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Modeling the binding mechanism of Alzheimer's $A\beta_{1-42}$ to nicotinic acetylcholine receptors based on similarity with snake α-neurotoxins☆

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ABSTRACT

For over a decade, it has been known that amyloid β (Aβ) peptides of Alzheimer's disease bind to the nicotinic α7 acetylcholine receptor (AChR) with picomolar affinity, and that snake α-neurotoxins competitively inhibit this binding. Here we propose a model of the binding mechanism of Aβ peptides to α7-AChR at atomic level. The binding mechanism is based on sequence and structure similarities of Aβ residues with functional residues of snake α-neurotoxins (ATX) in complex with AChR. The binding mechanism involves residue $^{A\beta}$K28 (similar to ^{ATX}R32) which forms cation/π interactions in the acetylcholine binding site, and residues $^{A\beta}$G29-$^{A\beta}$I32 [GAII] (similar to ^{ATX}G33-^{ATX}I36 [GTII]) which form an intermolecular β-sheet with residues $^{\alpha7}$F189-$^{\alpha7}$E191 of AChR. Through these interactions, we propose that the AChR serves as a chaperone for Aβ conformational changes from α- to β-hairpin. The interactions which block channel opening provide fundamental insight into Aβ neurotoxicity and cognition impairment, that could contribute to pathogenic processes in Alzheimer's disease, thus paving the way for structure based therapies.

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of proteins and protein fragments in the brain, progressive neuronal loss, inflammation, and the gradual and inevitable decline of memory and cognition. Much effort has been invested in finding a cure for the disease and understanding its causative origins. Major milestones include the isolation of amyloid β peptides from plaques, and the demonstration of abnormal tau phosphorylation in tangles. These milestones have led to the amyloid hypothesis proposing that amyloid fibrils and plaques in the brain were the drivers of the disease, while more recent versions of the hypothesis suggest small soluble aggregates of Aβ peptides as the primary impetus of disease progression.

Amyloid β peptides (Aβ) are derived from the Amyloid Precursor Protein (APP) through sequential cleavage by various proteolytic enzymes such as aspartyl protease, β-secretase and presenilin-dependent β-secretase (De Strooper, 2000). Aβ vary in length up to 42 amino acid residues and bind to neuronal α7-AChR with pico- to femtomolar affinity (Wang et al., 2000a,b). This binding leads to intraneuronal accumulation of complexes between α7-AChR and $A\beta_{1-42}$ (Nagele et al., 2002), blocking of α7-AChR channels (Liu et al., 2001), cholinergic neurotransmission defects (Lee and Wang, 2003), Aβ fibrillization as well as fast tau phosphorylation (Wang et al., 2003), and eventually neuronal cell death (Wang et al., 2000a), all contributing to the progression of Alzheimer's disease. Importantly, the exact binding mechanism between AChR and $A\beta_{1-42}$ is unknown to date and to our knowledge no molecular model has been proposed so far. Aβ fibrillization involves formation of dimers and small oligomers followed by growth into protofibrils and fibrils via a complex multistep-nucleated polymerization that eventually forms Aβ plaques or deposits (De Strooper, 2000). The events leading up to polymerization, and in particular the initial nucleation and conversion of Aβ remains elusive in spite of recent molecular dynamics (MD) studies (Straub and Thirumalai, 2011).

Nicotinic acetylcholine receptors (AChR) are a family of ligand-gated pentameric ion channels (Lindstrom, 1995; Le Novere and Changeux, 1995; Dajas-Bailador and Wonnacott, 2004; Kalamida et al., 2007). The main function of the AChR family is to transmit signals of the neurotransmitter acetylcholine at neuromuscular junctions and in the central and peripheral nervous systems (Steinlein, 1998). To date, 17 different subunits (α1–10, β1–4, δ, ε,

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Abbreviations: AChR, acetylcholine receptor; αAChR, acetylcholine receptor α-subunit; AD, Alzheimer's disease; Aβ, amyloid β; $A\beta_{1-42}$, 42 amino acid amyloid β peptide, DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA; $A\beta_{1-40}$, same as Aβ1–42 without two last residues, IA; $^{A\beta}$X, amino acid residues of amyloid β; ^{BTX}X, residues of α-bungarotoxin (PDB ID 1L4W), representing long α-neurotoxin; ^{ATX}X, residues of atratoxin (PDB ID 1VB0), representing short α-neurotoxins; $^{\alpha7}$X, residues of the AChR are indicated with a superscript α1, α7, γ, or δ distinctive of the subunit type.

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γ) have been identified in human which can combine to generate many subtypes of homo- and heteropentameric AChR with different physiologies, pharmacologies, and anatomical distributions (Lindstrom, 1995; Le Novere and Changeux, 1995; Dajas-Bailador and Wonnacott, 2004; Kalamida et al., 2007). Two major subtypes exist in the brain, namely those comprised of α7 and those consisting of α4β2. AChR also bind a variety of agonists such as nicotine, cyticine and epibatidine, and antagonists such as D-tubocurarine, lophotoxins, Aβ peptides, and last but not least snake α-neurotoxins.

