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Preformed β-amyloid fibrils are destabilized by coenzyme Q_{10} in vitro

Kenjiro Ono [a], Kazuhiro Hasegawa [b], Hironobu Naiki [b], Masahito Yamada [a,*]

[a] Department of Neurology and Neurobiology of Aging, Kanazawa University Graduate School of Medical Science, Kanazawa 920-8640, Japan
[b] Department of Pathology, Fukui University, Fukui 910-1193, CREST of Japan Science and Technology Corporation, Kawaguchi 332-0012, Japan

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Abstract

Inhibition of the formation of β-amyloid fibrils (fAβ), as well as the destabilization of preformed fAβ in the CNS, would be attractive therapeutic targets for the treatment of Alzheimer's disease (AD). We reported previously that nordihydroguaiaretic acid (NDGA) and wine-related polyphenol, myricetin (Myr), inhibit fAβ formation from Aβ and destabilize preformed fAβ in vitro. Using fluorescence spectroscopic analysis with thioflavin T and electron microscopic studies, we examined the effects of coenzyme Q_{10} (CoQ_{10}) on the formation, extension, and destabilization of fAβ at pH 7.5 at 37 °C in vitro. We next compared the anti-amyloidogenic activities of CoQ_{10} with NDGA and Myr. CoQ_{10} dose-dependently inhibited fAβ formation from amyloid β-peptide (Aβ), as well as their extension. Moreover, it destabilized preformed fAβs. The anti-amyloidogenic effects of CoQ_{10} were slightly weaker than those of NDGA and Myr. CoQ_{10} could be a key molecule for the development of therapeutics for AD.

Keywords: Alzheimer's disease; Coenzyme Q_{10}; β-Amyloid fibrils; Thioflavin T; Electron microscopy

Alzheimer's disease (AD) is the most common cause of dementia in aged humans, affecting ~200 million people worldwide [1]. The disease is characterized by extracellular deposition of amyloid β-peptide (Aβ) in senile plaques, intracellular appearance of neurofibrillary tangles, and synaptic and neuronal loss [2]. Considerable effort is currently directed toward the development of anti-amyloid therapeutics as possible strategies to prevent or treat AD. Using a nucleation-dependent polymerization model to explain the mechanism of the formation of Alzheimer's β-amyloid fibrils (fAβ) in vitro [3,4], we previously found that nordihydroguaiaretic acid (NDGA) and rifampicin (RIF) inhibit fAβ formation dose-dependently in vitro [5]. Moreover, we reported that they also destabilize fAβ(1–40) and fAβ(1–42) in a concentration-dependent manner, based on fluorescence spectroscopic analysis with thioflavin T (ThT) and electron microscopic studies [6].

A large number of studies indicate that oxidative injury may play a role in the development of AD [7]. Many antioxidant compounds, such as vitamin E [8], NDGA [9], and nicotine [10], have been demonstrated to protect the brain from in vitro Aβ toxicity. Recently, we showed that the wine-related polyphenols such as myricetin (Myr), and the lipophilic antioxidant, vitamin A, dose-dependently inhibit formation and extension of fAβ(1–40) and fAβ(1–42), as well as destabilizing preformed fAβs in vitro [11,12]. As similar to vitamin A, coenzyme Q_{10} (CoQ_{10}) is well known to be an important lipid-soluble antioxidant [13]. Soderberg et al. [14] found increased levels of CoQ_{10} in most regions of the brains of patients with AD. A recent study by Bustos et al. [15] found no significant difference in plasma CoQ_{10} levels between patients with AD and controls. Although Imagawa et al. [16] reported a marked improvement in mental state of two sisters with genetically confirmed AD after a combined therapy with CoQ_{10}, iron, and vitamin B6, no large clinical studies assessing the cognitive effect of oral supplementation of CoQ_{10} in AD have

* Corresponding author. Fax: +81 76 234 4253.
E-mail address: m-yamada@med.kanazawa-u.ac.jp (M. Yamada).

