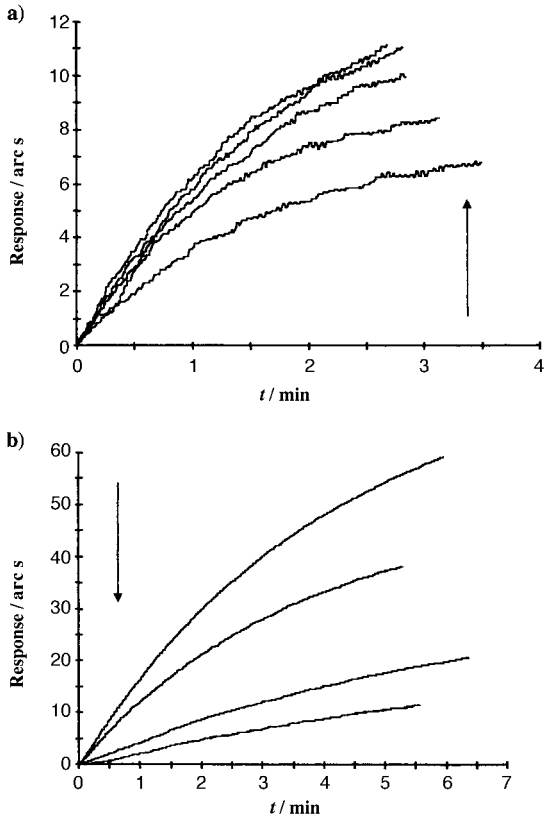


Figure 2. *a) Overlaid biosensor association traces showing the interaction between 3′→5′ βCP_{1-15} (from lower to upper trace in the direction of the arrow: 2, 5, 10, 20, 40 $\mu g\,mL^{-1}$) and $A\beta_{1-40}$ (3.98 μM) immobilised on CMD cuvette. Interactions were studied in phosphate-buffered saline (pH 7.4) containing Tween-20 (0.05%) at 25 °C using an IAsys resonant mirror biosensor as described previously.[19, 21] b) Overlaid association traces showing the extent of interaction between polyclonal antibody 2FI2 (2.4 $\mu g\,mL^{-1}$) and CMD-immobilised $A\beta_{1-40}$ (3.98 μM) in the presense of 3′→5′ βCP_{1-15} (from upper to lower trace in the direction of the arrow: 0, 2, 10, 20 $\mu g\,mL^{-1}$).*

$A\beta_{1-40}$ was investigated with the biosensor. Although 2FI2 is able to bind $A\beta_{1-40}$ tightly ($K_d = 4.2 \pm 0.8$ nM), the interaction was found to be disrupted by 3′→5′ βCP_{1-15} in a concentration-dependent manner, which is consistent with competitive inhibition of binding (Figure 2b). RO βCP_{1-15} was unable to do the same. Subsequently, we were able to perform a control experiment to show that 3′→5′ βCP_{1-15} was not able to interact with immobilised 2FI2 antibody directly (results not illustrated), therefore the ability of 3′→5′ βCP_{1-15} to inhibit 2FI2 – $A\beta_{1-40}$ interactions could not be due to a direct interaction between 3′→5′ βCP_{1-15} and 2FI2 but only to the specific sense – complementary peptide interaction between 3′→5′ βCP_{1-15} and $A\beta_{1-40}$.

Given the difficulties of obtaining quantitative information from biosensor data, ELISA assays were performed to try and obtain some quantitative information. Initially, the specificity of the 2FI2 antibody affinity was assessed in preliminary ELISA experiments. $A\beta_{1-15}$, βCP_{1-15}, 3′→5′ βCP_{1-15}, HP-βCP_{1-15} and RO βCP_{1-15} were absorbed at a fixed concentration (6 μg mL^{-1}) in different wells of the same microtitre plate, then serial double dilutions of 2FI2 were introduced across the plate followed by additions of alkaline phosphatase conjugate and a chromogenic substrate (*p*-nitrophenylphosphate). The 2FI2 antibody was confirmed to bind significantly only to $A\beta_{1-15}$, to give a measurable absorbance at A_{405} in the ELISA test (Figure 3a).

Subsequently, in the first main set of ELISA experiments $A\beta_{1-15}$, βCP_{1-15}, 3′→5′ βCP_{1-15}, HP-βCP_{1-15} and RO βCP_{1-15} were adsorbed at a fixed concentration (9 μg mL^{-1}) in different wells of another microtitre plate and incubated with different concentrations of $A\beta_{1-15}$. The amount of $A\beta_{1-15}$ "captured" by each adsorbed peptide in each well was then estimated by using fixed dilutions (1:100) of 2FI2 and the linked alkaline phosphatase conjugate. Only the 3′→5′ βCP_{1-15} complementary peptide was able to capture enough $A\beta_{1-15}$ to give significant absorbance values at A_{405} in the ELISA test (Figure 3b); this is in keeping with the results of the biosensor assay analysis.

