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The N-terminal fragment of human islet amyloid polypeptide is non-fibrillogenic in the presence of membranes and does not cause leakage of bilayers of physiologically relevant lipid composition

Lucie Khemtémourian [a,*,1], Maarten F.M. Engel [a,b,2], Rob M.J. Liskamp [c], Jo W.M. Höppener [b,d], J. Antoinette Killian [a]

[a] Department of Chemical Biology and Organic Chemistry, Institute of Biomembranes, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
[b] Department of Metabolic and Endocrine Diseases, Division of Biomedical Genetics, University Medical Center Utrecht, P.O. Box 85090, 3508 AB Utrecht, The Netherlands
[c] Medicinal Chemistry and Chemical Biology, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, The Netherlands
[d] Netherlands Metabolomics Center, University Medical Center Utrecht, P.O. Box 85090, 3508 AB Utrecht, The Netherlands

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ABSTRACT

Human islet amyloid polypeptide (hIAPP) forms amyloid fibrils in pancreatic islets of patients with type 2 diabetes mellitus (DM2). The formation of hIAPP fibrils has been shown to cause membrane damage which most likely is responsible for the death of pancreatic islet β-cells during the pathogenesis of DM2. Previous studies have shown that the N-terminal part of hIAPP, $hIAPP_{1-19}$, plays a major role in the initial interaction of hIAPP with lipid membranes. However, the exact role of this N-terminal part of hIAPP in causing membrane damage is unknown. Here we investigate the structure and aggregation properties of $hIAPP_{1-19}$ in relation to membrane damage *in vitro* by using membranes of the zwitterionic lipid phosphatidylcholine (PC), the anionic lipid phosphatidylserine (PS) and mixtures of these lipids to mimic membranes of islet cells. Our data reveal that $hIAPP_{1-19}$ is weakly fibrillogenic in solution and not fibrillogenic in the presence of membranes, where it adopts a secondary structure that is dependent on lipid composition and stable in time. Furthermore, $hIAPP_{1-19}$ is not able to induce leakage in membranes of PC/PS or PC bilayers, indicating that the membrane interaction of the N-terminal fragment by itself is not responsible for membrane leakage under physiologically relevant conditions. In bilayers of the anionic lipid PS, the peptide does induce membrane damage, but this leakage is not correlated to fibril formation, as it is for mature hIAPP. Hence, membrane permeabilization by the N-terminal fragment of hIAPP in anionic lipids is most likely an aspecific process, occurring via a mechanism that is not relevant for hIAPP-induced membrane damage *in vivo*.

1. Introduction

Amyloid formation has been implicated in a wide range of human diseases including Alzheimer's disease, Parkinson's disease, and type 2 diabetes mellitus (DM2). DM2 is characterized histopathologically by the presence of fibrillar amyloid deposits in the pancreatic islets of Langerhans (islet amyloid). Amyloid cytotoxicity, most likely related to membrane damage, is thought to be an early mechanism involved in death of insulin-producing islet β-cells in DM2 [1]. The main component of islet amyloid, and the actual fibril-forming molecule, is a 37 amino acid peptide called human islet amyloid polypeptide (hIAPP) or amylin, which is produced and secreted together with insulin by the pancreatic islet β-cells. The normal physiological role of hIAPP is not entirely clear, but it is believed to play a role as a hormone in gastric emptying, suppression of food intake, and glucose homeostasis [2–4].

It is clear from several reports that hIAPP is able to interact with membranes [5–10]. This interaction promotes the formation of amyloid fibrils and leads to membrane damage. However, the details of the nature of this interaction are still unknown. Recent publications suggest that full-length hIAPP has a pronounced ability to insert into lipid monolayers, and that it is the N-terminal part of hIAPP and not the amyloidogenic region that mediates the insertion of the peptide into membranes [6,7,11]. It is not clear whether the N-terminal part of hIAPP is involved only in membrane insertion, or whether it also directly contributes to membrane damage. Two recent studies, using membranes composed of anionic lipids only, indicate that $hIAPP_{1-19}$ induces membrane disruption to a near identical extent as full-length

Abbreviations: CD, circular dichroism; DMSO, dimethyl sulfoxide; hIAPP, human islet amyloid polypeptide; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; LUV, large unilamellar vesicle; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPS, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine; ThT, Thioflavin T

* Corresponding author. Tel.: +33 1 44 27 38 20; fax: +33 1 44 27 38 43.
E-mail address: lucie.khemtemourian@upmc.fr (L. Khemtémourian).
[1] Present address: UPMC Univ Paris 06, UMR 7203 CNRS-UPMC-ENS, Laboratoire des Biomolécules, 4 place Jussieu, 75005 Paris, France.
[2] Present address: Astbury Centre for Structural Molecular Biology and Institute of Molecular and Cellular Biology, University of Leeds, Leeds LS2 9JT, United Kingdom.

