Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 1160–1167
Bioorganic & Medicinal Chemistry

Inhibitory activity of stilbenes on Alzheimer's β-amyloid fibrils in vitro

Céline Rivière,[a] Tristan Richard,[a] Lysiane Quentin,[a] Stéphanie Krisa,[b] Jean-Michel Mérillon[b] and Jean-Pierre Monti[a,*]

[a]*Laboratoire de physique et biophysique, Université de Bordeaux 2, 146 rue Léo Saignat, 33076 Bordeaux cedex, France*
[b]*Laboratoire de biotechnologie végétale GESVAB EA 3675, Institut des Sciences de la Vigne et du Vin, Université de Bordeaux 2, 146 rue Léo Saignat, 33076 Bordeaux cedex, France*

Received 24 April 2006; revised 21 September 2006; accepted 29 September 2006
Available online 1 October 2006

Abstract—Polymerization of the amyloid β-peptide (Aβ) has been identified as one of the major characteristics of Alzheimer's disease (AD). Thus, finding molecules to prevent the aggregation of Aβ could be of therapeutic value in AD. We describe an original routine in vitro assay to search for inhibitors of Aβ(25–35) fibril formation which uses UV–visible measurements and electron microscopy (EM). In particular, this routine assay was used to examine the effects of stilbenes, a well-known polyphenol class, as inhibitors of Aβ fibril formation. The inhibitory properties of resveratrol (RES), piceid (PIC), resveratrol diglucoside (DIG), piceatannol (PIA), astringine (AST), and viniferin (VIN) were characterized and compared. RES and PIC effectively and dose-dependently inhibited Aβ polymerization while other polyphenols exerted less inhibition. Although the mechanism of anti-amyloidogenic activity is still unknown, these results support the hypothesis that stilbenes could be of therapeutic value in AD.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder which is a cause of dementia in the aged population.[1–3] The decline in cognition and memory is accompanied by different pathological signals with progressive deposition of senile plaques and neurofibrillary tangles leading to neuronal degeneration.[4] One of the characteristics of AD is the accumulation of extracellular amyloid-β peptide (Aβ) derived from the transmembrane glycoprotein β-amyloid precursor protein (βAPP) via a proteolytic cleavage. Two proteases, β- and γ-secretase, can cleave βAPP.[5] Unlike α-secretase which cleaves APP into non-toxic amyloid APPα, β-secretase gives rise to toxic amyloid Aβ.[6,7] The two predominant Aβ peptide variants are constituted by 40 or 42 amino acids.[8] They promote pro-inflammatory responses and activate neurotoxic pathways, leading to dysfunction and death of brain cells.[9] Studies have demonstrated that when aggregated in fibrillar form, Aβ has neurotoxic effects in cell culture and in vivo.[3,4,10–12] Moreover, others reports suggest that the toxicity lies in soluble oligomeric intermediates rather than in the insoluble fibrils that accumulate.[13–20] Thus, finding molecules to prevent the aggregation of Aβ could be of therapeutic value in AD. Consequently, the discovery of substances by employing simple and reliable in vitro assays could be promising.

Since Renaud et al.'s study on the French paradox, polyphenols have received much attention.[21] Polyphenols are natural substances present in beverages obtained from plants, fruits, and vegetables such as olive oil, red wine, and tea. They are known to have wide-ranging properties on health as an antioxidant effect[22,23] and for preventing various diseases like carcinogenesis[24] and AD.[25] Moreover, one of the major properties of polyphenols is that they interact with peptides and proteins.[26–30] Such interactions might be important at the biological level in general[28,29,31–33] and particularly in AD. Recent studies have shown that polyphenols like curcumin have potent anti-amyloidogenic activities in vitro[34,35] and in vivo.[36]

Keywords: Alzheimer's disease; β-Amyloid fibrils; Polyphenols; Stilbenes.
* Corresponding author. Tel.: +33 0 5 5757 1792; fax: +33 0 5 5757 4563; e-mail: jean-pierre.monti@physique.u-bordeaux2.fr

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.09.069

In this study, we describe an original routine in vitro assay to search for inhibitors of Aβ fibril formation which uses UV–visible measurements and electron microscopy (EM). We used the Aβ25–35 amino-acid peptide ($A\beta_{25-35}$) that preserves the properties of neurotoxicity and aggregation.[37,38] The effects of stilbenes as inhibitors of Aβ fibril formation were examined. Among the various groups of polyphenols,[25] stilbenes are particularly interesting because these molecules are known for their biological activities and potent peptide-binding effects.[26,28,29,32,39] Moreover, recent studies have shown that resveratrol which belongs to this group had protective effect of PC12 cells from β-amyloid toxicity.[40,41]

2. Results

2.1. β-amyloid polymerization

First, to evaluate the sensitivity limits, absorption measurements were performed with different final concentrations in peptide: 10, 50, 100, and 200 μM. The $A\beta_{25-35}$ concentrations 100 and 200 μM showed very good reproducibility of measurements (see below the error bars in Figs. 1, 2, and 4). At 50 μM the reproducibility decreased and it was lost at 10 μM. Thus, 100 μM was chosen as working concentration.

To find the optimal incubation temperature, absorption measurements were performed at 15 and 37 °C. Both polymerization kinetics of Aβ led to the same equilibrium but incubation at 37 °C led to equilibrium much more rapidly than at 15 °C (data not shown). Moreover, a low evaporation was observed in the samples incubated at 37 °C which could have led to measurement errors. Thus, to avoid a too fast kinetic and to prevent sample evaporation, all kinetics were measured at 15 °C.