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been performed. Here, we examined the effects of CoQ_{10} to inhibit the formation, extension of fAβ(1–40) and fAβ(1–42), as well as to destabilize fAβs at pH 7.5 at 37 °C in vitro, using fluorescence spectroscopy with ThT and electron microscopy. We also compared its anti-amyloidogenic and fibril-destabilizing effects with those of NDGA and Myr.

Materials and methods

Preparation of Aβ and fAβ solutions. Aβ(1–40) (a trifluoroacetate salt, Peptide Institute, Osaka, Japan) and Aβ(1–42) (a trifluoroacetate salt, Peptide Institute) were dissolved by brief vortexing in a 0.02% ammonia solution at a concentration of 500 μM (2.2 mg/mL) and 250 μM, respectively, in a 4 °C room and stored at −80 °C before assaying (fresh Aβ(1–40) and Aβ(1–42) solutions). fAβ(1–40) and fAβ(1–42) were formed from the fresh Aβ(1–40) and Aβ(1–42) solutions, respectively, sonicated, and stored at 4 °C as described elsewhere [17].

Fresh, non-aggregated fAβ(1–40) and fAβ(1–42) were obtained by extending sonicated fAβ(1–40) or fAβ(1–42) with fresh Aβ(1–40) or Aβ(1–42) solutions, respectively, just before the destabilization reaction [6,11,12,18]. The reaction mixture was 600 μL and contained 10 μg/mL fAβ(1–40) or fAβ(1–42), 50 μM Aβ(1–40) or Aβ(1–42), 50 mM phosphate buffer, pH 7.5, and 100 mM NaCl. Measurement of the fluorescence of ThT showed that the extension reaction proceeded to equilibrium after incubation at 37 °C for 3–6 h under non-agitated conditions. In the following experiment, the concentration of fAβ(1–40) and fAβ(1–42) in the final reaction mixture was regarded as 50 μM.

Fluorescence spectroscopy, electron microscopy, and polarized light microscopy. A fluorescence spectroscopic study was performed on a Hitachi F-2500 fluorescence spectrophotometer as described elsewhere [19]. Optimum fluorescence measurements of fAβ(1–40) and fAβ(1–42) were obtained at the excitation and emission wavelengths of 445 and 490 nm, respectively, with the reaction mixture containing 5 μM ThT (Wako Pure Chemical Industries, Osaka, Japan) and 50 mM glycine–NaOH buffer, pH 8.5. Electron microscopic and polarized light microscopic studies of the reaction mixtures were performed as described elsewhere [17].

Polymerization assay. Polymerization of Aβ with or without fAβ added as seeds was assayed as described elsewhere [5]. Briefly, the reaction mixture contained 50 μM Aβ(1–40), or 25 or 50 μM Aβ(1–42), 0 or 10 μg/mL fAβ(1–40) or fAβ(1–42), 0–50 μM CoQ_{10}, NDGA, or Myr (Sigma Chemical, St. Louis, MO), 1% dimethyl sulfoxide (DMSO), 50 mM phosphate buffer, pH 7.5, and 100 mM NaCl.

Thirty-microliter aliquots of the mixture were put into oil-free PCR tubes (Takara Shuzo, Otsu, Japan). These tubes were then put into a DNA thermal cycler (PJ480, Perkin-Elmer Cetus, Emeryville, California). Starting at 4 °C, the plate temperature was elevated at maximal speed, to 37 °C. Incubation times ranged between 0 and 8 days as indicated in each figure. The tubes were not agitated during the reaction. Five microliter aliquots from each tube in triplicate were subjected to fluorescence spectroscopy and the mean of the three measurements was determined. In the ThT solution, the concentration of CoQ_{10}, NDGA, and Myr examined in this study was diluted up to

