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Apricot Carotenoids Possess Potent Anti-amyloidogenic Activity in Vitro

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ABSTRACT: Alzheimer's disease (AD) is the most common form of dementia and is characterized by the progressive accumulation of amyloid β protein (Aβ) in areas of the brain. There has been an increased interest in screening for food-grade anti-amyloidogenic compounds in foodstuffs. The purpose of this study was to screen and identify bioactive compounds with anti-amyloidogenicity in apricot fruits using synthetic A$\beta_{1\text{-}42}$. The anti-amyloidogenicity was investigated using thioflavin T fluorescence assay, electron microscopy, and dot blotting analysis. The carotenoid fraction from apricot showed strong inhibitory effects against oligomer and fibril formation of Aβ and fibril-destabilizing effects. Among the peaks in the HPLC chromatogram, lutein showed the strongest inhibitory effect on Aβ fibril formation. The inhibitory effect was dependent on the number and portion of hydroxyl groups on both sides of carotenoids. These findings suggest that lutein in fruits may be useful as a preventive agent for amyloid-associated diseases.

KEYWORDS: amyloid β protein, anti-amyloidogenicity, carotenoids, lutein

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia, a neurologic disease characterized by impairment of mental ability severe enough to interfere with normal activities of daily living.[1,2] AD is characterized by the progressive accumulation of amyloid fibrils composed of the amyloid β protein (Aβ) in areas of the brain serving cognitive functions such as memory and language.[3] Aβ is a peptide consisting of 40−42 amino acids and is derived by β- and γ-secretase cleavage from the Aβ precursor protein (APP) containing approximately 700 amino acid residues, a type I transmembrane protein.[4] A$\beta_{1\text{-}42}$ is more hydrophobic than A$\beta_{1\text{-}40}$ and is most closely linked with AD pathogenesis.[5] Increases in the concentration of Aβ in the course of the disease with subtle effects on synaptic efficacy will lead to gradual increase in the load of amyloid plaques and progression in cognitive impairment.

Under a certain environment, amyloids self-assemble into β-sheet-rich structures (cross-β conformation) characterized by the transformation of soluble proteins/peptides into aggregated fibrils.[6] According to numerous studies on Aβ, suppression or prevention of transition from monomeric to oligomeric and polymeric forms has emerged as an effective therapeutic approach in the development of anti-amyloid therapy.[5,7,8] There has been increased interest in screening for food-grade anti-amyloidogenic agents in naturally occurring compounds. Recent studies have demonstrated that polyphenols such as resveratrol and curcumin are capable of inhibiting amyloid fibril formation in vitro,[9,10] where it is assumed that some of them interact directly with the amyloid-forming sequences in amyloidogenic proteins. Furthermore, it was shown recently that oral administration of phenolic compounds, including curcumin, rosmarinic acid, and myricetin, prevented the development of AD pathology by inhibiting the Aβ aggregation pathway in an AD transgenic mouse model.[11] These findings suggest natural polyphenols could be a candidate therapeutic molecule for inhibiting Aβ aggregation.

Many epidemiological studies suggest that increased fruit consumption decreases the risk of several degenerative diseases including atherosclerosis and heart and brain disorders.[12,13] Stuerenburg et al.[14] reported the statistically significant association between plasma β-carotene levels and cerebrospinal fluid concentrations of total Tau, A$\beta_{1\text{-}40}$, and A$\beta_{1\text{-}42}$ in Alzheimer's disease patients. Ono et al.[15] demonstrated that vitamin A and β-carotene could exhibit anti-amyloidogenic effects by directly inhibiting the formation of Aβ fibrils as well as by scavenging reactive oxygen species. These findings support the postulation that β-carotene or carotenoid-rich fruits and vegetables could be useful for the prevention of AD, and other carotenoids are also expected to show anti-amyloidogenic effects. However, the effects of various natural carotenoids contained in fruits and vegetables against amyloidogenicity are little known.

Apricot (*Prunus armeniaca* L.) is one of the most important commercial fruits and preferable in the worldwide markets. Apricot fruits, a rich natural source of provitamin A, contain much more carotenoid than other fruits.[16] In the present study, we screened and identified the bioactive compounds with amyloidogenicity in apricot fruits using synthetic A$\beta_{1\text{-}42}$ in vitro. To evaluate the anti-amyloidogenic activity, their inhibitory effects on the formation of A$\beta_{1\text{-}42}$ oligomers and fibrils were measured using thioflavin T (ThT) fluorescence assay and dot blot analysis with anti-oligomer antibody.

