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The effects of organic solvents on the membrane-induced fibrillation of human islet amyloid polypeptide and on the inhibition of the fibrillation

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ABSTRACT

The organic solvent dimethylsulphoxide (DMSO) and 1,1,1,3,3,3-hexafluoro-2-isopropanol (HFIP) have been widely used as a pre-treating agent of amyloid peptides and as a vehicle for water-insoluble inhibitors. These solvents are left in many cases as a trace quantity in bulk and membrane environments with treated amyloid peptides or inhibitors. In the present work, we studied the effects of the two organic solvents on the aggregation behaviors of human islet amyloid polypeptide (hIAPP) and the performances of an all-D-amino-acid inhibitor D-NFGAIL in preventing hIAPP fibrillation both in bulk solution and at phospholipid membrane. We showed that the presence of 1% *v/v* DMSO or HFIP decreases the rate of fibril formation of hIAPP at the lipid membrane rather than accelerates the fibril formation as what happened in bulk solution. We also showed that the presence of 1% *v/v* DMSO or HFIP impairs the activity of the inhibitor at the lipid membrane surface dramatically, while it affects the efficiency of the inhibitor in bulk solution slightly. We found that the inhibitor inserts into the lipid membrane more deeply or with more proportion in the presence of the organic solvents than it does in the absence of the organic solvents, which may hinder the binding of the inhibitor to hIAPP at the lipid membrane. Our results suggest that the organic solvents should be used with caution in studying membrane-induced fibrillogenesis of amyloid peptides and in testing amyloid inhibitors under membrane environments to avoid incorrect evaluation to the fibrillation process of amyloid peptides and the activity of inhibitors.

1. Introduction

The amyloid deposits of proteins have been associated with many aging and degenerative diseases including Alzheimer's Disease, Parkinson's Disease, and Type II Diabetes [1–5]. There have been a large amount of investigations on the structures of amyloid fibrils, mechanisms of fibril formation as well as inhibitions of amyloidogenesis by inhibitors. In these studies, organic solvents, such as dimethylsulphoxide (DMSO) and 1,1,1,3,3,3-hexafluoro-2-isopropanol (HFIP), have been widely used to treat synthetic peptides so as to disassociate pre-existing aggregates. Besides, DMSO has also been widely used as a vehicle for water insoluble inhibitors [6–11]. Some assays of amyloid peptides were performed in bulk solutions or in membrane environments by diluting a stock solution of amyloid peptides in DMSO or HFIP directly. In these cases, the organic solvents were left as a trace quantity in bulk or membrane environments (in most cases the organic solvents were not more than 2.5% *v/v*) [12,13]. The presence of a trace quantity of HFIP in bulk solution has been demonstrated to accelerate the formation of fibrillar aggregates [14].

The interactions of DMSO with lipid membrane surfaces were investigated by experimental measurements and molecular dynamics simulations, and a number of features which are significant with respect to the effects of DMSO on membrane structure and function were revealed [15–21]. DMSO molecules occupy a position just beneath the lipid headgroups and affect lipid membrane structure by displacing water, reducing bilayer thickness, increasing headgroup area, inducing water pore formation, etc. The perturbation of HFIP to phospholipid membranes was demonstrated to be pronounced. HFIP has a high affinity for liposomal membranes [22]. Even low concentration of HFIP (0.01%–0.06% *v/v*) can adversely affect membranes and alter their permeability for ions [23,24]. Therefore, it can be supposed that the presence of organic solvent at lipid membranes would change the micro-environments of both amyloid peptides and inhibitors, interfere with fibril growth of amyloidogenic peptides at membrane surfaces and affect the binding of inhibitors to amyloid peptides. Moreover, the organic solvents, even at a low concentration, also can exert an effect on the folding of amyloid peptides [14,25–27]. Although a facilitating role of a trace quantity of HFIP for the amyloid fibrillation in bulk solution was reported, the effects of organic solvents on the aggregation behaviors of amyloid peptides at lipid membranes and more importantly on the inhibitory efficiency of amyloidogenic inhibitors at lipid membranes were almost ignored in previous studies. If the solvent effects really exist, the introduction of the organic solvents in the assays

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could lead to an incorrect estimation to the aggregation behavior of an amyloid peptide and to the activity of an inhibitor.

In this study, we detected the effects of the organic solvents on the aggregation behaviors of human islet amyloid polypeptide (hIAPP) and on the performances of an all-D-amino-acid inhibitor in blocking fibril formation of hIAPP either in bulk solution or at the phospholipid membrane surface using thioflavin-T (ThT) fluorescence spectroscopy, atomic force microscopy (AFM), transmission electron microscopy (TEM) and far-ultraviolet circular dichroism (CD).

2. Materials and methods

2.1. Materials

Synthetic hIAPP (1–37) with an amidated C-terminus and an oxidized disulfide between Cys2 and Cys7 was obtained from Shanghai Sci. Pept. Biol. Technol. Co., Ltd (Shanghai, China). The sample purity was greater than 95%. The short peptide composed of an all-D-amino-acid, D-NFGAIL, with purity greater than 98% was purchased from APeptide Co. Ltd (Shanghai, China). The phospholipids 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (POPG) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). DMSO (99%), HFIP (99.5%) and other chemical agents were obtained from Sigma-Aldrich (St. Louis, MO, USA). The peptides and chemical agents were used as purchased without further treatment.