The effect of the complementary peptides upon the interaction between $A\beta_{1-40}$ and 2FI2 was then studied in a second main set of ELISA competition experiments. In these, the ability of 2FI2 to interact with immobilised $A\beta_{1-40}$ was studied in the presence and absence of all the complementary and control peptides described above. The total amount of antibody bound to immobilised $A\beta_{1-40}$ (*B*) in the presence of various concentrations of each peptide was expressed as a fraction of the total amount of antibody available (B_0; Figure 3c). Only complementary peptide 3′→5′ βCP_{1-15} proved able to inhibit antibody binding to immobilised $A\beta_{1-40}$, a result completely consistent with the foregoing ELISA and biosensor data. In this case, data were analyzed according to Equation (1), where IC_{50} is the concentration of peptide required to reduce 2FI2 binding to immobilised $A\beta_{1-40}$ by 50%, [*L*] is the concentration of peptide and K_i is the inhibition constant.

$$IC_{50}/K_d = 1 + [L]/K_i \quad (1)$$

However, Equation (1) may simplify to Equation (2). This is applicable when [*L*] is small compared to K_i. By using Equation (2), a dissociation constant, K_d, of 2.4 ± 1.2 μg mL^{-1} (1.5 ± 0.8 μM, FWt 1631) was estimated for the interaction of 3′→5′ βCP_{1-15} with immobilised $A\beta_{1-40}$. The magnitude is completely consistent with previously reported dissociation constant values involving other sense – complementary peptide interactions.[18–21]).

$$IC_{50}/K_d = 1 \quad (2)$$

Evidence to the effect that Aβ peptides are neurotoxic following fibrilisation has led to the development of several in vitro assays designed to measure the degree of fibrilisation and neurotoxicity.[22, 23] In this case, the dissociation-enhanced lanthanide fluoro-immunoassay (DELFIA) of Schoket et al.[22] was used to evaluate the ability of 3′→5′ βCP_{1-15} and the control peptide RO-βCP_{1-15} to inhibit fibrilisation. The DELFIA assay is a simple ELISA-like assay performed with an antibody that specifically recognises the aggregated form of Aβ in preference to the nonaggregated state. Inhibition of Aβ fibrilisation leads to a proportional decrease in antibody fluorescence response. Fibrilisation-dependent neurotoxicity tests were also performed in parallel with an IMR32 cell line.[23] These were simple cell-