MATERIALS AND METHODS

Materials. Synthetic A$\beta_{1\text{-}42}$ was purchased from Peptide Institute Inc. (Osaka, Japan). Apricot fruits of the variety 'Showa' (*P. armeniaca* L. Showa) were kindly provided by Chikuma Industry Support Center

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(Nagano, Japan). ThT was obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Standard carotenoids were purchased from Extrasynthese (Genay, France). Anti-Aβ antibody (NT), anti-oligomer antibody (A11), and goat anti-rabbit IgG labeled with horseradish peroxidase were purchased from Invitrogen (Camarillo, CA) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. Pierce Western Blotting Substrate was purchased from ThermoFisher Scientific (Waltham, MA). All other chemicals were of analytical reagent grade.

Preparation of Carotenoid Fraction from Apricot (CFA). The preparation of CFA was performed at room temperature as described in Khachik et al.[17] with a slight modification. Briefly, 100 g of apricot fruits (*P. armeniaca* L. Showa) was cut into small pieces and rinsed with 200 mL of methanol. The samples were filtered with suction, thoroughly ground with methanol on the filter, and then air-dried on paper towels for 5−10 min in a fume hood to remove all of the alcohol. Subsequently, the carotenoid fraction was extracted with diethyl ether (3 × 80 mL) until the residue was white. The total ether extract was dried over anhydrous sodium sulfate and evaporated under reduced pressure, and the residue was dissolved in ethanol. The extract was saponified with 5% ethanolic potassium hydroxide solution to remove fatty acids and then extracted with hexane/diethyl ether (1:1, v/v). The resulting organic layer was evaporated to dryness and dissolved in an appropriate volume of ethanol. This solution was stored as CFA in a −80 °C freezer until use. CFA was quantified usingthe peak area ratio of the HPLC chromatograms (described later), calibrated with a β-carotene standard. CFA diluted in ethanol was applied to the following experiments at final concentrations of 1 and 5 μg/mL.

Preparation of Aβ Fibrils. $A\beta_{1-42}$ was dissolved in 0.1% ammonia solution at a final concentration of 250 μM, separated into aliquots, and then stored at −80 °C until use. For preparing amyloid fibrils, aliquots of Aβ were diluted to 25 μM in 50 mM phosphate buffer, pH 7.5, containing 100 mM NaCl. The Aβ solution was then sealed in a microcentrifuge tube and incubated at 37 °C for 24 h with various concentrations of the samples. For the determination of fibril-destabilizing effects, Aβ was incubated at 37 °C for 24 h, and the obtained Aβ fibrils were then incubated with various concentrations of the samples.

ThT Fluorescence Assay. Amyloid fibril formation was monitored using a ThT binding assay. A 6 μL aliquot of the sample was added to 1.2 mL of ThT solution containing 50 mM glycine−NaOH buffer, pH 8.5, at a final concentration of 5 μM, and then the mixture was vortexed briefly. The fluorescence was determined using an FP-6200 spectrofluorometer (JASCO, Tokyo, Japan). Excitation and emission wavelengths were 446 and 490 nm, respectively. Sample fluorescence was determined by measuring four times and subtracting the fluorescence of a ThT blank. Ethanol or tetrahydrofuran (THF)/ethanol (1:9, v/v) was used as control.

Electron Microscopy. Transmission electron microscopy was used to characterize the structural morphology of Aβ fibrils both in the presence and in the absence of CFA. $A\beta_{1-42}$ (25 μM) or $A\beta_{1-42}$ fibrils were incubated with or without 5 μg/mL CFA at 37 °C. Aliquots (5 μL) of the sample were spotted onto collodion-coated 400 mesh copper grids. The grids were negatively stained with 1% phosphotungstic acid and visualized with a JEM-1400 microscope (JEOL, Tokyo, Japan) operating with an accelerating voltage of 80 kV.

