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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

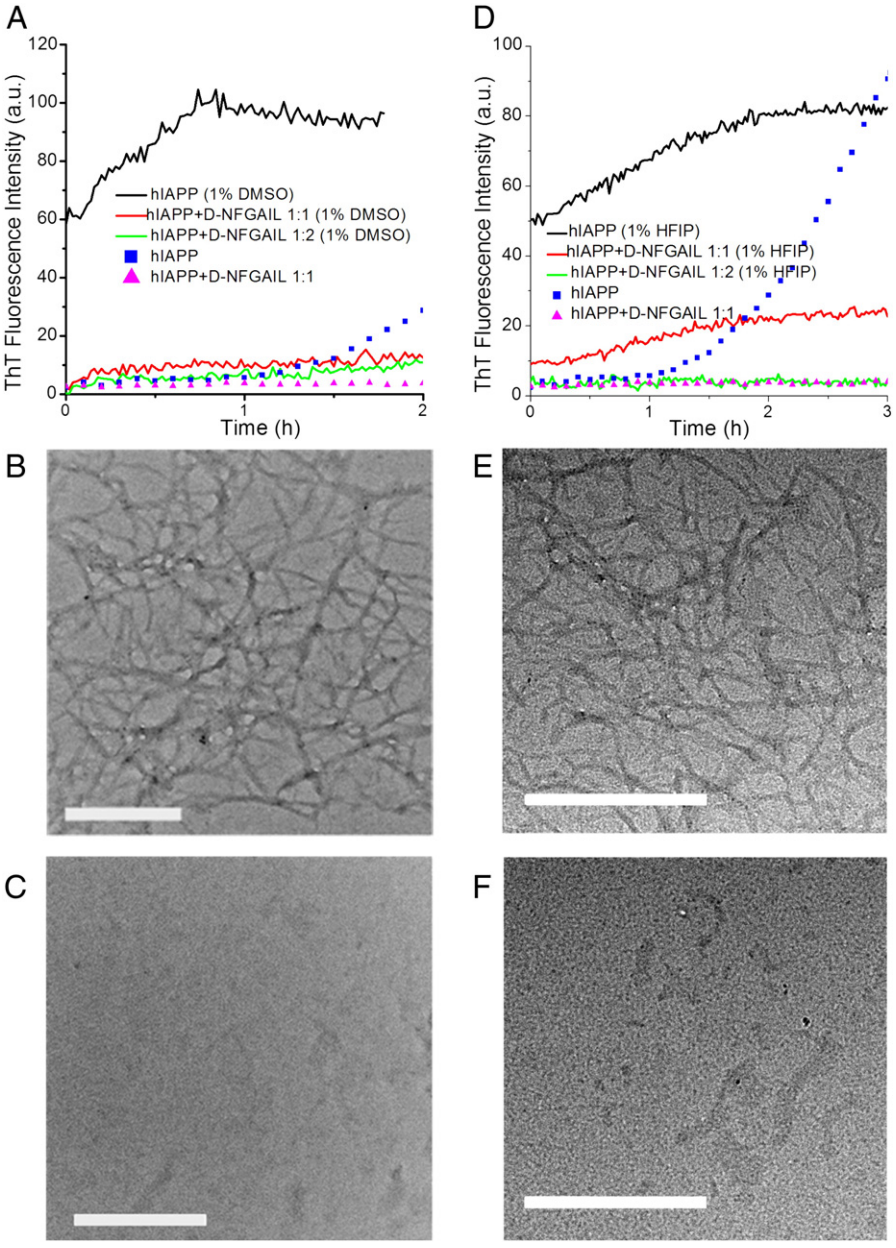

Fig. 1. The ThT monitored kinetic processes of aggregation of hIAPP alone and hIAPP mixed with different molar ratios of D-NFGAIL in bulk solution containing 1% *v*/*v* DMSO (A), the TEM images of hIAPP incubated alone (B) and incubated with an equimolar D-NFGAIL (C) in bulk solution containing 1% *v*/*v* DMSO for 24 h, and the corresponding results from 1% *v*/*v* HFIP containing solutions (D–F). The scale bars in all images are 500 nm.

formation of amyloid fibrils of hIAPP in bulk solution with 1% *v*/*v* DMSO (Fig. 1B). The morphology of the fibrils formed in the presence of 1% *v*/*v* DMSO was similar to that formed in the absence of the solvent [28], suggesting that the growth pattern of hIAPP fibrils is not significantly affected by the organic solvent.

An all-D-amino-acid inhibitor D-NFGAIL that has been confirmed to be efficient in preventing the formation of hIAPP fibrils [28] was used to detect the effects of the organic solvents on the performance of the inhibitor. When an equimolar amount of inhibitor was added in the hIAPP bulk solution with 1% *v*/*v* DMSO, the ThT fluorescence intensity was dramatically decreased (Figs. 1A and S1) and no amyloid fibrils were detected by the TEM image (Fig. 1C). This indicates that the potency of the inhibitor in blocking hIAPP fibril formation is almost not affected by 1% *v*/*v* DMSO, or the interaction between the inhibitor and hIAPP is not significantly affected by the organic solvent. Similar results were also obtained for hIAPP in bulk solution with 1% *v*/*v* HFIP (Figs. 1D–F and S1).