α-Neurotoxins derived from snake venom bind to AChR and competitively inhibit acetylcholine binding, thereby preventing the depolarizing action on postsynaptic membranes, and blocking neuronal transmission (Samson et al., 2002). α-Neurotoxins are divided into two groups according to their length, namely short α-neurotoxins such as atratoxin (ATX) comprising 61 residues, and long α-neurotoxins such as α-bungarotoxin (BTX) consisting of 74 amino acids. The binding mechanism of BTX to AChR was determined in our group using NMR spectroscopy at atomic level (Samson et al., 2002). In that study, we showed how BTX fits snugly into the acetylcholine binding site of AChR thereby blocking neuronal transmission.

In this study, we show that $A\beta_{1-42}$ and α-neurotoxins share surprising sequence and structural similarities. To our knowledge this is the first report of such similarities between α-neurotoxins and $A\beta_{1-42}$. The similarities are pronounced largely in functional residues of α-neurotoxins that bind the AChR. Based on the similarities and interactions of BTX with α1-AChR we propose a binding mechanism of $A\beta_{1-42}$ to α7-AChR. To the best of our information, this is the first publication of a molecular model of $A\beta_{1-42}$ in complex with α7-AChR. The model may also serve as a template for the interaction of Aβ peptides with other neuronal AChR subtypes such as the $\alpha_4\beta_2$, and $\alpha_7\beta_2$ pentamers. Finally, we suggest that AChR interactions stabilize Aβ refolding into β-rich structures. These interactions which inhibit AChR provide novel insight into Alzheimer's disease and pave the way for designing potential therapeutic drugs capable of disrupting $A\beta_{1-42}$ interactions with AChR.

1. Materials and methods

1.1. Sequence alignment and homology modeling

The sequence of $A\beta_{1-42}$ was aligned with those of short and long α-neurotoxins obtained from the Uniprot databank (http://www.uniprot.org/) using the ClustalW multiple sequence alignment tool with default values (Thompson et al., 1994). Similarly, the acetylcholine binding protein (AChBP) sequence was aligned with those of α1, α7, β, γ, and δ subunits of the AChR.

Our α7-AChR model was based on the structure of AChBP (PDB ID 1I9B (Brejc et al., 2001)). Since single AChBP subunits consist of 210 amino acids, the α7 subunits were delimited to this size. The α7-AChR was assumed to be a homopentamer. For most of the sequence, the alignment was straightforward requiring no insertion or deletions. Such segments were considered structurally conserved regions, in which the conformation of the polypeptide chain is unchanged. Random loops were generated where insertion or deletions occurred, using Pymol. No backbone–backbone clashes were observed. Side chains exhibiting steric clashes with other side chain or backbone atoms were manually assigned with an alternative rotamer conformation using Pymol.

The model of $A\beta_{1-42}$ in long α-neurotoxin conformation was based on the structure of BTX (PDB ID 1L4W) (residues of α-bungarotoxin; representing long α-neurotoxin, are indicated with a superscript BTX (^{BTX}X)). Since the homology with $A\beta_{1-42}$ is pronounced particularly in finger II of the toxin, the model was delimited to this region. The modeling process was similar to that of α7-AChR.

1.2. Docking

To dock five $A\beta_{1-42}$ molecules into the α7-AChR model, the structure of α1-AChR in complex with BTX (PDB ID 1LK1 (Samson et al., 2002)) was used as a template. Structurally conserved regions of α7-AChR were superimposed onto those of α1-AChR, and residues $^{A\beta}$K28-$^{A\beta}$I32 of $A\beta_{1-42}$ were superimposed onto residues ^{BTX}R36-^{BTX}V40 of BTX.

1.3. PDB structure search

To find PDB structures with glycine repeats, the Protein Segment Finder (PSF) search engine was used (Samson and Levitt, 2009).

2. Results

2.1. Alzheimer's $A\beta_{1-42}$ sequence and structure is similar to snake α-neurotoxins

The sequence alignment of long α-neurotoxins and $A\beta_{1-42}$ is shown in Table 1. The sequence similarity is pronounced principally in functional regions of the toxin that bind AChR, namely finger II residues ^{BTX}W28 ($^{A\beta}$F20), ^{BTX}D30 ($^{A\beta}$E22), and ^{BTX}R36-^{BTX}L42 ($^{A\beta}$K28-$^{A\beta}$L34). Of particular interest are the