Immuno-dot Blotting. $A\beta_{1-42}$ (25 μM) was incubated with or without 5 μg/mL CFA at 37 °C, and 5 μL of the sample mixture containing Aβ (0.56 μg) was spotted onto nitrocellulose membrane and air-dried for 30 min. The membrane was blocked with 3% (w/v) bovine serum albumin in phosphate-buffered saline (PBS) for 60 min at room temperature and washed three times with PBS containing 0.05% (w/v) Tween 20. The membrane was incubated with rabbit anti-oligomer antibody A11 (1:2500 dilution) or anti-Aβ sequence antibody (NT) (1:5000 dilution) for 60 min at room temperature. After three washings with PBST, the membrane was incubated with anti-rabbit IgG−horseradish peroxidase (1:5000 dilution) for 60 min at 37 °C. The membrane was developed in Pierce Western Blotting Substrate for 1 min following washes with PBS containing 0.05% (w/v) Tween 20. The immunoreactive proteins were visualized using an ImageQuant LAS 4000 mini (GE Healthcare, Tokyo, Japan) by enhanced chemiluminescence.

Isolation and Structural Analysis. The CFA was analyzed by a HPLC system consisting of a gradient pump (UV-2089, Jasco, Tokyo, Japan) and a UV detector (UV-2075) on an YMC carotenoid C30 column (4.6 × 250 mm, 5 μm; YMC Co. Ltd., Japan). The initial solvent composition consisted of 90% methanol, 5% water, and 5% methyl *tert*-butyl ether (MTBE). The solvent composition changed in a linear gradient to 95% methanol and 5% MTBE at 12 min. During the next 8 min, the solvent composition was changed to 86% methanol and 14% MTBE. After reaching this concentration, the solvent was gradually changed to 75% methanol and 25% MTBE at 30 min. Final composition was reached at 50 min and consisted of 50% methanol and 50% MTBE. Flow rate was 1.0 mL/min. The column was maintained at 30 °C, and the elution profiles were monitored at 455 nm with a UV detector. The eluates were collected separately, concentrated by evaporation, and lyophilized. The structures of carotenoid in each peak were identified by their retention time on HPLC, UV spectra, and ESI-MS. The UV−visible spectra were recorded with a PowerScan HT (DS Pharma Biomedical, Osaka, Japan) in methanol. The mass spectra were recorded using a JMS-700 mass spectrometer (JEOL, Tokyo, Japan).

Statistical Analysis. The results were expressed as the mean ± SD. Statistical evaluation was carried out using an unpaired Student's *t* test or one-way analysis of variance (ANOVA followed by Scheffe's multiple-range test) with Statcel software ver. 2.0 (OMS-Publishing, Saitama, Japan).

RESULTS

Effects of CFA on Aβ Fibril Formation. CFA was prepared by solvent extraction of diethyl ether, removal of lipids through saponification, and subsequent purification of the carotenoids by solvent extraction with hexane/diethyl ether (1:1, v/v). The carotenoid yield was approximately 0.7 mg from 100 g of apricot fruits.

We first investigated whether fibril formation of $A\beta_{1-42}$ was inhibited by CFA using the ThT fluorescence assay. ThT specifically binds to the hydrophobic grooves on the surface of the β-sheet,[18] and thus the ThT fluorescence has been used as a quantitative indicator of amyloid fibril formation. Figure 1A shows the time course of ThT fluorescence for $A\beta_{1-42}$ incubated at 37 °C in the absence or presence of CFA (final concentrations of 1 and 5 μg/mL). ThT fluorescence of $A\beta_{1-42}$ incubated alone increased rapidly from 2 to 4 h and reached a plateau after 12 h. This is consistent with the well-known polymerization model of amyloidogenic proteins, which is characterized by an initial lag phase, followed by an elongation phase, and a final equilibrium phase.[19] In contrast, the addition of CFA resulted in a significant and dose-dependent decrease in the ThT fluorescence, indicating effective inhibition of amyloid fibril formation. We further investigated the fibril-destabilizing effects of CFA (Figure 1B). As a result, the fluorescence dramatically decreased and reached the same level as achieved by 24 h of incubation with each sample in Figure 1A. Especially, inhibition of fibril formation and destabilization of preformed fibrils were almost completely achieved by the addition of 5 μg/mL CFA. The inhibitory effect on Aβ fibril formation and morphology were further studied by transmission electron microscopy. When Aβ alone was incubated for 24 h, clear amyloid fibrils were observed (Figure 2A). In contrast, incubation of Aβ with 5 μg/mL CFA for 24 h did not result in