3.2. Effects of the organic solvents on hIAPP fibrillation and inhibitor activity at the phospholipid membrane surface

The ThT fluorescence assay showed that the introduction of 1% *v*/*v* DMSO in the SUV solution postponed the formation of hIAPP fibrils as the lag phase increased from ~5 min in the absence of DMSO to ~20 min in the presence of 1% *v*/*v* DMSO (Figs. 2A and S2). The difference between the morphologies of hIAPP aggregates in the presence and in the absence of DMSO was also observed by the AFM image (Fig. 2B). Besides the linear structure, the peptide also formed the fibrils of circular structure at the membrane in the presence of DMSO.

With the addition of an equimolar amount of inhibitor D-NFGAIL, the time dependence of the ThT fluorescence for hIAPP fibrillation at the lipid membrane treated with 1% *v*/*v* DMSO was significantly different from that in the absence of DMSO. Whereas the increase in the ThT fluorescence intensity was completely suppressed at the 1:1 ratio of inhibitor:hIAPP in the absence of DMSO (also see ref. [28]), it was

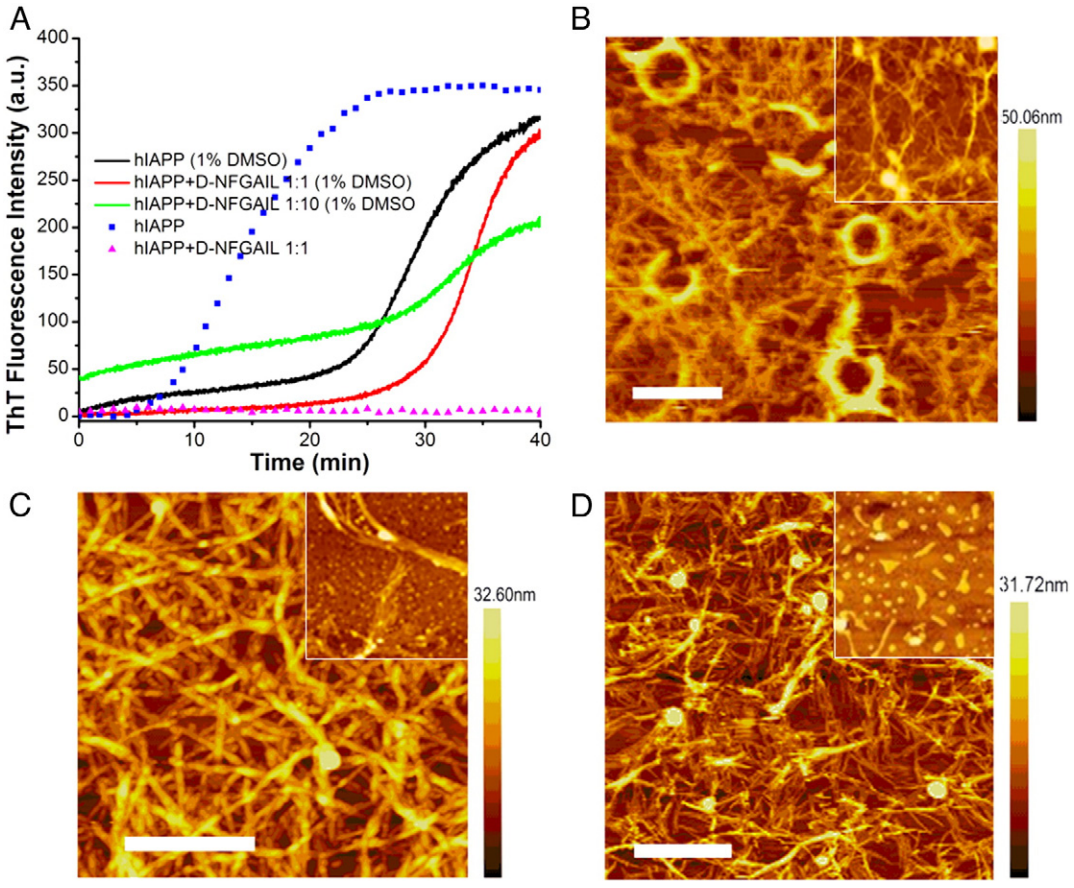

Fig. 2. The aggregation behaviors of hIAPP at the lipid membrane surface containing 1% *v*/*v* DMSO. (A) The ThT monitored kinetic processes of aggregation of hIAPP alone and hIAPP mixed with different molar ratios of D-NFGAIL; (B–D) the AFM images of hIAPP incubated with liposome in the absence and presence of inhibitor for 50 min: (B) hIAPP alone, (C) D-NFGAIL:hIAPP 1:1, (D) D-NFGAIL:hIAPP 3:1. The ratios of lipid:hIAPP are 150:1 in the ThT assays and 25:1 in the AFM assays. The inserts in the AFM images (B–D) are the parallel results obtained in the absence of DMSO. The scale bars in all images are 1 μm.