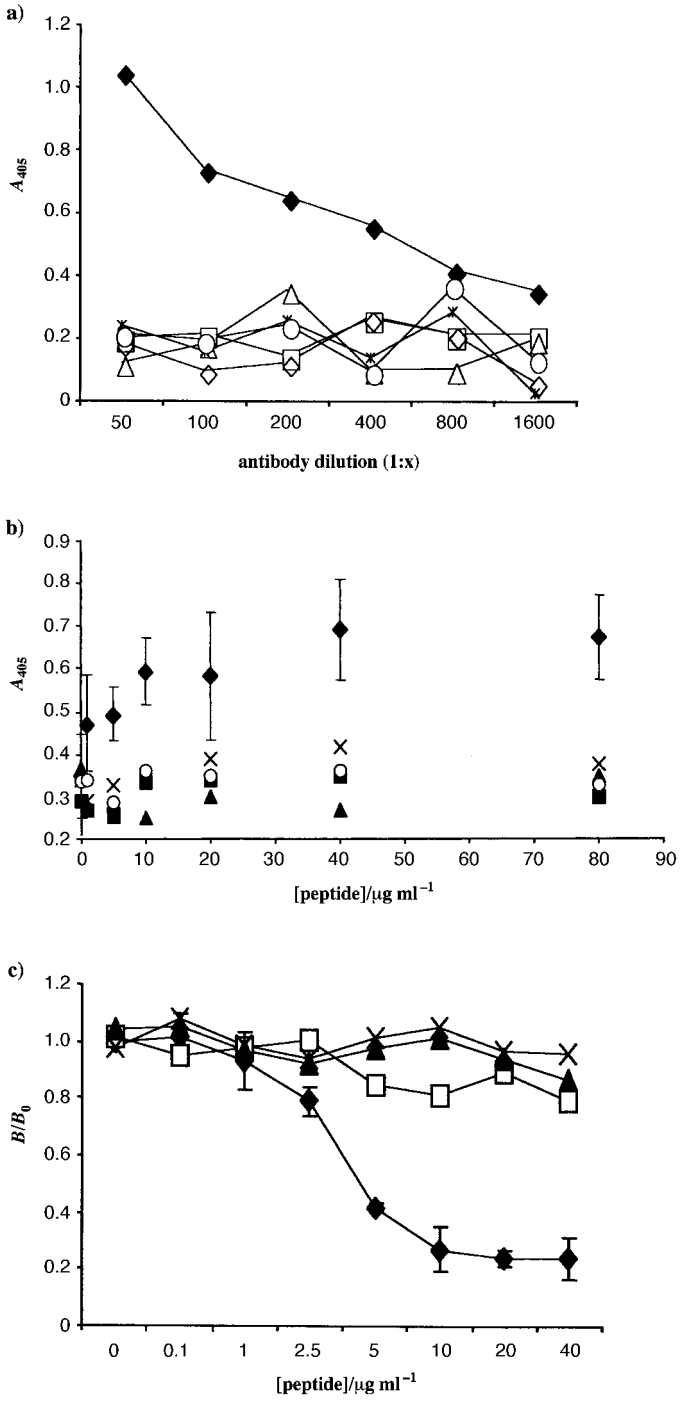


Figure 3. *a) Effect of antibody dilution on the ELISA response of ExtrAvidin alkaline phosphatase conjugate following the binding of the indicated dilutions of antibody 2FI2 to either $A\beta_{1-15}$ (◆), βCP_{1-15} (◇), 3′ →5′ βCP_{1-15} (□), HP βCP_{1-15} (∗) or RO βCP_{1-15} (△) immobilised (6 μg mL^{-1}) on microtitre plates. Response without immobilised peptide is also shown (○) b) ELISA response showing extent of interaction between $A\beta_{1-15}$ and immobilised (9 μg mL^{-1}) $A\beta_{1-15}$ (○), βCP_{1-15} (■), 3′ →5′ βCP_{1-15} (◆), HP-βCP_{1-15} (▲) or RO-βCP_{1-15} (×) as a function of $A\beta_{1-15}$ concentration in solution. Antibody 2FI2 detects $A\beta_{1-15}$ captured by immobilised peptide and extent of binding is determined by the level of ExtrAvidin alkaline phosphatase conjugate enzymatic activity. c) ELISA competition experiment to demonstrate the effect of the indicated concentrations of βCP_{1-15} (▲), 3′ →5′ βCP_{1-15} (◆), HP-βCP_{1-15} (×) or RO-βCP_{1-15} (□) on the interaction between 2FI2 and $A\beta_{1-40}$ immobilised (9 μg mL^{-1}) on microtitre plates at 25 °C in PBS-T buffer. The K_d characterising the interaction between 3′ →5′ βCP_{1-15} and immobilised $A\beta_{1-40}$ was determined from Equation (2).*

survival and -viability tests. The results show that 3′ →5′ βCP_{1-15} was able to block the vast majority of Aβ fibrilisation and, as a result, the vast majority of Aβ fibrilisation-dependent neurotoxicity (both at 10 μg mL^{-1} concentration; Table 2). The control peptide RO βCP_{1-15} was unable to block aggregation and even proved neurotoxic to the IMR32 cell line in the absence of Aβ peptides. In the light of the data described, 3′ →5′ βCP_{1-15} most likely blocks Aβ fibrilisation and, hence, neurotoxicity by interacting directly with Aβ peptides, thereby sterically preventing the growth of the Aβ aggregates that are known to be neurotoxic.[3, 10]

Table 2. *Summary of DELFIA fibrilisation assay and IMR32-cell neurotoxicity data.*[22, 23]

Peptide	Inhibition of Aβ fibrilisation in DELFIA assay [%]	Inhibition of Aβ neurotoxicity in IMR32-cell assay [%]
3′ →5′ βCP_{1-15}	91 ± 5	83 ± 10
RO βCP_{1-15}	9.5 ± 4	n.d.[a]

[a] RO βCP_{1-15} peptide was itself neurotoxic in the IMR32-cell assay.