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hIAPP but that it does not form any fibrils, even after 15 days of incubation [12,13]. This would suggest that fibrillogenesis is not required for membrane damage. This is unexpected since many reports have shown that amyloid-induced membrane disruption is directly related to the propensity to form fibrils, not only for hIAPP but also for other amyloidogenic proteins, such as Aβ in Alzheimer's disease [8,9,14–17]. Unfortunately, comparison of published results is hampered by the use of different lipid compositions in various studies.

To gain further insight into the nature of the initial step of hIAPP-membrane interactions, including the potential importance of lipid composition, and to shed light on seemingly contradicting results in the literature, we performed a biophysical study of $hIAPP_{1-19}$, which corresponds to the N-terminal part of full-length hIAPP that is involved in the initial steps of membrane interactions [6], in lipid bilayers of different composition. In particular, we examined the aggregational behaviour, the effect on membrane integrity and the structure of $hIAPP_{1-19}$ in comparison with full-length hIAPP. For these studies we used mixtures of the zwitterionic lipid phosphatidylcholine (PC) and the anionic lipid phosphatidylserine (PS) in a 7:3 ratio to mimic the membranes of pancreatic islet cells [18] and we used bilayers composed of pure PC and PS, respectively, to obtain further insight into the importance of electrostatic interactions, and to allow comparison with previously reported studies on the membrane interaction of $hIAPP_{1-19}$ [13].

2. Materials and methods

2.1. Materials

Full-length hIAPP with an amidated C-terminus and disulfide bridge was obtained from Bachem AG (Bubendorf, Switzerland). The fragment $hIAPP_{1-19}$ was synthesised using Fmoc chemistry on a Tentagel S RAM resin as described previously [19,20]. Linear $hIAPP_{1-19}$ was dissolved in aqueous DMSO and oxidized with air to the corresponding disulfide [21]. The peptide was purified by reverse phase high-performance liquid chromatography (HPLC). Purity of both peptides was higher than 95% as determined by analytical HPLC and the masses of the peptides were confirmed with MALDI-TOF mass spectrometry. 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS) were obtained from Avanti Polar Lipids (Alabaster, AL). Thioflavin T (ThT) was obtained from Sigma.

2.2. Preparation of peptide samples

hIAPP and $hIAPP_{1-19}$ peptides were dissolved at a concentration of 1 mM in hexafluoro-2-propanol (HFIP) and incubated for at least 1 h. Next, HFIP was evaporated with dry nitrogen gas followed by vacuum desiccation for at least 30 min. The resulting peptide film was then dissolved in DMSO for electron microscopy (EM), membrane leakage experiments and ThT assay and in phosphate buffer for the CD experiments. For the membrane leakage experiments, the ThT assay, and for the EM experiments we used the same concentration of DMSO (2.5%) and hence were able to compare for the leakage experiments and the ThT assays the shape of the curve and the lag time.

2.3. Preparation of LUVs

LUVs were prepared of POPC, POPS or POPC/POPS in a 7:3 molar ratio. Stock solutions of POPC and POPS in chloroform at concentrations of 20–30 mM were used alone or mixed in a 7:3 molar ratio in a glass tube. The solvent was evaporated with dry nitrogen gas yielding a lipid film that was further dried under vacuum for at least 30 min. Lipid films were hydrated in 10 mM Tris–HCl, 100 mM NaCl (pH 7.4) during at least 30 min, at a lipid concentration of 10 mM. The lipid suspensions were subjected to 10 freeze-thaw cycles, at temperatures of approximately −80 and 40 °C, and subsequently extruded 10 times through 0.2 μm pore size filters (Anotop 10, Whatman, Maidstone, U.K.). The phospholipid content of lipid stock solutions and vesicle preparations was determined as inorganic phosphate according to Rouser et al. [22]. Calcein-containing LUVs were made using the same protocol, except for the following adaptations. The buffer for hydration of the lipid films was replaced with a buffer containing 70 mM calcein and 10 mM Tris–HCl (pH 7.4). Free calcein was separated from the calcein-filled LUVs using size-exclusion column chromatography (Sephadex G-50 fine) and elution with 10 mM Tris–HCl, 100 mM NaCl (pH 7.4).

2.4. Thioflavin T assay

The kinetics of fibril formation was measured using the fluorescence intensity increase upon binding of the fluorescent dye ThT to fibrils. A plate reader (Spectrafluor, Tecan, Salzburg, Austria) and standard 96-well flat-bottom black microtiter plates in combination with a 430 nm excitation filter and a 535 nm emission filter were used as described previously [14]. The ThT assay in the presence of membranes was started via addition of 5 μL of a 0.2 mM peptide stock solution in DMSO to 195 μL of a mixture of 10 μM ThT, LUVs (45 μM lipids) and 10 mM Tris–HCl, 100 mM NaCl (pH 7.4). The ThT assay in solution was performed using the same method but without the addition of the LUVs. The microtiter plate was shaken for 9 s, directly after addition of all components but not during the measurement.