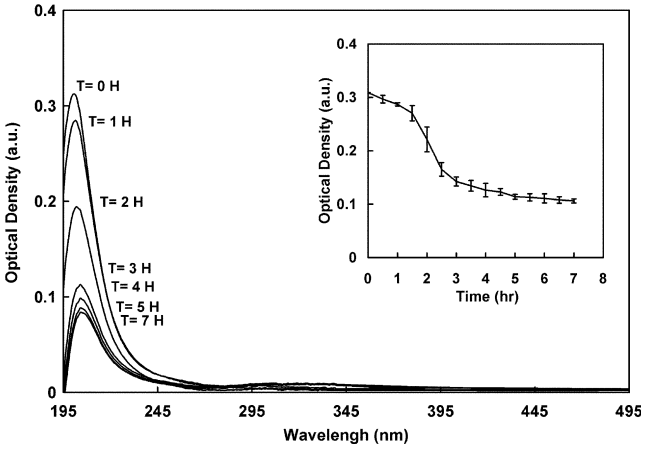


Figure 1. Absorbance spectra of Aβ versus time at 15 °C. Reaction mixture containing 100 μM of $A\beta_{25-35}$, 10 mM phosphate buffer, pH 7.2, and 10 μM MeOH. The inset presents a recording every 10 min at 200 nm to show the typical lag phase required for nucleation. For clarity, only standard deviations (SD for three independent experiments) for points at 30 min are represented.

As shown in Figure 1 (for clarity only seven spectra are presented), the $A\beta_{25-35}$ absorption spectra presented a maximum at 200 nm, this wavelength corresponding to the absorption of the peptide bond.[42] In the inset, we present a recording every 10 min to show the typical lag phase required for nucleation. The sigmoidal curve is consistent with the nucleation-dependent polymerization model.[43] The nucleation time varied between 30 and 50 min according to the prepared samples. The absorption maximum decreased as incubation times increased from 0 to 4–5 h, finally leading to equilibrium after 4–5 h, according to the prepared samples. The absorption decrease was likely due to the peptide bond hidden in the macromolecular structures (see Section 3).

To verify this hypothesis, the kinetics of $A\beta_{25-35}$ and bradykinin (BK) were compared because BK which is a nonapeptide does not aggregate.[44] Figure 2A shows the incubation kinetics of $A\beta_{25-35}$ and BK at 200 nm. For ease of comparison, the initial absorbance of BK and that of $A\beta_{25-35}$ are aligned. Whereas BK signal was constant, the $A\beta_{25-35}$ curve decreased and led to

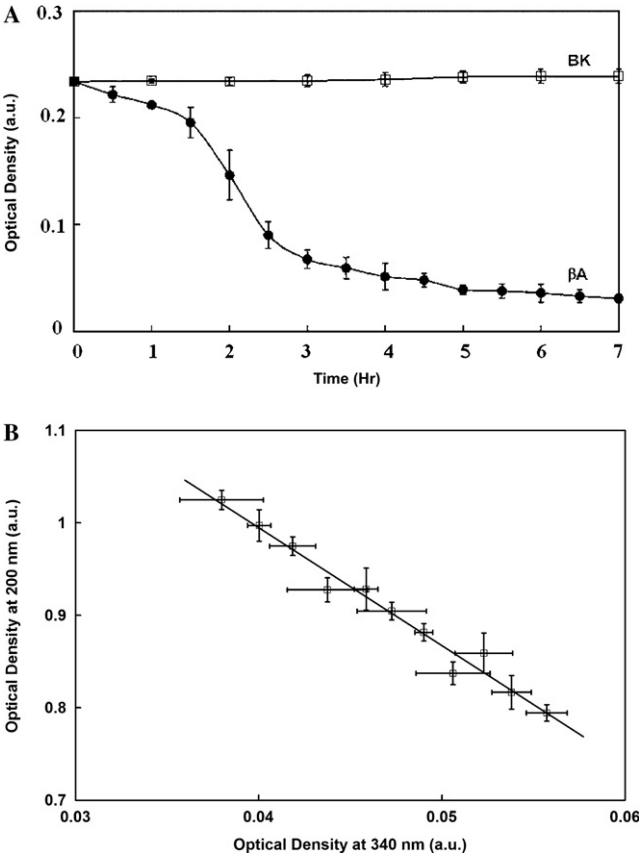


Figure 2. (A) Comparison of OD variations between bradykinin (BK) and $A\beta_{25-35}$ at 200 nm according to time. Reactions mixtures containing 10 mM phosphate buffer, 10 μL MeOH, pH 7.2, and 100 μM $A\beta_{25-35}$ or 100 μM BK were incubated at 15 °C for 7 h. For ease of comparison, the initial absorbance of BK and that of $A\beta_{25-35}$ are aligned. (B) Comparison of OD variations at 200 and 340 nm in experimental turbidity conditions (see text). Means and SD of three independent experiments are shown.

