Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 324 (2004) 64–69
BBRC
www.elsevier.com/locate/ybbrc

β-Amyloid-derived pentapeptide $RIIGL_a$ inhibits $A\beta_{1–42}$ aggregation and toxicity☆

Lívia Fülöp[a,*], Márta Zarándi[a], Zsolt Datki[a], Katalin Soós[a], Botond Penke[a,b]

[a] *Department of Medical Chemistry, University of Szeged, Dóm tér 8, Szeged, Hungary*
[b] *Protein Chemistry Research Group, Hungarian Academy of Sciences, Szeged, Hungary*

Received 30 August 2004
Available online 21 September 2004

Abstract

Pr-$IIGL_a$, a derivative of the tetrapeptide β-amyloid 31–34 ($A\beta_{31–34}$), exerts controversial effects: it is toxic in a neuroblastoma culture, but it protects glial cells from the cytotoxic action of $A\beta_{1–42}$. For an understanding of this phenomenon, a new pentapeptide, $RIIGL_a$ was synthetized, and both compounds were studied by different physicochemical and biological methods. Transmission electron microscopic (TEM) studies revealed that Pr-$IIGL_a$ forms fibrillar aggregates, whereas $RIIGL_a$ does not form fibrils. Congo red binding studies furnished the same results. Aggregated Pr-$IIGL_a$ acts as a cytotoxic agent in neuroblastoma cultures, but $RIIGL_a$ does not display inherent toxicity. $RIIGL_a$ co-incubated with $A\beta_{1–42}$ inhibits the formation of mature amyloid fibres (TEM studies) and reduces the cytotoxic effect of fibrillar $A\beta_{1–42}$. These results indicate that $RIIGL_a$ is an effective inhibitor of both the aggregation and the toxic effects of $A\beta_{1–42}$ and can serve as a lead compound for the design of novel neuroprotective peptidomimetics.

Keywords: β-Amyloid 31–34; Amyloid aggregation; Aggregation inhibitor; Toxic aggregates; Transmission electron microscopy; Alzheimer's disease

One of the main pathological hallmarks of Alzheimer's disease (AD) is the presence of amyloid plaques in the affected brain. These specific assemblies contain amphipathic molecules consisting of 39–43 residues, the amyloid-β peptides (Aβ-s), derived from the amyloid precursor protein (APP). The Aβ-s have a high tendency to aggregate by forming oligomers, protofibrils, and fibrils.

A number of mechanisms have been put forward to explain the cytotoxicity induced by Aβ aggregates. Extracellular aggregates may activate a series of false signal transduction pathways that can lead to apoptosis. Many receptors involved in this process have already been identified, e.g., the scavenger receptors (expressed in microglia and macrophages [1,2]), the receptor for advanced glycation end-products [3,4], α_2-macroglobular protein [5,6], apolipoprotein E [7], the endoplasmic reticulum amyloid binding protein [8], and the α7 subunit containing nicotinic acetylcholine receptors [9,10]. Another proposal is that the aggregates affect the membrane structure by opening ion-channels causing depolarization, which leads to the dysregulation of signal transduction [11,12]. Additionally, the fibrillar aggregates can induce oxidative stress by generating free radicals, resulting in mitochondrial dysfunction [13,14].

Controversy has recently arisen as to whether the fibrillar aggregate is the only toxic form involved in Aβ-mediated cytotoxicity. Diffusible Aβ oligomers [15–18] (also known as ADDLs) or protofibrils [19] likewise exhibit noteworthy biological effects. The 'aggregation

☆ *Abbreviations:* Aβ, β-amyloid; BSB, β-sheet breaker; CR, congo red; DMS, dimethyl sulphide; FBS, fetal bovine serum; MEM, Dulbecco's modified Eagle's medium; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; RA, all-*trans*-retinoic acid; TEM, transmission electron microscopy.

* Corresponding author. Fax: +36 62 545971.
E-mail address: fulopl@index.hu (L. Fülöp).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.09.024

inhibitors' [20–23] (or β-sheet breakers) should be able to arrest and reverse the folding of the Aβ peptide into a fibrillar β-sheet structure, hindering formation of the neurotoxic aggregational states of Aβ.

In this study, we report on the synthesis and investigation of a novel aggregation inhibitor with a structure analogous to that of $A\beta_{31-35}$, which plays important roles in Aβ aggregation [24,25] and cytotoxicity [26,27]. We have already reported that Pr-$IIGL_a$, a close analogue of $A\beta_{31-34}$, was able to antagonize the $A\beta_{1-42}$-mediated toxicity effectively both in vitro in glial cells [28] and in vivo [29]. However, in some in vitro experiments in our laboratory, Pr-$IIGL_a$ proved to be neurotoxic (unpublished observations). For an understanding of the controversial effects of Pr-$IIGL_a$, we have further examined its aggregation and biological effectiveness, with the aim of designing an effective inhibitor of Aβ neurotoxicity without any self-toxicity. Our strategy to design compounds to inhibit $A\beta_{1-42}$ aggregation was based on the tetrapeptide IIGL (which interacts with Aβ-s) linked to a further solubilizing amino acid residue. We chose the cationic arginine (R) for this purpose and thus, as an analogue of the $A\beta_{31-34}$ sequence, the pentapeptide $RIIGL_a$ was synthetized and studied by means of different physicochemical methods and in in vitro bioassays.