Table 1
Multiple sequence alignment of Alzheimer's $A\beta_{1-42}$ and long snake α-neurotoxins. Shown are the sequences of α-neurotoxins named according to their UniProt accession ID and of $A\beta_{1-42}$. The alignment was performed using ClustalW multiple sequence alignment (Thompson et al., 1994). Identical residues are marked with asterisks (*), conserved residues with double dots (:), and semi-conserved residues with single dots (·). Note the sequence similarity of $^{A\beta}$F19-$^{A\beta}$L34 and ^{BTX}M27-^{BTX}L42 (highlighted in gray) which are both known to adopt β-hairpin conformations. Of special interest, is the similarity of $^{A\beta}$K28-$^{A\beta}$I33 and ^{BTX}R36-^{BTX}V40 (highlighted in black) which in the latter form multiple interactions with the acetylcholine receptor.

```
        ---Finger I---          ------Finger II------    ---Finger III---   ---Tail---
D2N121  IVCHTTATSPISAVTCPPGENLCYRKMWCDAFCSSRGKVVELGCAATCPSKKPYEEVTCCSTDKCNPHPKQRPG--74
D2N122  IVCHTTATSPISAVTCPPGENLCYRKMWCDALCSSRGKVVELGCAATCPSKKPYEEVTCCSTDKCNPHPKQRPG--74
P60616  IVCHTTATSPISAVTCPPGENLCYRKMWCDVFCSSRGKVVELGCAATCPSKKPYEEVTCCSTDKCNPHPKQRPG--74
D2N117  IVCHATATSPISAVTCPPGENLCYRKMWCDAFCSSRGKVVELGCAATCPSKKPYEEVTCCSTDKCNPHPKQRPG--74
D2N120  IVCHTTTASPISAVTCPPGENLCYRKMWCDAFCSSRGKVVELGCAATCPSKKPYEEVTCCSTDKCNPHPKQRPG--74
D2N116  LLCHTTSTSPISTVTCPSGENLCYTKMWCDAFCSSRGKVIELGCVATCPQPKPYEEVTCCSTDKCNPHPKQRPG--74
A1IVR8  LLCYKTP-SPINAETCPPGENLCYTKMWCDAWCSSRGKVIELGCAATCPSKKPYEEVTCCSTDKCNPHPKQRPG--73
C5ILC5  LLCYKTP-SPINAETCPPGENLCYTKMWCDAWCSSRGKVIELGCAATCPSKKPYEEVDCCSTDNCNPHPKLRP---72
A1IVR7  LLCYKTP-SPINAETCPPGENLCYTKMWCDAWCSSRGKVVELGCAATCPSKKPYEEVTCCSTDKCNPHPKQRPD--73
A1IVR9  LLCYKTP-IPINAETCPPGENLCYTKMWCDIWCSSRGKVVELGCAATCPSKKPYEEVTCCSTDKCNPHPKQRPD--73
P34073  VICYRKYT--NNVKTCPDGENVCYTKMWCDGFCTSRGKVVELGCAATCPIRKPGNEVKCCSTNKCNHPPKRKKRRP74
Aβ1-42  --------DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA-------------------------42
                        :      ::.:  .:.:* :: *  ...
```

Table 2
Multiple sequence alignment of Alzheimer's $A\beta_{1-42}$ and short snake α-neurotoxins. Shown are the sequences of α-neurotoxins named according to their UniProt accession ID and of $A\beta_{1-42}$. The alignment was performed using ClustalW multiple sequence alignment (Thompson et al., 1994). Identical residues are marked with asterisks (*), conserved residues with double dots (:), and semi-conserved residues with single dots (·). Note the sequence similarity of $^{A\beta}$F20-$^{A\beta}$I32 and ^{ATX}Y24-^{ATX}I36 of short α-neurotoxins (highlighted in gray) which are both known to adopt β-hairpin conformations. Of special interest, is the similarity of $^{A\beta}$K28-$^{A\beta}$I32 and ^{ATX}R32-^{ATX}I36 (highlighted in black) which in the latter forms multiple interactions with the acetylcholine receptor.

```
                -Finger I-            ----Finger II----     Finger III   -Tail-
P01427          LECHNQQSSQPPTTKTCSGETNCYKKWWSDHRGTIIERGCGCPKVKPGVNLNCCRTDRCNN 61
P59275          LECHNQQSSQTPTTKTCSGETNCYKKWWSDHRGTIIERGCGCPKVKPGVNLNCCRRDRCNN 61
P59276          LECHNQQSSQAPTTKTCSGETNCYKKWWSDHRGTIIERGCGCPKVKPGVNLNCCRTDRCNN 61
P60773          LECHNQQSSQAPTTKTCSGETNCYKKWWSDHRGTIIERGCGCPKVKPGVKLNCCRTDRCNN 61
P60772          LECHNQQSSQAPTTKTCSGETNCYKKWWSDHRGTIIERGCGCPKVKPGVKLNCCTTDRCNN 61
P60774          LECHNQQSSQAPTTKTCSGETNCYKKWWSDHRGTIIERGCGCPKVKPGVKLNCCTTDRCNN 61
P01426          LECHNQQSSQPPTTKTCPGETNCYKKVWRDHRGTIIERGCGCPTVKPGIKLNCCTTDKCNN 61
P01425          LECHNQQSSQPPTTKSCPGDTNCYNKRWRDHRGTIIERGCGCPTVKPGINLKCCTTDRCNN 61
P01424          MECHNQQSSQPPTTKTCPGETNCYKKQWSDHRGTIIERGCGCPSVKKGVKINCCTTDRCNN 61
P34075          KICYNQPSSQHPTTKACPGEKNCYRKQWSDHRGTIIERGCGCPTVKPGVKLHCCTTEKCNN 61
P01422          MICHNQQSSQPPTIKTCPGETNCYKKRWRDHRGTIIERGCGCPSVKKGVGIYCCKTNKCNN 61
P01423          MICHNQQSSQRPTIKTCPGETNCYKKRWRDHRGTIIERGCGCPSVKKGVGIYCCKTDKCNN 61
P25675          MICHNQQSSQPPTIKTCPGETNCYKKQWRDHRGTIIERGCGCPSVKKGVGIYCCKTDKCNN 61
P01420          MICYKQQSLQFPITTVCPGEKNCYKKQWSGHRGTIIERGCGCPSVKKGIEINCCTTDKCNN 61
P01421          MICYKQRSLQFPITTVCPGEKNCYKKQWSGHRGTIIERGCGCPSVKKGIEINCCTTDKCNN 61
Aβ(1-42)        ----DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA--------------- 42
                    .   : .       .   : :   .::*:**    *   :
```