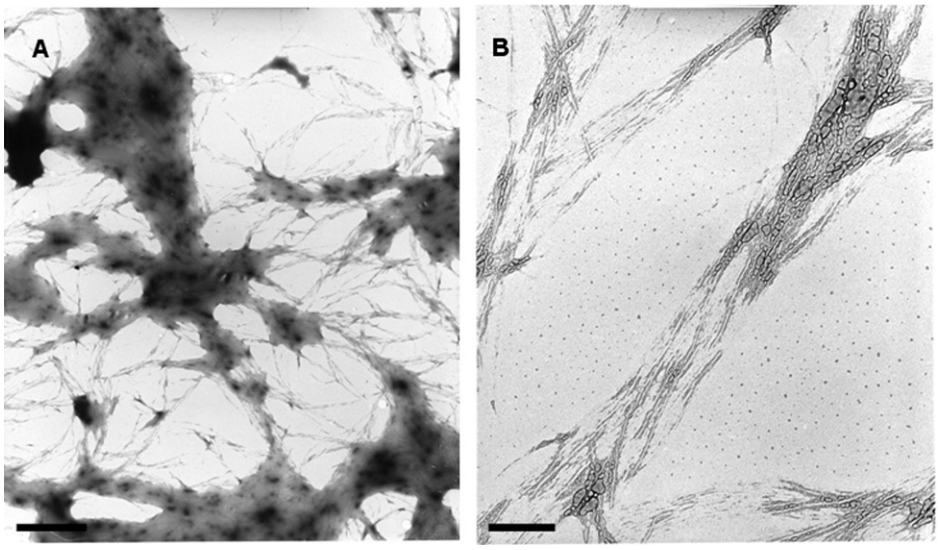


Figure 3. Electron micrographs of fibrils of $A\beta_{25-35}$ alone. (A) The bar represents 5 μm. (B) The bar represents 500 nm.

an equilibrium. Thus, Aβ fibril formation involved a decrease of the maximum signal at 200 nm in the absorption spectrum.

To confirm that the decrease in signal at 200 nm was correlated to the aggregate formation, we performed classical turbidity measurements at 350 nm.[45] Since measuring turbidity is a less sensitive method, the final concentration of $A\beta_{25-35}$ was 0.5 mM for both wavelengths. Figure 2B shows the good correlation between both methods with a correlation coefficient equal to 0.987. Therefore, the polymerization kinetic of $A\beta_{25-35}$ can be monitored by absorption measurements at 200 nm, thus allowing lower concentrations of amyloid fragment and polyphenols to be used.

To observe $A\beta_{25-35}$ fibrils in our conditions, electron microscopic studies were performed. After incubation of $A\beta_{25-35}$ at 15 °C, clear fibril extension was observed (Fig. 3A and B).

2.2. Curcumin inhibitory assays as control

Curcumin (CUR) was used as a reliable reference because this anti-amyloidogenic polyphenol was studied by Ono et al. in other experimental conditions.[35] The effects of CUR on the Aβ incubation were studied under the same conditions of temperature (15 °C) and measurement as above. For example, comparison of $A\beta_{25-35}$ incubation kinetics with and without 10 and 20 μM CUR showed an increase in the final equilibrium level (Fig. 4). Thus CUR induced the inhibition of fibril formation, as demonstrated by Ono et al.[35]

In all cases, $A\beta_{25-35}$ polymerization led to a plateau 5 h after incubation. Thus, to verify the dose-dependent inhibition of Aβ fibril formation by CUR, the relative absorbance variation (ΔA) at 6 h after incubation was measured. The CUR inhibition percentages on Aβ fibril formation (I%) were calculated as follows:

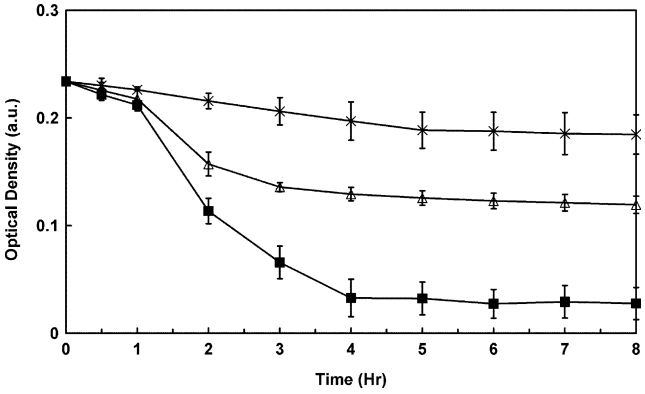


Figure 4. Curcumin (CUR) effects on the kinetics of $A\beta_{25-35}$ polymerization measured at 200 nm. Reactional mixtures containing 100 μM of $A\beta_{25-35}$, 10 mM phosphate buffer, pH 7.2, and 10 μM MeOH with 0 (■), 10 μM (Δ) or 20 μM (×) of CUR were incubated at 15 °C for 8 h. Means and SD of three independent experiments are shown.

$$I\% = \frac{\Delta A_{\text{control}} - \Delta A}{\Delta A_{\text{control}}} \times 100$$

The inhibition percentages of Aβ fibril formation increased when the CUR concentration increased, inducing a dose-dependent effect. On the basis of these data, the EC_{50} value calculated for CUR was 10 μM (Table 1), a value one order of magnitude greater than

Table 1. EC_{50} of curcumin (CUR), resveratrol (RES), and piceid (PIC) for formation inhibition of $A\beta_{25-35}$ fibrils

Polyphenols	EC_{50} (μM)
Curcumin	10.0
Resveratrol	5.6
Piceid	4.7

EC_{50} defined as the concentrations of CUR, PIC or RES to inhibit the formation $A\beta_{25-35}$ fibrils to 50% of the control value. EC_{50} was calculated by sigmoidal curve fitting of the data. Reaction mixtures containing 100 μM of $A\beta_{25-35}$, 10 mM phosphate buffer, pH 7.2, 10 μM MeOH, and 0, 0.1, 1, 10, and 50 μM CUR, PIC, or RES were incubated at 15 °C for 6 h.

that already reported.[35] This could be due to a difference in the Aβ fragment used in this work and in Ono et al. study and/or to a difference in the techniques used to detect the polyphenol inhibitory effect (see Section 3).