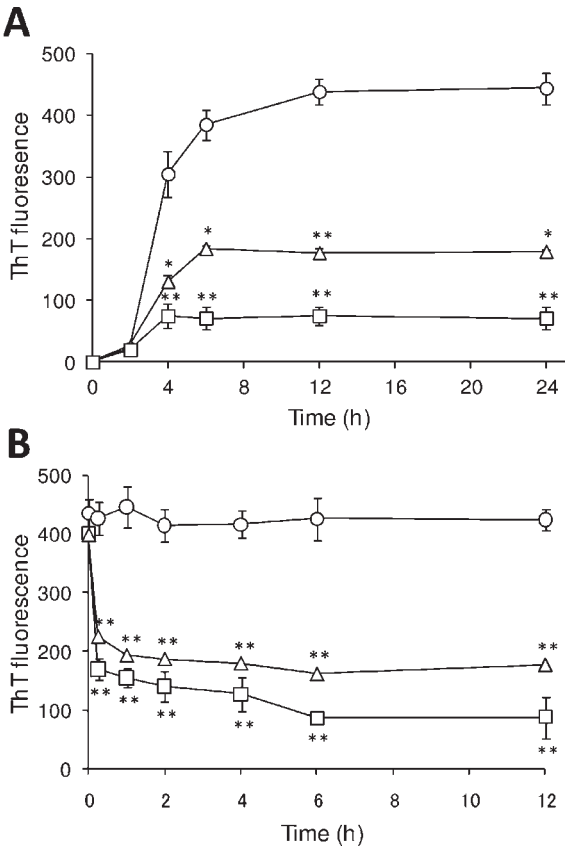

Figure 1. Effects of carotenoid fraction from apricot (CFA) on the time course of Aβ fibril formation (A) and destabilization (B). A$\beta_{1\text{-}42}$ (25 μM) was incubated at 37 °C in 50 mM phosphate buffer, pH 7.5, in the presence of 0 (○), 1 (△), and 5 (□) μg/mL CFA. ThT fluorescence was measured over a period of 24 h. For the destabilizing effects, A$\beta_{1\text{-}42}$ (25 μM) was incubated at 37 °C for 24 h in 50 mM phosphate buffer, pH 7.5, and the resulting Aβ fibrils were then incubated at 37 °C in the presence of 0 (○), 1 (△), and 5 (□) μg/mL CFA. ThT fluorescence was measured over a period of 12 h. Data are presented as the mean ± SD in quadruplicates from two separate experiments. *, $p < 0.01$, and **, $p < 0.001$, when compared with A$\beta_{1\text{-}42}$ only.

