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Interfacial Aβ fibril formation is modulated by the disorder-order state of the lipids: The concept of the physical environment as amyloid inductor in biomembranes

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ABSTRACT

The behavior of amphiphilic molecules such as lipids, peptides and their mixtures at the air/water interface allow us to evaluate and visualize the arrangement formed in a confined and controlled surface area. We have studied the surface properties of the zwitterionic DPPC lipid and Aβ(1–40) amyloid peptide in mixed films at different temperatures (from 15 to 40 °C). In this range of temperature the surface properties of pure Aβ(1–40) peptide remained unchanged, whereas DPPC undergoes its characteristic liquid-expanded → liquid-condensed bidimensional phase transition that depends on the temperature and lateral pressure. This particular property of DPPC makes it possible to dynamically study the influence of the lipid phase state on amyloid structure formation at the interface in a continuous, isothermal and abrupt change on the environmental condition. As the mixed film is compressed the fibril-like structure of Aβ(1–40) is triggered specifically *in the liquid-expanded region*, independently of temperature, and it is selectively excluded from the well-visible liquid condensed domains of DPPC. The Aβ amyloid fibers were visualized by using BAM and AFM and they were Thio T positive. In mixed DPPC/Aβ (1–40) films the condensed domains (in between 11 mN/m to 20 mN/m) become irregular probably due to the fibril-like structures is imposing additional lateral stress sequestering lipid molecules in the surrounding liquid-expanded phase to self-organize into amyloids.

1. Introduction

The enzymatically misprocessing of the amyloid precursor protein (APP) produces the Aβ amyloid peptide isoforms Aβ(1–40) and Aβ(1–42) [1]. The observation of self-aggregated Aβ peptides in the form of compact β-sheet fibrils in the extracellular environment is one of the hallmarks of Alzheimer's disease (AD) [1–3]. However, the aggregation process carried out close to the membrane lipids may be associated to early events that have not been clearly understood yet and they could play a role in the triggering the neurotoxic forms leading to AD. In this connection, either Aβ(1–40) or Aβ(1–42) segments is forming part of the transmembrane portion of APP; thus the interactions of Aβ peptides with the membrane lipids are important [4] since the lipid phase plays a determining role in the structural properties of the membrane [5,6], that could drive the amyloid fibers formation. Even when, molecular simulation studies support the idea that the Aβ amyloid at the membrane surface favors the aggregation probably due to the diffusion occurs in two dimensions [7]. However, a mere high surface concentration of confined Aβ amyloid is not enough for fibril formation and a liquid-expanded lipid phase is required as inductor [8,9].

Additionally, by using infrared reflection absorption spectroscopy (IRRAS) in adsorption experiments into negatively charged lipids such as DPPS, DPPG and DMPG were used to evaluate the influence of lipid monolayers in both: the lipid packing and peptide conformation. In this way, the Aβ amyloid peptide desorbs from the negatively charged monolayer when the subphase is buffer [10]. Similarly, occur when the lipid monolayer is formed by zwitterionic phospholipid such as DPPC. However, when the subphase is pure water the Aβ amyloid peptides kept

Abbreviations: DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine; ThT, Thioflavin T; POPC, 1-palmitoyl-2-oleoyl-phosphatidylcholine; (Aβ), amyloid peptides; PAP, percentage of peptide area proportion in the mixed peptide/lipid film.

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adsorbed to the lipid monolayer in a β-sheet conformation [10]. In contrast with the above idea, others researchers have found that both factors: the lipid charge and lipidic phase disturb the membrane/amyloid interaction and may not only alter the Aβ amyloid aggregation state and the membrane structure [11,12] but also others amyloidogenic proteins such as medin, associated with amyloid deposits in the medial layer of human arteries [13]. However, it is discussed the role of zwitterionic lipids such as DPPC in the Aβ amyloid insertion, claiming a low lipid density as a requirement [14,15] but not at 25–30 mN/m [16] which is the range taken as the equivalent lateral packing of bilayers [17,18]. In this connection, recently works by Ahyayauch et al. showed that the lipids in the liquid-disorder state can facilitate the Aβ(1–42) binding into bilayers and monolayers [19,20].