2.2. Preparation of solutions

Stock solutions of a certain quantity of hIAPP in DMSO or HFIP were prepared by solubilizing synthetic hIAPP powder in the organic solvent and sonicating the mixture in water bath for 2 min. The DMSO and HFIP solutions of the all-D-amino-acid inhibitor were prepared similarly before each experiment. A 25 mM phosphate buffer with 25 mM NaCl at pH 7.4 was prepared and used for all experiments.

2.3. Small unilamellar lipid vesicles (SUVs)

POPC and POPG with the molar ratio of POPC:POPG 7:3 were dissolved in chloroform/methanol (2:1 *v*/*v*) and gently dried under a dry nitrogen flux. The lipid film was vacuum-desiccated overnight. The dry lipid film was rehydrated using phosphate buffer and bath-sonicated for 1 h. The liposome was used immediately after preparation.

2.4. Thioflavin-T fluorescence spectroscopy

The samples of 15 μM hIAPP in bulk solutions containing 1% *v*/*v* organic solvent or the samples of 15 μM hIAPP in liposome solutions containing various quantities of organic solvent (0.05%–1% in *v*/*v*) were prepared. The ThT of 20 μM was added in all samples. The lipid concentration of 2.25 mM (lipid:hIAPP = 150:1) was used. The samples for testing the performance of inhibitor were prepared by mixing different quantities of inhibitor stock solution with a certain quantity of hIAPP stock solution and then adding buffer or freshly prepared liposome solution in the mixtures immediately. The time dependence of fluorescence intensity was monitored immediately after the sample preparation using an RF-5301PC spectrofluorophotometer (Shimadzu, Japan) at an emission wavelength of 482 nm and an excitation wavelength of 440 nm. The spectra were recorded at room temperature without shaking. All experiments were repeated at least twice by individually prepared samples to ensure the results reproducible.

2.5. Transmission electron microscopy

The samples of 5 μL used in ThT assays were deposited on a 300-mesh Formvar-carbon coated copper grid (Shanghai, China) for 2 min at room temperature. Excess samples were removed using filter paper followed by washing twice with 10 μL Milli-Q water. Then, the samples were air-dried overnight and stained with 1% freshly prepared uranyl formate. The samples were observed under a transmission electron microscope (JEM-2100F, JEOL Co., Ltd., Japan) operating at an accelerating voltage of 200 kV.

2.6. Far-ultraviolet circular dichroism

Far-UV CD spectra were measured at room temperature under a constant flow of nitrogen gas by using a PMS-450 spectropolarimeter (Biologic, France). A 0.1 mm quartz cuvette was used for all CD spectra. Data were recorded from 260 to 190 nm with 1 nm sampling interval. The samples used in CD experiments were prepared similarly to those used in ThT assays except the addition of ThT dye. The final spectra were the average of three repeated experiments and the background (the CD spectrum of the sample without hIAPP) was subtracted.

2.7. Atomic force microscopy

AFM was performed as previously described [28]. Briefly, the hIAPP stock solution was mixed with freshly prepared SUV solution (POPC:POPG 7:3) at a 25:1 molar ratio of lipid:peptide and at a 1% *v*/*v* concentration of HFIP or DMSO. The mixture was incubated at room temperature for 30 min. The liposome solution of 20 μL was then deposited on freshly cleaved mica for 20 min and allowed to heat at 65 °C for 5 min to promote fusion and formation of planar lipid bilayers [29]. All images were recorded using SPA-300 AFM instrument (Seiko Instruments Inc., Japan) in a contact mode.

2.8. Acrylamide fluorescence quenching experiment

The quenching experiments of phenylalanine (Phe) fluorescence were performed on an RF-5301PC spectrofluorophotometer at room temperature using acrylamide as a quencher. The SUV samples of 15 μM hIAPP and those of 15 μM all-D-amino-acid inhibitor in the absence and presence of 1% *v*/*v* DMSO or HFIP were used, and the lipid:peptide ratio was fixed at 150:1 in all these experiments. The fluorescence was excited at 256 nm and the emission was scanned from 270 to 400 nm. Three scans were collected and averaged. The fluorescence spectrum of liposome alone as the background was subtracted. A series of fluorescence spectra were measured after serial addition of small aliquots of freshly prepared acrylamide solution (5 M) into the peptide-incorporated SUV solution. The relation of the fluorescence intensity with the concentration of the quencher [Q] was determined by the Stern–Volmer equation [30]:

$$F_0/F = 1 + K_{sv}[Q] \quad (1)$$

where F_0 and F are the fluorescence intensity in the absence and presence of the quencher, respectively, and K_{sv} is the quenching constant.

3. Results and discussion

3.1. Effects of the organic solvents on hIAPP fibrillation and inhibitor activity in bulk solution

The fibrillation behaviors of hIAPP in bulk solution were monitored by the ThT fluorescence assay in the absence and presence of organic solvents. In the presence of 1% *v*/*v* DMSO, the ThT fluorescence displayed a higher initial intensity and the intensity increased with time to a plateau in ~1 h. A characteristic lag phase that existed in the ThT assay of hIAPP in bulk solution without the organic solvent (~1.5 h) was not observed (Figs. 1A and S1). This suggests that the early aggregation of hIAPP before the formation of fibrils is promoted by the presence of 1% *v*/*v* DMSO. The TEM image confirmed the