similarity of residue ^{BTX}R36 ($^{A\beta}$K28), located on the tip of finger II, which inserts into the acetylcholine binding site (Samson et al., 2002), the similarity of residues ^{BTX}V39 ($^{A\beta}$I31), ^{BTX}V40 ($^{A\beta}$I32), ^{BTX}L42 ($^{A\beta}$L34) which form an intermolecular β-sheet with residues $^{\alpha 1}$Y189 and $^{\alpha 1}$Y190 of the AChR, and the similarity of ^{BTX}G37 ($^{A\beta}$G29) which serves as a small flexible spacer. Also similar, are residues at the base of finger II, such as ^{BTX}W28 ($^{A\beta}$F20) and ^{BTX}D30 ($^{A\beta}$E22) which form multiple interactions with γ or δ subunits of AChR (Samson et al., 2002). This double register motif is typical of β-sheets interacting through one face only. Conveniently, the disulfide bound cysteine residues ^{BTX}C29 and ^{BTX}C33, at the tip of finger II, are replaced by $^{A\beta}$A21 and $^{A\beta}$G25 in the amyloid β-hairpin that allow the residue backbones to come equidistantly close. Finally, both finger II of α-neurotoxins (Samson et al., 2002) and $A\beta_{1-42}$ (Hoyer et al., 2008) adopt a similar β-hairpin conformation with backbone RMSD values of 1.27 Å for the segment ^{BTX}W28-^{BTX}L42 ($^{A\beta}$F20-$^{A\beta}$L34). Overall, the sequence and structure similarity of long α-neurotoxin finger II and $A\beta_{1-42}$ is impressive as it is surprising.

Also striking is the $A\beta_{1-42}$ sequence similarity with short α-neurotoxins shown in Table 2. As with long α-neurotoxins, the similarity is especially pronounced in functional regions that interact with AChR, namely finger II residues ^{ATX}Y24 ($^{A\beta}$F20), ^{ATX}K26 ($^{A\beta}$E22), ^{ATX}F28 ($^{A\beta}$V24), ^{ATX}S30 ($^{A\beta}$S26), and ^{ATX}R32-^{ATX}I36 ($^{A\beta}$K28-$^{A\beta}$I32). Strikingly, $A\beta_{1-42}$ sequence $^{A\beta}$K28-$^{A\beta}$I32 (KGAII) is highly similar to finger II sequence ^{ATX}R32-^{ATX}I36 (RGTII) which interacts with the α-subunit of AChR (Samson et al., 2002). Interestingly, residues at the base of finger II namely ^{ATX}Y24 ($^{A\beta}$F20), and ^{ATX}K26 ($^{A\beta}$E22), ^{ATX}F28 ($^{A\beta}$V24), ^{ATX}S30 ($^{A\beta}$S26), display a double register motif, typical of β-sheets interacting with one face only like ATX in complex with AChR. Finally, both finger II of α-neurotoxins (Samson et al., 2002) and $A\beta_{1-42}$ (Hoyer et al., 2008) adopt a similar β-hairpin conformation with backbone RMSD values of 1.79 Å for the segment ^{ATX}W27-^{ATX}I36 ($^{A\beta}$D23-$^{A\beta}$I32). On the whole, the sequence and structure similarity of short α-neurotoxin finger II and $A\beta_{1-42}$ is remarkable as it is unexpected.

2.2. Binding mechanism of $A\beta_{1-42}$ to AChR

Based on sequence similarity of $A\beta_{1-42}$ and α-neurotoxins and the experimental finding that BTX competitively inhibits $A\beta_{1-42}$ binding to AChR (Wang et al., 2000a), there is strong evidence that binding to AChR occurs in the same site and through similar interactions. Shown in Figs. 1 and 2 are the secondary structures of $A\beta_{1-42}$ interacting with α7-AChR, based on those of ATX and BTX. In both cases, $A\beta_{1-42}$ folds into a β-hairpin in which residues $^{A\beta}$A30-$^{A\beta}$I32 form an intermolecular β-sheet with $^{\alpha 7}$F189-$^{\alpha 7}$E191. In the long toxin conformation (Fig. 1), $A\beta_{1-42}$ β-hairpin strands $^{A\beta}$F20-$^{A\beta}$D23 are opposite $^{A\beta}$K28-$^{A\beta}$I31 according to the alignment with BTX finger II. In the short toxin conformation (Fig. 2), $A\beta_{1-42}$ hairpin strands $^{A\beta}$E22-$^{A\beta}$S26 are opposite $^{A\beta}$G29-$^{A\beta}$G33 according to the alignment with ATX finger II. The short toxin sequence similarity of Aβ (Fig. 2) is more remarkable than that of the long