Materials and methods

Peptide synthesis. All peptides were synthetized manually in the solid phase. Details of the $A\beta_{1-42}$ synthesis procedure have been reported elsewhere. Briefly, the peptide was manually synthetized on preloaded Fmoc-Ala-Tentagel SRAM resin (Rapp Polymere GmbH) with the use of Fmoc chemistry and DCC/HOBt coupling. The crude peptide was cleaved off the resin with a mixture of TFA/water and purified on a Delta-PAK C4 semipreparative column (300 × 47 mm, 15 μm 300 Å).

$A\beta_{25-35a}$, $A\beta_{31-35a}$, LPFFD, $RIIGL_a$, and Pr-$IIGL_a$ were synthetized manually on MBHA resin with the use of standard Boc chemistry and DCC/HOBt coupling. The peptides were cleaved off with HF in the presence of 8% dimethyl sulphide (DMS) and 2% anisole. Crude products were purified on a Knauer semipreparative HPLC system equipped with a Phenomenex Jupiter C18 (250 × 21 mm, 10 μm 300 Å) semipreparative column. Fractions were checked on a Hewlett–Packard analytical HPLC apparatus, using a Phenomenex Jupiter C18 (250 × 4 mm, 5 μm 300 Å) analytical column. Mass spectra were taken on a FinniganMat TSQ 7000 mass spectrometer in ES–MS positive ion mode.

Viability assay. An improved and convenient MTT test was earlier developed in our laboratory [30]. Differentiated SHSY-5Y neuroblastoma cells (purchased from Sigma–Aldrich, Hungary) were plated on a 96-well plate and cultured in a humidified 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (MEM): F-12 (1:1) with phenol red, to which L-glutamine, penicillin, streptomycin, MEM, non-essential amino acids, 10% fetal bovine serum (FBS), 0.5% DMSO, and 10 μM all-*trans*-retinoic acid (RA) as differentiating agent were added. After 8 days, the supernatant was removed with a pipette and a new medium (with 2% FBS), free from phenol red, DMSO, and RA, was added to each cell. The cells were then treated with peptide solutions containing either pre-aggregated, fibrillar $A\beta_{1-42}$ alone ($c = 10$ μM) or a mixture of $A\beta_{1-42}$ ($c = 10$ μM) and one of the short peptides: LPFFD (used as a reference standard), $RIIGL_a$ or Pr-$IIGL_a$ ($c = 50$ μM). After incubation for 24 h at 37 °C, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2 H-tetrazolium bromide (MTT) solution was added to the wells and the incubation was continued for a further 3 h. The formazan crystals formed were dissolved in a DMSO/EtOH 4:1 (v/v) mixture. Absorbance was measured with a 96-well plate ELISA reader at 550 nm, with a reference filter set to 620 nm. All MTT assays were triplicated and each measurement involved seven parallels.

Congo red binding assay. For the congo red (CR) binding studies, a modification of the method of Klunk et al. [31] was applied. The peptide solutions used for this assay were the same as for the MTT, with the single exception that the peptides were dissolved in PBS at pH 7.4 instead of in the cell culture medium. Samples were incubated for one day at 37 °C. The CR contents of the aggregated peptide-CR suspensions were measured photometrically at 550 nm (bound CR) with an ELISA plate reader. All measurements were repeated 7 times.

TEM experiments. For the studies of $A\beta_{25-35a}$, $A\beta_{31-35a}$, $RIIGL_a$, and Pr-$IIGL_a$ aggregation, the peptides were dissolved in dd water with constant pipetting for 2 min (concentration: 1 mg/ml for $A\beta_{25-35a}$, $A\beta_{31-35a}$, and $RIIGL_a$, and 0.5 mg/ml for Pr-$IIGL_a$) and sonicated for 10 min. Samples were incubated from 1 to 6 days at 37 °C. For the aggregation inhibition experiments, $A\beta_{1-42}$ was dissolved in dd water by the same methodology, either alone ($c = 100$ μM) or together with $RIIGL_a$ ($c_{A\beta1-42} = 100$ μM, $c_{RIIGLa} = 500$ μM). The samples were incubated from 1 to 6 days at 37 °C.