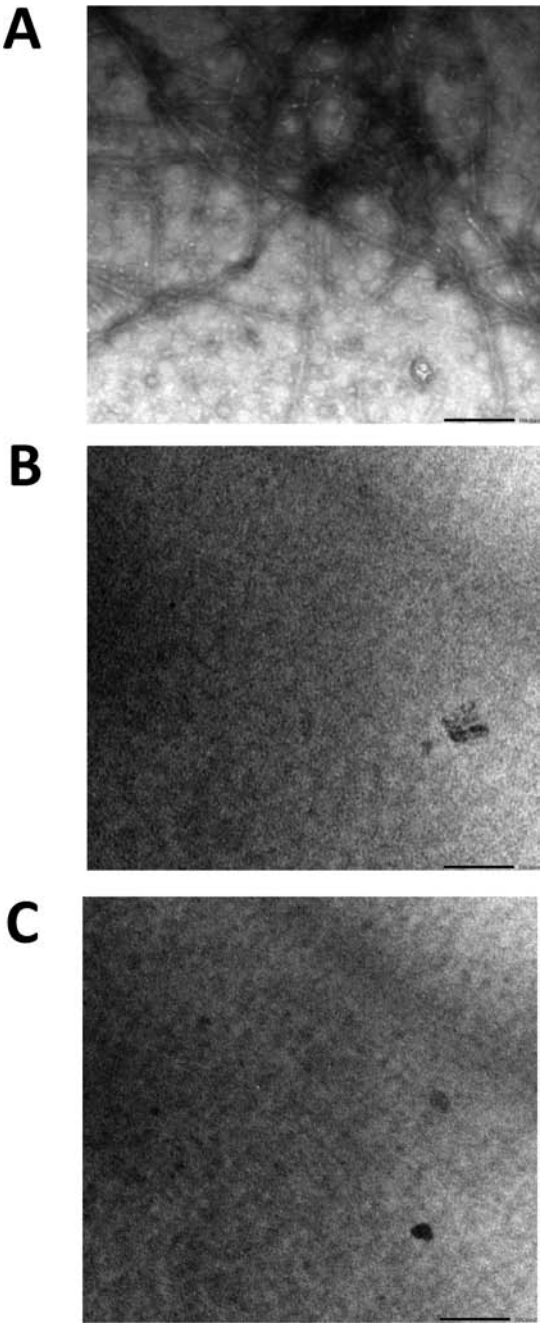

Figure 2. Electron micrographs after 24 h of incubation of Aβ without (A) and with CFA (B) and 12 h of incubation of pretreated Aβ with CFA (C). A$\beta_{1\text{-}42}$ (25 μM) was incubated at 37 °C for 24 h in 50 mM phosphate buffer, pH 7.5, in the absence (A) and presence of 5 μg/mL CFA (B). For the destabilizing effects, A$\beta_{1\text{-}42}$ (25 μM) was incubated at 37 °C for 24 h in 50 mM phosphate buffer, pH 7.5, and the resulting Aβ fibrils were then incubated at 37 °C in the presence of 5 μg/mL CFA (C). The samples were negatively stained with 1% phosphotungstic acid. The scale bar indicates 100 nm.

fibril formation (Figure 2B). Aβ fibrils were not also observed in the 12 h incubation of the Aβ fibrils with 5 μg/mL CFA (Figure 2C).

Aβ aggregates to form oligomers, protofibrils, and fibrils en route to the deposition of amyloid plaques associated with AD. It has recently been demonstrated that soluble Aβ oligomers are more toxic than the monomeric form and their mature fibrils.[20,21] We then investigated the inhibitory effect of CFA on Aβ oligomer formation. After incubation of Aβ in the absence and presence of 5 μg/mL CFA, the supernatants were collected and assayed by dot blot analysis with an Aβ and its oligomer-specific antibodies NT and A11. The NT antibody recognizes the N terminus of Aβ, and the A11 antibody recognizes prefibrillar Aβ oligomers and does not react with Aβ monomer or fibrils. NT-positive Aβ was present during the 24 h incubation period, whereas A11-positive Aβ oligomers were formed from 0 to 4 h (Figure 3). On the other hand, Aβ oligomers were not observed when CFA was incubated with Aβ. These results indicated that CFA can significantly inhibit Aβ oligomer formation.

Identification of More Effective Carotenoid Compounds. We next investigated what type of carotenoid compound is more effective in the inhibition of Aβ fibril formation. HPLC chromatograms of CFA showed five peaks (Figure 4A). No marked difference was observed in HPLC chromatograms between before and after saponification in CFA preparation and between CFA obtained from other varieties such as 'Harcot', 'Heiwa', and 'Shinshu-oomi' (data not shown). Quantification of the total