2.3. Stilbene effects on $A\beta_{25-35}$ polymerization

To evaluate the effects of polyphenols, the inhibition percentages of Aβ fibril formation due to various polyphenols were compared to that of CUR (10 μM) as control. We chose the concentration 10 μM because this value was the EC_{50} of CUR in our experimental conditions (Fig. 5). On the one hand, ellagic acid (EA) did not inhibit Aβ fibril formation so it could be used as negative control. However, resveratrol (RES) and its monoglucoside (piceid, PIC) exerted a potent inhibitory effect on Aβ fibril formation which was greater than that of CUR. On the other hand, catechin (CAT), piceatannol (PIA), astringin (AST), resveratrol diglucoside (DIG), and resveratrol dimer (viniferin, VIN) had less significant inhibitory activities. Therefore, the anti-amyloidogenic activity of the molecules studied is in the following order: RES ≈ PIC > CUR > DIG ≈ AST ≈ VIN ≈ PIA ≈ CAT > EA.

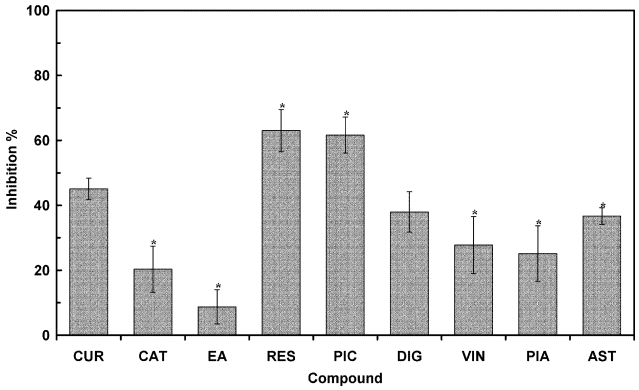


Figure 5. Polyphenol $A\beta_{25-35}$ fibril inhibition compared to that of CUR. Reaction mixtures containing 100 μM $A\beta_{25-35}$, 10 mM phosphate buffer, pH 7.2, and 10 μL MeOH with 10 μM CUR, CAT, EA, RES, PIC, DIG, VIN, PIA, or AST were incubated at 15 °C. Means and SD of three independent experiments are shown. *Significantly different from the CUR inhibitory effect ($p < 0.05$).

To reinforce these results, the EC_{50} of RES and PIC were calculated (Table 1). Table 1 shows that both polyphenols had a greater inhibitory activity on fibril formation (about twofold) than CUR. For example, during incubation of Aβ in the presence of 10 μM resveratrol (Fig. 6), only small bulk aggregates were visible and no characteristic Aβ fibrils were observed in the electron micrographs. Thus, resveratrol completely inhibited Aβ fibril formation (Fig. 7).

3. Discussion

The formation of insoluble Aβ deposits in the brain is a pathological hallmark of AD. If the hypothesis that the neurotoxicity of Aβ is mediated by amyloid fibril formation is correct,[3,4,10–12] inhibition of Aβ fibril formation might slow progression or prevent the disease. However, more recent studies have shown that fibrils are not the only neurotoxic structures and that Aβ also assembles into soluble forms like small oligomers and protofibrils, which could be responsible for neurotoxicity.[13–20] Thus, screening for compounds that reduce Aβ oligomerization or fibrillization could be discrete targets for treatment, as suggested by Selkoe.[3]

In this report we propose an original method based on peptide bond absorbance measurement by UV–visible spectrometry to screen compounds that might prevent fibril formation. Our results show that fibril formation involves a decrease in peptide bond absorbance that might be due to the screen formed on the peptide bond during Aβ fibril polymerization and the gradual

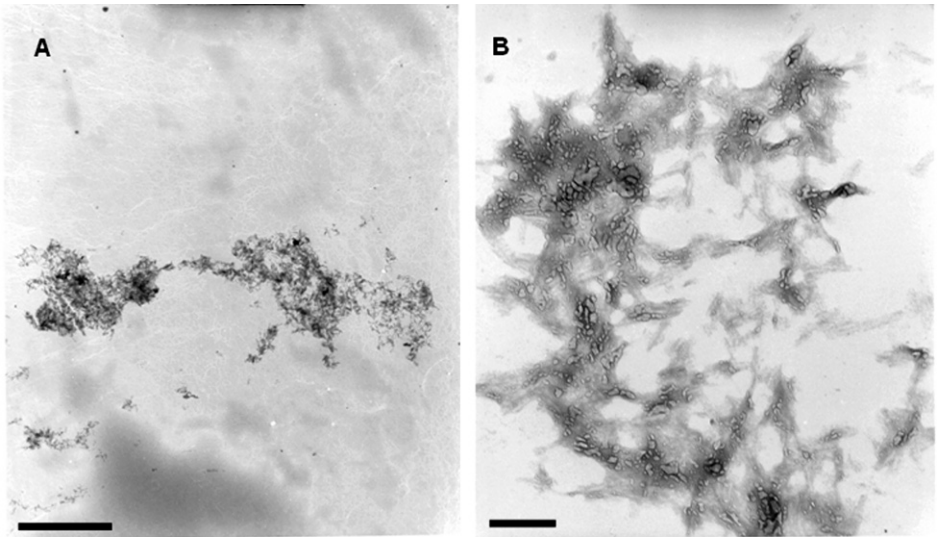
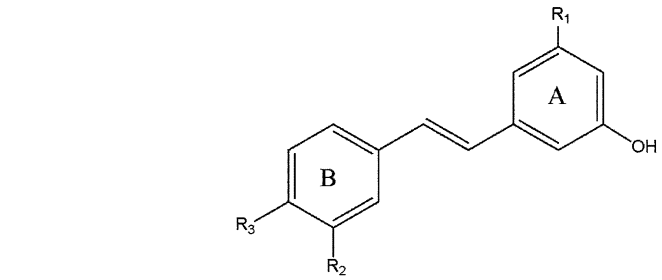
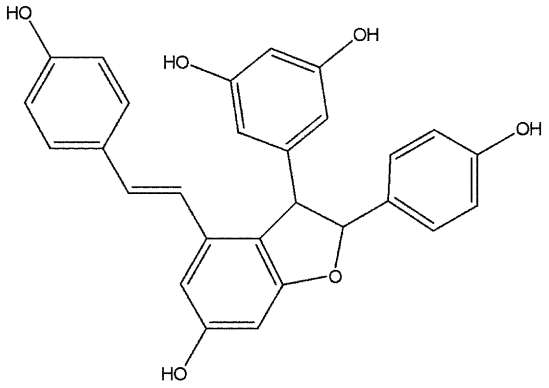