The singular success of 3′ →5′ βCP_{1-15} is striking. Alone amongst the complementary peptides synthesised, this peptide was found to interact specifically with $A\beta_{1-40}$ and $A\beta_{1-15}$ in resonant mirror biosensor and ELISA assays. Moreover, this interaction was strong and specific enough to compete with the binding of an anti-$A\beta_{4-17}$ antibody (2FI2) to $A\beta_{1-40}$. These properties appear to be sufficient for 3′ →5′ βCP_{1-15} to be an effective inhibitor of Aβ fibrilisation and, hence, neurotoxicity. The failure of other complementary peptides to do similarly is difficult to interpret, although our most recent evidence shows that bonefide complementary peptides can vary substantially in their affinities for a corresponding sense peptide (K_d values may vary from high mM to low μM) with even the most modest of sequence differences between them.[24] Therefore, it should not be too surprising that only one out of the six complementary peptides designed and synthesised should have the ability to interact efficiently with $A\beta_{1-40}$ and behave as a biological inhibitor of Aβ effects.

Conclusion

In summary, complementary peptide 3′ →5′ βCP_{1-15} is shown to bind specifically to $A\beta_{1-40}$ and, in the process, to inhibit Aβ fibrilisation and neurotoxicity. This peptide could form the basis of a therapeutic approach against AD. The absolute specificity of this process is amply demonstrated by the failure of any other complementary peptide or the RO βCP_{1-15} control peptide to behave similarly. A BLAST search conducted with the amino acid residue sequence of 3′ →5′ βCP_{1-15} was unable to find any similar sequence within the SwissProt database. This very uniqueness could be helpful for therapeutic development and is a potent demonstration of the application of complementary peptides in generating new leads for drug discovery, in particular for diseases of protein misfolding.

Experimental Section

Materials: All materials unless otherwise stated were the purest possible grade from Sigma – Aldrich (UK). Milli Q water was used throughout (> 10 MΩ cm resistivity).

Peptide synthesis: Peptides were synthesised by standard methods on a Schimadzu PSSM-8 multi-peptide synthesiser or on an Advanced Chemtech 348 Omega multiple peptide synthesiser with N-terminal 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acids (Novabiochem, UK) and a Rink Amide MBHA solid-phase resin (Novabiochem, UK). Following deprotection and cleavage from the resin, peptides were desalted by gel filtration (2 × 28 cm, P2 biogel (Bio-Rad, UK)) eluting with 0.1 % aqueous trifluoroacetic acid (TFA). Final purification was effected by reversed-phase high-pressure liquid chromatography (HPLC; Vydac C18 column (Hichrom, UK)] with a Gilson HPLC system) eluting with a linear gradient of acetonitrile in 0.1 % aqueous TFA. Following freeze drying, peptides were either stored at − 20 °C under N_2 in anhydrous conditions or else stored as stock solutions (10 mg mL^{-1}) in dimethylsulfoxide (DMSO) under N_2 in anhydrous conditions. The identity of all peptides was confirmed before use by quantitative amino acid analysis and by positive- or negative-ion fast atom bombardment mass spectrometry (FAB-MS) as appropriate. Purity was judged to be > 95 % by reversed-phase HPLC.

Resonant mirror biosensor analysis: Binding analyses were performed on an IAsys plus biosensor (Affinity Sensors, UK). The peptide Aβ_{1-40} or appropriate control proteins (BSA or blank surface) were immobilised on CMD cuvettes over a period of 30 – 120 min in 10 mM sodium phosphate buffer at pH 4.75 by using a standard *N*-hydroxysuccinimide/1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (NHS/EDC) procedure that we had used previously.[19] Before use, aliquots of each complementary or control peptide stock solution in DMSO were diluted into phosphate-buffered saline (pH 7.4) containing 0.05 % Tween-20 (PBS-T) to create dilution series ranging in concentration from 2 – 40 μg mL^{-1} (final peptide concentrations). Each dilution series was maintained at 25 °C and used within a few hours of preparation. Measurements were obtained to try and characterise the interactions between these various concentrations of complementary or control peptides (2 – 40 μg mL^{-1}) and CMD-immobilised Aβ_{1-40} (3.98μM; approximately 3.5 ng mm^{-2}) or BSA (2.17μM). Each set of measurements involving a given peptide and a given immobilised protein, was carried out at 25 °C in PBS-T buffer (unless otherwise stated). For each measurement, the association phase was typically 250 s. After the dissociation phase a 2 min washing step (with 10 mM HCl) was used to regenerate the cuvette before the next measurement was obtained. Where necessary, derivatised cuvettes were stored overnight at 4 °C with a covering of parafilm. All experiments were performed at least in duplicate.