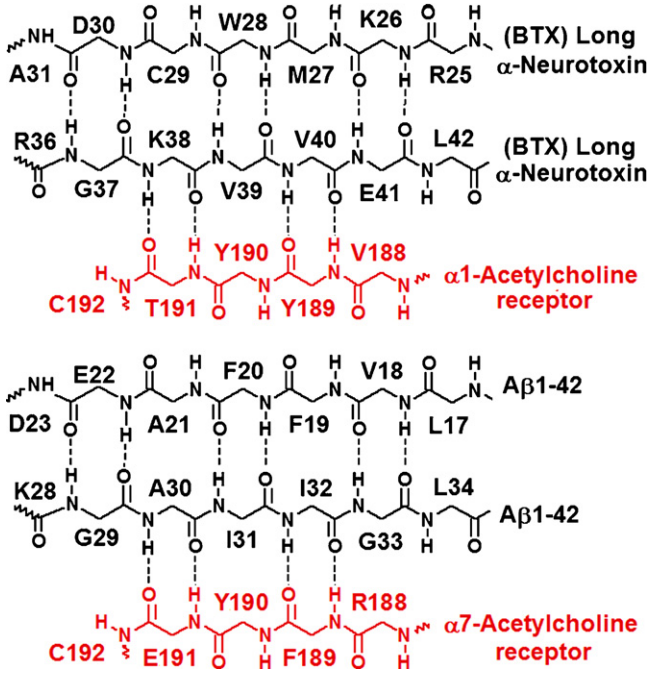

Fig. 1. Secondary structure of long α-neurotoxins and $A\beta_{1-42}$ interacting with AChR. Shown on top is the secondary structure of BTX (PDB ID 1L4W) (in black) in complex with α1-AChR (in red) (Samson et al., 2002). Shown on the bottom is the predicted secondary structure of $A\beta_{1-42}$ (in black) in complex with α7-AChR (in red). The figure was prepared using ChemSketch.

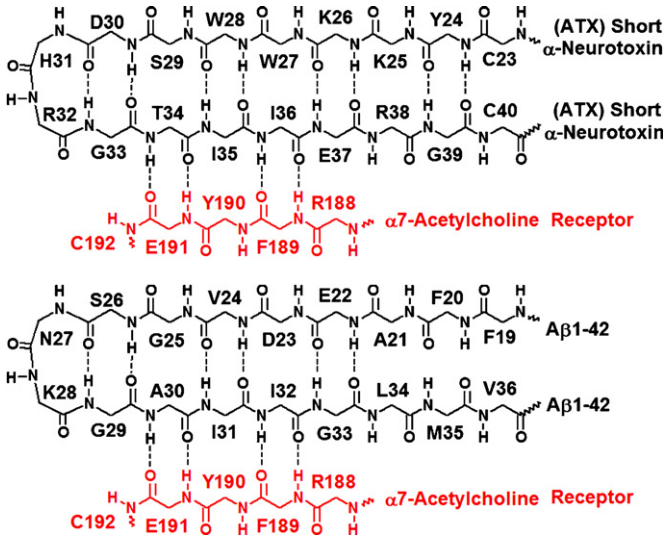

Fig. 2. Secondary structure of short α-neurotoxins and $A\beta_{1-42}$ interacting with AChR. Shown on top is the secondary structure of ATX (PDB ID 1VB0 (Lou et al., 2004)) (in black) in complex with α7-AChR (in red) (Samson et al., 2002). Shown on the bottom is the predicted secondary structure of $A\beta_{1-42}$ (in black) in complex with α7-AChR (in red). The figure was prepared using ChemSketch.

toxin (Fig. 1), as there are more similarities. The two conformations do not preclude one another, and equilibrium between the two states through a β-hairpin register shift is thinkable. This register shift could lead to more than two conformations with different β-strand pairings like that of PDB IDs 2OTK and 2BEG. In all conformations, the intermolecular β-sheet register does not shift, and residues $^{A\beta}$A29-$^{A\beta}$I30-$^{A\beta}$I31 remain opposite residues $^{\alpha7}$F189-$^{\alpha7}$Y190-$^{\alpha7}$E191 alike short and long neurotoxins. The various Aβ conformations could exhibit different binding constants and toxicity to the AChR, thus accounting for the affinity controversies in the literature (Wang et al., 2000a). Also important

Table 3
Interactions between $A\beta_{1-42}$ and α7-acetylcholine receptor.

$A\beta_{1-42}$	α7-Subunit of AChR
E22	Y190
D23	E211
V24	Y190
G25	G189
S26	W77 Y190
N27	L37 S38 L39 W77 Q79 L141
K28	W77 Q79 L141 Y115 W171 Y190 Y217
G29	Y190 E191
A30	Y190 E191
I31	F189 Y191 E191
I32	F189 Y190 E191 C192
G33	S188
L34	F189

is the length of the amyloid peptide (i.e. $A\beta_{1-40}$ and $A\beta_{1-42}$) which could influences the secondary structure and composition of ADDL. These issues should be addressed experimentally for higher certainty, and such investigations are currently underway in our laboratory.

A homology derived model of human homopentameric α7-AChR in complex with five $A\beta_{1-42}$ molecules in long α-neurotoxin conformation is shown in Fig. 3. The model is based on the NMR structure of α1-AChR in complex with two BTX molecules (PDB ID 1LK1 (Samson et al., 2002)). The difference in ligand stoichiometry arises from the fact that α1-AChR has two ligand binding sites (2 α-subunits) whereas α7-AChR has five of them (5 α-subunits). Other neuronal combinations of AChR subunits, such as the hetero-pentameric α4β2, and α7β2 have also been reported, and Aβ binding is expected to occur in a similar fashion at the α-subunit. We constricted our AChR model to the homopentameric α7 form as it was shown experimentally to bind amyloid peptides. The $A\beta_{1-42}$ hairpin forms multiple interactions with the α7-AChR ligand binding site, all summarized in Table 3. Most notable of the interactions is that of $^{A\beta}$K28 which inserts into the acetylcholine binding site and forms cation/π interactions with aromatic