Following the method of Walsh et al. [32], 10 μL peptide samples were applied to 400 mesh carbon-coated copper grids (Electron Microscopy Sciences, Washington, PA), fixed with 0.5% (v/v) glutaraldehyde solution, washed three times with 10 μL droplets of dd water, and stained with 2% (w/v) uranyl acetate. Specimens were studied by using a Philips CM 10 transmission electron microscope at 100 kV, routinely at magnifications of 25,000× and 46,000×.

Results

Self-aggregation of Aβ fragments and derivatives

The abilities of some short Aβ fragments and new fragment analogues to form well-ordered aggregates under our experimental conditions were examined by TEM using negative staining visualization. Fig. 1 presents TEM images of $A\beta_{25-35a}$ (A), $A\beta_{31-35a}$ (B), $RIIGL_a$ (C), and Pr-$IIGL_a$ (D). Fig. 1A reveals that $A\beta_{25-35a}$ forms a huge number of short, rod-like aggregates with an average diameter of 6 nm and a length in the interval 20–120 nm. These dimensions are typical for protofibrils, and we could find mature fibrils in the sample only after a prolonged incubation time (several weeks, unpublished data). $A\beta_{31-35a}$ and $RIIGL_a$ in a relatively high concentration do not aggregate even after 6 days, as there are no signs of aggregates in pictures 1B and C. Unexpectedly, Pr-$IIGL_a$ exhibited a very strong propensity to aggregation, as demonstrated in pictures 1D1d and 1D6d. After incubation for 1 day (1D1d), the peptide forms wide flakes at most 200 nm long, with a diameter of 20–30 nm. After 6 days (1D6d), mature, well-ordered strands 2–3 μm long and 10–40 nm wide are formed, and the short aggregates disappear completely from the sample.

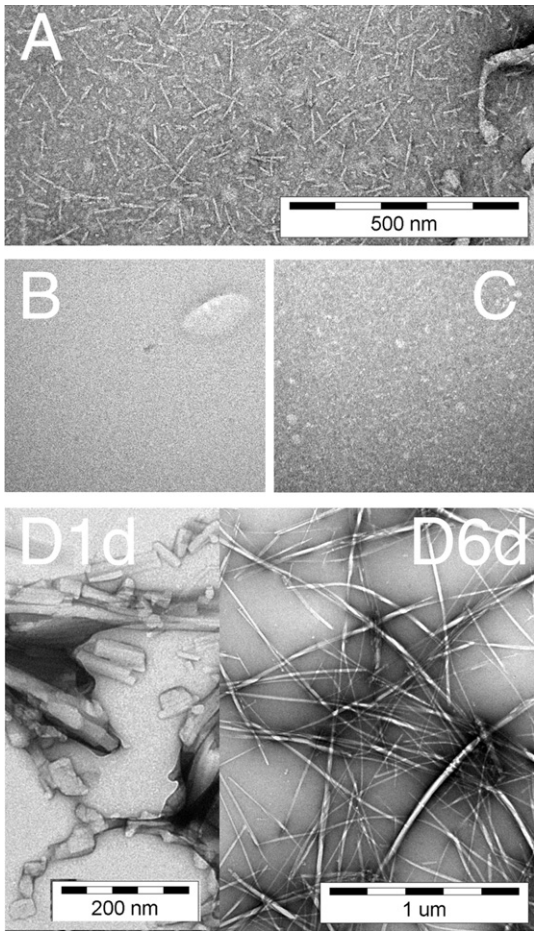

Fig. 1. TEM images of the fibril formation of $A\beta_{25-35a}$ (A), $A\beta_{31-35a}$ (B), $RIIGL_a$ (C), and Pr-$IIGL_a$ (D1d and D6d). The peptides were dissolved in dd water ($c = 1$ mg/mL) and incubated at 37 °C for 6 days, except for Pr-$IIGL_a$, where the concentration of the sample was 0.5 mg/mL and image D1d was taken after incubation for 1 day at 37 °C.

Effects of LPFFD, Pr-$IIGL_a$, and $RIIGL_a$ on the aggregation state of $A\beta_{1-42}$

The CR test was used to study whether the short Aβ fragments and fragment analogues are able to influence the process of synthetic $A\beta_{1-42}$ aggregation. The CR-binding ability of the peptides was examined both alone and on co-incubation with $A\beta_{1-42}$ for 1 day at 37 °C. Fig. 2 indicates that LPFFD ('Soto peptide' applied as a reference standard) and $RIIGL_a$ do not aggregate, whereas the increase in absorbance (characteristic of CR bound to a β-sheet) demonstrates that Pr-$IIGL_a$ can form aggregates with a β-pleated sheet structure within the duration of the experiment. As compared with the fibrillization of $A\beta_{1-42}$ itself, each of the short peptides used can decrease the absorbance of the CR-Aβ fibril complex in a 5-fold molar excess. In our experiment, $RIIGL_a$ was the most effective inhibitor of the binding of CR to $A\beta_{1-42}$ fibrils.