2.5. Electron microscopy

Peptides and LUVs were incubated under the same conditions as in the thioflavin T assay. Aliquots (20 μL) of this mixture were adsorbed onto glow-discharged carbon-coated 300-mesh copper grids for 2 min. The grids were then blotted, and dried. Next, the grids were negatively stained for 45 s on 2% uranyl acetate, and again, blotted and dried. The grids were examined using a Technai 12 electron microscope operating at 120 kV.

2.6. Membrane leakage experiments

A plate reader and standard 96-well transparent microtiter plates in combination with a 485 nm excitation filter and a 535 nm emission filter were used as described previously [14]. The leakage assay was started by adding 5 μL of a 0.2 mM peptide stock solution in DMSO to 195 μL of a mixture of calcein containing LUVs (100 μM lipid, peptide: lipid 1:20) and 10 mM Tris–HCl, 100 mM NaCl (pH 7.4). The DMSO concentration of all samples was matched to 2.5% (v/v). The microtiter plate was shaken for 9 s, directly after addition of all components and not during the measurement. The maximum leakage at the end of each measurement was determined with Triton X-100 as reported previously [14].

2.7. CD spectroscopy

CD spectra were recorded on a Jasco 810 spectropolarimeter (Jasco Inc., Easton, MD) over the wavelength range 190–270 nm. Measurements were taken in cells with a path length of 0.1 mm at room temperature in 10 mM phosphate buffer (pH 7.4) and in buffer containing either POPC, POPS or POPC/POPS (7:3) LUVs. Measurements were taken every 0.2 nm at a scan rate of 20 nm/min. Each spectrum reported is the average of five scans after subtraction of the baseline spectrum of the buffer and vesicles without peptide. Peptide concentrations were 25 μM in buffer and 50 μM in the presence of lipids (1:20 peptide:lipid ratio). In order to estimate the peptide secondary structure content, an analysis of relevant CD spectra was carried out using the CDFriend software [23] and the CDPro2 program [24]. Both analyses gave the same results (less than 5% difference).

3. Results

3.1. hIAPP$_{1-19}$ is weakly fibrillogenic in solution, but not fibrillogenic in the presence of membranes

The amino acid sequences of full-length hIAPP and hIAPP$_{1-19}$ are shown in Fig. 1. We first investigated whether hIAPP$_{1-19}$ can form amyloid fibrils in the absence of membranes by using transmission electron microscopy (TEM) and we followed the kinetics of fibril formation using the amyloid specific thioflavin (ThT) binding assays [25]. These results are shown in Fig. 2A–C. Fig. 2A shows that full-length hIAPP forms fibrils within 1 day of incubation in buffer. The fibrils exhibited the typical morphology of amyloid fibrils with widths between 10 and 15 nm. For hIAPP$_{1-19}$ at the same concentration, even after 4 days of incubation, only a few fibrils were observed (Fig. 2B). The population of hIAPP$_{1-19}$ fibrillar assemblies consisted of thin fibrils with a width of 6–10 nm that, contrary to full-length hIAPP, do not appear to have a strong propensity to cluster. As shown in Fig. 2C, analysis of the kinetics of fibril formation in the absence of vesicles yields an S-shaped curve for full-length hIAPP, which is a well-known characteristic of hIAPP fibril formation [26]. In contrast, the data do not show any increase in time in fluorescence for hIAPP$_{1-19}$, supporting the notion that hIAPP$_{1-19}$ is only weakly fibrillogenic.

The same experiments were carried out in the presence of vesicles composed of POPC/POPS in a molar ratio of 7:3 (Fig. 2D–F). Full-length hIAPP forms fibrils within a day of incubation, with a similar fibril morphology as in the absence of membranes (Fig. 2D), whereas even after 15 days of incubation with POPC/POPS vesicles, hIAPP$_{1-19}$ does not form fibrils (Fig. 2E). The ThT assays are in agreement with the TEM results, as they confirmed the absence of hIAPP$_{1-19}$ fibrils after 12 days (Fig. 2F). For full-length hIAPP, the ThT assays show an increase in fluorescence related to fibril formation and an S-shaped curve, as shown previously. Qualitatively similar results were observed with LUVs composed of only POPC or only POPS (data not shown). These results demonstrate that hIAPP$_{1-19}$ is weakly fibrillogenic in solution and not fibrillogenic in the presence of membranes, suggesting that in this case, contrary to full-length peptide, the membranes in fact inhibit fibril formation.