ELISA protocols: Set 1: Peptides Aβ_{1-15}, βCP$_{1-15}$, 3′ →5′ βCP$_{1-15}$, HP-βCP$_{1-15}$ or RO-βCP$_{1-15}$ were diluted from DMSO stock solutions (10 mg mL^{-1}) into 0.1 M sodium carbonate buffer (pH 9.6; final peptide concentration 9 μ mL^{-1}). Aliquots (200 μL well^{-1}) were then added to individual wells of a 96-well microtitre plate and incubated for 24 h at 4 °C. After washing the wells with water, egg albumin solution (1 mg mL^{-1} in 0.1 M sodium carbonate buffer, pH 9.6) was added (200 μL well^{-1}) to block unreacted sites. After 1 h, the wells were washed three times with PBS-T buffer prior to the addition of Aβ_{1-15} aliquots (200 μL well^{-1}). In the set of wells corresponding to each immobilised peptide in turn, final Aβ_{1-15} concentration covered the range 0 – 80 μg mL^{-1}. Following overnight incubation at 4 °C, the plate was washed three times with PBS-T buffer, 2FI2 antibody added (1:100 dilution in PBS-T) and incubation continued for a further 1 h. After washing the wells with PBS-T and then water, ExtrAvidin alkaline phosphatase conjugate (1:400 dilution in 0.2 M tris(hydroxymethyl)aminomethane-HCl (Tris-HCl), pH 9) was added (200 μL well^{-1}) followed by a final incubation of 1 h. The plate was washed three times with water and then 0.2 M Tris-HCl, pH 9, before the addition of *p*-nitrophenylphosphate (*p*-NPP) (1 mg mL^{-1} in 0.2 M Tris-HCl, pH 9). After 30 min, the enzymatic reaction was quenched with 3 M sodium hydroxide in 50 mM Tris-buffered saline (TBS; pH 8.0) and the plate read in a Biotek EL-900 plate reader at 405 nm. All measurements were repeated in triplicate and values used were the recorded means. This set of experiments was repeated three times.

Set 2: Aβ_{1-40} was diluted from stock solution into 0.1 M sodium carbonate buffer (pH 9.6; final peptide concentration 9 μg mL^{-1}). Aliquots (200 μL well^{-1}) were then added to individual wells of a 96-well microtitre plate and incubated for 24 h at 4 °C. After washing the wells with water, egg albumin solution (1 mg mL^{-1} in 0.1 M sodium carbonate buffer, pH 9.6) was added (200 μL well^{-1}) to block unreacted sites. After 1 h, the wells were washed three times with PBS-T buffer prior to the addition of aliquots (200 μL well^{-1}) containing 2FI2 antibody (1:400 dilution in PBS-T) and a complementary or control peptide (namely βCP$_{1-15}$, 3′ →5′ βCP$_{1-15}$, HP-βCP$_{1-15}$ or RO-βCP$_{1-15}$). Final peptide concentrations covered the range 0 – 40 μg mL^{-1}. After 1 h incubation at 25 °C, the plate was washed with PBS-T and then water followed by the addition of ExtrAvidin alkaline phosphatase conjugate (1:400 dilution in 0.2 M Tris-HCl, pH 9) (200 μL well^{-1}) and a further incubation period of 1 h. The plate was washed three times with water and then 0.2 M Tris-HCl (pH 9), and then *p*-nitrophenylphosphate (*p*-NPP; 1 mg mL^{-1} in 0.2 M Tris-HCl, pH 9) was added. After 10 min, the enzymatic reaction was quenched with 3 M sodium hydroxide in 50 mM TBS (pH 8.0) and the plate read in a Biotek EL-900 plate reader at A_{405}. All measurements were repeated in triplicate and the values used were the recorded means. This set of experiments was repeated three times.

DELFIA and toxicity assay: An aliquot of Aβ_{1-40} stock solution (2 mg mL^{-1}) in aqueous acetic acid (0.1 %) was diluted into PBS-T buffer. Similarly, aliquots of complementary or control peptide (namely 3′ →5′ βCP$_{1-15}$ or RO-βCP$_{1-15}$) stock solutions (10 mg mL^{-1}) in DMSO were diluted into the same buffer. Thereafter, fixed aliquots (5μL) of either diluted peptide were combined with fixed aliquots (50μL) of diluted Aβ_{1-40} in PBS-T (final Aβ_{1-40} concentration 50 μg mL^{-1}; other peptides 10 μg mL^{-1}) and resulting mixtures incubated for 16 h at 37 °C. Aggregation products were detected by immunoassay according to previously published protocols.[22] Toxicity assays were performed as described previously.[23] All measurements were repeated in triplicate and values used were the recorded means.

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