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Inhibition of amyloid beta-induced synaptotoxicity by a pentapeptide derived from the glycine zipper region of the neurotoxic peptide

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ABSTRACT

A major characteristic of Alzheimer's disease is the presence of amyloid beta (Aβ) oligomers and aggregates in the brain. Aβ oligomers interact with the neuronal membrane inducing perforations, causing an influx of calcium ions and increasing the release of synaptic vesicles that leads to a delayed synaptic failure by vesicle depletion. Here, we identified a neuroprotective pentapeptide anti-Aβ compound having the sequence of the glycine zipper region of the C-terminal of Aβ ($G_{33}LMVG_{37}$). Docking and Förster resonance energy transfer experiments showed that $G_{33}LMVG_{37}$ interacts with Aβ at the C-terminal region, which is important for Aβ association and insertion into the lipid membrane. Furthermore, this pentapeptide interfered with Aβ aggregation, association, and perforation of the plasma membrane. The synaptotoxicity induced by Aβ after acute and chronic applications were abolished by $G_{33}LMVG_{37}$. These results provide a novel rationale for drug development against Alzheimer's disease.

1. Introduction

Alzheimer's disease is a progressive, neurodegenerative pathology, which affects the central nervous system in the elderly, causing cognitive and behavioral disorders. A major histopathological feature of this disease is the presence of macroscopic structures in the brain parenchyma called senile plaques, which consist mainly of oligomers of amyloid beta (Aβ) (Kawahara et al., 2000). These oligomers can directly alter the plasma membrane through the formation of a pore-like structure that allows the passage of calcium ions (Arispe et al., 1993; Sepulveda et al., 2010). The mechanism of this membrane insertion is largely unknown, but the early calcium entry increases the release of synaptic vesicles in hippocampal neurons, thus facilitating neurotransmission (Parodi et al., 2009). Chronic application of Aβ, in contrast, reduces the number of synaptic vesicles leading to neurotransmission failure (Parodi et al., 2009).

The Aβ protein sequence contains amino acids with properties like metal coordination (Nakamura et al., 2007), aggregation (Armstrong et al., 2011), and membrane insertion (Kim, 2005) (Fig. 1A). It is widely believed that the hydrophobic C-terminal of Aβ interacts with the lipid bilayer and the N-terminal contains polar, charged residues facing the solvated pore formed by Aβ oligomers (Jang et al., 2008). Though most of the small peptides used to block Aβ toxicity were focused on the N-terminal sequence of Aβ, the C-terminal region has been largely ignored. The physiopathological effect of Aβ pores was demonstrated using NA7 (EVHHQKL), a minipeptide derived from the N-terminus of Aβ, that blocked calcium and cell toxicity mediated by Aβ (Arispe et al., 2008; Sepulveda et al., 2010). The C-terminal region, in contrast, is important for association and insertion of Aβ into the lipid membrane, and G33 and G37 have been described as important in the process of Aβ association with artificial lipid membranes (Hung et al., 2008). Furthermore, the pathogenic effects of Aβ have been attributed to the hydrophobic C-terminal stretch of 14 amino acids that is also thought to be the seed for aggregation (Jarrett and Lansbury, 1993). Within this region, 3 repeat GxxxG motifs encompassing Aβ residues G25–G37 (G25xxxG29xxxG33xxxG37) promote Aβ aggregation independent of the hydrophobic residues in the x positions (Kim and Hecht, 2006). Interestingly, these

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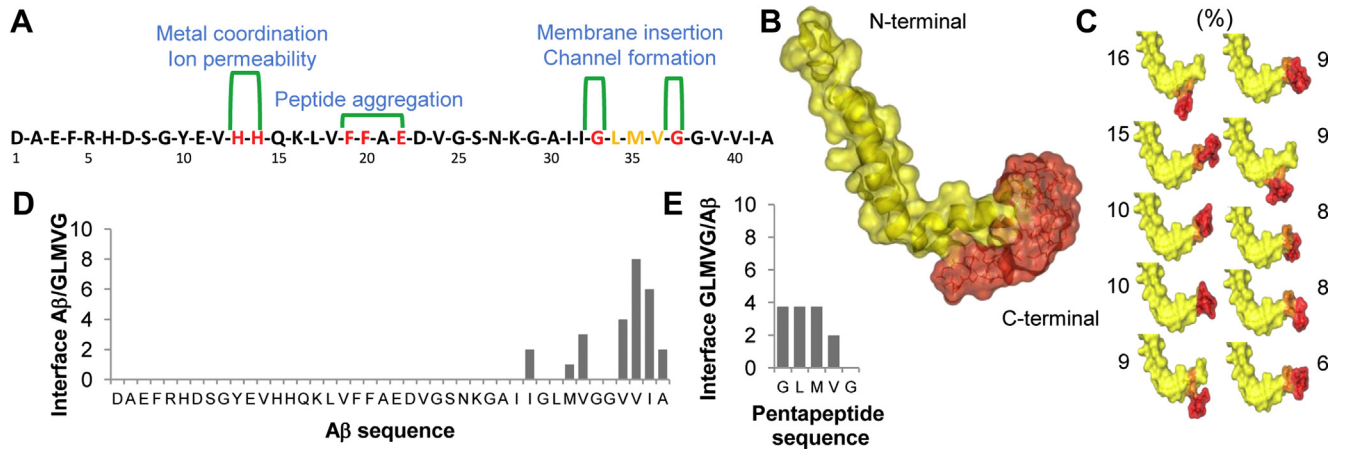

Fig. 1. Interaction of GLMVG with Aβ as predicted using molecular docking. (A) Sequence of $A\beta_{1-42}$ with colored critical residues. (B) Representative complex between Aβ (yellow) and the pentapeptide GLMVG (red) showing different regions of interaction. (C) Clustering of potential sites showing percentage of relative abundance (upper numbers). (D) and (E) Amino acids that comprise the interaction surface of Aβ and GLMVG for the 10 clusters.

glycines might be important in the formation of ion-permeable Aβ channels (Kim, 2005) and they can promote dimerization of the amyloid precursor protein (Barnham et al., 2006; Kienlen-Campard et al., 2008; Munter et al., 2007).