3.2. hIAPP$_{1-19}$ does not induce membrane leakage under physiological membrane compositions

Next, membrane leakage experiments were performed under the same conditions as used for fibril formation. Membrane damage was assayed quantitatively by analyzing the extent of leakage of a fluorescent dye (calcein) from LUVs. As shown in Fig. 3A, full-length hIAPP induces significant leakage in POPC/POPS LUVs to about 80% of the total vesicle content. The process of leakage is characterized by an S-shaped curve with a lag time of approximately 2 h. In contrast to full-length hIAPP, hIAPP$_{1-19}$ is not able to induce significant membrane damage (only <5% leakage) using the same experimental conditions. With POPC LUVs, a qualitatively similar result is observed; an S-shaped curve is obtained for full-length hIAPP, but now with a smaller extent of leakage (~25%) and a longer lag time of approximately 8 h (Fig. 3B), while hIAPP$_{1-19}$ does not significantly affect barrier properties of the membrane (<5% leakage). In the presence of POPS LUVs different results are obtained: both peptides, full-length hIAPP and hIAPP$_{1-19}$, induce considerable leakage during the first hours and then a plateau is reached (Fig. 3C). Importantly, a lag phase is not observed in this system, and the leakage increases exponentially from the start of the incubation. In addition, we should note that in none of our experiments we reached the maximum value of 100% leakage. The reason is that the plateau value for leakage depends on the peptide concentration and on the lipid:peptide ratio. We have used a peptide concentration of 5 μM and a lipid:peptide ratio of 9:1, similar to our previous studies [14,20], but not sufficient to reach 100% leakage. As has been published, a high peptide concentration of 50 μM hIAPP$_{1-37}$ combined with a low lipid:peptide ratio of 4:1 is required to obtain 100% leakage [27].

peptides	Sequences
hIAPP$_{1-37}$	KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY-NH$_2$
hIAPP$_{1-19}$	KCNTATCATQRLANFLVHS-NH$_2$

Fig. 1. The amino acid sequence of full-length hIAPP and hIAPP$_{1-19}$. Both peptides have an intramolecular disulfide bridge and an amidated C-terminus.

3.3. Like hIAPP$_{1-37}$, hIAPP$_{1-19}$ adopts an α-helical conformation in the presence of negatively charged lipids

We used CD spectroscopy to investigate the secondary structure of full-length hIAPP and hIAPP$_{1-19}$ in the absence and presence of membranes at the start of the incubation. The CD spectra of full-length hIAPP and of hIAPP$_{1-19}$ freshly dissolved in 10 mM phosphate buffer at 25 μM are nearly identical, displaying a peak with negative ellipticity at 200 nm that is characteristic of a random coil conformation (Fig. 4A). Even at a high peptide concentration of 50 μM, hIAPP$_{1-19}$ is unstructured, whereas at this concentration hIAPP$_{1-37}$ starts showing β-sheet structure (results not shown). This is in agreement with the lower amyloidogenic potential of hIAPP$_{1-19}$, as observed by EM (Fig. 2).

The same experiments were conducted in the presence of membranes. At these short incubation times, full-length hIAPP adopts a mixture of mostly α-helical and random coil structure in the presence of all lipids, but with the highest α-helical content in PS (Fig. 4B). In the presence of negatively charged lipid (POPC/POPS mixtures and POPS), the spectrum of hIAPP$_{1-19}$ displays negative ellipticity at 208 and 222 nm, characteristic of an α-helical backbone structure (Fig. 4C). However, without negatively charged lipids (POPC only), hIAPP$_{1-19}$ shows a spectrum indicative of random coil conformation (Fig. 4C), similar to that of hIAPP$_{1-19}$ in solution.

3.4. hIAPP$_{1-19}$ retains its initial conformation upon incubation with lipid membranes, while full-length hIAPP undergoes a conformational rearrangement

To investigate the relationship between fibril formation and membrane leakage by full-length hIAPP and hIAPP$_{1-19}$, we performed CD measurements to analyse the conformational changes of the peptides after a few hours of incubation in the presence of membranes. Fig. 5 shows that in the presence of POPC/POPS or POPC, while full-length hIAPP adopts a β-sheet structure, hIAPP$_{1-19}$ retains respectively its α-helical conformation and its random coil configuration during at least 2 h. In the presence of POPS both peptides retain an α-helical structure for few hours (Fig. 5). The α-helical structure of hIAPP$_{1-19}$ persisted even after 1 day (not shown). These results provide evidence that PS-containing membranes promote an α-helical conformation in membrane-bound hIAPP. In addition, in the presence of the tested lipid membranes, hIAPP$_{1-19}$ does not change its conformation in time.