This ability of $RIIGL_a$ was proven by TEM experiments as well. Fig. 3 depicts a series of TEM images of the aggregation process when $A\beta_{1-42}$ was incubated alone or in the presence of $RIIGL_a$ ($c_{A\beta 1\text{-}42}$:c_{RIIGLa} = 1:5) at 37 °C for several days. The zero time controls contained no fibrils, but only sheet-like, amorphous clusters of the lyophilized peptide which could not fully dissolve (data not shown). After incubation for 1 day, both samples (Fig. 3, A1d and B1d) contained fibrillar aggregates, but with differences. $A\beta_{1-42}$ alone formed irregular, lumpy, occasionally branching protofibrils 10–100 nm long and 6–8 nm wide, and some 200–400 nm long smooth, straight fibrils as well (Fig. 3, A1d). In the sample containing a mixture of $A\beta_{1-42}$ and $RIIGL_a$ (Fig. 3, B1d), these structures were again present, but only a few mature amyloid strands were seen as compared with the situation for $A\beta_{1-42}$ alone. The difference in the aggregation process between the two samples was much more pronounced after 6 days: in this period, pure $A\beta_{1-42}$ formed predominantly mature fibres 2–3 μm long (Fig. 3, A6d), whereas co-incubation with $RIIGL_a$ completely inhibited the fibril formation of $A\beta_{1-42}$ (3B6d). In this sample, sparsely distributed small, irregular protofibrils 10–40 nm long and amorphous, three-dimensional, non-fibrillar microaggregates with a diameter of 0.5–2 μm were present (3B6d, insert).

Cell viability studies

The viability of SHSY-5Y human neuroblastoma cells differentiated with RA was determined in an

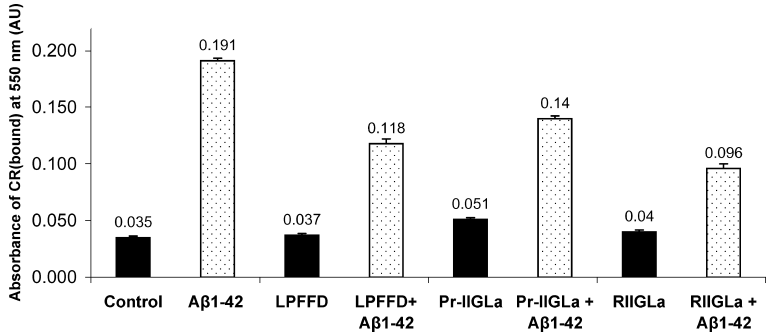

Fig. 2. CR binding of LPFFD, Pr-$IIGL_a$, and $RIIGL_a$. The peptides were incubated in PBS at 37 °C for 1 day either alone ($c = 50$ μM) or together with $A\beta_{1-42}$ ($c = 10$ μM). The absorbance of the CR–peptide complex was measured at 550 nm. All experiments were repeated 7 times.

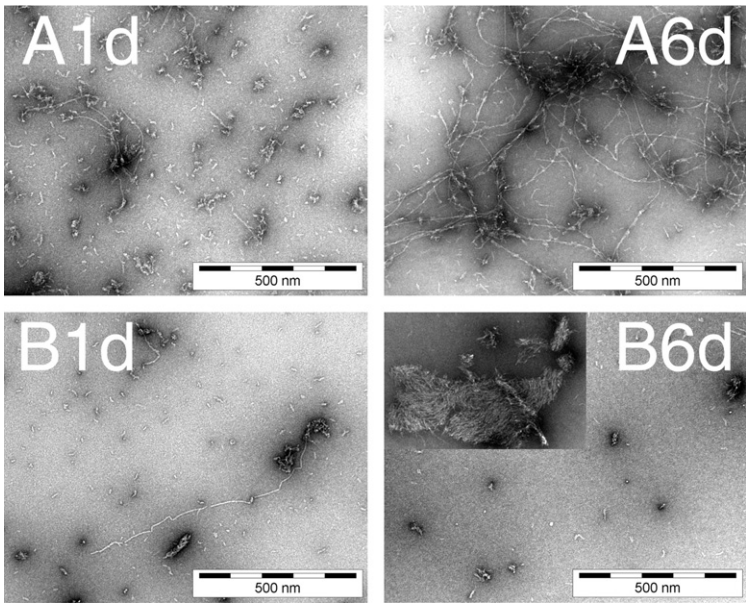

Fig. 3. Effect of $RIIGL_a$ on the aggregation of $A\beta_{1–42}$. Aβ was incubated either alone ($c = 100$ μM) at 37 °C for 1 day (A1d) or 6 days (A6d), or together with $RIIGL_a$ ($c = 500$ μM) (B1d and B6d, respectively).