By using Langmuir monolayers, in the present work we have found that Aβ amyloid peptide is able to form fibril-like structures from 1 mN/m to 20 mN/m only *in the fluid phase* of DPPC even in between the lipid condensed domains as the film is compressed and goes from the liquid-expanded to liquid-condensed state. We obtained images showing how the Aβ amyloid fibril-like structures distort the condensed domains inducing a different irregular shape compared to pure lipid probably due a lipid extraction process to stabilize de Aβ amyloid fibers. We pretend highlight that the lipid phase at the interface is an important supramolecular arrangement that support and give the framework for the formation of Aβ amyloid fibril-like structures. We hypothesized that if equivalent molecular conditions are given at the neuron membrane, they may trigger the initial steps in the pathogenesis of the AD by changing membrane homeostasis before the typical plaques appeared in the brain.

2. Material and methods

2.1. Chemicals

Aβ(1–40) (H-D^{1}AEFRHDSGYEVHHQKLVFFAEDVG25SNKGAIIGLM VGGVV-OH^{40}), MW = 4329.86, purity by HPLC > 95.9 % according to the manufacturer, were purchased from Bachem AG, U.S.A. (www.bachem.com). The subphase was NaCl 145 mM. Water was obtained from a Milli-Q system (Millipore, USA) with a conductivity <0.085 μS·cm^{-1} and a surface tension of 72 mN/m. NaCl from Merck (Gottingen, Germany) was roasted in an appropriate oven at 400–450 °C for at least 4 h to eliminate small amounts of surfactant impurities. 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC), were purchased from Avanti Polar Lipids, USA. Dimethyl sulfoxide (DMSO) and chloroform were from Merck, Göttingen, Germany.

2.2. π-Area isotherms and film balance setting

Langmuir-film experiments were performed essentially as described previously [9,21]. Subphase was unbuffered NaCl 145 mM, pH 5.6. The Teflon trough has a volume of 180 ml. The temperature was set as it is specified ±0.5 °C with a thermostatized circulating Haake bath. Aβ (1–40) peptide and the DPPC lipid were dissolved in pure DMSO or pure chloroform, respectively, into stock solutions of 10 mM. Individual diluted daily working spreading solutions were freshly prepared to ~1 mM. For mixed monolayers, Aβ(1–40) peptide and lipid were premixed from their respective individual solvent solutions at the desired proportion just before spreading. The spreading of 15–30 μl of the mixture onto the subphase interface was performed with a Hamilton syringe. Surface pressure was determined with a Pt plate using the Wilhelmy method. The continuous compression was achieved through an automatic, simultaneous, and symmetric movement of both barriers that confined the film. The total film area was continuously recorded using the software provided by KSV Instruments® Ltd. The usual compression rate was adjusted to 10 mm^{2}.min^{-1}, lower barrier speeds did not alter the results. The surface potential or ΔV was measured with a vibrating plate collocated at the surface and coupled to a reference electrode and setting to cero in the free surfactant subphase before spreading the film forming components. The percentage of peptide area proportion in the mixed peptide/lipid film (PAP) is defined as the corresponding proportional covered area (in percentage) by protein in the mixed film calculated from their individual molecular areas taken at a same lateral pressure. The PAP gives a better dimension of the proportion of the peptide covered surface area with respect to the lipid than the individual mole fractions when their respective molecular weights are very dissimilar with a high difference in the individual molecular areas [9,21,22].

2.3. Transferred films experiment for atomic force and fluorescence microscopy

Compressed monolayers up to a desired surface pressure were transferred to mica support (Nano-Tec V-1 grade Muscovite of 10 mm diameter and 0.15–0.21 mm thickness, from Innovative Microscopy Suppliers, Argentina); the mica support is packed in several layers which were used by removing one layer per experiment. The mica face was rinsed with distilled water before transferring the surface material. Langmuir monolayers were then transferred to the support by dipping the mica, perpendicular oriented to the trough previously submerged, at the smallest speed (1 mm·min^{-1}) keeping the surface pressure constant with a surface barostat coupled to the barriers of the KSV equipment. Before transferring, the monolayer was allowed to stabilize (~2 min). Each monolayer of either pure components or their mixtures were first compressed up to 20 mN/m before transferring to the mica support.

2.4. Atomic force microscopy

AFM measurements were performed on a multimode atomic force microscope, SPM, Agilent Technology 5500, USA. We used the mica support as described above. It was used in contact-mode scanning to measure the transferred the Aβ(1–40) peptide/lipid mixed monolayers and their respective controls (pure DPPC lipid o pure Aβ(1–40) peptide). All of the experiments were carried out at room temperature. Resolution images of 512 × 5.

12 pixels were collected at a scanning rate in between 0.3 and 0.4 ln·s^{-1}. The height and error-signal (vertical deflection), friction, and topography images were taken simultaneously.