4. Discussion

Recent studies have shown that the ability of amyloidogenic peptides to induce disruption of lipid bilayers is correlated with the toxicity of such peptides [8,28–30]. Studies on model membranes have shown that membrane permeabilization by amyloidogenic peptides is highly dependent on the lipid composition of the membrane [7,11]. Therefore, an understanding of lipid specificity of amyloid peptide-lipid interactions can lead to a better understanding of the mechanism of amyloid cytotoxicity. In the present study we have investigated and compared the aggregational behaviour in relation to membrane damage

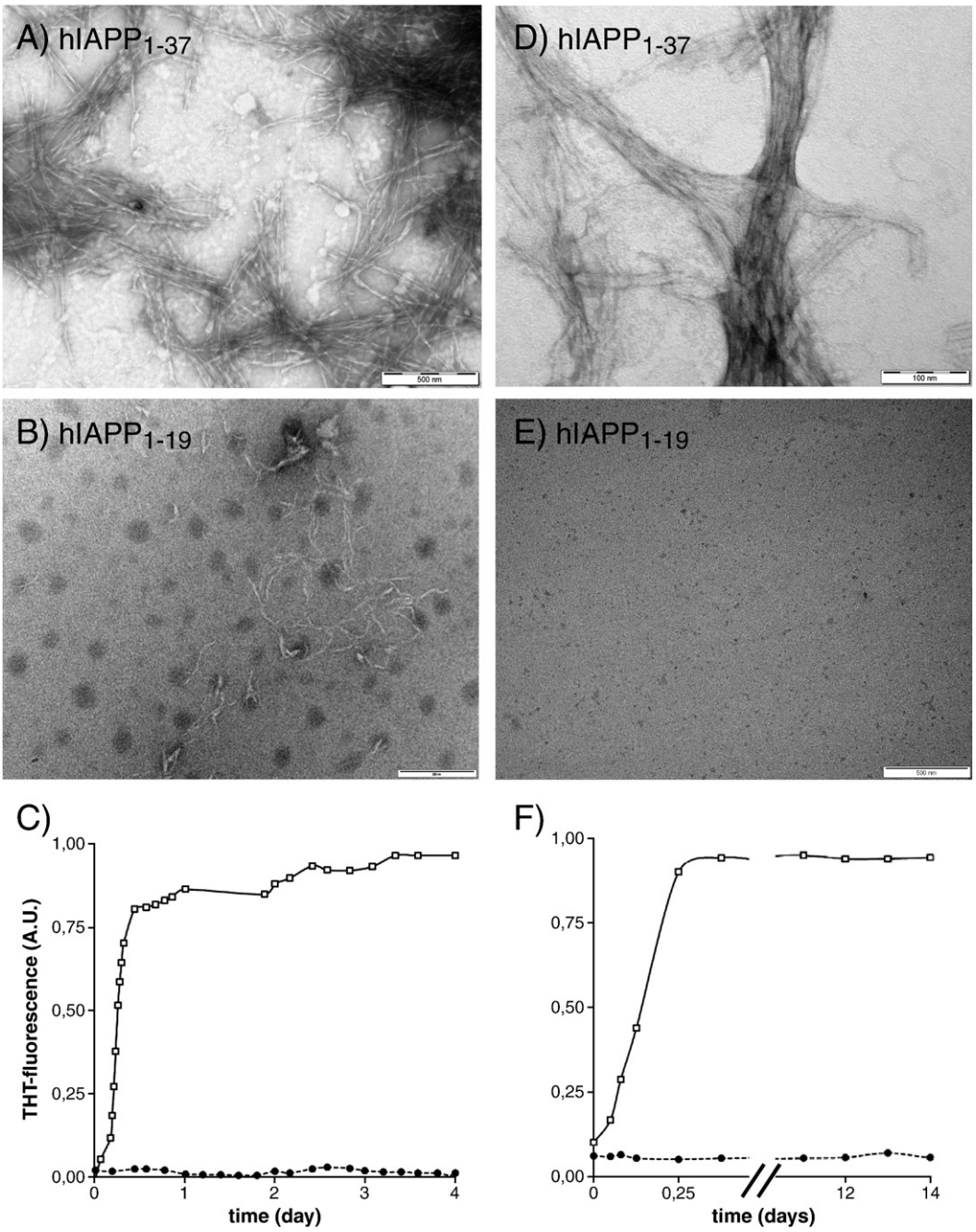

Fig. 2. Negatively stained electron microscopy images of full-length hIAPP and $hIAPP_{1-19}$ at 5 μM. (A) Full-length hIAPP after 1 day of incubation in solution and (D) in presence of POPC/POPS LUVs. (B) $hIAPP_{1-19}$ after 4 days of incubation in solution and (E) after 15 days of incubation in the presence of POPC/POPS LUVs. Scale bars are 500 nm except in (E) where it is 100 nm. ThT fluorescence of fibril formation of full-length hIAPP (solid with open squares) and $hIAPP_{1-19}$ (dashed with filled circles) at 5 μM. (C) Fibril formation in solution and (F) in the presence of POPC/POPS LUVs (peptide:lipid ratio 1:9).

