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Nonfibrous β-structured aggregation of an Aβ model peptide (Ad-2α) on GM1/DPPC mixed monolayer surfaces

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Abstract

Adsorption and aggregation of transformed peptides and proteins onto the cell membrane surface is commonly associated with forms of amyloidosis such as Alzheimer's disease and prion disease. To address dynamic features of these pathological phenomena molecularly, the in situ Ad-2α model peptide deposition on glycolipid-containing monolayers was studied by using a 9 MHz quartz-crystal microbalance (QCM). The Ad-2α peptide has two amphiphilic α-helix segments, each modified with a 1-adamantanecarbonyl group at the N-terminal as a hydrophobic defect. The peptide folds in a 2α-helix structure in the bulk solution. In the presence of mixed monolayers of glycolipids (GM1, asialo-GM1, GM3, or LacCer) and/or dipalmitoyl phosphatidylcholine (DPPC) laminated on the QCM plate, the peptide deposition and the conformational change to β-structure on the monolayers were accelerated. The adsorption kinetics and the amount of Ad-2α were dependent on the sort and contents of the glycolipid in the DPPC matrix. Although the Ad-2α peptide adsorbs onto most of the glycolipid membranes as monolayer coverage, it adsorbed largely onto the GM1/DPPC (30/70 mol%) mixed monolayer with characteristic kinetic behaviors. The accumulation of β-structured nonfibrous aggregations was confirmed by AFM and fluorescence microscopy with Thioflavin T (ThT).

Keywords: Amyloid peptide; Quartz-crystal microbalance; Ganglioside mixed monolayers; Thioflavin T; AFM

1. Introduction

The misfolding of proteins often causes protein aggregation [1]. β-Amyloid peptides, prion proteins, α-synuclein, and other amyloidogenic proteins trigger Alzheimer's disease (AD) [2,3], prion disease, Parkinson's disease, etc. The transformation of peptides from α-helix to β-sheet is a crucial step of protein misfolding [4]. This type of amyloidogenic proteins has an inherent isoform [5,6]. For example, α-rich cellular prion protein (PrP^{C}), transforms to a β-rich disease-specific isomer (PrP^{Sc}) [5] that causes prion disease, and PrP^{C} can be converted to a β-rich structure reversibly [7]. Not only this structural changeability but also the agglutinative aspect of peptides plays a crucial role in amyloidosis incidence.

A β-amyloid (Aβ) peptide is an important constituent of amyloid plaque, which is pathologically associated with AD. While A$\beta_{1\text{-}40}$ (Aβ_{40}) is a component, another derivative from the same precursor protein (amyloid precursor protein; APP) A$\beta_{1\text{-}42}$ (Aβ_{42}) is the major component of amyloid plaque. Two additional hydrophobic amino acid residues (Ile41 and Ala42) of Aβ_{42} at the carbonyl terminus enhance the cohesive property of the peptide compared to Aβ_{40} [8]. In contrast, the replacement of hydrophobic residues reduces the amyloidogenecity of the peptide [9]. Corresponding to this chemical aspect, genetic studies have also shown that Aβ_{42} has strong amyloidogenecity [10]. Although amyloid fibril formation of amyloid Aβ peptide (Aβ) has historically been believed to be related to the onset of AD, the significance of the fibril structure is not clear. For instance, in Parkinson's disease, a kind of amyloidosis, it is mentioned that the fibrous isoform of α-synuclein is inert [11].

Although the amyloidogenecity of the peptide is closely related to its chemical structure as described above, many studies have indicated that the cell surface on which Aβ deposits aggregate also plays as a crucial role [12,13]. Many studies using