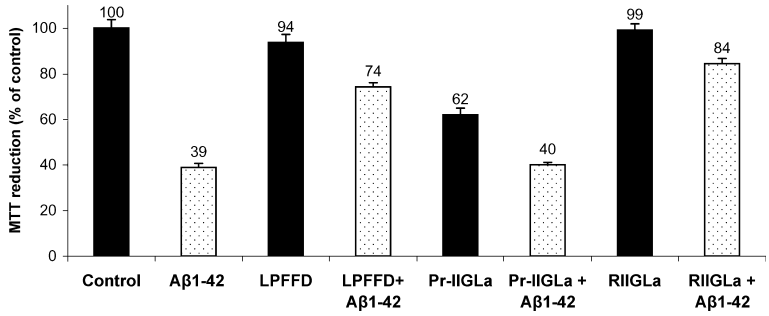

Fig. 4. Cell viability of differentiated SHSY-5Y neuroblastoma cells treated with LPFFD, $Pr\text{-}IIGL_a$ or $RIIGL_a$ ($c = 50$ μM) alone or co-incubated with $A\beta_{1–42}$ ($c = 10$ μM). Measurements were triplicated, with seven parallels within each trial. Viabilities are expressed as percentages of the untreated control.

MTT assay by monitoring the mitochondrial reduction activity (Fig. 4). As compared with the control, $A\beta_{1–42}$ was able to reduce the cell viability to 39%, while LPFFD (the reference standard) and $RIIGL_a$ in 50 μM concentration did not alter the mitochondrial activity significantly. In contrast, $Pr\text{-}IIGL_a$ itself proved to be toxic, reducing the viability to 62%. When these short peptides were co-incubated with $A\beta_{1–42}$, $Pr\text{-}IIGL_a$ did not exert any protecting effect, whereas both LPFFD and $RIIGL_a$ were able to inhibit the cytotoxic effect of $A\beta_{1–42}$ to a considerable extent (Fig. 4).

Discussion

The toxicity of Aβ peptides depends strongly on their aggregational state. The amyloid cascade hypothesis [33,34] presumes that Aβ aggregates in contact with the neuronal membrane induce a series of biophysical and biochemical malfunctions which can finally lead to neuritic dysfunction and cell death. It has not yet been fully ascertained which aggregational state of Aβ is mostly responsible for the neuronal damaging effect. Several groups have reported the cytotoxic action of small diffusible oligomers [15–18] (ADDLs) or protofibrils [19], instead of the large, plaque-forming fibrillar aggregates. However, it is still a promising therapeutic strategy to find suitable molecules which can influence the process of Aβ aggregation. A number of attempts have already been made to design such compounds, the 'aggregation inhibitors.' Many of them are peptides, peptidomimetics or non-peptidic small molecules (recently reviewed by Talaga [35]). Among the peptide-like inhibitors, the most frequently investigated ones contain the $A\beta_{17–20}$ sequence, LVFF, as a self-recognition motif

with a structural modification which diminishes the propensity of the inhibitor to self-aggregate. As examples, charged residues built into the molecule ($KLVFFK_6$ [36,37]) or a Val replacement by Pro (LPFFD [38,39]) enable the peptides to serve as potent aggregation inhibitors without any detectable self-aggregation.

Numerous studies have already shown that fragment 31–35 plays an important role in the toxicity of Aβ [26,27]. $A\beta_{25-35}$ has been extensively studied [24,40–42], as it is highly cytotoxic and can adopt a well-ordered conformation leading to fibril formation. These findings have been confirmed by TEM experiments, and Bond et al. [24] recently revealed the exact conformations of $A\beta_{25-35}$ and $A\beta_{31-35}$ by X-ray diffraction. They found that both peptides exhibit a reverse turn at Gly33; $A\beta_{31-35}$ thereby adopts a reverse turn conformation, as previously concluded by our group [43]. The data that have emerged so far point to the 31–35 residue being an interesting target in the design of putative peptide inhibitors. We earlier reported that Pr-$IIGL_a$, an analogue of $A\beta_{30-34}$, was able to prevent the $A\beta_{1-42}$-induced long-term elevation of the intracellular Ca^{2+} concentration in glial cells in vitro [28]. Moreover, Pr-$IIGL_a$ was able to attenuate the excitotoxic action of $A\beta_{1-42}$ on the cholinergic neurons of the rat magnocellular nucleus basalis [29]. These in vivo experiments also evinced that Pr-$IIGL_a$ exhibited agonistic effects resembling the excitatory action of $A\beta_{1-42}$. Moreover, the in vivo administration of the tetrapeptide derivative required the application of a strong chaotropic agent (TFA) as solvent, which destroys ordered peptide conformations. Our TEM results have proven that Pr-$IIGL_a$ displays a strong tendency to aggregate in aqueous solution, by forming highly ordered, fibrillar aggregates within a relatively short time. The tetrapeptide can also attenuate the formation of the complex CR–$A\beta_{1-42}$, possibly by adhering to the binding sites of the Aβ strands. In the MTT assay, Pr-$IIGL_a$ exerted a moderate cytotoxic effect on a neuroblastoma cell culture, while it did not affect the $A\beta_{1-42}$-mediated toxicity.