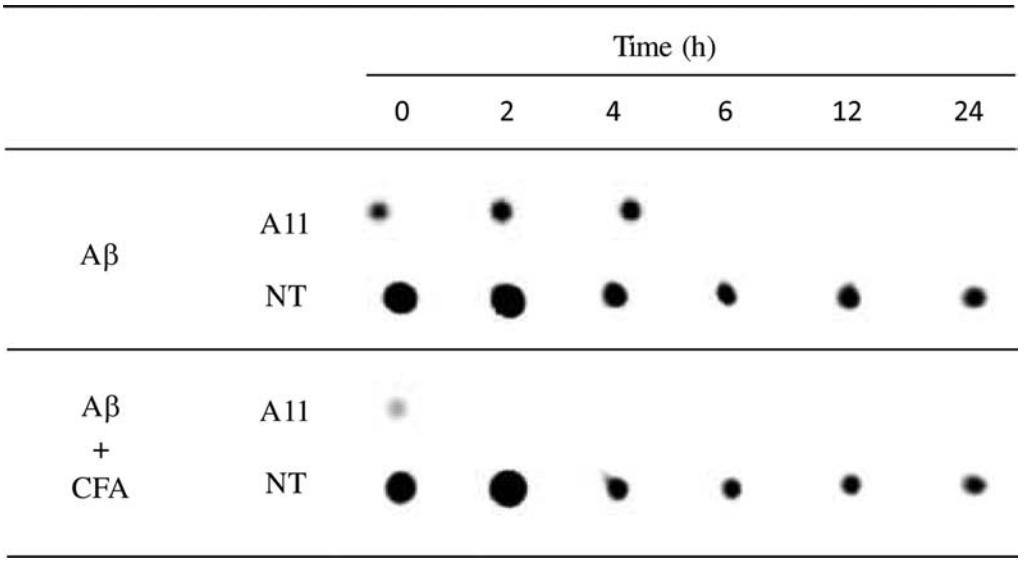

Figure 3. Dot blot analysis using anti-Aβ and anti-oligomer antibodies. A$\beta_{1\text{-}42}$ (25 μM) was incubated at 37 °C for the indicated times with and without 5 μg/mL CFA. Each sample was spotted on a nitrocellulose membrane and probed with the conformation-specific antibody A11 (specific for Aβ oligomers) and the sequence-specific antibody NT (specific for the N terminus of Aβ).

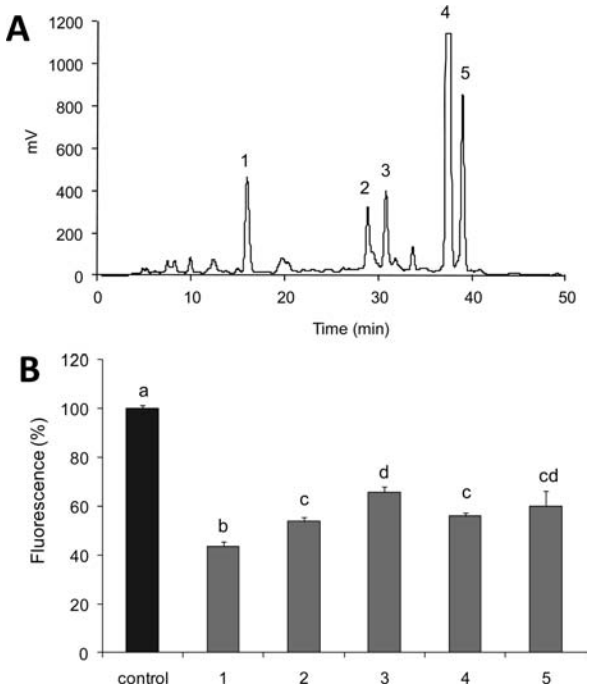

Figure 4. HPLC chromatograms of CFA (A) and inhibitory effects of each peak on Aβ fibril formation (B). Peaks: 1, lutein; 2, β-cryptoxanthin; 4, β-carotene. A$\beta_{1\text{-}42}$ (25 μM) was incubated at 37 °C for 24 h in 50 mM phosphate buffer, pH 7.5, in the presence of 5 μg/mL peaks of CFA separated by HPLC, and ThT fluorescences were determined. Results are shown as the percent fluorescence of controls (taken to be 100%). Data are presented as the mean ± SD in quadruplicates from two separate experiments.

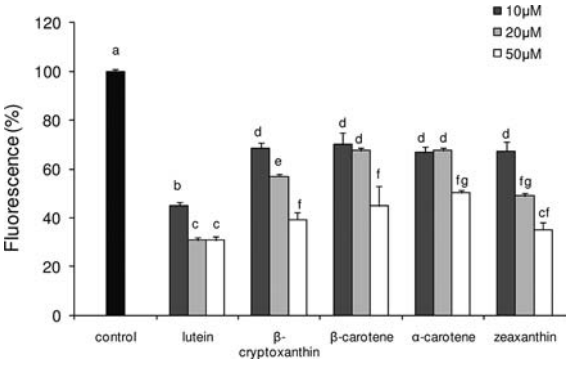

Figure 5. Effect of commercial carotenoids on Aβ fibril formation. A$\beta_{1\text{-}42}$ (25 μM) was incubated at 37 °C for 24 h in 50 mM phosphate buffer, pH 7.5, in the presence of 10, 20, and 50 μM commercial carotenoids, and ThT fluorescences were determined. Results are shown as the percent fluorescence of controls (taken to be 100%). Data are presented as the mean ± SD in quadruplicates from two separate experiments.