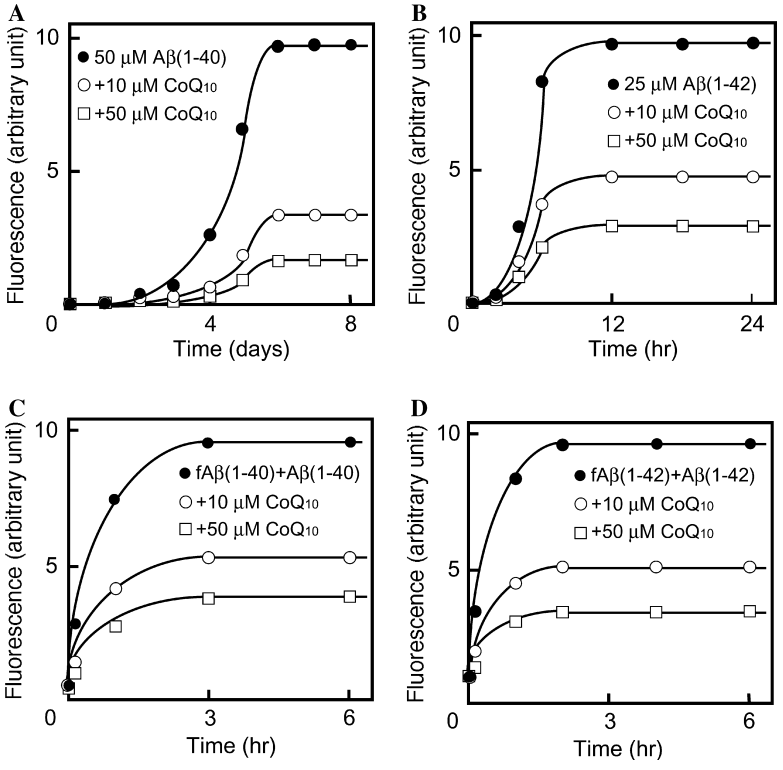

Fig. 1. CoQ_{10} inhibits the formation of fAβ. Effects of CoQ_{10} on the kinetics of formation of fAβ(1–40) (A) and fAβ(1–42) (B) from fresh Aβ(1–40) and Aβ(1–42), respectively. The reaction mixtures containing 50 μM Aβ(1–40) (A) or 25 μM Aβ(1–42) (B), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0 (●), 10 (○), or 50 μM (□) of CoQ_{10} were incubated at 37 °C for the indicated times. Each figure shows a representative pattern of three independent experiments. (C,D) Effects of CoQ_{10} on the kinetics of fAβ(1–40) and fAβ(1–42) extension. The reaction mixtures containing 10 μg/mL sonicated fAβ(1–40) (C) or fAβ(1–42) (D), 50 μM Aβ(1–40) (C) or Aβ(1–42) (D), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0 (●), 10 (○), or 50 μM (□) of CoQ_{10} were incubated at 37 °C for the indicated times. Each figure shows a representative pattern of three independent experiments.

1/200 of that in the reaction mixture. We confirmed that these compounds did not quench ThT fluorescence at the diluted concentration (data not shown).

Measurement of fibril-destabilizing activity. Destabilization of fAβ was assayed as described elsewhere [6]. Briefly, the reaction mixture contained 25 μM fresh fAβ(1–40) or fAβ(1–42), 0–50 μM CoQ_{10}, NDGA, or Myr, 1% DMSO, 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 1%(wt/vol.) polyvinyl alcohol (Wako Pure Chemical Industries) to avoid the aggregation of fAβ and the adsorption of fAβ onto the inner wall of the reaction tube during the reaction.

After being mixed by pipetting, triplicate 5-μL aliquots of the reaction mixture were subjected to fluorescence spectroscopy and 30-μL aliquots were put into PCR tubes. The reaction tubes were then transferred into a DNA thermal cycler. Starting at 4 °C, the plate temperature was elevated at maximal speed to 37 °C. Incubation times ranged between 0 and 6 h as indicated in each figure. The reaction tubes were not agitated during the reaction. Five microliter aliquots from each tube in triplicate were subjected to fluorescence spectroscopy and the mean of the three measurements was determined. At the diluted concentration, CoQ_{10}, NDGA, and Myr did not compete with ThT for fAβ at either 4 °C or 37 °C for 1 min (data not shown).