Our present aim was to design a new effective inhibitor of Aβ neurotoxicity without the inherent toxicity and self-aggregation ability of Pr-$IIGL_a$. The hydrophobic character of this peptide decreases markedly when the propionyl group is replaced by the cationic arginine. This replacement results $RIIGL_a$, which exhibits improved solubility in aqueous media without the use of a chaotropic solvent, and a strongly reduced propensity to self-aggregation, as proven by TEM and CR-binding studies. In the CR and MTT experiments, the well-known inhibitor peptide LPFFD, designed by Soto et al. [38,39], as a classic 'beta sheet breaker' (BSB), was chosen as reference compound. As expected, in our experiments LPFFD did not aggregate and inhibited the binding of CR to amyloid aggregates and also the toxic effect of $A\beta_{1-42}$ in the MTT assay. The new compound $RIIGL_a$ behaved similarly. It significantly reduced the $A\beta_{1-42}$-induced cytotoxicity, possibly by interacting with the toxic forms involved in the Aβ aggregation. $RIIGL_a$ inhibited the fibrillization of $A\beta_{1-42}$ completely within 6 days, as demonstrated by TEM.

It is concluded that $A\beta_{31-34}$ can serve as a lead motif for the design of an inhibitor of Aβ aggregation, but the propionyl group (mimicking Ala30 in the sequence of $A\beta_{1-42}$) increases the hydrophobicity of the peptide, leading to extended self-aggregation and toxicity. When the highly polar Arg was built into the molecule, the new pentapeptide, $RIIGL_a$, inhibited $A\beta_{1-42}$ aggregation with high efficacy. Further studies will focus on the in vivo behavior of the $RIIGL_a$. Via rational modification of the sequence, the resulting peptidomimetics may exhibit enhanced enzyme resistance and possess the ability to permeate the blood–brain barrier while retaining the original biological activity of $RIIGL_a$.

Acknowledgments

This work was supported by the following Grants: OTKA T034895, OTKA T038236, EU-5 'LIPIDIET' (QLRT-2001-00172), and NKFP 1/027/2001.

References

[1] J. El Khoury, S.E. Hickman, C.A. Thomas, L. Cao, S.C. Silverstein, J.D. Loike, Scavenger receptor-mediated adhesion of microglia to β-amyloid fibrils, Nature 382 (1996) 716–719.
[2] D.M. Paresce, R.N. Ghosh, F.R. Maxfield, Microglia cells internalize aggregates of the Alzheimer's disease amyloid β-protein via a scavenger receptor, Neuron 17 (1996) 553–565.
[3] S.D Yan, X. Chen, A.M. Schmidt, J. Brett, G. Goodman, Y.S. Zou, C.W. Scott, C. Caputo, T. Frappier, M.A. Smith, G. Perry, S.H. Yen, D. Stern, RAGE in Alzheimer's disease: a receptor mediated amyloid-β peptide-induced oxidant stress and neurotoxicity, and microglial activation, Nature 382 (1996) 685–691.
[4] S.D. Yan, H. Zhu, J. Fu, S.F. Yan, A. Roher, W.W. Tourtellotte, T. Rajavashisth, X. Chen, G.C. Godman, D. Stern, A.M. Schmidt, Amyloid-beta peptide-receptor for advanced glycation endproduct interaction elicits neuronal expression of macrophage-colony stimulating factor: a proinflammatory pathway in Alzheimer's disease, Proc. Natl. Acad. Sci. USA 94 (1997) 5296–5301.
[5] Y. Du, B. Ni, M. Glinn, R.C. Dodel, K.R. Bales, Z. Zhang, P.A. Hyslop, S.M. Paul, α_2-Macroglobular protein as a β-amyloid peptide-binding plasma protein, J. Neurochem. 69 (1997) 299–305.
[6] M. Narita, D.M. Holtzman, A.L. Schwartz, G. Bu, α_2-Macroglobulin complexes with and mediates the endocytosis of β-amyloid peptide via cell surface low-density lipoprotein receptor-related protein, J. Neurochem. 69 (1997) 1904–1911.
[7] T. Pillot, M. Goethals, J. Najib, C. Labeur, L. Lins, J. Chambaz, R. Brasseur, J. Vandekerckhove, M. Rosseneu, β-Amyloid peptide interacts specifically with the carboxy-terminal domain of human apolipoprotein E: relevance to Alzheimer's disease, J. Neurochem. 72 (1999) 230–237.
[8] S.D. Yan, J. Fu, C. Soto, X. Chen, H. Zhu, F. Al-Mohanna, K. Collison, A. Zhu, E. Stern, T. Saido, M. Tohyjama, S.

