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ATP-independent inhibition of amyloid beta fibrillation by the endoplasmic reticulum resident molecular chaperone GRP78

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ABSTRACT

Neuronal cell death induced by an accumulation of amyloid beta (Aβ) peptides, which are pathogenic molecules for Alzheimer's disease, is closely related with endoplasmic reticulum (ER) stress. In the ER stress condition, part of the ER resident chaperones is known to be translocated to another cellular location, such as the cell surface. The ER chaperone 78-kDa glucose-regulated protein (GRP78), which shows ATP-dependent chaperone activity, also shows translocation to the cell surface. In this study, we examined the influence of GRP78 on Aβ fibrillation in the presence or absence of ATP. We revealed that a small amount of GRP78 effectively inhibited fibrillation of Aβ fragments. Intriguingly, the fibrillation inhibition by GRP78 was confirmed in the absence of ATP, suggesting GRP78 exhibited ATP-independent interaction with Aβ fragments.

1. Introduction

Amyloid beta (Aβ) peptides are widely known as pathogenic molecules for Alzheimer's disease (AD) [1]. They form a fibrillar assembly consisting of intermolecular β-sheet formation [2]. Aβ aggregates found in hippocampal senile plaques are believed to induce neural cell death, resulting in short-term memory impairment through hippocampal denaturation [3]. Aβ cleaved from the transmembrane amyloid precursor protein (APP) is released into extracellular space. Subsequently, amyloid aggregation is caused by deposition of Aβ fragments and structural change of Aβ on the cell surface, such as with lipid rafts [4]. Neuronal cell death induced by Aβ accumulation in the AD is closely related with endoplasmic reticulum (ER) stress [5]. A translocation of the ER chaperone to another cellular location, such as the cell surface, in the ER stress condition was recently found [6], raising the possibility of interaction between Aβ fragments and ER chaperones. Our previous report indicated that ER resident chaperones such as calreticulin, protein disulfide isomerase, and ERp57 effectively inhibited Aβ fibrillation [7]. This implied that ER resident chaperones exhibited good interaction with Aβ fragments.

The 78-kDa glucose-regulated protein (GRP78), referred to as BiP or HSPA5, is one of the ER resident molecular chaperones, and serves multiple roles in maintaining cell viability [8,9]. The major function of GRP78 is facilitating transportation of new-born proteins and their folding assistance. GRP78 is included in the heat shock protein 70 (Hsp70) protein family, and the amino acid sequence of Hsp70 is highly conserved. Similar to Hsp70, both the ATPase and substrate recognition domains are contained within GRP78 [10]. Binding and hydrolysis of ATP play crucial roles in preventing misfolding and aggregation of nascent polypeptides [11–13]. In other words, GRP78 shows ATP-dependent chaperone activity for inhibiting protein aggregation. A number of studies have reported decrease of Aβ40 and Aβ42 accompanying interaction between GRP78 and APP [14,15]. Moreover, translocation of GRP78 to the cell surface was found in the ER stress condition [16,17], suggesting the possibility that GRP78 interacts with Aβ fragments. In the present study, we examined the influence of GRP78 addition on Aβ fibrillation in the presence or absence of ATP. Our results indicated that a small amount of GRP78 effectively inhibited Aβ elongation. We also confirmed fibrillation inhibition by GRP78 in the absence of ATP, suggesting GRP78 exhibited ATP-independent interaction with Aβ fragments.

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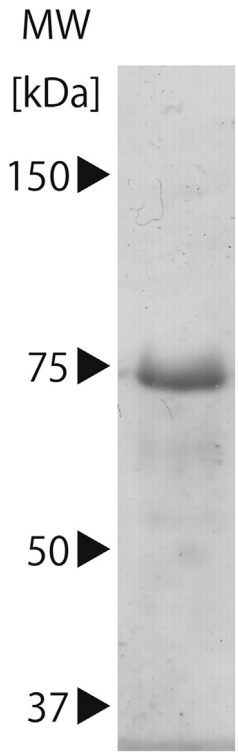

Fig. 1. SDS-PAGE profiles of recombinant human GRP78.