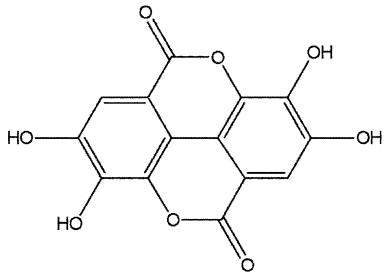
Figure 6. Electron micrographs of $A\beta_{25-35}$ after the end of RES inhibitory activity measurements (A) The bar represents 5 μm. (B) The bar represents 500 nm. CUR inhibitory effect showed the same electron micrographs.



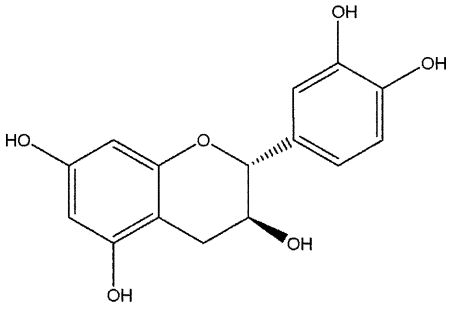
	R_1	R_2	R_3	
1	OH	H	OH	**resveratrol**
2	OGlc	H	OH	**piceid**
3	OGlc	H	OGlc	**resveratrol diglucoside**
4	OH	OH	OH	**piceatannol**
5	OGlc	OH	OH	**astringine**



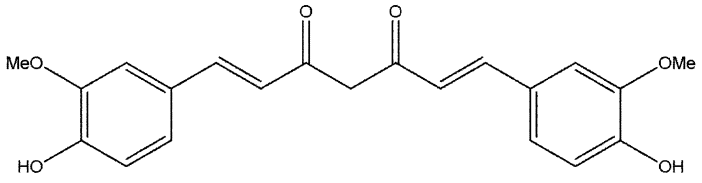
ε-viniferin



ellagic acid



catechine



curcumine

Figure 7. Polyphenol structures.

decrease in the free Aβ concentration. Thus, measuring peptide bond absorbance allows the direct monitoring of fibril formation by simple and reliable in vitro assays. However, are the anti-fibrillization effects observed here related to the anti-neurotoxic effect? Studies have shown that the neurotoxic effect could also be due to soluble oligomers. Two explanations can be given for this. First, the molecular association leading to macromolecular structures like fibrils involves a burying of the peptide bond in the core of these structures,[46,47] and thus the UV–visible absorbance decrease. The soluble oligomers which include spherical particles of 2.7–4.2 nm in diameter and protofibrils[20,48] are also macromolecular forms and likely have the same behavior as fibrils in relation to UV absorbance. Second, in a recent work, resveratrol protected PC12 cells against the apoptosis caused by Aβ, a result in agreement with our in vitro test (see below).[41] Moreover, a recent study showed that Aβ fibrils contain an intrinsic toxicity which is correlated with their morphology.[47] In conclusion, measuring peptide

bond absorbance provides a simple and reliable in vitro assay for monitoring fibril inhibition, without recourse to an intermediate like thioflavin T as in fluorescence measurements.[49]

To verify whether our methodology allows the identification of fibril inhibitors, the inhibitory properties of CUR on fibril formation were evaluated because a recent study using fluorescence spectroscopy indicated that CUR inhibits fibril aggregation.[35] In agreement with that finding, CUR dose-dependently inhibited fibril formation in our experimental conditions. Nevertheless, the Ec_{50} value calculated for CUR was one order of magnitude greater than that in Ono's work. There could be two reasons for this. First, for reasons of cost we used the $A\beta_{25-35}$ fragment, which is known to preserve the properties of neurotoxicity and aggregation,[37,38] whereas Ono et al. used the 1–40 and 1–42 fragments. Second, those authors investigated inhibition by indirect measurement of thioflavin T fluorescence.

Recent epidemiological studies performed in a relatively large cohort suggest that the moderate consumption of red wine, which contains a high amount of polyphenols, appears to be associated with a diminished risk of macular degeneration, AD, cognitive deficits, and cerebral infarction.[50–52] The affinity of polyphenols for peptides and proteins could provide a possible explanation for these observations. Moreover, resveratrol, a representative member of the stilbene group, could protect PC12 cells against the apoptosis caused by Aβ,[40,41] even though the findings in those papers were contradictory concerning the protective effect of polyphenol as tested with the fragment $A\beta_{25-35}$. Indeed, Aβ toxicity leads to a decrease in tetrazolium redox dye (MTT test) and the inhibition of MTT reduction was observed with $A\beta_{1-41}$ and β_{25-35} in both studies. Conte et al. noted no protection by RES with regard to the inhibition of MTT reduction by $A\beta_{25-35}$, while Jang et al. observed an attenuation of $A\beta_{25-35}$-induced apoptotic cell death by RES. However, these results do not call into question the in vitro data presented in this study.

