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Strikingly different effects of cholesterol and 7-ketocholesterol on lipid bilayer-mediated aggregation of amyloid beta (1-42)

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ABSTRACT

Oxidized cholesterol has been widely reported to contribute to the pathogenesis of Alzheimer's disease (AD). However, the mechanism by which they affect the disease is not fully understood. Herein, we aimed to investigate the effect of 7-ketocholesterol (7keto) on membrane-mediated aggregation of amyloid beta (Aβ-42), one of the critical pathogenic events in AD. We have shown that when cholesterol is present in lipid vesicles, kinetics of Aβ nuclei formation is moderately hindered while that of fibril growth was considerably accelerated. The partial substitution of cholesterol with 7keto slightly enhanced the formation of Aβ-42 nuclei and remarkably decreased fibril elongation, thus maintaining the peptide in protofibrillar aggregates, which are reportedly the most toxic species. These findings add in understanding of how cholesterol and its oxidation can affect Aβ-induced cytotoxicity.

1. Introduction

The aggregation of soluble monomeric amyloid beta (Aβ) peptide into fibrils is one of principal pathogenic events in the pathogenesis of Alzheimer's disease (AD), the most common neurodegenerative illness of late life [1]. According to the prevalent model, Aβ aggregation is a nucleation-dependent polymerization process, including two main steps: (i) nucleus formation and (ii) fibril elongation [2]. In the first step, soluble monomeric Aβ, which is generated from the amyloidogenic cleavage of a transmembrane amyloid precursor protein, undergoes a misfolding from random coil to β-sheet structure [3] and/or adopts a conformational switching from mainly α-helical conformation to β-sheet-enriched conformation under various conditions [4]. The β-sheet conformation is prone to self-aggregation, producing dimers, trimmers, and eventually nuclei (n-mers) [2]. In the next step, the formed Aβ nuclei trigger the formation of intermediate oligomers or protofibrils [5], and finally insoluble fibrils. The fibrils, together with other biomolecules, form extracellular neuritic plaques that are recognized as hallmarks of AD [1]. Cell membranes have been reported to serve as an aggregation matrix for Aβ seeding and for facilitating fibrillar Aβ formation [6]. Accumulating evidence shows that Aβ toxicity significantly depends on the aggregated state. Intermediate species including oligomers and protofibrils are reportedly more toxic than soluble monomers and mature fibrils [7,8]. Therefore, controlling the Aβ aggregation and the formation of neurotoxic species has become one of the emerging therapeutic strategies in the treatment of AD [9,10].

It has been reported that the oxidation of cholesterol, a prominent structural component and property modulator of membranes [11], accelerates the development of AD [12]. Cholesterol is susceptible to cellular oxidation induced by enzymes or reactive oxygen species (ROS), generating various oxidized derivatives including 24(S)-hydroxycholesterol (24(S)OH), 25-hydroxycholesterol (25OH) 7-ketochoelsterol (7keto), and 7α/β- hydroxycholesterol (7α/βOH). Possessing one or more supplementary oxygen groups such as hydroxyl, carbonyl, and epoxide, these compounds are more hydrophilic than cholesterol. They also differ from cholesterol in three-dimensional shape as well as orientation in membranes [13]. Increasing evidence is now pointing that oxidized cholesterols play important role in facilitating Aβ generation and accumulation [14,15], Aβ/membrane interaction [16–18], and neuron death [19]. However, there is little evidence to date on their effects on membrane-mediated aggregation of Aβ. In this study, we aimed to investigate the impact of 7-ketocholesterol (7keto)-containing model membranes on Aβ − 42 aggregation. 7-keto is a major product of reactive oxygen species (ROS)-caused oxidation of cholesterol [13]. The presence of this sterol in membrane changes membrane physical properties such as fluidity [20,21], thus altering the interaction of

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membrane with proteins [16]. We have recently reported the high ability of 7keto to facilitate Aβ insertion into lipid bilayers [17,18]. Since membrane-mediated aggregation of Aβ-42 is dependent on membrane lipid composition including cholesterol [22–24], we hypothesized that the partial substitution of cholesterol with this oxysterol in membrane is able to affect Aβ aggregation, thereby leading to the formation of neurotoxic species and triggering Aβ toxicity. Unravelling how 7keto influences Aβ aggregation on lipid bilayer is important to understand the impact of cholesterol oxidation in Aβ-induced toxicity.

2. Materials and methods

2.1. Materials

1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and cholesterol were purchased from Avanti Polar Lipids (USA). 7-ketocholesterol (7keto) was from Sigma-Aldrich (USA). Amyloid beta protein (Human, 1–42) (Aβ-42) and Hilyte Fluor™ 488-labelled (λex = 503 nm, λex = 528 nm) Aβ-42 were obtained from Peptide Institute Inc. (Japan) and Anaspec, Inc. (USA), respectively. Thioflavin T (ThT), chloroform, Tris(hydroxymethyl)aminomethane (Tris), and methanol were purchased from Tokyo Chemical industry co., Takara Bio Inc., Kanto-Chemical, and Nacalai Tesque (Japan), respectively. Deionized water was obtained using an ultraviolet water purification system (Millipore S.A.S, France).