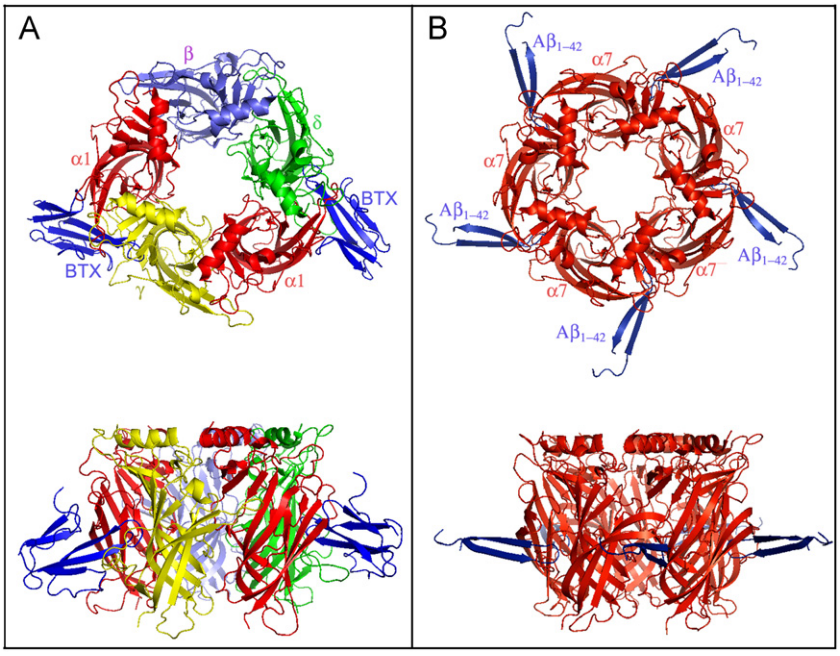

Fig. 3. Models of AChR in complex with snake α-neurotoxins and Alzheimer's $A\beta_{1-42}$. Shown are top and side views of models of (A) α1-AChR in complex with BTX (PDB ID 1LK1 (Samson et al., 2002)) and (B) human α7-AChR in complex with $A\beta_{1-42}$. The latter model is based on the former, by superimposing α7-AChR residues $^{\alpha7}$Y210-$^{\alpha7}$C212 onto α1-AChR residues $^{\alpha7}$Y190-$^{\alpha7}$C192, and $A\beta_{1-42}$ residues $^{A\beta}$G29-$^{A\beta}$I32 onto BTX residues ^{BTX}G37-^{BTX}V40. Only $A\beta_{1-42}$ residues $^{A\beta}$H14-$^{A\beta}$G36 are shown. The figure was prepared using Pymol.

residues $^{\alpha7}$W77, $^{\alpha7}$Y115, $^{\alpha7}$W171, $^{\alpha7}$Y210 and $^{\alpha7}$Y217 paving the binding site (Fig. 4). In this fashion, $^{A\beta}$K28 sterically occludes acetylcholine binding, and blocks channel opening. This interactions is homologous to that formed by ^{BTX}R36 in the ligand binding site of AChR (Samson et al., 2002). Interestingly, lysine (i.e. $^{A\beta}$K28), acetylcholine, and arginine (^{BTX}R36) are capable of mimicking each other since they possess a positively charged ammonium head linked through an aliphatic chain to a carbonyl tail (Fig. 4). Also notable is the antiparallel intermolecular β-sheet formed between $A\beta_{1-42}$ residues $^{A\beta}$A30-$^{A\beta}$I32 and AChR residues $^{\alpha7}$F189-$^{\alpha7}$E191 which accounts for the picomolar affinity (Wang et al., 2000a). This intermolecular β-sheet is homologous to that formed between BTX residues ^{BTX}K38-^{BTX}V40 and AChR residues $^{\alpha1}$Y189-$^{\alpha1}$T191 (Samson et al., 2002).

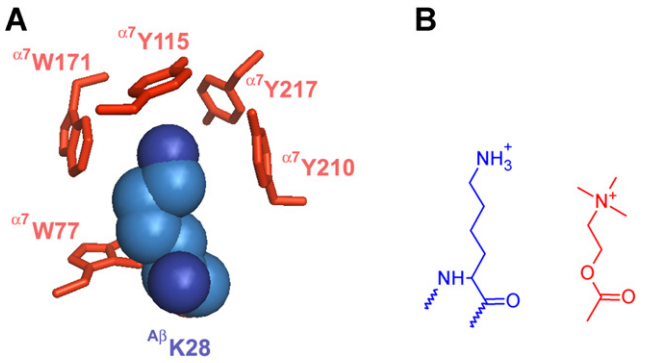

Fig. 4. Predicted interaction of Aβ in the AChR binding site. (A) Shown is $^{A\beta}$K28 which protrudes into the AChR binding site, and occludes acetylcholine binding. The ammonium group of $^{A\beta}$K28 forms cation/π interaction with aromatic residues, $^{\alpha7}$Y115, $^{\alpha7}$W171, $^{\alpha7}$Y210, and $^{\alpha7}$Y217 lining the acetylcholine binding site. (B) Lysine (i.e. $^{A\beta}$K28) and acetylcholine are homologous in that they have a positively charged head linked through an aliphatic chain to a carbonyl tail.