Other analytical procedures. Protein concentrations of the supernatants of the reaction mixtures after centrifugation were determined by the method of Bradford [20] with a protein assay kit (Bio-Rad Laboratories, Hercules, CA). The Aβ(1–40) solution quantified by amino acid analysis was used as the standard. The statistical significance of the data was analyzed by the linear least squares fit. The effective concentration EC_{50} was defined as the concentration of CoQ_{10}, NDGA, or Myr to inhibit the formation or extension of fAβs to 50% of the control value, or the concentration to destabilize fAβs to 50% of the control value. EC_{50} was calculated by the sigmoidal curve fitting of the data, using Igor Pro ver.4 (WaveMetrics, Lake Oswego, OR, USA).

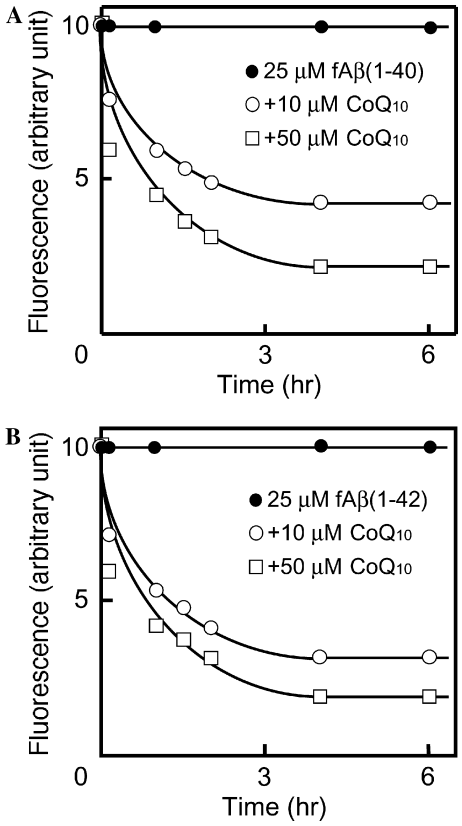

Fig. 2. Effects of CoQ_{10} on the kinetics of destabilization of fAβ(1–40) (A) and fAβ(1–42) (B). The reaction mixtures containing 25 μM fAβ(1–40) (A) or fAβ(1–42) (B), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0 (●), 10 (○), or 50 μM (□) of CoQ_{10} were incubated at 37 °C for the indicated times. Each figure shows a representative pattern of three independent experiments.

Results and discussion

As shown in Figs. 1A and B, when fresh Aβ(1–40) or Aβ(1–42) was incubated at 37 °C, the fluorescence of ThT followed a characteristic sigmoidal curve. This curve is consistent with a nucleation-dependent polymerization model [3]. When Aβ(1–40) was incubated with 10 and 50 μM CoQ_{10}, the final equilibrium level decreased dose-dependently (Fig. 1A). A similar effect of CoQ_{10} was observed with Aβ(1–42) (Fig. 1B). As shown in Figs. 1C and D, when fresh Aβ(1–40) or Aβ(1–42) was incubated with fAβ(1–40) or fAβ(1–42), respectively, at 37 °C, the fluorescence increased hyperbolically without a lag phase and proceeded to equilibrium much more rapidly than without seeds (compare Figs. 1A and B with C and D). This curve is consistent with a first-order kinetic model [19]. When Aβ(1–40) and fAβ(1–40) were incubated with CoQ_{10}, the final equilibrium level decreased (Fig. 1C). A similar effect of CoQ_{10} was observed for the extension of fAβ(1–42) (Fig. 1D). We observed that 50 μM CoQ_{10} inhibited the extension of sonicated fAβ(1–40) and fAβ(1–42) electron-microscopically (data not shown).

As shown in Figs. 2A and B, the fluorescence of ThT was almost unchanged during the incubation of fresh fAβ(1–40) or fAβ(1–42) at 37 °C without additional molecules. On the other hand, the ThT fluorescence decreased immediately after addition of CoQ_{10} to the reaction mixture. After incubation of 25 μM fresh fAβ(1–40) with 50 μM CoQ_{10} for 1 h, many short, sheared fibrils were observed (Fig. 3B). At 6 h, the number of fibrils was reduced markedly, and small amorphous aggregates were occasionally observed (Fig. 3C). Similar morphology was observed when 25 μM fresh fAβ(1–42) was incubated with 50 μM CoQ_{10} (data not shown).

