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Localization of amyloid beta (Aβ1-42) protofibrils in membrane lateral compartments: Effect of cholesterol and 7-Ketocholesterol

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ABSTRACT

Cholesterol plays an important role in the interaction of Alzheimer's amyloid beta (Aβ) with cell membranes, an important event in Aβ-induced cytotoxicity. However, it is not fully understood how cholesterol influences the association of Aβ with membrane lateral compartments. We have shown that by modulating membrane fluidity, cholesterol decreased peptide localization in solid-ordered domains and increased that in liquid-ordered domains. It changed the amount of Aβ associating with liquid-disordered (Ld) phase with different tendencies depending on the composition of heterogeneous membrane systems. 7-Ketocholesterol, an oxidized derivative of cholesterol, majorly enhanced the fluidity of and Aβ interaction with Ld phase. These findings are useful for clarifying the impact of cholesterol and its oxidation in Aβ-induced toxicity.

1. Introduction

The aggregation and cytotoxicity of amyloid beta (Aβ), a key factor in Alzheimer's disease (AD), are mediated by the interaction of the peptide with cell membranes [1,2]. Accumulating evidences have indicated that Aβ is able to specifically bind to some membrane components such as monosialotetrahexosylganglioside (GM1) [3,4] or directly insert into membrane's lipid bilayer [5,6]. This interaction triggers a cascade of harmful processes including formation of ion-permeable amyloid pores [7], interference of receptor activities [8], and oxidation of membrane lipids [9]. Therefore, a clear understanding of the condition and mechanism of Aβ/membrane interaction is important to unravel Aβ-induced cytotoxicity during AD's pathogenesis.

Cholesterol, an essential structural constituent and property modulator of cell membranes [10], has been widely implicated in Aβ/membrane interaction [11,12]. However, it is not fully understood how cholesterol affects the interaction of Aβ with membrane lateral compartments. An important membrane micro-domain, the lipid raft, is enriched in sphingolipids and cholesterol [13]. Recent studies have indicated that cholesterol specifically binds to Aβ [14] and accelerates the specific interaction between sphingolipid GM1 with the peptide [15–17], thus promoting the recruitment of Aβ in lipid rafts. In addition, some authors have suggested that the peptide can selectively adsorb in or insert into lipid bilayer of membrane lateral compartments depending on its aggregated states and the structure of these compartments [18,19]. Since cholesterol remarkably mediates the structure and properties of membrane domains, the sterol can influence the association of Aβ with the lipid bilayer of these domains.

This study aims to investigate the effect of cholesterol and its oxidized derivative, 7-Ketocholesterol (7keto), on the association of protofibrillar Aβ-42, the reportedly highly neurotoxic amyloid species [20,21], with heterogeneous membranes' phases. 7keto is the major product of cholesterol oxidation induced by reactive oxygen species [22] and is shown to significantly affect membrane properties and interaction with Aβ [23–25]. We employed a heterogeneous cell-sized liposome system, composed of cholesterol, equal concentrations of unsaturated phospholipid and saturated phospholipid. This ternary model membrane tends to form a liquid-disordered (Ld) phase coexisting with solid-ordered (So) phase at low cholesterol fraction (0–10%) or liquid-ordered (Lo)

Abbreviations: 7keto, 7-Ketocholesterol; Aβ, amyloid beta; AD, Alzheimer's disease; Fig, Figure; GM1, monosialotetrahexosylganglioside; GP, generalized polarization; Ld, liquid-disordered; Lo, liquid-ordered; So, solid-ordered

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phase at higher cholesterol levels (10–45%) [26]. Ld and So are considered as liquid–crystalline and gel states of biological membranes, respectively, whist Lo is lipid raft-like domains [27].

2. Materials and methods

2.1. Materials

1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and cholesterol (Chol) were purchased from Avanti Polar Lipids (USA). Water-soluble cholesterol, 7-ketocholesterol (7keto), methyl-β-cyclodexin (MβCD), and bovine serum albumin (BSA) were obtained 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 from Peptide Institute Inc. (Japan) and Anaspec, Inc. (USA), respectively. Roswell Park Memorial Institute 1640 (RPMI1640) medium, fetal bovine serum (FBS), Rhodamine b 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine triethylammonium salt (Rho-DHPE) (λ_{ex} = 560 nm, λ_{em} = 580 nm), Alexa Fluor® 555 conjugated cholera toxin subunit B (CT-B) (λ_{ex} = 555 nm, λ_{em} = 565 nm), 6-dodecanoyl-2-dimethylaminonaphthalene (Laurdan) (λ_{ex} = 363 - nm, λ_{em} = 497 nm) were from Invitrogen (USA). Jurkat human leukemic T cell line (T cell) was from Riken cell bank (Japan). All other reagents were of analytical grade.