3. Discussion

3.1. Refolding of $A\beta_{1-42}$ is stabilized by AChR

The structure of native and free Aβ is α-helical (i.e. PDB IDs 1IYT, 1BA4, 2LFM, 1AML, etc.) while that of fibrillar Aβ is in β-hairpin conformation. Interestingly, most of the native structures show a kink in the helical structure around residue $^{A\beta}$K28 (Fig. 5). This helix breaking kink is intrinsic in all Aβ, and probably serves as a starting point for conformational transition from α- to β-structure. Once bound to the AChR, Aβ is stabilized in its refolded β-hairpin conformation through an semi-induced fit mechanism involving antiparallel intermolecular β-sheet interactions with AChR (Fig. 5). Conveninently, the helix breaking kink is located around residue $^{A\beta}$K28 which can serve as an anchor for AChR binding through insertion into the acetylcholine binding site. Such anchoring and semi-induced fit is facilitated by the presence of 5 glycine residues of Aβ which provide the necessary flexibility to undergo conformation changes. Interestingly, a PDB search for structures with glycine repeats every 4 residues, $(GXXX)_4$, like that found in $A\beta_{1-42}$ resulted in mostly α-helices that need to be tightly packed, flexible, and undergo secondary structure changes.

It is also interesting to note that even after oligomerization, $^{A\beta}$K28 of the ultimate Aβ unit remains solvent accessible (Fig. 5), as if to retain the capacity of interacting with AChR and acnhoring in the acetylcholine binding site. This is in line with a study by Lambert et al. which find Aβ-derived diffusible ligands (ADDL) to be potent central nervous system neurotoxins (Lambert et al., 1998).

It is unclear, if Aβ peptides are prone to undergo the conformational transition from α- to β-hairpins autonomously (Straub and Thirumalai, 2011), or if binding to AChR or Aβ oligomers is required for lowering the energetic barrier between the conformation states (Dziewczapolski et al., 2009). Molecular dynamics predictions show that only small Aβ segments can fold into β-structures autonomously (Straub and Thirumalai, 2011), yet experimental evidence show that the Aβ interaction with α7-AChR is crucial for AD progression (Dziewczapolski et al., 2009). In any case, it is safe to assume that the antiparallel β-sheet formed between Aβ and the AChR lowers the energetic barrier for structural conversion of Aβ peptides from α- to β-structure. With or without the assistance of AChR, Aβ peptides are believed to zip together to form long β-hairpins. Such "zipping" mechanisms are common in protein structural conversions, and were postulated for polar zippers by Perutz (1995), steric zippers of amyloid-like fibrils (Nelson et al., 2005), and recently with β-sheet elongation of prion proteins (Samson and Levitt, 2011).

3.2. Similarities and differences of α-neurotoxins and $A\beta_{1-42}$

In this study we deal with the similarities of $A\beta_{1-42}$ and α-neurotoxins, however there are several differences too. For instance, α-neurotoxins bind AChR with nanomollar affinity while $A\beta_{1-42}$ binds AChR with picomolar affinity (Wang et al., 2000a,b; Samson et al., 2002). The large affinity differences arise from α-neurotoxins interacting through three "fingers" and a "tail", whereas $A\beta_{1-42}$ interacts through one β-hairpin "finger" only. Also k_{on} of $A\beta_{1-42}$ is lower than that of snake toxins. This is because, unlike α-neurotoxins that are constrained by several disulfide bonds, Aβ binding is conformation dependent. This illustrates the importance of the disulfide bonds in α-neurotoxins, without which binding would also be conformation dependent and less efficient. The disulfide bonds which rigidify the protein backbone skeleton also prevent the toxins from forming fibrils like $A\beta_{1-42}$ which is more flexible due to glycine repeats.

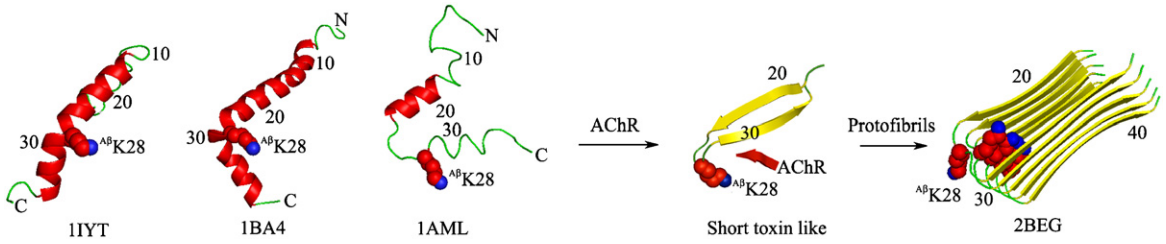

Fig. 5. AChR assists Aβ folding into β-hairpins. Shown on the left are three structures of Aβ peptides in equilibrium between the helix-kink-helix and α-hairpin conformations (PDB IDs 1IYT (Crescenzi et al., 2002), 1BA4 (Coles et al., 1998) and 1AML (Sticht et al., 1995)). Upon complex formation with the AChR, $^{A\beta}$K28 inserts into the acetylcholine binding site (see Fig. 4) and the α-hairpin becomes a β-hairpin through an induced fit mechanism driven by intermolecular β-sheet formation. Finally, the β-hairpins oligomerizes into neurotoxic protofibrils (PDB ID 2BEG (Lührs et al., 2005)). Note that $^{A\beta}$K28 of the ultimate protofibril (or ADLL) monomer remains solvent accessible to bind the AChR, and that its β-strand can still form an intermolecular β-strand.