The aim of the present study was to develop a new anti-Aβ compound based on a pentapeptide having the sequence $G_{33}LMVG_{37}$ and that was able to block the aggregation and the synaptoxicity induced by Aβ. In addition, Förster resonance energy transfer (FRET) and docking studies with monomer (Crescenzi et al., 2002), fibril (Luhrs et al., 2005), and the crystallographically resolved structure of a partial sequence of Aβ (Streltsov et al., 2011), support the idea of a direct interaction of Aβ with the $G_{33}LMVG_{37}$ peptide. The inhibition of these pathophysiological processes by a small peptide, similar to those used in cancer, endocrinological diseases, and neurological disorders (Bidwell, 2012; Dodel et al., 2010; Herrero et al., 2012), could be beneficial in altering the course of AD.

2. Methods

2.1. Peptide preparation and storage

Human $A\beta_{1-42}$ labeled with 6-carboxyfluorescein (FAM; green fluorescence) or 6-carboxytetramethylrhodamine (TAMRA; red fluorescence) at its N-terminus, and unlabeled peptides were purchased from Anaspec. $A\beta_{1-42}$ was dissolved in hexafluoroisopropanol (HFIP, 10 mg/mL) or dimethyl sulfoxide (DMSO, 10 mg/mL) and stored in aliquots at −20 °C. For the preparation of Aβ aggregates (80-μM), aliquots of 5 μL were added to 137.5 μL ultrapure water in an Eppendorf tube. After 10–20-minute incubation at room temperature, the samples were centrifuged at 14,000*g* for 15 minutes, and the supernatant fraction was transferred to a new Eppendorf tube. To form Aβ oligomers, the samples were stirred at 500 rpm using a Teflon-coated microstir bar for 24–48 hours at room temperature (approximately 22 °C) and stored at 4 °C until required.

The pentapeptides (GLMVG, ILMVL, GPMVG, GLMVG-FAM, MVGGV, GSNKG, and GAIIG) were dissolved in DMSO to a final concentration of 10 mM, and then aliquoted and stored at −20 °C.

2.2. Electrophysiology

Electrophysiological recordings in whole-cell and perforated patch clamp were carried out using the patch clamp technique as previously described (Peters et al., 2011). Briefly, culture media was changed for an external solution containing (in mM): 150 NaCl, 5.4 KCl, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 10 glucose and 10 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.4). The internal solution consisted of (in mM): 120 KCl, 2.0 $MgCl_2$, 2 Adenosine-5′-triphosphate-Na_2, 10 1,2-bis(o-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid (BAPTA), 0.5 guanosine-5′-triphosphate (GTP), 10 HEPES (pH 7.4). The holding potential was fixed at −60 mV and postsynaptic currents were acquired at 50-μs intervals using a Digidata 1200 board, an inverted microscope (Nikon, Eclipse, TE200-U) and pClamp10 software (Axon Instruments, Inc). Recording pipettes were pulled from borosilicate glass (WPI, Sarasota, FL, USA) in a horizontal puller (Sutter Instruments, Novato, CA, USA) having a resistance between 4 and 6 MΩ. For synaptic activity recordings, data were analyzed using the Minianalysis software (Synaptosoft version 6.0.7) to obtain the frequency, amplitude, and decay time values. Perforated recordings were obtained as previously described (Sepulveda et al., 2010). Briefly, Aβ was added to the pipette internal solution and a 5-mV pulse was used to monitor the formation of the perforation.

2.3. Immunogold and transmission electronic microscopy

Five microliters of Aβ, at a concentration of 50 μM, were applied to carbon-coated Formvar grids (Origen). Nonspecific immunoreactivity was blocked with 3% bovine serum albumin (BSA) for 30 minutes at room temperature and incubated with the primary antibody anti-Aβ (1:50; Santa Cruz Biotechnology) for 1 hour. A secondary 5-nm gold-conjugated anti-mouse IgG antibody was used at a 1:20 dilution for 30 minutes. Samples were fixed with a 2% glutaraldehyde solution for 5 minutes. Aβ aggregates were stained with 5 μL of 0.2 % (wt/vol) phosphotungstic acid and the grid was air-dried. Samples were examined using a JEOL 1200 EX II electronic microscope.

2.4. Immunofluorescence

Hippocampal neurons plated in 35-mm dishes were washed in phosphate buffered saline (PBS) (pH 7.4) and fixed with cold methanol (−20 °C) for 10 minutes. Then, the dish was washed again in PBS and neurons were permeabilized and blocked for 30 minutes with PBS and Triton X-100 0.1%, and BSA 10%, respectively.