still observed after a slightly longer lag phase in the presence of 1% *v*/*v* DMSO (Figs. 2A and S2). The addition of even ten times of inhibitor in the SUV solution of hIAPP with 1% *v*/*v* DMSO only resulted in a smaller change in the kinetic behavior of hIAPP aggregation, but did not prevent the increase in the ThT fluorescence intensity. The severe impairing of DMSO to the efficiency of the inhibitor at the lipid membrane was also confirmed by the AFM images, where abundant fibrils of hIAPP were always observed at the inhibitor:hIAPP ratios from 1:1 to 20:1 in the presence of 1% *v*/*v* DMSO (Fig. 2C and D, also see Fig. S3 for the AFM images at the inhibitor:hIAPP ratios of 10:1 and 20:1). In contrast, the formation of fibrils was prevented largely by the inhibitor at inhibitor:hIAPP of 1:1 and blocked completely at inhibitor:hIAPP of 3:1 (see the inserts in Fig. 2C and D, also see ref. [28]). We also measured the AFM images of the inhibitor alone in the SUV solution with 1% *v*/*v* DMSO after the same incubation time (Fig. S4). No any apparent aggregates were observed on the AFM images, even when the inhibitor concentration was as high as 300 μM (corresponding to the inhibitor concentration used in the assay of inhibitor:hIAPP 20:1). This confirmed that the fibrils appearing in the AFM images resulted from the hIAPP but not the inhibitor.

The ThT assay of hIAPP at the lipid membrane treated with 1% *v*/*v* HFIP showed a gradual increase in the fluorescence intensity without an initial delay (Figs. 3A and S2). The intensity reached to the plateau after ~40 min. In contrast, the ThT fluorescence intensity rose steeply after a delay of ~5 min and reached to the plateau after ~25 min in the absence of HFIP (Fig. 3A). This indicates that the early aggregation of hIAPP is promoted by 1% *v*/*v* HIFP at the membrane surface, but the growth rate of the fibers is reduced at the membrane treated with 1% *v*/*v* HFIP. The presence of 1% *v*/*v* HFIP also affected the morphology of hIAPP fibrils formed at the lipid membrane. Although abundant fibrils of hIAPP were detected by AFM image after 50 min incubation at the membrane with 1% *v*/*v* HFIP (Fig. 3B), it was noted that the fibrils formed at the lipid membrane with 1% *v*/*v* HFIP seems thicker and shorter than those formed in the absence of the solvent (the insert in Fig. 2B).

More importantly, the introduction of 1% *v*/*v* HFIP at the lipid membrane impaired the activity of the all-D-amino-acid inhibitor in blocking the formation of fibrils dramatically. The addition of the inhibitor in the liposome solution with 1% *v*/*v* HFIP only resulted in a dose dependent extension of the lag phase in the ThT fluorescence spectra (Fig. 3A), whereas the increase in the ThT fluorescence intensity of hIAPP incorporated with SUVs alone was completely suppressed at the molar ratio of inhibitor to hIAPP for 1:1. The increase in the ThT fluorescence intensity of hIAPP at the lipid membrane in the presence of 1% *v*/*v* HFIP was not suppressed even at the molar ratio of inhibitor to hIAPP for 10:1. The adverse effect of HFIP on the inhibitory efficiency was clearly observed in the AFM images of hIAPP reconstructed at the membrane surface treated with 1% *v*/*v* HFIP. Abundant short and straight fibers were observed at the inhibitor:hIAPP ratios from 1:1 to 10:1 (Figs. 3C, D and S5). The fibrils appearing in the AFM images were attributed unambiguously to the amyloid peptides but not the inhibitor, as the inhibitor alone did not form fibrils in the same conditions (see Fig. S6).

3.3. Effects of the organic solvents on the structure of hIAPP at the lipid membrane

The effects of DMSO and HFIP on the structure of hIAPP at the membrane surface were analyzed by the CD spectroscopy. Our previous