content of carotenoid peaks revealed peak 1, 10.7%; peak 2, 6.6%; peak 3, 8.3%; peak 4, 38.7%; and peak 5, 15.7%. Among them, lutein (peak 1), β-cryptoxanthin (peak 2), and β-carotene (peak 4) were identified by comparing their retention times and UV spectra with those of pure standard. The molecular masses were then confirmed by ESI-MS analysis. Each peak was concentrated by evaporation, lyophilized, and dissolved in ethanol, and this solution was assayed by the ThT fluorescence assay. All of the examined peaks showed the inhibition of Aβ fibril formation, with activity in the following order: peak 1 (lutein) > peak 2 (β-cryptoxanthin) > peak 4 (β-carotene) > peak 5 (unknown) > peak 3 (unknown) (Figure 4B). Furthermore, the inhibitory effect of commercial carotenoids, including lutein, β-cryptoxanthin, β-carotene, and their structural homologues, α-carotene and zeaxanthin, was investigated to study the structure−activity relationship of carotenoids (Figure 5). The carotenoids were dissolved in THF/ethanol (1:9, v/v). All of the examined carotenoids dose-dependently inhibited the formation of Aβ fibrils. Values between 10, 20, and 50 μM (final concentration) were used to determine the half-maximal inhibitory concentration (IC_{50}), which is the molar concentration of carotenoid required to reduce the fluorescence by 50%. The activity was in

the following order: lutein > zeaxanthin > β-cryptoxanthin > β-carotene = α-carotene. IC_{50} values were 9.1, 18.5, 21.3, 40.4, and 43.2 μM, respectively. These results corresponded to those shown in Figure 4B, and β-carotene, a major component of CFA, did not show any dramatic effects compared to lutein.

DISCUSSION

The present study demonstrated that the carotenoid fraction from apricot fruits exhibited potent anti-amyloidogenic and fibril-destabilizing effects in vitro. Furthermore, lutein showed the highest effect among the components of the carotenoid fraction.

Amyloidogenic proteins contain common structural features, which are characterized by a β-sheet parallel to the fibril axis, with their extended protein strands perpendicular to the axis. They consist of a pair of β-sheets, with the facing side chains of the two sheets interdigitated in a steric zipper. Sawaya et al.[22] have demonstrated that GGVVIA ($Aβ_{37-42}$) forms a parallel sheet, whereas the overlapping segment MVGGVV ($Aβ_{35-40}$) forms antiparallel sheets. The formation of ordered β-sheet structures is predominantly a consequence of the formation of highly directional interchain hydrogen bonds. Therefore, the inhibition and destabilization of Aβ amyloidogenic aggregates will be caused by a steric interference in β-sheet interaction.

Lutein consists of chains with eight conjugated double bonds containing closed rings at each end of the chain. Two hydroxyl groups on each side are considered to be important for the inhibition of Aβ formation. This suggests that the inhibitory effect of lutein on Aβ fibrillogenesis may be caused by the interaction strength via hydrogen bonding between the hydroxyl group of lutein and the acceptor/donor groups of Aβ. Indeed, the inhibitory effect of carotenoids on Aβ fibrillogenesis was observed in accordance with the number of hydroxyl groups (lutein > β-cryptoxanthin > β-carotene) as shown in Figure 5. On the other hand, zeaxanthin, which is a stereoisomer of lutein and differs only in the location of a hydroxyl group, showed a lower inhibitory effect than lutein. Thus, it is apparent that not only the number but also the portion of hydroxyl groups on carotenoid will affect the inhibitory effect on Aβ fibril formation. However, the inhibitory effect was observed in β-carotene and α-carotene, containing no hydroxyl group, suggesting that the hydrophobic chain of polyene units itself also plays an important role in the inhibition of Aβ fibrillogenesis via the hydrophobic interaction. Taken together, these findings indicate that the distribution of positive- and negative-charged regions in the hydrophilic areas of both sides and a hydrophobic center unit must be important for the anti-amyloidogenic activity.

Recent evidence suggests that soluble Aβ oligomers are more toxic for neurons than are the monomers or fibrils, and increased levels of these Aβ oligomers were found to kill mature neurons in hippocampal brain regions even at nanomolar concentration.[23] This suggests that the degree of assembly of Aβ into cytotoxic oligomeric aggregates will be a trigger for AD pathophysiology. CFA showed the fibril-destabilizing effects in vitro (Figure 1B). According to the results of dot blot analysis, small amount of oligomers observed at 0 h disappeared after incubation of CFA (Figure 3), suggesting the oligomer-destabilizing effect. Therefore, the inhibitory effect of CFA on Aβ oligomerization is a valuable aspect of anti-amyloid agents.