2.2. Lipid vesicle preparation

DOPC (DOPC only), Chol (DOPC/cholesterol = 50/50 M ratio), and 7keto (DOPC/cholesterol/7keto = 50/40/10 M ratio) lipid vesicles were prepared following natural swelling method [25]. Lipid mixture was dissolved in chloroform/methanol (2/1, v/v) in a glass tube at the final concentration of 0.2 mM. The solvent was subsequently removed by evaporating the tube under a gentle nitrogen stream and drying in a desiccator for 3 h, resulting in a thin lipid film at the bottom of tube. The film was swollen with Tris buffer (20 mM, pH = 7.4) overnight at 37 °C to form lipid vesicles. A phase-contrast microscopy (Olympus BX50, Japan) was employed to estimate the vesicle formation.

2.3. Aβ-42 incubation

Aβ-42 was incubated following the method as reported previously [26]. First, 200 μM Aβ-42 solutions were prepared by dissolving Aβ-42 powder in 0.02% ammonia and stored at − 80 °C. The peptide solution was then diluted and incubated in the absence or presence of lipid vesicles (lipid vesicles/peptide = 5/4, v/v) in Tris buffer (20 mM, pH 7.4) at 80 μM concentration in various incubation periods (0 h, 6 h, 12 h, 24 h, 36 h, and 48 h). This concentration has been shown to provide quantitative data as well as characterize molecular events [27]. Since aggregation kinetics of the peptide is known to be influenced by various factors including concentration [28], we have maintained the parameters in subsequent experiments. This enables us to compare our findings, and also build up new understandings.

2.4. Measurement of Aβ-42 aggregation

The aggregation of Aβ-42 was assessed by ThT fluorescence assay [26]. The peptide incubated in different conditions was diluted in 20 mM Tris buffer at 20 μM concentration and subsequently added into 5 μM ThT solution contained in a transparent cell. The cell was immediately placed in FP-6500 spectrofluophotometer (Jasco, Japan) to detect ThT fluorescence after an excitation at 450 nm and an emission at 483 nm.

2.5. Kinetic analysis of Aβ-42 aggregation

The kinetics of Aβ-42-42 aggregation was analysed using the autocatalytic reaction model reported by Sabaté et al. [2]. ThT fluorescence intensity data was fitted to this model using equation $f = \rho\{\exp[(1+\rho)kt] - 1\}/\{1 + \rho\exp[(1+\rho)kt]\}$ where f is the fraction of fibrillar form; $k = k_e a, k_e$ is elongation rate constant, a is the initial concentration of Aβ-42 in the solution; $\rho = k_n/k$, k_n is nucleation rate constant.

2.6. Characterization of Aβ-42 aggregate morphology

Atomic force microscopy (AFM) was used to image and characterize the morphology of Aβ − 42 aggregates derived from the incubation of Aβ-42 alone or with lipid vesicles. In order to prepare AFM samples, 5 μM of Aβ-42 solution was uniformly spread and immobilized in a mica plate (Furuuchi Chemical Co., Shinagawa, Tokyo, Japan). Then, the mica was washed three times with 50 μl of deionized water and was dried under the vacuum condition. The sample was measured by AFM (SPA400-SPI 3800, Seiko Instruments Inc., Japan) equipped with a calibrated 20 μm xy-scan, 10 μm z-scan range PZT-scanner and a scanning silicon nitride tip (SI-DF3, spring constant = 1.6 N/m, frequency resonance = 28 kHz, Seiko Instruments Inc.) in a dynamic force mode (DFM). All AFM operations were performed in an automated moisture control box with 30–40% humidity at room temperature. The length and height of Aβ-42 aggregates were analysed using Image J and SPI software, respectively [18].

3. Results

3.1. Effect of cholesterol- and 7keto-containing model membranes on the kinetics of Aβ-42 aggregation

First, Thioflavin T (ThT) assay was employed to investigate the effect of cholesterol-containing and 7keto-containing lipid vesicles on Aβ-42 aggregation kinetics in comparison with Aβ-42 aggregation in buffer solution. This assay is a common analytical method for detecting the degree of amyloid fibrillation. Its principle is based on the ability of ThT to show the enhanced fluorescence emission at 483 nm wavelength upon the binding to the β-sheet of Aβ-42 peptide, while that of free ThT is observed at 445 nm [26]. We correlated the fluorescence intensity at 483 nm with the extent of fibrils in solution, as a function of time. Fig. 1 shows time course curves of fibrillar Aβ-42 formation from monomers in the absence and presence of three lipid vesicle systems. The peptide in the absence of lipid vesicles (also in buffer) exhibited a typical

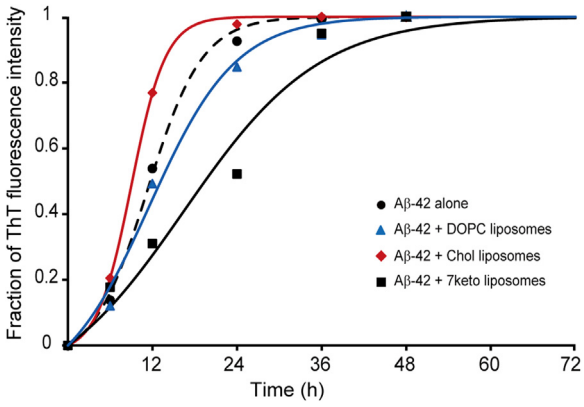

Fig. 1. Time course curves of Aβ-42 aggregation in the absence (black, dash) and presence of DOPC vesicles (blue), DOPC/Chol (50/50) vesicles (red), and DOPC/Chol/7keto (50/40/10) vesicles (black, solid). Each point is the average value of three independent experimental measurements. Lines are obtained from fitting the experimental data to the equation of the autocatalytic reaction model [3]. Aβ-42 was incubated at 80 μM in Tris buffer (20 mM, pH 7.4).