Our findings show that resveratrol and its glucoside, piceid, could be promising inhibitors. Their inhibitory effects are twofold greater than that of CUR (Table 1). The structure of RES, which is more compact than that of CUR or nordihydroguaiaretic acid (NDGA), is close to the latter, which have anti-amyloidogenic activity. Binding could be induced by hydrophobic interactions between polyphenol rings and the hydrophobic region of Aβ, thus blocking associations between Aβ molecules and inhibiting fibril formation. These interactions could be reinforced by the H-bond between the hydroxyl group of phenolic rings and some donor/acceptor groups of Aβ, as observed for other peptides.[28–30] Similarly, Tomiyama et al. already suggested hydrophobic interactions between Aβ and rifampicin (RIF) where the blocking of the association between both molecules was due to interactions between the RIF lipophilic chain and the hydrophobic region of Aβ.[53,54]

Piceatannol (PIA), which has only one hydroxyl in addition to RES on the B ring, has anti-amyloidogenic activity lower than that of RES. This could be due to binding between RES and the specific site of free Aβ, thereby inhibiting Aβ fibril formation. Indeed, in PIC, the presence of sugar on ring A did not modify its anti-amyloidogenic activity. Thus, ring B seems to be involved in the interaction between either RES or PIC and free Aβ peptide. This hypothesis could be in agreement with the results obtained with DIG, which presented a weaker inhibitory activity than that of RES and PIC. However, further structural studies are essential to elucidate the mechanisms by which RES and PIC inhibit fibril formation.

Concerning viniferin (VIN), our results indicate that this resveratrol dimer does not significantly inhibit fibril polymerization, so its bulk and rigidity block the interaction with free Aβ and lead to less anti-amyloidogenic activity. These results are in agreement with Ono's results obtained with RIF and tetracycline (TC) which are large molecules with a weak anti-amyloidogenic activity.[35]

Taken together, these results suggest at first sight that small structures like RES or CUR could be effective anti-amyloidogenic molecules and bulk structures like RIF or VIN might be less active. Nevertheless, the results obtained with PIA showing a low anti-amyloidogenic activity with a structure similar to that of RES, together with Ono's results with tannic acid (TA), a large molecule, that has a high anti-amyloidogenic activity, are not in favor of this simple structure–activity relationship.[55] However, TA is a flexible bulk molecule with rings containing numerous hydroxyl functions. Thus, TA might have three interactions: (i) hydrophobic interactions; (ii), conformational mutual adaptation owing to the flexibility of Aβ and TA and allowing them to adopt a steric complementarity and to create additional van der Waals bonds; and (iii) additional H-bonds owing to the numerous hydroxyl groups.

While RES and PIC could be important molecules for therapeutic development, the mechanism of anti-amyloidogenic activity is unknown so a molecular approach to these interactions is now required.

4. Experimental

4.1. Synthetic peptides and polyphenols

Peptides $A\beta_{25-35}$ and bradykinin (BK) were purchased from Bachem California (Torrance, CA) and were used without further purification. The sequence of $A\beta_{25-35}$ peptide is GSNKGAIIGLM, corresponding to residues of the human wild type sequence $A\beta_{1-40}$. Curcumin (CUR) was purchased from Bachem. Resveratrol (RES), catechin (CAT), and ellagic acid (EA) were ordered from Extrasynthese (Genay, France). Piceatannol (PIA) and astringine (AST) were purchased from Sigma–Aldrich (Saint-Louis). Piceid (PIC), resveratrol diglucoside (DIG), and viniferin (VIN) were extracted

from *vitis vinifera* cell cultures.[56] Polyphenol purity was controlled by HPLC measurements.

4.2. Preparation of solutions

Stock solution of 1 mM was prepared by solubilizing the lyophilized $A\beta_{25-35}$ peptide by brief vortexing in sterile water at 4 °C, then by sonication for 1 min. The peptide stock solution was aliquoted and stored at −20 °C. All steps were carried out at 4 °C to prevent $A\beta_{25-35}$ polymerization.

Polyphenols were solubilized in MeOH solution to a concentration of 1 mg/ml. The polyphenol stock solution was diluted to obtain aliquots with concentrations between 0.1 and 100 μM, then stored at −20 °C.

4.3. Measurement of inhibitory activity by UV–visible spectroscopy

To study the kinetic of $A\beta_{25-35}$ polymerization alone, experiments were carried out by using a reactional mixture containing 80 μL phosphate buffer (10 mM final concentration) and 10 μL $A\beta_{25-35}$ (100 μM final concentration), pH 7.2. When $A\beta_{25-35}$ was added to the buffer solution, we performed sonication for 1 min to avoid any peptide aggregation. 10 μL MeOH was added to the solution to have the same conditions for the experiments with polyphenols. For turbidity measurements, the $A\beta_{25-35}$ final concentration was 0.5 mM.

To study the inhibitory activity of polyphenols, experiments were carried out by using a reactional mixture containing 80 μL phosphate buffer (10 mM final concentration), 10 μL MeOH containing 0.1, 1, 10, 20, and 50 μM final concentration of polyphenols and 10 μL $A\beta_{25-35}$ (100 μM final concentration), pH 7.2. All steps were carried out at 4 °C to prevent $A\beta_{25-35}$ polymerization.