Ogawa, A. Roher, D. Stern, An intracellular protein that binds amyloid β-peptide and mediates neurotoxicity in Alzheimer's disease, Nature 389 (1997) 689–695.
[9] H.Y. Wang, D.H.S. Lee, M.R. D'Andrea, P.A. Peterson, A.B. Reitz, β-Amyloid$_{1–42}$ binds to α7 nicotinic acetylcholine receptor with high affinity: implications for Alzheimer's disease pathology, J. Biol. Chem. 275 (2000) 5626–5632.
[10] H.Y. Wang, D.H.S. Lee, C.B. Davia, R.P. Shank, Amyloid peptide A$\beta_{1–42}$ binds selectively and with picomolar affinity to α7 nicotinic acetylcholine receptors, J. Neurochem. 75 (2000) 1155–1161.
[11] N. Arispe, E. Rojas, H.B. Pollard, Alzheimer disease amyloid-β protein forms calcium channels in bilayer membranes: blockade by tromethamine and aluminium, Proc. Natl. Acad. Sci. USA 90 (1993) 561–571.
[12] M.X. Lin, T. Mirzabekov, B.L. Kagan, Channel formation by a neurotoxic prion protein fragment, J. Biol. Chem. 272 (1997) 44–47.
[13] C. Behl, J.B. Davis, R. Lesley, D. Schubert, Hydrogen peroxide mediates amyloid-β toxicity, Cell 77 (1994) 817–827.
[14] L.J. Hsu, Y. Sagara, A. Arroyo, E. Rockenstein, A. Sisk, M. Mallory, J. Wong, T. Takenouchi, M. Hashimoto, E. Masliah, α-Synuclein promotes mitochondrial deficit and oxidative stress, Am. J. Pathol. 157 (2000) 401–410.
[15] M.P. Lambert, A.K. Barlow, B.A. Chromy, C. Edwards, R. Freed, M. Liosatos, T.E. Morgan, I. Rozovsky, B. Trommer, K.L. Viola, P. Wals, C. Zhang, C.E. Finch, G.A. Krafft, W.L. Klein, Diffusible, nonfibrillar ligands derived from A beta(1–42) are potent central nervous system neurotoxins, Proc. Natl. Acad. Sci. USA 95 (1998) 6448–6453.
[16] K.N. Dahlgren, A.M. Manelli, W.B. Stine, L.K. Baker, G.A. Krafft, M.J. LaDu, Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability, J. Biol. Chem. 277 (2002) 32046–32053.
[17] R. Bhatia, H. Lin, R. Lal, Fresh and globular amyloid beta protein (1–42) induces rapid cellular degeneration: evidence for AβP channel-mediated cellular toxicity, FASEB J. 14 (2000) 1233–1243.
[18] T. Pillot, B. Drouet, S. Queillé, C. Labeur, J. Vandekerckhove, M. Rosseneu, M.P. Raymond, J. Chambaz, The nonfibrillar amyloid β-peptide induces apoptotic neuronal cell death: involvement of its C-terminal fusogenic domain, J. Neurochem. 73 (1999) 1626–1634.
[19] D.M. Walsh, D.M. Hartley, Y. Kusumoto, Y. Fezoui, M.M. Condron, A. Lomakin, G.B. Benedek, D.J. Selkoe, D.B. Teplow, Amyloid β-protein fibrillogenesis, J. Biol. Chem. 274 (1999) 25945–25952.
[20] J. Ghanta, C.L. Shen, L.L. Kiessling, R.M. Murphy, A strategy for designing inhibitors of β-amyloid toxicity, J. Biol. Chem. 271 (1996) 29525–29528.
[21] C.M.M. Pallitto, J. Ghanta, P. Heinzelman, L.L. Kiessling, R.M. Murphy, Recognition sequence design for peptidyl modulators of β-amyloid aggregation and toxicity, Biochemistry 38 (1999) 3570–3578.
[22] C. Soto, Plaque buster strategies to inhibit amyloid formation in Alzheimer's disease, Mol. Med. Today 5 (1999) 343–350.
[23] C. Soto, Unfolding the role of protein misfolding in neurodegenerative diseases, Nat. Rew. Neurosci. 4 (2003) 49–60.
[24] J.P. Bond, S.P. Deverin, H. Inouye, O.A. El-Agnaf, M.M. Teeter, D.A. Kirschner, Assemblies of Alzheimer's peptides A$\beta]_{25–35}$ and A$\beta_{31–35}$: reverse turn conformation and side-chain interactions revealed by X-ray diffraction, J. Struct. Biol. 141 (2003) 156–170.
[25] R. Liu, C. McAllister, Y. Lyubchenko, M.R. Sierks, Residues 17–20 and 30–35 of beta-amyloid play critical roles in aggregation, J. Neurosci. Res. 75 (2004) 162–171.