of full-length hIAPP and of $hIAPP_{1-19}$ in lipid membranes of different composition (PC only, PS only and a mixture of PC/PS). We have made the following key observations: i) $hIAPP_{1-19}$ does not form fibrils in the presence of membranes of varying lipid composition as used in this study, whereas full-length hIAPP does, ii) $hIAPP_{1-19}$ does not induce leakage of membranes without or with a limited content of anionic lipids (PC or PC/PS 7/3), whereas again full-length hIAPP does, iii) $hIAPP_{1-19}$ does induce leakage in membranes composed of anionic lipids (PS) only, but this is not accompanied by fibril formation, and iv) $hIAPP_{1-19}$ has a conformation that remains stable for several days upon incubation with LUVs of varying lipid composition. These findings are discussed below, together with potential implications for islet amyloid formation and for the cytotoxic mechanism of action of hIAPP.

The EM, CD and ThT fluorescence studies of $hIAPP_{1-19}$ reveal several interesting features. The EM results suggest that in solution this peptide is markedly less fibrillogenic than full-length hIAPP. Only a few fibrils of $hIAPP_{1-19}$ were found in different samples after several days of incubation. These results are consistent with our ThT fluorescence and CD data that show no increase in ThT signal after a few days of incubation, and predominantly random coil structure for $hIAPP_{1-19}$, even at high peptide concentrations. In the presence of different membranes (POPC, POPS and POPC/POPS) our EM experiments did not show any evidence at all of fibril formation for $hIAPP_{1-19}$, even after 15 days of incubation. In addition, the ThT fluorescence did not increase during longer incubation times and CD spectroscopy indicated the absence of β-sheet structure. Hence, these experiments

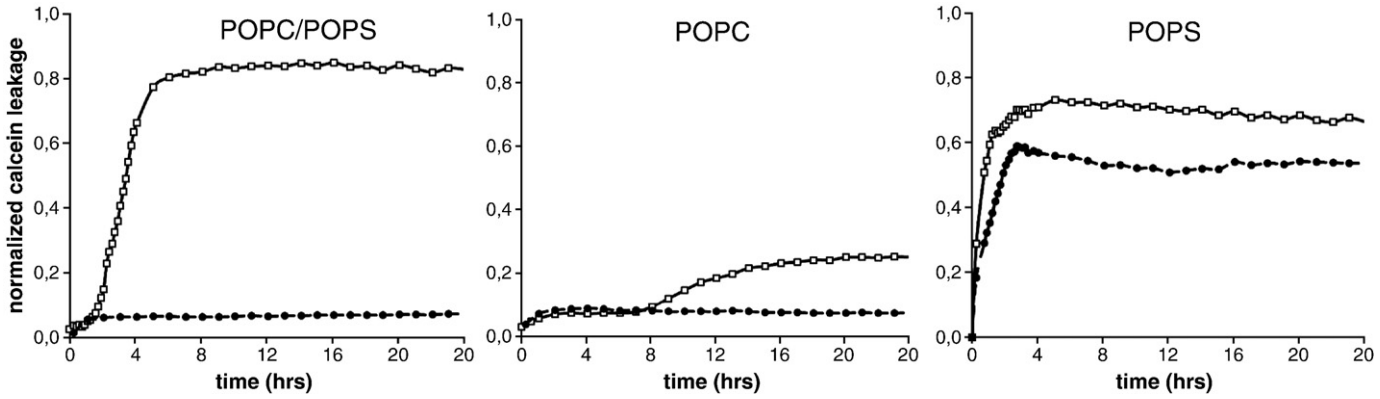

Fig. 3. Kinetics of membrane permeabilization induced by 5 μM full-length hIAPP (solid with open squares) and 5 μM $hIAPP_{1-19}$ (dashed with filled circles). The peptides were added to calcein containing POPC/POPS (7:3) LUVs (left), POPC LUVs (middle), and POPS LUVs (right) at $t = 0$ (peptide:lipid ratio 1:20). Leakage shown after 1 day is the average of three experiments with three different peptide stock solutions, of which each experiment was performed in triplicate. The maximum leakage, after complete disruption of all vesicles by Triton, was set at 1.

strongly suggest that membranes inhibit fibril formation of $hIAPP_{1-19}$. Our result is consistent with the recent notion, based on simulation studies, that highly fibrillogenic peptides form fibrils more rapidly in the presence of lipid vesicles than in their absence, whereas the opposite is observed for peptides of low fibrillogenicity, where fibril formation is slower in the presence of lipid vesicles [15].