UV–visible spectroscopy was performed on a Cary 300 bio UV–visible spectrophotometer. First, the UV–visible spectra of polyphenol alone were recorded by using the spectrometer in the range of 190–600 nm at 15 °C. Then, optimal measurements of $A\beta_{25-35}$ were recorded by using the spectrometer in the range of 190–600 nm at 15 °C, to control for any artifacts due to polyphenol aggregation. Then, polymerization kinetics were monitored at 200 nm between 0 and 10 h, this wavelength corresponding to the absorption of peptide bond.[42] Data were collected 6 h after incubation. For each inhibitory experiment, one sample containing $A\beta_{25-35}$ alone and another containing the polyphenol alone were used in parallel as control in the same experimental conditions. Moreover, to rule out any influence due to polyphenol absorbance, their UV–visible spectra were subtracted from the $A\beta_{25-35}$ absorption spectra. At least three independent measurements were made for all cases. All results are presented with means and standard deviation.

EC_{50} was calculated by using a least-square fitting technique to match the experimental data with a sigmoidal curve. EC_{50} was the effective concentration of polyphenol inhibiting the formation of Aβ fibrils to 50% of the control value.

4.4. Electron microscopy

The samples are taken again 5 min after measuring the kinetics of the inhibitory activity of $A\beta_{25-35}$. 5 μL of reaction mixtures was applied to carbon-coated collodium film on 200 or 400 mesh copper grid, negatively stained with 2% (w/v) uranyl acetate, and viewed on a Philips CM 10 transmission electron microscope operating at 100 kV (SERCOMI, Bordeaux, France).

Acknowledgments

We are grateful to SERCOMI for Electron Microscopy. We are grateful to Dr. Ray Cooke for reading this manuscript. Financial support for this study was supported in part by the 'Conseil Régional d'Aquitaine'.

References and notes

1. Gelmacher, D. S.; Whitehouse, P. J. *N. Engl. J. Med.* **1996**, *335*, 330.
2. Mayeux, R.; Sano, M. *N. Engl. J. Med.* **1999**, *341*, 1670.
3. Selkoe, D. J. *Nature* **1999**, *399*, A23.
4. Yankner, B. A. *Neuron* **1996**, *16*, 921.
5. Selkoe, D. J. *Trends Cell Biol.* **1998**, *8*, 447.
6. Dumery, L.; Bourdel, F.; Soussan, Y.; Fialkowsky, A.; Viale, S.; Nicolas, P.; Reboud-Ravaux, M. *Pathol. Biol.* **2001**, *49*, 72.
7. Mandel, S.; Youdim, M. B. *Free Radic. Biol. Med.* **2004**, *37*, 304.
8. Naslund, J.; Schierhorn, A.; Hellman, U.; Lannfelt, L.; Roses, A. D.; Tjernberg, L. O.; Silberring, J.; Gandy, S. E.; Winblad, B.; Greengard, P.; Norstedt, C.; Terenius, L. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 8378.
9. Pereira, C.; Agostinho, P.; Moreira, P. I.; Cardoso, S. M.; Oliveira, C. R. *Curr. Drug. Targets CNS Neurol. Disor.* **2005**, *4*, 383.
10. Pike, C. J.; Walencewicz, A. J.; Glabe, C. G.; Cotman, C. W. *Brain Res.* **1991**, *563*, 311.
11. Pike, C. J.; Burdick, D.; Walencewicz, A. J.; Glabe, C. G.; Cotman, C. W. *J. Neurochem.* **1993**, *13*, 1676.
12. Lorenzo, A.; Yankner, B. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12243.
13. Lambert, M. P.; Barlow, A. K.; Chromy, B. A.; Edwards, C.; Freed, R.; Liosatos, M.; Morgan, T. E.; Rozovsky, I.; Trommer, B.; Viola, K. L.; Wals, P.; Zhang, C.; Finch, C. E.; Krafft, G. A.; Klein, W. L. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 6448.
14. Walsh, D. M.; Hartley, D. M.; Kusumoto, Y.; Fezoui, Y.; Condron, M. M.; Lomakin, A.; Benedek, G. B.; Selkoe, D. J.; Teplow, D. B . *J. Biol. Chem.* **1999**, *274*, 25945.
15. Bhatia, R.; Lin, H.; Lal, R. *FASEB J.* **2000**, *14*, 1233.
16. Zhu, Y. J.; Lin, H.; Lal, R. *FASEB J.* **2000**, *14*, 1244.
17. Klein, W. L.; Krafft, G. A.; Finch, C. E. *Trends Neurosci.* **2001**, *24*, 219.
18. Kim, H. J.; Chae, S. C.; Lee, D. K.; Chromy, B.; Lee, S. C.; Park, Y. C.; Klein, W. L.; Krafft, G. A.; Hong, S. T. *FASEB J.* **2003**, *17*, 118.
19. Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*, 353.
20. Kayed, R.; Head, E.; Thompson, J. L.; McIntire, T. M.; Milton, S. C.; Cotman, C. W.; Glabe, C. G. *Science* **2003**, *300*, 486.