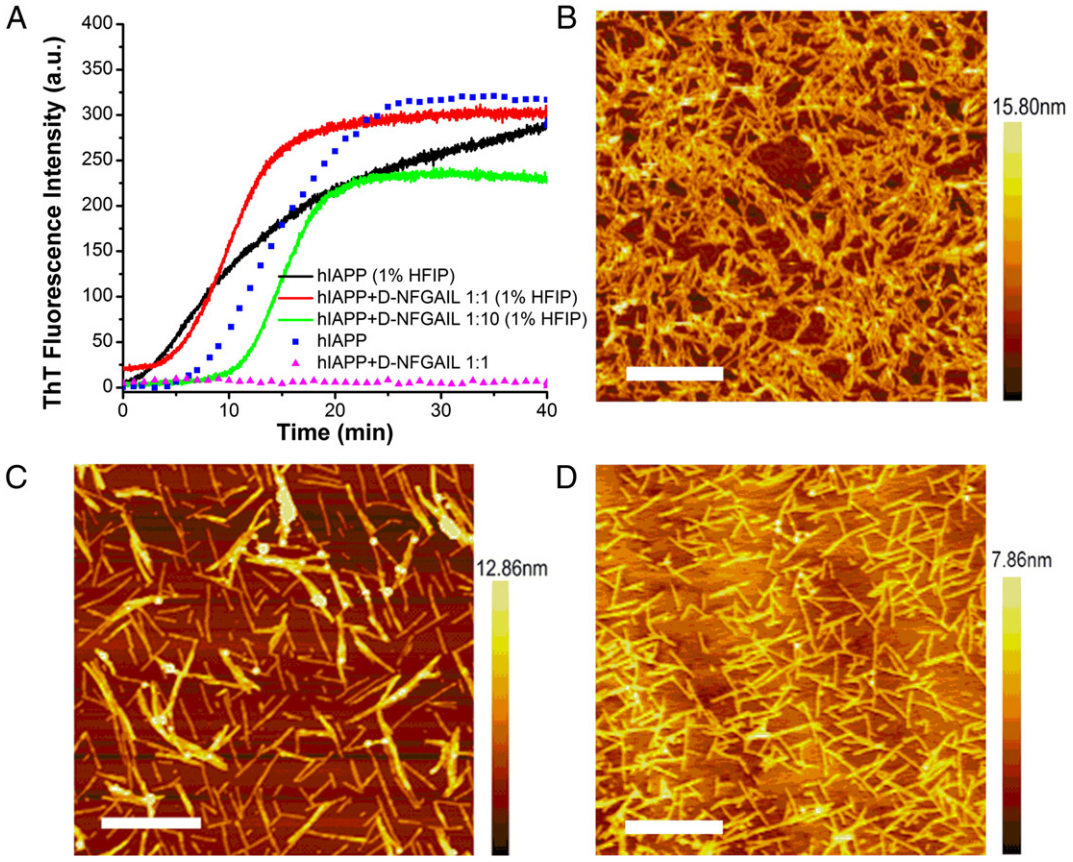

Fig. 3. The aggregation behaviors of hIAPP at the lipid membrane surface containing 1% *v*/*v* HFIP. (A) The ThT monitored kinetic processes of aggregation for hIAPP alone and hIAPP mixed with different molar ratios of D-NFGAIL; (B–D) the AFM images of hIAPP incubated with liposome in the absence and presence of inhibitor for 50 min: (B) hIAPP alone, (C) D-NFGAIL:hIAPP 1:1, (D) D-NFGAIL:hIAPP 3:1. The ratios of lipid:hIAPP are 150:1 in the ThT assays and 25:1 in the AFM assays. The scale bars in all images are 1 μm.

study indicated that hIAPP forms an α-helix structure at the initial incubation time at the membrane without the organic solvent [28]. The CD results in the present study showed that the organic solvents have an effect on the initial structure of hIAPP at the membrane. In the absence of inhibitor, hIAPP formed either a β-sheet structure at the membrane with 1% *v*/*v* DMSO or an α-helix structure at the membrane with 1% *v*/*v* HFIP at an initial incubation time (Fig. 4). The peptide formed a β-sheet structure after 45 min incubation under both conditions, which is similar to the case of hIAPP at the membrane without the organic solvent. In the presence of the inhibitor, the initial structures of hIAPP at the membrane with 1% *v*/*v* DMSO depended on the ratio of inhibitor:hIAPP, e.g., a β-sheet structure at a 1:1 ratio and an α-helix structure at a 10:1 ratio, while the initial structures of hIAPP at the membrane with 1% *v*/*v* HFIP were α-helix at all inhibitor:hIAPP ratios studied. Despite having the different initial structures, hIAPP aggregated at the membranes containing the organic solvents to form fibrils with a β-sheet structure after 45 min incubation. In contrast, the initial α-helix structure is stabilized and the conversion from an α-helix to a β-sheet is blocked by the all-D-amino-acid inhibitor at the membrane without the organic solvent [28]. As a result, the fibrillation of hIAPP is inhibited. The CD results indicate that the potency of the inhibitor in specific stabilization of an α-helix structure or α-helical intermediates of hIAPP at the membrane surface is abolished by the presence of DMSO and HFIP.

3.4. Effects of the organic solvents on the interactions of either inhibitor or hIAPP with the lipid membrane

Although the organic solvents impose effects on the structures of hIAPP monomers or oligomeric intermediates at phospholipid membrane, the structural change may not be a direct cause that leads to the loss in the activity of the all-D-amino-acid inhibitor. The fibril formation from either the α-helical intermediates or from the β-sheet intermediates can be disrupted by the D-peptide in the absence of the organic solvents [28]. A more direct factor for the invalidation of the inhibitor could originate from the obstruction of the inhibitor binding with hIAPP at the lipid membrane disturbed by the organic solvents. As we know, the presence of DMSO or HFIP in lipid membrane is able to change the property of membrane. Thus, the interactions of hIAPP and inhibitor with the lipid membrane or the insertion of the two peptides in the membrane should be influenced. In order to examine the effects of the organic solvents on the insertion of hIAPP and the D-peptide inhibitor in the membrane, we performed the acrylamide fluorescence quenching experiments for hIAPP and the inhibitor in liposome solution separately.