Upon initial interaction with membranes, full-length hIAPP adopts an α-helical structure. A transition of α-helical to β-sheet structure is observed in membranes without or with limited content of anionic lipid (PC and PC/PS). This transition results in fibril formation that is correlated to membrane damage. This transition has been observed for many amyloidogenic proteins. Indeed, the Aβ peptide (aggregating in Alzheimer's disease) and the N-terminal part of α-synuclein (aggregating in Parkinson's disease) adopt an α-helical structure before transformation to β-sheet structure [31,32]. For $hIAPP_{1-19}$, a different behaviour was observed: the structure of the peptide is stable in time (random coil in PC and α-helical in PC/PS membranes, respectively) and no transition to β-sheet structure occurs. In addition, this peptide does not form fibrils and does not induce membrane leakage of PC and PC/PS membranes. In membranes composed of anionic lipids (PS) only, both peptides adopt an α-helical structure that is stable for a few hours and this is associated with membrane damage. Hence, both peptides seem to induce membrane damage that is not correlated to fibril formation. This notion is further supported by the absence of a lag phase in kinetic experiments, as explained in the next paragraph.

Previously, for full-length hIAPP a correlation was found between fibril formation and peptide-induced membrane damage [14]. By studying the kinetics of hIAPP-induced membrane damage in relation to hIAPP fibril growth, it was found that the kinetic profile of hIAPP-induced membrane damage is characterized by a lag phase and a sigmoidal transition, which matches the kinetic profile of hIAPP fibril growth. Moreover, it was found that pre-formed fibrils do not induce membrane damage. It was thus postulated that growth of hIAPP fibrils at the membrane surface causes hIAPP-induced membrane damage [14], in agreement with simulation experiments [15]. Our present data show that $hIAPP_{1-19}$, although being implicated in membrane insertion of hIAPP, is not able to form fibrils in the presence of membranes and, as expected, does not induce leakage of membranes composed of PC or PC/PS 7/3. These results are thus consistent with the hypothesis that under physiological conditions, the processes of fibril formation and membrane damage are causally related [14]. On the other hand, in POPS LUVs both $hIAPP_{1-19}$ and full-length hIAPP induce leakage that is characterized by an immediate, exponential increase without a lag phase. Importantly, CD data show that under these conditions both peptides remain helical for few hours, indicating that the observed leakage is not related to β-sheet formation that is characteristic of amyloid fibrils. Similar results

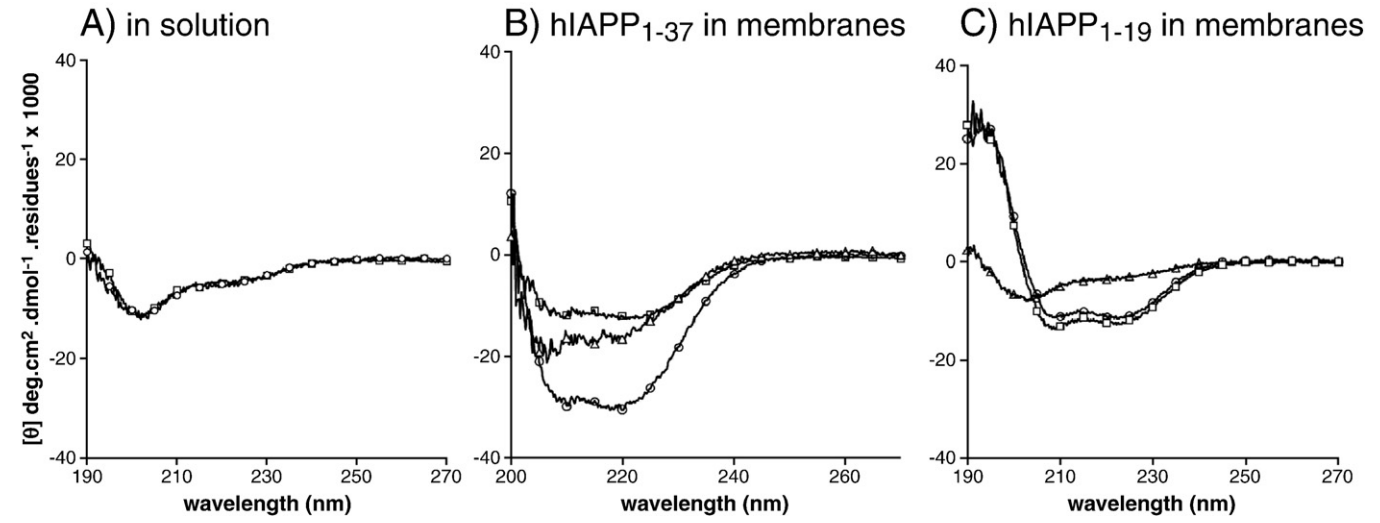

Fig. 4. (A) CD spectra at 25 μM concentrations of $hIAPP_{1-19}$ (squares) and full-length hIAPP (diamonds) in 10 mM phosphate buffer (pH 7.4). CD spectra at 50 μM concentrations of full-length hIAPP (B) and of $hIAPP_{1-19}$ (C) in the presence of POPC LUVs (triangles), POPC/POPS 7:3 LUVs (squares) and POPS LUVs (circles) (peptide:lipid ratio 1:20).