After incubation with 50 μM CoQ_{10} for 6 h, fAβ (1–40) and fAβ(1–42) were stained with Congo red much more weakly than fresh fAβ(1–40) and fAβ(1–42). However, they all showed orange-green birefringence under polarized light. This means that a significant amount of intact fAβ(1–40) and fAβ(1–42) still remains in the mixture after the reaction. When the protein concentration of the supernatant after centrifugation at 4 °C for 2 h at $1.6 \times 10^4 g$ was measured by the Bradford assay, no proteins were detected in the supernatant. This implies that although these agents could destabilize fAβ(1–40) and fAβ(1–42) to visible aggregates (Fig.

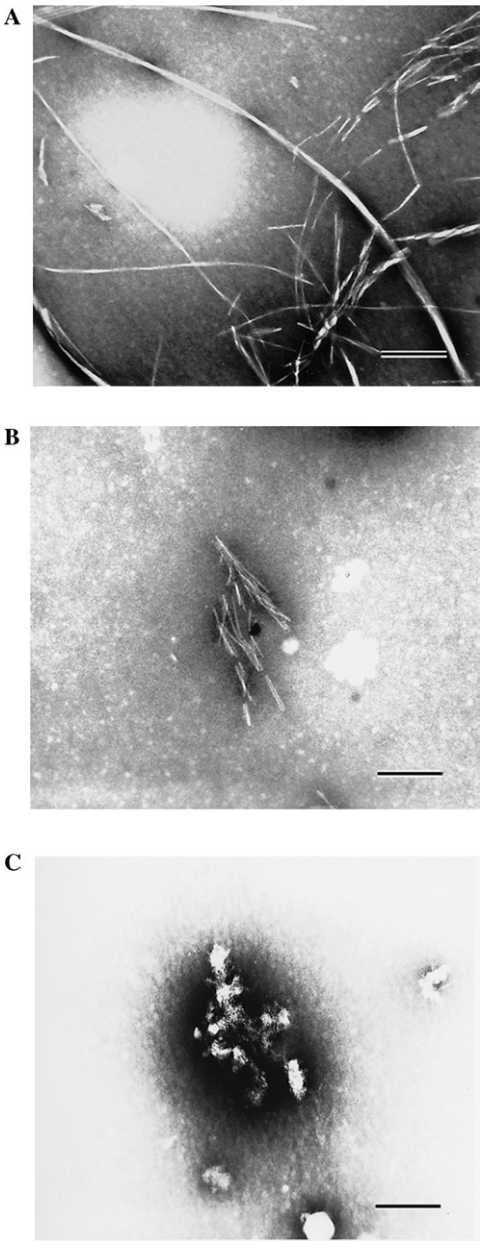

Fig. 3. Electron micrographs of destabilized fAβ(1–40). The reaction mixture containing 25 μM fAβ(1–40), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 50 μM CoQ_{10} was incubated at 37 °C for 0 h (A), 1 h (B) or 6 h (C). Scale bars indicate a length of 250 nm.

3C), they could not depolymerize fAβ(1–40) and fAβ(1–42) to monomers or oligomers of Aβ(1–40) and Aβ(1–42). When fresh 50 μM Aβ(1–40) or Aβ(1–42) was incubated with the pellet at 10 μg/mL, no increase in the fluorescence was observed for 6 h. This implies that destabilized fAβ(1–40) and fAβ(1–42) could not function as seeds.

We calculated EC_{50}, the concentrations of NDGA, Myr, or CoQ_{10} to inhibit the formation or extension of fAβs to 50% of the control value, or the concentrations to destabilize fAβs to 50% of the control value, by the sigmoidal curve fitting of the data as shown (Table 1). The overall activity of the molecules examined was in the order of: NDGA = Myr > CoQ_{10}.