The emission spectra of Phe residue in inhibitor were monitored at a serial concentration of acrylamide. As shown in Fig. 5, the introduction of 1% *v*/*v* DMSO or 1% *v*/*v* HFIP in the liposome solution of inhibitor led to an evident increase in the fluorescence intensity before the addition of acrylamide. With the addition of acrylamide, the fluorescence intensity was much more quickly quenched in the absence of the organic solvents than it was in the presence of the organic solvents. The K_{sv} values (or the slopes of the straight lines in Fig. 5D) obtained in the presence of the organic solvents were smaller than that obtained in the absence of the organic solvents by about one order of magnitude. One possibility to interpret this result is a deeper insertion of the inhibitor in the membrane in the presence of 1% *v*/*v* DMSO and HFIP. Another possibility is an increasing portioning of the inhibitor in the membrane. Either deeper insertion or more portioning in lipid membrane with 1% *v*/*v* DMSO or HFIP is clearly unfavorable for the interaction of the inhibitor with hIAPP.

Considering the fast aggregation of hIAPP at the lipid membrane, we performed the quenching experiments of the Phe emission at only one

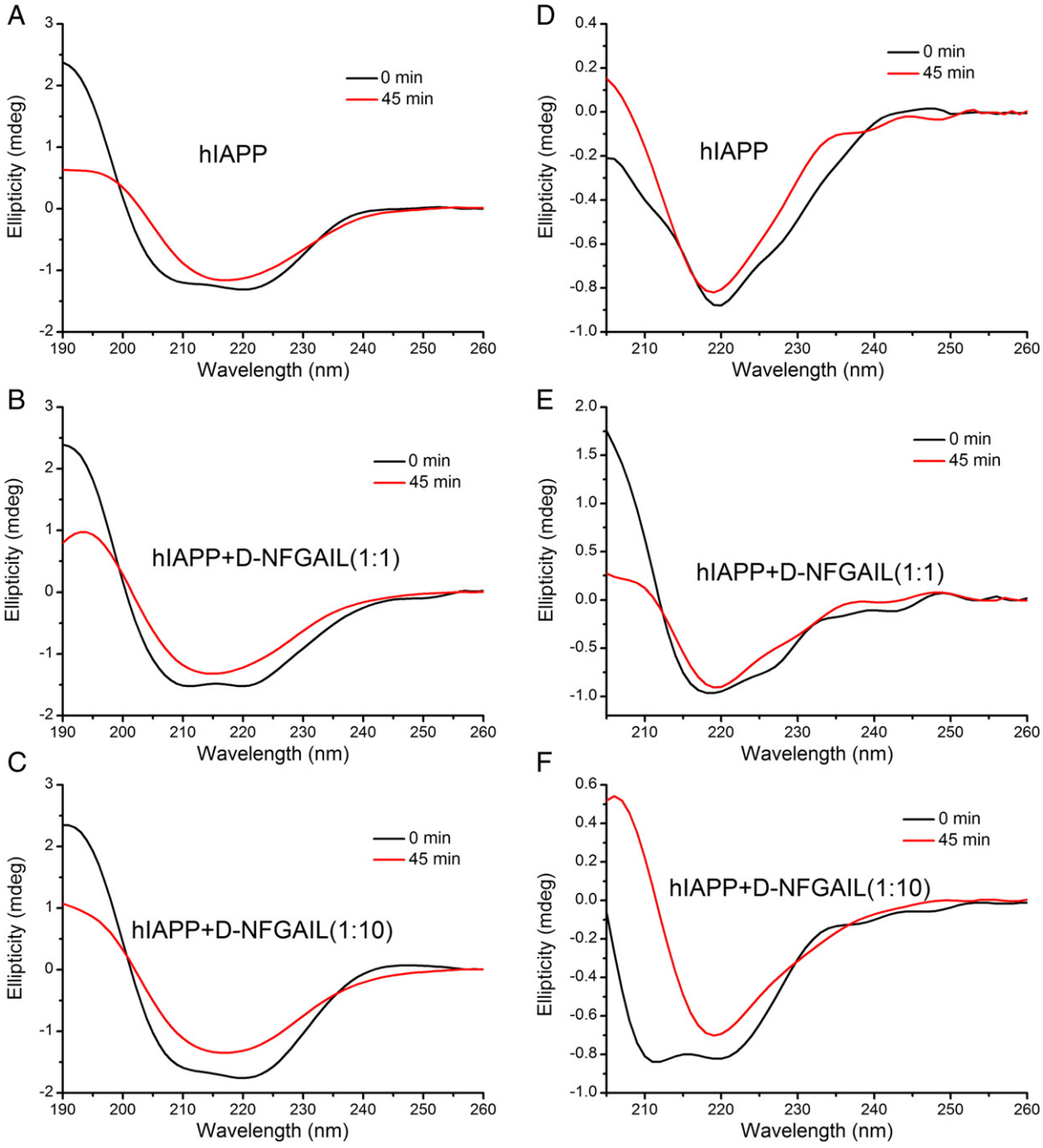

Fig. 4. The CD spectra of hIAPP alone, hIAPP mixed with D-NFGAIL at 1:1 and 1:10 ratios incubated at the lipid membrane containing 1% *v*/*v* HFIP (A–C) and 1% *v*/*v* DMSO (D–F) for 0 and 45 min.