2.2. Preparation of cell-sized heterogeneous liposomes

Cell-sized heterogeneous liposomes were prepared following natural swelling method [28]. So/Ld liposomes were prepared with two cholesterol concentrations, Chol 0% (DOPC/DPPC/Cholesterol = 50/50/0 M ratio) and Chol 10% (DOPC/DPPC/Cholesterol = 45/45/10 M ratio). Similarly, two Lo/Ld liposome systems were prepared, including Chol 20% (DOPC/DPPC/Cholesterol = 40/40/20 M ratio) and Chol 30% (DOPC/DPPC/Cholesterol = 35/35/30 M ratio). Higher cholesterol levels were not used because of difficulty in preparation of stable Lo/Ld cell-sized vesicles. In order to study the effect of 7keto, we used Chol 30% Lo/Ld liposomes because this cholesterol concentration is closer to that in cell membranes [10]. Cholesterol was replaced with 7keto at Chol/7keto (75/25) ratio. To visualize membrane lateral compartments, 0.05% (mol/mol) Rho-DHPE, a specific probe of DOPC, was added into lipid mixtures. The final lipid concentration was 0.1 mM.

2.3. Cell culture and visualization of lipid rafts

T cells were cultured in RPMI1640 medium supplemented with 10% (v/v) FBS. They were maintained in a humidified atmosphere with 5% (v/v) CO_2 at 37 °C. Lipid rafts were labelled by treating cells with 15 μg/ml CT-B [29] and 0.02% (v/v) BSA solution at 0 °C for 30 min, followed by an incubation at 37 °C for 10 min.

2.4. Preparation of protofibrillar Aβ

We prepared 200 μM Aβ-42 solutions by dissolving Aβ-42 powder in 0.02% (v/v) ammonia. Fluorescence-labelled Aβ and Aβ was mixed and diluted in Tris buffer (20 mM, pH 7.4) at 80 μM concentration, subsequently incubated at 37 °C for 12 h [18,25]. Aβ-42 conformation was measured by atomic force microscopic measurement (SPA400-SPI 3800, Seiko Instruments Inc., Japan), analysed using SpiceI32 and Image J. Fig. S2 shows that assembled species were largely protofibrillar with an average height of 3.18 nm and lengths in the range from 50 to 250 nm [30].

2.5. Modulation of membrane cholesterol and 7keto level

Membrane cholesterol of T cells was depleted by treating cells with 2 mM MβCD in non-serum RPMI1640 medium for 10 min at 37 °C [31]. In order to increase cholesterol content, cells were treated with 1 mM soluble cholesterol for 10 min at 37 °C. Membrane cholesterol level was measured using cholesterol cell-base detection assay kit (Cayman, USA) and presented in Fig. S3. 7keto was added to cell membranes by treating cells with 7keto solution at two concentrations (5 μM and 10 μM) for 10 min at 37 °C.

2.6. Observation of Aβ localization in model heterogeneous membranes and T cells

Fluorescence-labelled Aβ was added to liposome or T cell suspension at 5 μM final concentration. The resultant mixture was poured into a silicon well (0.1 mm) placed on a glass slide and used for confocal microscopy observation (Olympus FV-1000, Japan) at room temperature (~21.5 °C) within 2 min. This observation period is short enough to avoid effect of fluorescent quenching [25]. Membrane lateral compartments and Aβ localization were visualized after excitation of Rho-DHPE or CT-B and fluorescence-labelled Aβ, respectively.

2.7. Measurement of membrane fluidity

Fluidity of membrane lateral compartments was measured using excitation generalized polarization (GP) of Laurdan [32]. Liposomes were labeled with 0.2% (mol/mol) Laurdan. Fluorescent emission of Laurdan was detected at 421 and 519 nm using confocal scanning microscopy. Laurdan GP value was calculated following the formula $GP = (I_{421} + I_{519})/(I_{421} + I_{519})$, in which I_{421} and I_{519} are average fluorescent intensities of Laurdan detected at 421 and 519 nm, respectively [32].

3. Results and discussion

3.1. Cholesterol-modulated localization of Aβ in membrane lateral compartments

In So/Ld heterogeneous membranes without cholesterol, Aβ protofibrils localized in both So and Ld phase (Fig. 1A). The selective localization of Aβ in So domain as well as its random distribution in both So and Ld phases has been reported [18,19]. The presence of cholesterol in the vesicles decreased the amount of Aβ protofibrils partitioning in So domains, while that in Ld phase was significantly increased (Fig. 1A). This implies that the sterol is able to inhibit the interaction of the peptide with So and facilitate its association with Ld domains.

Fig. 1B shows that in Lo/Ld membranes containing low concentration of cholesterol (20%, mol/mol), Aβ localized mainly in Ld phase, in agreement with Morita et al. [18]. At a higher cholesterol level (30%, mol/mol), Aβ association in Ld phase was reduced. The peptide was able to associate with Lo domains although its amount was lower than that in Ld (Fig. 1B). This result in combination with So/Ld membrane study shows that cholesterol is a modulator of the interaction of protofibrillar Aβ with lipid bilayer of heterogeneous membranes. Briefly, cholesterol enabled the protofibrils to interact with Lo (raft-like) domains at a moderate level. On the other hand, loss of cholesterol strongly enhanced Aβ localization in So which can exist in lipid rafts of cell membranes deficient in the sterol. Cholesterol could increase or decrease Aβ association with Ld phase depending on the composition of heterogeneous membranes.