21. Renaud, S.; de Lorgeril, M. *Lancet* **1992**, *339*, 1523.
22. Hubbard, G. P.; Wolffram, S.; Lovegrove, J. A.; Gibbins, J. M. *Proc. Nutr. Soc.* **2003**, *62*, 469.
23. Scalbert, A.; Manach, C.; Mrand, C.; Remesy, C.; Jimenez, L. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 287.
24. Yang, C. S.; Prabhu, S.; Lau, J. *Drug Metab. Rev.* **2001**, *33*, 237.
25. Manach, C.; Scalbert, A.; Morand, C.; Remesy, C.; Jimenez, L. *Am. J. Clin. Nutr.* **2004**, *79*, 727.
26. Richard, T.; Verge, S.; Berke, B.; Vercauteren, J.; Monti, J. P. *J. Biomol. Struct. Dyn.* **2001**, *18*, 627.
27. Charlton, A. J.; Baxter, N. J.; Khan, M. L.; Moir, A. J.; Haslam, E.; Davies, A. P.; Williamson, M. P. *J. Agric. Food. Chem.* **2002**, *50*, 1593.
28. Verge, S.; Richard, T.; Moreau, S.; Nurich, A.; Merillon, J. M.; Vercauteren, J.; Monti, J. P. *Biochim. Biophys. Acta* **2002**, *1571*, 89.
29. Richard, T.; Vitrac, X.; Merillon, J. M.; Monti, J. P. *Biochim. Biophys. Acta* **2005**, *1726*, 238.
30. Murray, N. J.; Williamson, M. P.; Lilley, T. H.; Haslam, E. *Eur. J. Biochem.* **1994**, *219*, 923.
31. Haslam, E. *Biochem. J.* **1974**, *139*, 285.
32. Richard, T.; Delaunay, J. C.; Merillon, J. M.; Monti, J. P. *J. Biomol. Struct. Dyn.* **2003**, *21*, 379.
33. Loudet, C.; Khemtemourian, L.; Aussena, F.; Gineste, S.; Achard, M. F.; Dufourc, E. J. *Biochim. Biophys. Acta* **2005**, *1724*, 315.
34. Ono, K.; Yoshiike, Y.; Taskashima, A.; Hasegawa, K.; Naiki, H.; Yamada, M. *J. Neurochem.* **2003**, *87*, 172.
35. Ono, K.; Hasegawa, K.; Naiki, H.; Yamada, M. *J. Neurosc. Res.* **2004**, *75*, 742.
36. Yang, F.; Lim, G. P.; Begum, A. N.; Ubeda, O. J.; Simmons, M. R.; Ambegaokar, S. S.; Chen, P. P.; Kayed, R.; Glabe, C. G.; Frautschy, S. A.; Cole, G. M. *J. Biol. Chem.* **2005**, *280*, 5892.
37. Klegeris, A.; Walker, D. G.; McGeer, P. L. *Biochem. Biophys. Res. Commun.* **1994**, *199*, 984.
38. Pike, C. J.; Walencewicz-Wasserman, A. J.; Kosmoski, J.; Cribbs, D. H.; Glabe, C. G.; Cotman, C. W. *J. Neurochem.* **1995**, *64*, 253.
39. Wenzel, E.; Somoza, V. *Mol. Nutr. Food Res.* **2005**, *49*, 472, Review references therein.
40. Conte, A.; Pellegrini, S.; Tagliazucchi, D. *Brain Res. Bull.* **2003**, *62*, 29.
41. Jang, J. H.; Surh, Y. J. *Free Radic Biol. Med.* **2003**, *34*, 1100.
42. Snyder, L. R.; Kirkl, J. J.; Glajch, J. L. In *Pratical HPLC Method development*; Wiley Interscience Publication: New York, 1997.
43. Naiki, H.; Gejyo, F.; Nakakuki, K. *Biochemistry* **1997**, *36*, 6243.
44. Kotovych, G.; Cann, J. R.; Stewart, J. M.; Yamamoto, H. *Biochem. Cell Biol.* **1998**, *76*, 257.
45. Zurdo, J.; Guijarro, J. I.; Jimenez, J. L.; Saibil, H. R.; Dobson, C. M. *J. Mol. Biol.* **2001**, *311*, 325.
46. Petkova, A. T.; Ishii, Y.; Balbach, J. J.; Antzutkin, O. N.; Leapman, R. D.; Delaglio, F.; Tycko, R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 16742.
47. Luhrs, T.; Ritter, C.; Adrian, M.; Riek-Loher, D.; Bohrmann, B.; Dobeli, H.; Schubert, D.; Riek, R. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 17342.
48. Hartley, D. M.; Walsh, D. M.; Ye, C. P.; Diehl, T.; Vasquez, S.; Vassilev, P. M.; Teplow, D. B.; Selkoe, D. J. *J. Neurosci.* **1999**, *19*, 8876.
49. LeVine, H., 3rd *Protein Sci.* **1993**, *2*, 404.
50. Orgogozo, J. M.; Dartigues, J. F.; Lafont, S.; Letenneur, L.; Commenges, D.; Salomon, R.; Renaud, S.; Breteler, M. B. *Rev. Neurol.* **1997**, *153*, 185.
51. Obisesan, T. O.; Hirsch, R.; Kosoko, O.; Carlson, L.; Parrott, M. *J. Am. Geriatr. Soc.* **1998**, *46*, 1.
52. Commenges, D.; Scotet, V.; Renaud, S.; Jacqmin-Gadda, H.; Barberger-Gateau, P.; Dartigues, J. F. *Eur. J. Epidemiol.* **2000**, *16*, 357.
53. Tomiyama, T.; Shoji, A.; Kataoka, K.; Suwa, Y.; Asano, S.; Kaneko, H.; Endo, N. *J. Biol. Chem.* **1996**, *271*, 6839.
54. Tomiyama, T.; Asano, S.; Suwa, Y.; Morita, T.; Kataoka, K.; Mori, H.; Endo, N. *Biochem. Biophys. Res. Commun.* **1994**, *204*, 76.
55. Ono, K.; Hasegawa, K.; Naiki, H.; Yamada, M. *Biochim. Biophys. Acta* **2004**, *1690*, 193.
56. Waffo Teguo, P.; Fauconneau, B.; Deffieux, G.; Huguet, F.; Vercauteren, J.; Merillon, J. M. *J. Nat. Prod.* **1998**, *61*, 655.