In this study, we showed that CoQ_{10} dose-dependently inhibited fAβ formation from fresh Aβ, as well as destabilizing preformed fAβ in vitro. The anti-amyloidogenic and fibril-destabilizing effects of CoQ_{10} were slightly weaker than those of NDGA and Myr. Our previous systematic in vitro study indicated that the overall activity of the anti-amyloidogenic molecules may be in the order of: NDGA = wine-related polyphenols (Myr, morin, and quercetin) ≫ RIF = tetracyclines (TC) > poly(vinylsulfonic acid, sodium salt) = 1,3-propanedisulfonic acid, disodium salt > β-sheet breaker peptides (iAβ5) > nicotine [6,11,18]. Tomiyama et al. [21] suggested that RIF binds to Aβ by hydrophobic interactions between its lipophilic ansa chain and the hydrophobic region of Aβ, thus blocking the association between Aβ molecules that lead to fAβ formation. The anti-amyloidogenic activity of NDGA, TCs, small-molecule anionic sulfonates or sulfates, melatonin, iAβ5, and nicotine may also be related to the propensity to bind to the specific sites of Aβ [6,22–26]. Recently, we also showed that lipophilic vitamin, vitamin A, dose-dependently inhibits formation and extension of fAβ(1–40) and fAβ(1–42), as well as destabilizing preformed fAβs in vitro [12]. The overall activity of vitamin A and β-carotene was in the order of retinol = retinal > β-carotene > retinoic acid [12]. We speculated that the decrease in lipophilicity might reduce the binding affinity of retinoic acid to Aβ and/or fAβ to exhibit anti-amyloidogenic and fibril-destabilizing effects in vitro

Table 1
The effective concentrations (EC_{50}) of NDGA, Myr, and CoQ_{10} for the formation, extension, and destabilization of fAβ(1–40) and fAβ(1–42)

Compounds	Formation[a]		Extension[b]		Destabilization[c]	
	fAβ(1–40)	fAβ(1–42)	fAβ(1–40)	fAβ(1–42)	fAβ(1–40)	fAβ(1–42)
NDGA	0.21 μM	1.1 μM	0.22 μM	0.11 μM	0.99 μM	0.93 μM
Myr	0.37	0.51	0.17	0.16	1.8	0.95
CoQ_{10}	1.8	5.5	13	9.3	5.3	4.9

[a] The reaction mixtures containing 50 μM Aβ(1–40) or 25 μM Aβ(1–42), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0, 0.01, 0.1, 1, 10, and 50 μM NDGA, Myr, or CoQ_{10} were incubated at 37 °C for 7 days and 24 h, respectively.

[b] The reaction mixtures containing 10 μg/mL (2.3 μM) sonicated fAβ(1–40) or fAβ(1–42), 50 μM Aβ(1–40) or Aβ(1–42), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0, 0.01, 0.1, 1, 10, and 50 μM NDGA, Myr, or CoQ_{10} were incubated at 37 °C for 1 h.

[c] The reaction mixtures containing 25 μM fAβ(1–40) or fAβ(1–42), 50 mM phosphate buffer, pH 7.5, 100 mM NaCl, and 0, 0.01, 0.1, 1, 10, and 50 μM NDGA, Myr, or CoQ_{10} were incubated at 37 °C for 6 h.

[13]. Interestingly, CoQ_{10}, NDGA, wine-related polyphenols, RIF, melatonin, nicotine, and vitamin A have all been reported to have antioxidant activity [9,13,21,24,27,28]. CoQ_{10} with potent lipophilic and antioxidant motifs could bind specifically to Aβ and/or fAβ, inhibit fAβ formation, and/or destabilize preformed fAβ. Moreover, CoQ_{10} may prevent the development of AD, not only through scavenging reactive oxygen species, but also through directly inhibiting the deposition of fAβ in the brain. Although the exact mechanism of anti-amyloidogenic activity of CoQ_{10} is unclear, these compounds could be key molecules for the development of therapeutics for AD. Further studies, such as nuclear magnetic resonance experiments, are essential to reveal the exact structure–activity relationships for the antioxidants and other organic compounds which exhibit anti-amyloidogenic and fibril-destabilizing effects.

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