fixed concentration of acrylamide (100 mM) immediately after the sample preparation. The results showed that the introduction of 1% *v*/*v* HFIP in the liposome solution of hIAPP has little effect on the fluorescence intensity, while the addition of 1% *v*/*v* DMSO causes a decrease in intensity (Fig. 6). The quenching by acrylamide resulted in a decrease in the fluorescence intensities with a similar magnitude for the liposome samples of hIAPP either with or without the organic solvents (the value of F_0/F was 2.3 in the absence of organic solvent and the values were 1.8 and 1.7 in the presence of 1% *v*/*v* HFIP and DMSO, respectively), whereas the introduction of the organic solvents induced larger change in the quenching magnitude for the liposome samples of the inhibitor at 100 mM acrylamide (the F_0/F values for the three liposome samples of inhibitor were 9.9, 2 and 1.45). This suggests that the interaction of inhibitor with lipids was much more impacted by the organic solvents than the interaction of hIAPP with lipids. The increasing embedding or portioning of the inhibitor in the membrane may be a main and direct factor that leads to the activity losing of the all-D amino-acid inhibitor in preventing hIAPP fibrillation.

It was noteworthy that the results of the quenching assays for the hIAPP liposome samples containing 1% *v*/*v* organic solvents displayed the F_0/F values somewhat smaller than that of the liposome sample of hIAPP without the organic solvents. This implies that the presence of the organic solvents may induce a little deeper embedding of hIAPP in the membrane. This result can be used to explain why the hIAPP fibrillation at the lipid membrane was slowed by the introduction of the organic solvents. A little deeper insertion of hIAPP in the membrane in the presence of the organic solvents could decrease the interactions of hIAPP with themselves, and thus slow the growth rate of fibrils.

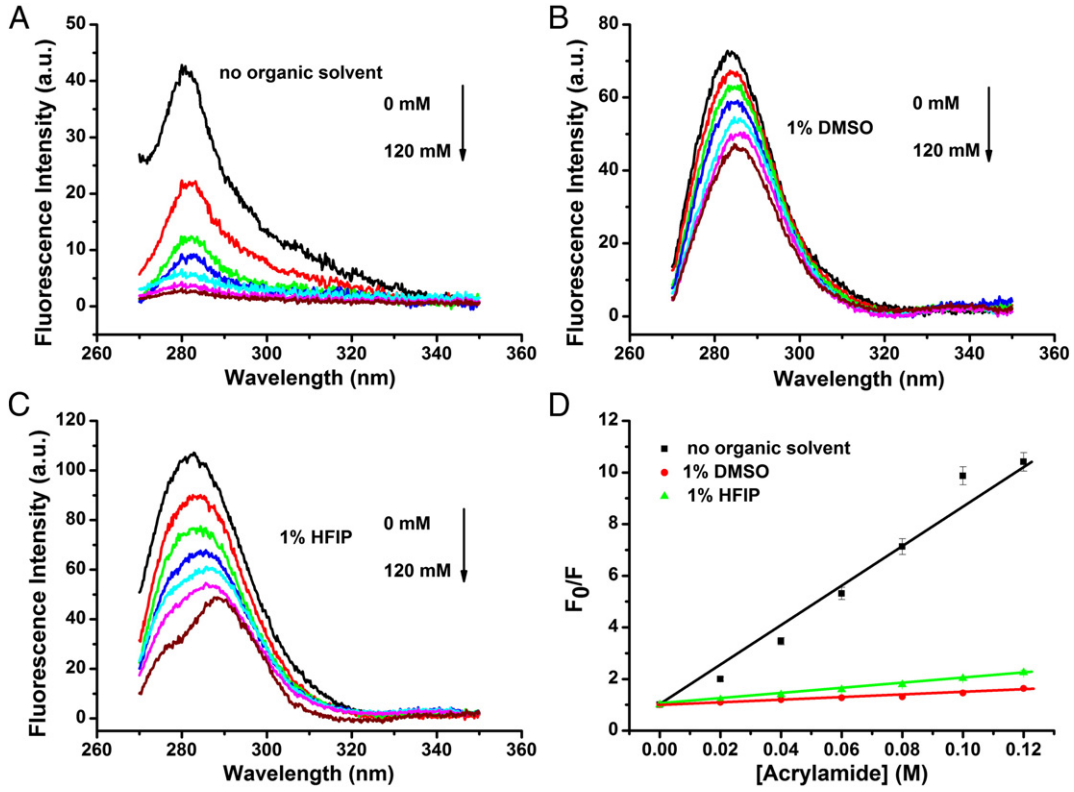

Fig. 5. The acrylamide fluorescence quenching spectra of the all-D-amino-acid inhibitor in pure liposome solution (A), in the liposome solution containing 1% *v*/*v* DMSO (B) and 1% *v*/*v* HFIP (C), as well as the Stern–Volmer plots obtained from the spectral data (D).