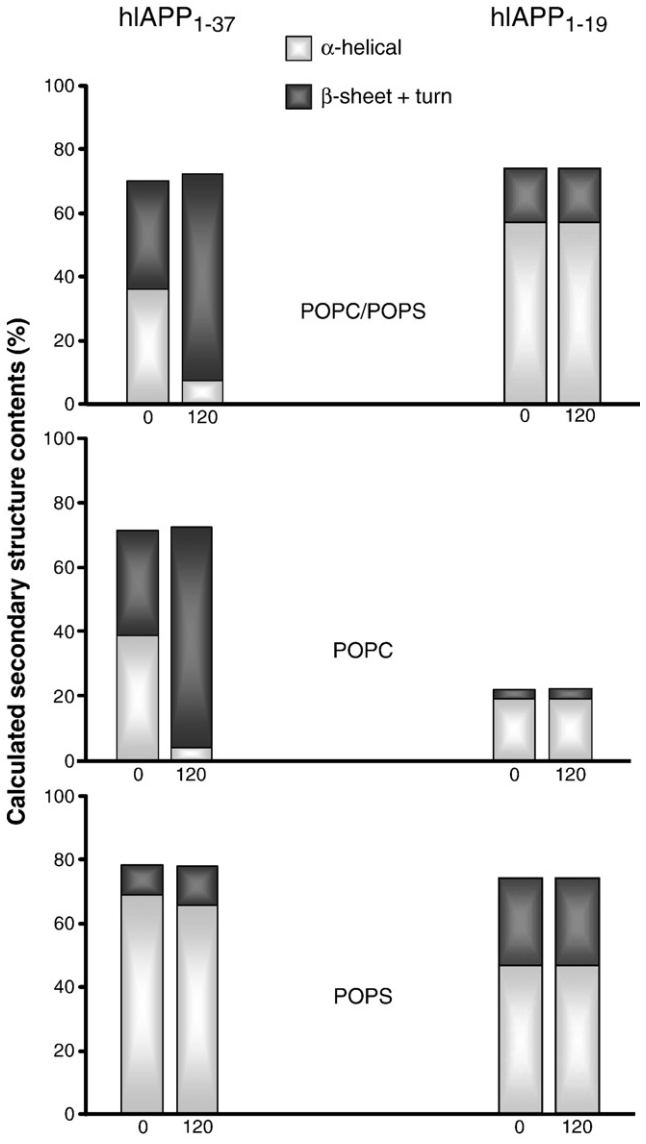

Fig. 5. Calculated secondary structure contents of 50 μM hIAPP$_{1-19}$ and 50 μM full-length hIAPP in the presence of POPC/POPS LUVs (top), POPC LUVs (middle) and POPS LUVs (bottom) obtained immediately after the addition of the LUVs and after 120 min of incubation with the LUVs (peptide:lipid ratio 1:20). Deconvolution of CD spectra was accomplished using the CDFriend software (see Materials and methods).

have been obtained by another research group, who showed that both full-length hIAPP and hIAPP$_{1-19}$ induce membrane disruption in anionic lipid vesicles and favor the formation of negative curvature, indicating that these peptides may induce the formation of pores by the induction of excess membrane curvature [12,33]. Our results show that both peptides, full-length hIAPP$_{1-37}$ and hIAPP$_{1-19}$, insert into anionic membranes with an α-helical structure, suggesting that they could damage the membrane via another mechanism, possibly a carpet type as reported for antimicrobial peptides [34].

In conclusion, our data suggest that in membranes without or with a limited content of anionic lipids (PC or PC/PS 7/3) the N-terminal part of hIAPP is not involved in fibril formation, nor in fibril growth mediated membrane leakage. However, in anionic lipid (PS) membranes, both full-length hIAPP$_{1-37}$ and hIAPP$_{1-19}$ induce membrane damage that is not correlated to fibril formation. The different behaviour of the peptides in the presence of membranes composed of negatively charged lipids may be related to a strong tendency for a fibril formation inhibiting, parallel orientation of the α-helical 1–19 fragment at the membrane [35]. The different results found in the literature are most likely due to the lipid membrane composition. We should note that the use of an 'extreme' system such as membranes composed for 100% of anionic lipid could provide information which helps to understand peptide/lipid interactions and the role of electrostatic interactions. However, it does not reflect peptide behaviour in physiologically relevant conditions.

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