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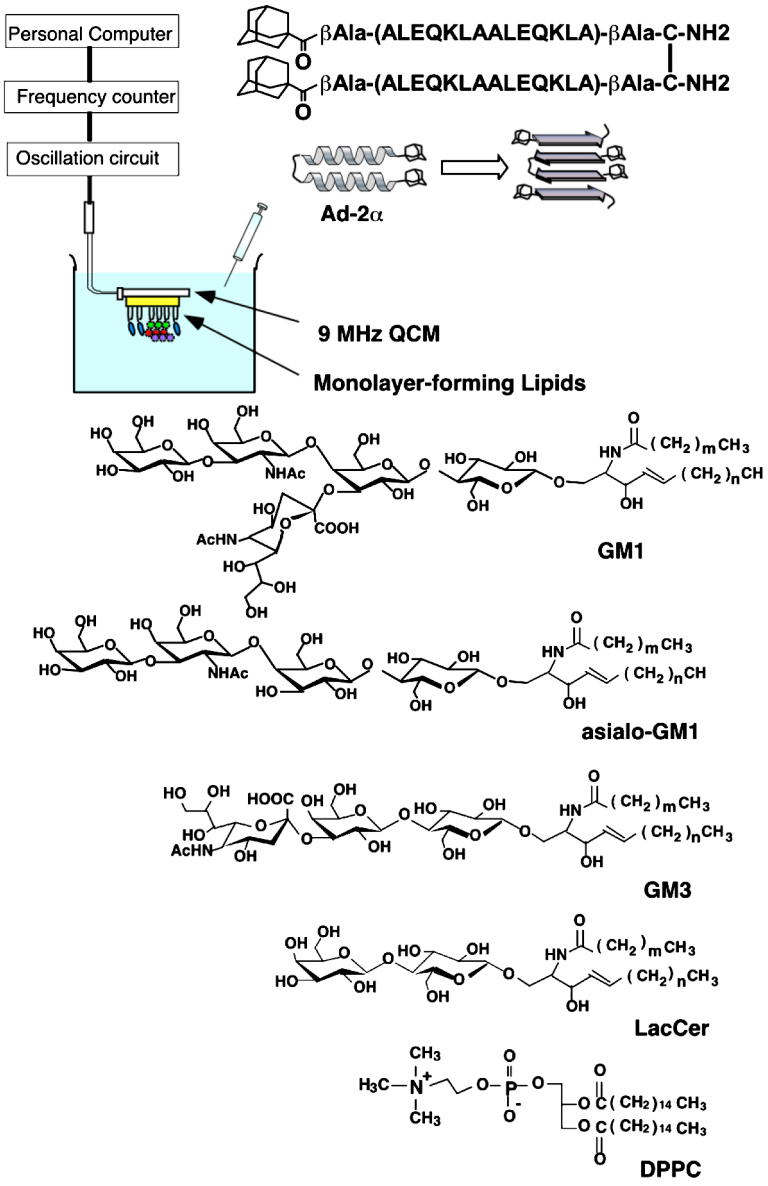

Fig. 1. An experimental setup for QCM measurements and chemical structures of the Ad-2α peptide with a schematic representation of α–β transformation and monolayer-forming glycolipids (GM1, asialo-GM1, GM3, or LacCer) and phospholipids (DPPC).

model cell membrane systems show that GM1 ganglioside-containing membranes particularly enhance Aβ affinity for membrane surfaces [14,15]. Furthermore, it has been shown that GM1 plays an important role in the structural transition of the peptide [16–18], aggregation [19,20], and amyloid fibril formation [21,22]. However, the relevance of amyloid fibrils, plaque, or other types of structure formation to Aβ association with cell surfaces is not clear. Furthermore, only a few studies [23] have ever tried to establish the adsorption and aggregation kinetics of peptides on membrane surfaces.

To address the dynamics and structures of peptide assemblies on cell surfaces more deeply, we have investigated the interaction of immobilized monolayer surfaces with an Aβ model peptide in aqueous media. It is hoped that kinetic information on peptide adsorption and aggregation on cell surfaces will lead us to new forms of prevention and treatment for amyloidosis [24,25].

In this study, we chose a hydrophobic model peptide (AD-2α), composed of two amphiphilic α-helix segments, each modified with a 1-adamantanecarbonyl group at the N-terminal as a hydrophobic defect [26]. The peptide folds into a 2α-helix structure in the bulk solution at first, but the conformation may change gradually to a β-structure due to some outside effects. We were interested in the chemical aspect of this model peptide that mimics the Aβ_{42} structure. Therefore, in order to generalize the adsorption and aggregation properties of Aβ_{42} on cell surfaces, we have studied the kinetics and structure of this model peptide aggregation on model cell surfaces.

We transferred a mixed monolayer of glycolipids (GM1, asialo-GM1, GM3, or LacCer) and dipalmitoyl phosphatidylcholine (DPPC) onto an Au electrode plate of a 9-MHz quartz-crystal microbalance (QCM) to examine the dynamics of Ad-2α peptide binding onto the model membrane surface (see Fig. 1). The QCM technique has been utilized as a sensor for