There have been some reports on the absorption and bioavailability of dietary carotenoids in humans. Asai et al.[24] demonstrated that β-carotene and lutein in spinach were efficiently absorbed and markedly increased their levels in blood plasma during intervention weeks. Generally, the uptake of hydrophobic compounds such as carotenoid by intestinal epithelial cells has been thought to occur by simple diffusion,[25] and it was dependent on the lipophilicity of the molecules. The partition coefficients (log *P* values) of β-carotene and lutein were calculated to be 17.6 and 14.8, respectively, whereas the majority of phytochemicals have log *P* values <7.6,[26] suggesting a relatively high bioavailability of β-carotene and lutein. Indeed, the concentration of β-carotene in the rat brain reached a maximum of 0.11 μg/g after the intake of a diet supplement with 0.2% β-carotene for 70 days.[27] It is well established that lutein can transport across the blood−brain barrier into the central nervous system.[28] Therefore, lutein would be a potentially influential nutrient with regard to brain health and cognitive function in humans.

There is significant evidence that oxidative stress is a critical event in the pathogenesis of AD. In human neuroblastoma SH-SY5Y cells, H_2O_2-induced oxidative stress caused an increase in the levels of intracellular Aβ and a decrease in the protein levels of full-length APP and C-terminal APP fragment without affecting the expression level of APP.[29] Additionally, induction of oxidative stress by treatment with $H_2O_2/FeSO_4$ and 4-hydroxy-2-nonenal (HNE) caused a significant increase of intracellular Aβ levels in human NT2N neurons. It is well-known that carotenoids can act as efficient antioxidants, which can quench free radicals and thus prevent subsequent harmful oxidation.[30] Although the data are not shown here, CFA was shown to possess potent DPPH radical scavenging activity. Thus, the antioxidative effect of CFA could contribute to the prevention of Aβ accumulation induced by oxidative stress.

Aβ is formed through a series of enzymatic cleavages of a much larger protein called APP, initially by either α-secretase or β-secretase.[4] The product of β-secretase cleavage is cleaved further by γ-secretase to produce the $Aβ_{1-40/42}$ proteins. The nonamyloidogenic pathway involves the processing of APP by α-secretase, which cleaves within the Aβ domain of APP, thus precluding the generation of Aβ. Mangelsdorf et al.[31] have revealed that retinoic acid (RA), the active metabolite of vitamin A, regulates the expression of genes related to APP processing through its nuclear receptors such as the RA receptors (RARs) and retinoid X receptors (RXRs). Furthermore, Tippmann et al.[32] demonstrated that not only RA but also acitretin, the aromatic analogue of vitamin A, increases α-secretase activity in the brain by increasing the RAR transcription, which leads to an overall reduction of Aβ. Thus, carotenoids might exhibit anti-amyloidogenic activity, not only by directly inhibiting the formation of Aβ oligomers and fibrils but also by affecting the processing of APP through the nuclear receptors. It would be interesting to examine whether lutein might affect the transcriptional regulation of gene expression as an agonist of either RAR or RXR. Further animal studies will be required to ascertain the mechanisms underlying the anti-amyloidogenic activity of carotenoids in vivo.

In conclusion, the present study demonstrated that lutein in apricot fruits has potent anti-amyloidogenic activity in vitro. The anti-amyloidogenic activity was found to correlate with a hydrophobic region, including conjugated double-bond chains, and the number and portion of hydroxyl groups on each side of the molecule. These findings will contribute to the development of anti-amyloidogenic agents from naturally occurring materials.

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ABBREVIATIONS USED

Aβ, amyloid β protein; AD, Alzheimer's disease; APP, Aβ precursor protein; CAF, carotenoid fraction from apricots; HNE, 4-hydroxy-2-nonenal; MTBE, methyl *tert*-butyl ether; PBS, phosphate-buffered saline; RA, retinoic acid; RAR, RA receptors; RXRs, retinoid X receptors; THF, tetrahydrofuran; ThT, thioflavin T.

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