[26] B. Penke, L. Tóth, K. Soós, J. Varga, E.Z. Szabó, J. Márki-Zay, A. Baranyi, Amyloid peptides: the active center and the possible mechanism of action, starting and maintaining neuronal death, peptides, in: Proceedings of the 33rd European Peptide Symposium, Braga, 1994, pp. 101–102.
[27] X.Z. Yan, J.T. Qiao, Y. Dou, Z.D. Qiao, β-Amyloid peptide fragment 31–35 induces apoptosis in cultured cortical neurons, Neuroscience 92 (1999) 177–184.
[28] G. Laskay, M. Zarándi, J. Varga, K. Jost, A. Fónagy, C. Torday, L. Latzkovits, B. Penke, A putative tetrapeptide antagonist prevents β-amyloid induced long-term elevation of $[Ca^{2+}]_I$ in rat astrocytes, Biochem. Biophys. Res. Commun. 235 (1997) 479–481.
[29] T. Harkány, I. Ábrahám, G. Laskay, W. Timmerman, K. Jost, M. Zarándi, B. Penke, C. Nyakas, P.G.M. Luiten, Propionyl-IIGL tetrapeptide antagonizes β-amyloid excitotoxicity in rat nucleus basalis, Neuroreport 10 (1999) 1693–1698.
[30] Z. Datki, A. Juhász, M. Gálfi, K. Soós, R. Papp, D. Zádori, B. Penke, Method for measuring neurotoxicity of aggregating polypeptides with the MTT assay on differentiated neuroblastoma cells, Brain Res. Bull. 62 (2003) 223–229.
[31] W.E. Klunk, R.F. Jacob, R.P. Mason, Quantifying amyloid β-peptide (Aβ) aggregation using the congo red-Aβ (CR-Aβ) spectrophotometric assay, Anal. Biochem. 266 (1999) 66–76.
[32] D.M. Walsh, A. Lomakin, G.B. Benedek, M.M. Condron, D.B. Teplow, Amyloid β-protein fibrillogenesis, J. Biol. Chem. 272 (1997) 22364–22372.
[33] J.A. Hardy, G.A. Higgins, Alzheimer's disease: the amyloid cascade hypothesis, Science 256 (1992) 184–185.
[34] J.A. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297 (2002) 353–356.
[35] P. Talaga, β-Amyloid aggregation inhibitors for the treatment of Alzheimer's disease: dream or reality?, Mini Rev. Med. Chem. 1 (2001) 175–186.
[36] M.M. Pallitto, J. Ghanta, P. Heinzelman, L.L. Kiessling, R.M. Murphy, Recognition sequence design for peptidyl modulators of beta-amyloid aggregation and toxicity, Biochemistry 38 (1999) 3570–3578.
[37] C.W. Cairo, A. Strzelec, R.M. Murphy, L.L. Kiessling, Affinity-based inhibition of β-amyloid toxicity, Biochemistry 41 (2002) 8620–8629.
[38] C. Soto, M.S. Kindy, M. Baumann, B. Frangione, Inhibition of Alzheimer's amyloidosis by peptides that prevent β-sheet conformation, Biochem. Biophys. Res. Commun. 226 (1996) 672–680.
[39] C. Soto, E.M. Sigurdsson, L. Morelli, R.A. Kumar, E.M. Castano, B. Frangione, β-Sheet breaker peptides inhibit fibrillogenesis in rat brain model of amyloidosis: implications for Alzheimer's therapy, Nat. Med. 4 (1998) 822–826.
[40] A. Monji, H. Utsumi, T. Ueda, T. Imoto, I. Yoshida, S. Hashioka, K. Tashiro, N. Tashiro, Amyloid-beta protein (A beta) (25–35) associated free radical generation is strongly influenced by the aggregational state of the peptides, Life Sci. 70 (2002) 833–841.
[41] C.J. Pike, A.J. Walencewicz-Wasserman, J. Kosmoski, D.H. Cribbs, C.G. Glabe, C.W. Cotman, Structure–activity analyses of beta-amyloid peptides: contributions of the beta 25–35 region to aggregation and neurotoxicity, J. Neurochem. 64 (1995) 253–265.
[42] A. Urani, P. Romieu, F.J. Roman, T. Maurice, Enhanced antidepressant effect of sigma (1) receptor agonists in beta(25–35)-amyloid peptide-treated mice, Behav. Brain Res. 134 (2002) 239–247.
[43] I. Laczkó, S. Holly, Z. Kónya, K. Soós, J.L. Varga, M. Hollósi, B. Penke, Conformational mapping of amyloid peptides from the putative neurotoxic 25–35 region, Biochem. Biophys. Res. Commun. 205 (1994) 120–126.