Table 4
Multiple sequence alignment of neuronal AChR α-subtypes and a high affinity peptide elicited against α-neurotoxins.

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                          123456789012
α3-subtype (mouse)        IKYNCCEEIYQD
α3-subtype (human)        IKYNCCEEIYPD
α4-subtype (mouse)        RKYECCAEIYPD
α4-subtype (human)        RKYECCAEIYPD
α7-subtype (mouse)        KFYECCKEPYPD
α7-subtype (human)        RFYECCKEPYPD
High affinity peptide     RYYESSLEPYPD
                            *:.. * * *
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3.3. Similarities and differences of α3, α4, and α7 AChR subtypes

Our model shows the binding interactions of $A\beta_{1-42}$ with homopentameric human α7 AChRs, yet we believe that the same interactions apply to α4β2 AChRs (Wu et al., 2004). The interactions are almost identical in both α4 and α7 because they are formed principally with the acetylcholine binding site and the peptide backbone of the AChR cys loop hairpin. These interactions explain well how AChRs are inhibited by amyloid peptides. In addition, the interactions also explain why the mouse α4 subtype bind Aβ more effectively than mouse α3 and α7 in brain regions (Martin-Ruiz et al., 1999). The reason being that the mouse α4 subtype is more similar to the potent and high affinity peptides residues 1–4 (Scherf et al., 2001, 1997) elicited against α-neurotoxins than other mouse α-subunit types (Table 4). These four residues, 1–4, constitute the binding residues of the high affinity peptide with BTX. Intriguingly, for human AChR the contrary is expected, as the α7-subtype is more similar to the high affinity peptide than are α3 and α4 subtype residues 1–4. Our proposed model of the interaction of human nicotinic homopentameric α7-AChR should thus serve as a general model for $A\beta_{1-42}$ interactions with AChRs.

3.4. High $A\beta_{1-42}$ levels reduce cognition

The reduced cognition in Alzheimer's patients is mainly due to neuronal death. Yet intriguingly, cognitive dysfunction is related to amyloid concentration as it has been seen in postoperative patients (Evered et al., 2009). This cognitive dysfunction could be due to the rise of free Aβ oligomers (or ADDL) levels that inhibit cholinergic neurotransmission and induce a brain fog state. This is partially why prescription of acetylcholine esterase (AChE) inhibitors, such as neostigmine, is so beneficial in Alzheimer's patients as it elevates the effective acetylcholine agonist level, which competitively inhibit the Aβ antagonist binding. Alzheimer's disease is a multifaceted disorder and most likely there are a number of complex pathological processes interacting or independent from amyloid processes, such as tau pathology and inflammation, that lead to clinical AD. We do not claim that amyloid binding to AChR is the sole mechanism for cognitive impairment, rather a contributing factor.

3.5. Potential AD therapies

Recently, Heinemann and coworkers showed that, despite the presence of high amounts of Aβ deposits in the brain, deleting the α7-AChR in mice models of AD lead to protection from dysfunction of learning and memory (Dziewczapolski et al., 2009). And so, disrupting the $A\beta_{1-42}$ interaction with α7-AChR may represent a novel approach to reducing $A\beta_{1-42}$-mediated toxicity in AD. This study provides a detailed molecular model for the interaction between $A\beta_{1-42}$ and α7-AChR. Based on these interactions, two separate structure based therapies are currently underway in or laboratory. One therapy involves blocking the $A\beta_{1-42}$ binding site on the α7-AChR periphery (without blocking acetylcholine binding), and another entails blocking the α7-AChR binding site on $A\beta_{1-42}$. In both cases, the therapies would block interactions of $A\beta_{1-42}$ with AChR and attenuate Aβ-mediated neurotoxicity (Dziewczapolski et al., 2009). Attempts in this direction have been made, and peptides eliminating Aβ, such as PDB ID 2OTK, were engineered (Hoyer et al., 2008). Interestingly, Aβ of 2OTK forms an intermolecular β-sheet with the hapten molecule similarly and in agreement to those formed with AChR of our model (data not shown). Unfortunately, these peptides are ineffective in the treatment of AD as they probably resolubilize Aβ. We suggest designing small Aβ analogs that bind the segment $^{\alpha7}$F189-$^{\alpha7}$E191 of AChR without protruding into the acetylcholine biding site. Such analogs are currently being designed in our lab.

These therapies should come in addition to proteolytic enzyme inhibitors that block the synthesis of $A\beta_{1-42}$ from APP, as well as acetylcholine esterase (AChE) inhibitors which function by increasing the level of available acetylcholine in the synapse, that can compete with Aβ.

This study is much needed in a time where Alzheimer research is trapped because of the lack of hypothesis that can explain the underlying pathophysiology. The amyloid hypothesis has been discredited after its failure to explain why plaque elimination does not improve the mental condition of Alzheimer's patient. The tau hypothesis alone cannot explain Alzheimer's disease on its own, and neither can apolipoprotein E. The scientific community is indeed in need of good alternative hypotheses that can explain the underlying biology responsible for the symptoms of Alzheimer's diseases.

Conflict of interest statement

None declared.

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