2. Materials and methods

2.1. Materials

Ammonium solution and adenosine 5′-triphosphate disodium salt trihydrate (ATP) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Thioflavin T (ThT) was obtained from Tokyo Chemical Industry (Tokyo, Japan). Human Aβ (1–42) was obtained from Peptide Institute (Osaka, Japan). PVDF membrane was purchased from EMD Millipore (Billerica, MA, USA). Nitrocellulose membrane was obtained from Bio-Rad Laboratories (Hercules, CA, USA). Aβ antibody (6E10) was obtained from BioLegend (San Diego, CA, USA). Rabbit anti-mouse IgG conjugated with horseradish peroxidase was purchased from Abcam (Cambridge, UK). ECL Prime Western Blotting Detection Reagent was purchased from GE Healthcare (Little Chalfont, UK).

2.2. Expression and purification of recombinant GRP78

A cDNA encoding human GRP78 was cloned into pCold I expression plasmid (Takara Bio Inc., Otsu, Japan), which is designed to produce N-terminally (His)6-tagged proteins. The obtained plasmid was transformed into BL21 cells, and the recombinant proteins were expressed and purified using Ni-NTA agarose (QIAGEN GmbH, Hilden, Germany), as per the manufacturer's instructions. The purified GRP78 was submitted into an NAP-5 column (GE Healthcare) equilibrated with PBS buffer. GRP78 dissolved in PBS was then obtained.

2.3. Preparation of Aβ fibril

Purchased lyophilized Aβ was fully dissolved in 0.1% ammonium solution, and the solution was then centrifuged at 15,000 rpm for 3 h to remove seeds. Supernatants containing Aβ monomers were collected into microtubes and stored as Aβ monomer stock solutions at −80 °C. Concentration of Aβ monomers was measured using the Bradford protein assay, with bovine serum albumin as a control. Aβ monomer solutions were gently thawed on ice and diluted in PBS buffer containing GRP78 and ATP. The mixtures containing 20 μM Aβ were incubated for 24 h at 37 °C and without agitation.

2.4. Confirmation of Aβ fibrillation by ThT assay

Aβ fibrillation was assessed by ThT assay in accordance with previous reports [7]. Briefly, Aβ solution was mixed with PBS buffer containing 20 μM ThT, and the final Aβ concentration was adjusted to 0.5 μM. The mixture was incubated for 5 min at room temperature. Emission fluorescent intensity was monitored at 485 nm through excitation at 445 nm using a spectrofluorometer (LS-55; PerkinElmer Inc., Waltham, MA, USA).

2.5. Immunoblotting analysis

SDS-PAGE and native-PAGE of Aβ samples were performed using 14% Tris-Glycine gels. After gel electrophoresis, separated proteins were transferred to PVDF membranes, using a current constant of 100 mA for 1 h. Incubated Aβ samples were centrifuged at 15,000 rpm for 20 min for dot-blotting analysis. Supernatants and precipitates were collected separately, with precipitates resuspended in fresh PBS solution. Dot-blotting was performed by spotting each Aβ sample solution directly onto nitrocellulose membrane.

For immunodetection, membranes were blocked overnight at 4 °C with 5% skimmed milk in PBS. After blocking, membranes were incubated with a mouse monoclonal Aβ antibody (6E10, 1:2000) for 1 h at 37 °C, followed by secondary horseradish peroxidase-conjugated anti-mouse IgG (1:10000). Proteins were visualized using the ECL Prime Western Blotting Detection Reagent, as per the manufacturer's instructions.

2.6. Morphology observation using transmission electron microscopy

Samples were placed on carbon-coated copper grids. Excess sample was removed using filter paper, and the grids were air-

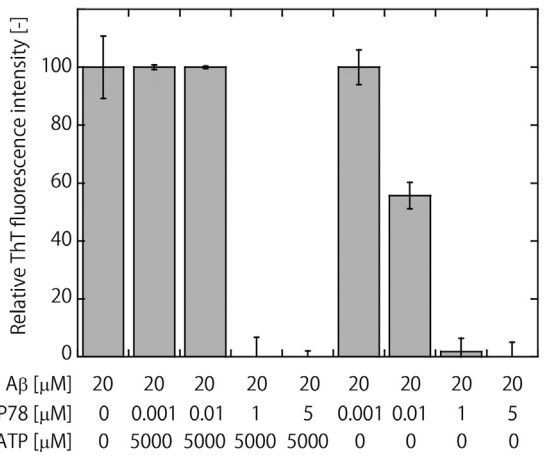

Fig. 2. Influence of GRP78 on Aβ fibrillation. Fibrillation was assessed using the ThT fluorescence method. Incubated samples containing chaperones with or without ATP were mixed with 20 μM ThT solution, and the final Aβ concentration adjusted to 0.5 μM. Emission fluorescent intensity was monitored at 480 nm by excitation at 445 nm, using a spectrofluorometer.

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