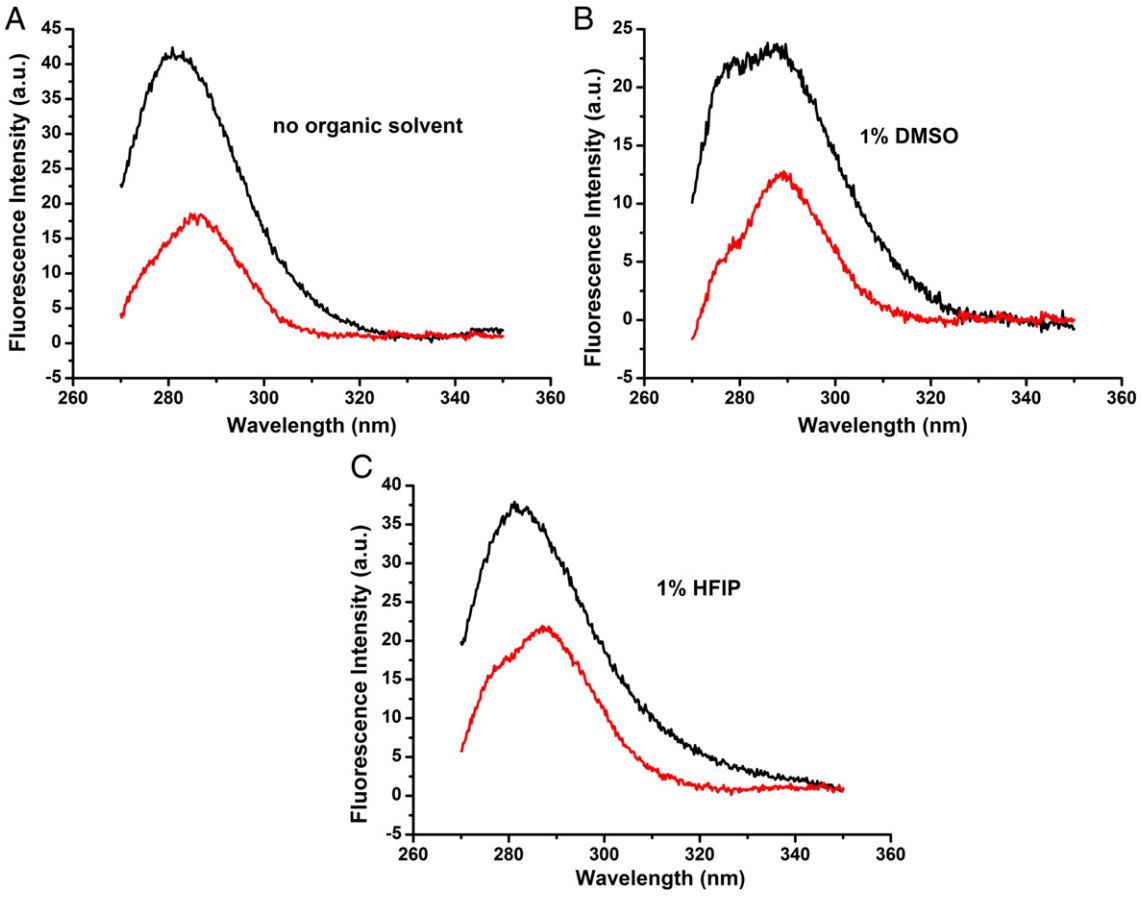

Fig. 6. The fluorescence spectra of hIAPP in pure liposome solution (A), in the liposome solution containing 1% *v*/*v* DMSO (B) and 1% *v*/*v* HFIP (C) before (black) and after (red) the addition of acrylamide.

3.5. The maximal concentrations of the organic solvents at which the hIAPP fibrillation and the efficiency of the inhibitor at the lipid membrane are not affected

The fibrillation of hIAPP at the lipid membrane mixed with various quantities of organic solvents was monitored using ThT fluorescence assays either in the absence or in the presence of the inhibitor D-NFGAIL (Figs. 7 and S7). The ThT fluorescence data showed that the changes in the kinetic processes of the hIAPP fibrillation induced by the organic solvents were decreased with the decrease in the amount of the solvents at the membrane. The kinetic processes of the hIAPP fibrillation in the presence of the organic solvents were close to that of the fibrillation of hIAPP alone when the amount of HFIP was decreased to 0.2% *v*/*v* and the amount of DMSO was decreased to 0.05% *v*/*v*. Interestingly, the activity of the D-peptide inhibitor in preventing the formation of hIAPP fibrils at the lipid membrane was increased as the concentrations of the organic solvents were decreased. The fluorescence intensity was fully suppressed at about 0.2% *v*/*v* HFIP, while a small extent of fluorescence increase was still observed even at 0.05% *v*/*v* DMSO. The maximal concentrations of the organic solvents obtained in the absence and presence of the inhibitor were very close, suggesting that the perturbation of the organic solvents to lipid membrane may play a more important role in changing the hIAPP fibrillation and the inhibitor performance than the effects by the interactions of the solvents with the peptides (hIAPP and inhibitor). In addition, it was clearly showed that the maximal concentration of DMSO is lower than that of HFIP. This indicates that DMSO strengthens the interactions of the D-peptide inhibitor and hIAPP with the lipid membrane more effectively than HFIP does.

The maximal allowed concentrations of organic solvents may differ in different conditions, depending on such as the property of a peptide and an inhibitor, the composition of membrane, the concentration of each component, and the environment or matrix. The mechanism and extent of the role of organic solvent in disturbing amyloidogenesis and inhibitor activity may be also different in different cases. Nevertheless, the effects of organic solvents should be evaluated in the experiments with respect to amyloid peptides if organic solvents are used, especially in the experiments involved in liposome.

4. Conclusion

Our results in this study demonstrate that the presence of a trace amount of organic solvents alters both the kinetics of hIAPP fibrillation and the binding of inhibitor with the amyloid peptide at the lipid membrane surface considerably. In this case, the fibril growth of hIAPP cannot be disrupted efficiently by the inhibitor. The increasing insertion depth or proportion of the D-peptide inhibitor in the lipid membrane embedded with organic solvents may be a key factor for the inefficiency of the inhibitor. This study reveals that the presence of the organic solvents could introduce deviation in studying the kinetic behaviors of amyloid peptides and more importantly could lead to an opposite conclusion in estimating the efficiency of inhibitors of amyloid peptides. 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.

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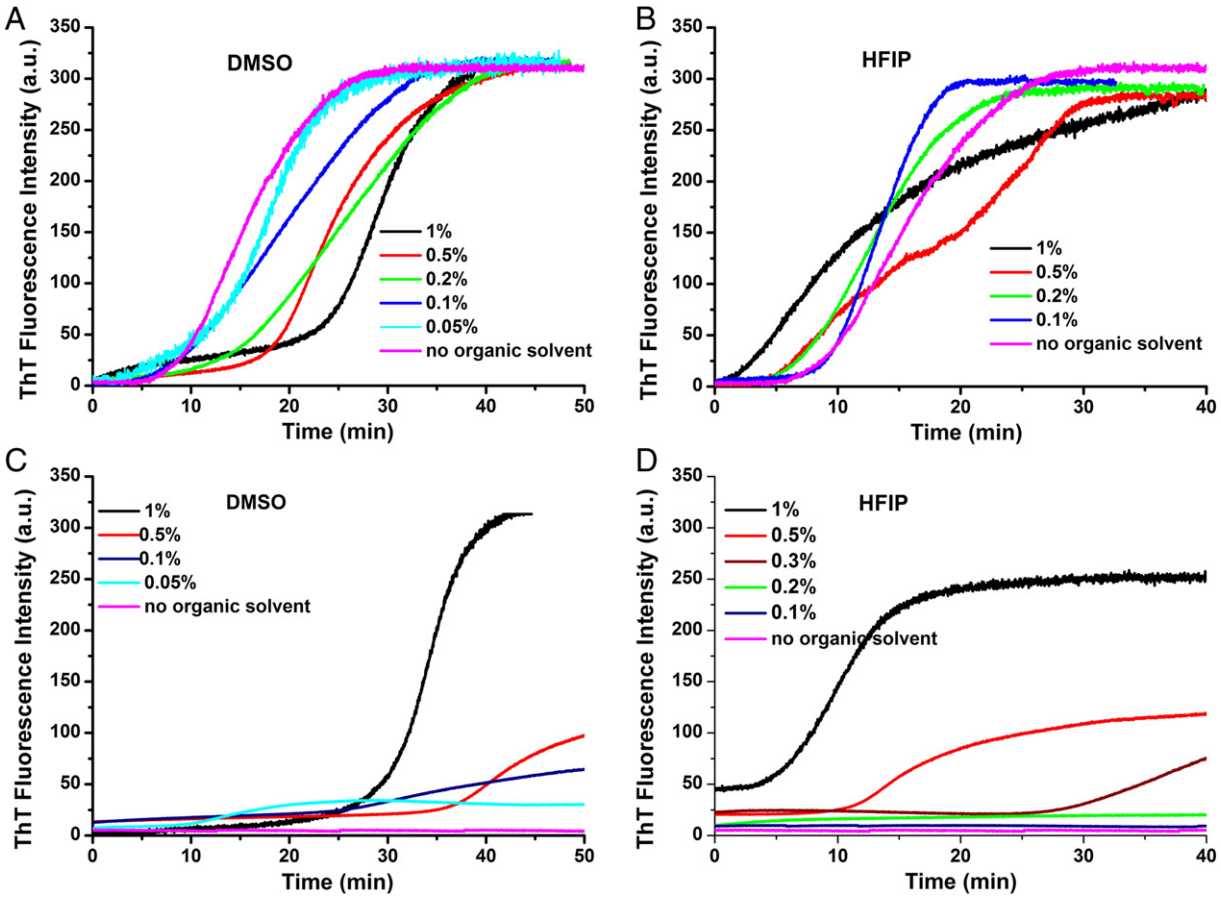

Fig. 7. The ThT monitored kinetic processes for hIAPP fibrillation at the lipid membrane containing various amounts of DMSO and HFIP in the absence of inhibitor (A and B) and in the presence of an equimolar D-peptide inhibitor (C and D).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamem.2014.09.002.

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