2.5. Brewster angle microscopy

Monolayers were spread over a Langmuir film balance, as described above, and the films were directly observed along all the π-area isotherm using a BAM coupled to an EP3 Imaging Ellipsometer equipment (Accurion, Göttingen, Germany) with a 20× objective (Nikon, Japan, NA 0.35). For each imaging acquisition, the monolayer was compressed by forward compression steps of 5 mN/m. During imaging acquisition (5–20 s), the lateral pressure did not exhibit any detectable variation. Three to five images were sequentially taken for each surface pressure, in order to have pictures of different regions of the same monolayer. Results acquired with BAM were compared with those images obtained by using AFM and FM.

2.6. Imaging analysis

The fibril-like structures were visualized by FM by adding a drop of aqueous freshly prepared 10 μM ThT probe added to the transferred monolayer onto the mica support and leaving to dry off for 5 min. Excitation wavelength was selected with a cutting filter of 450 nm, and the emission was taken at 482 nm. Negative controls were done with either pure ThT, pure lipid o pure Aβ peptide subjected to the same procedure (Fig. 4A). As a positive control, we tested preformed fibrils of α-synuclein formed in bulk condition according to Gallea and coworkers [23] and treated in the same way with ThT by using a

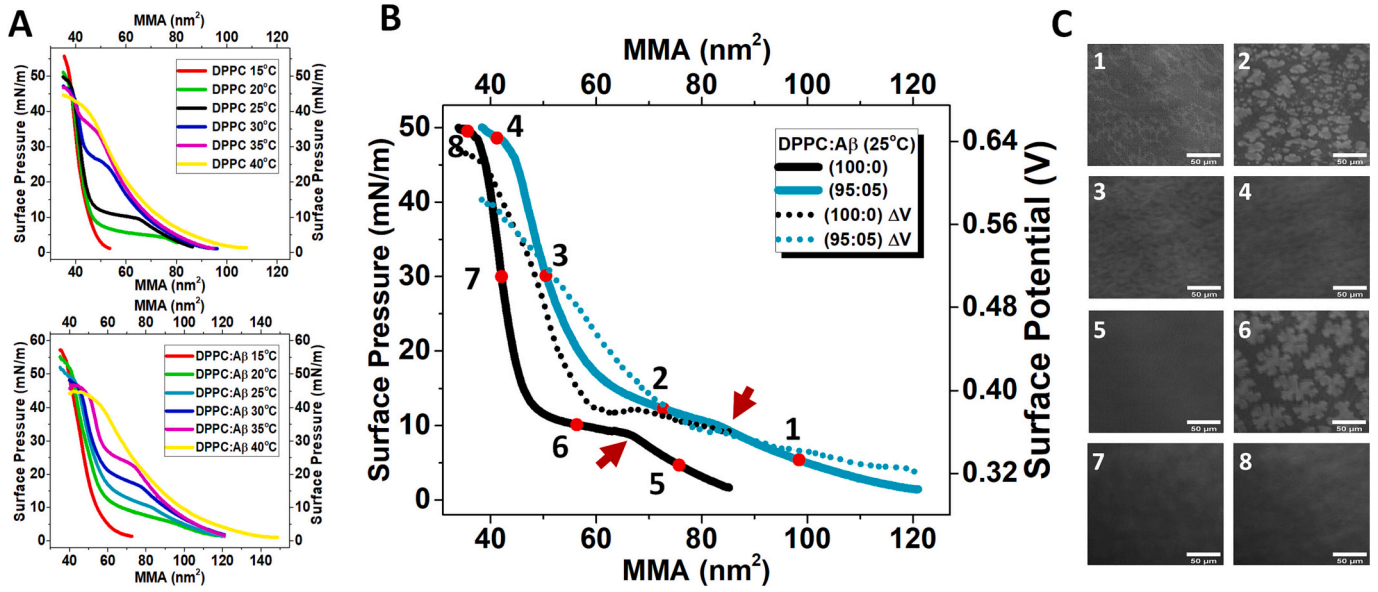

Fig. 1. Surface behavior and BAM imaging of pure DPPC lipid and DPPC/Aβ(1–40) mixed films at different temperatures. A: Temperature dependence isotherms of pure DPPC lipid (upper part) and DPPC/Aβ(1–40) (95:05) mixture (lower part). The temperature is indicated by the color in the inserts. B: Isotherms (solid line) and surface potential curves (dotted line) of pure DPPC lipid (black) and DPPC/Aβ(1–40) (95:05) mix (blue) at 25 °C. Red arrows point out the beginning of the transition from liquid-expanded to liquid-condensed phase. Each red dot marks the surface pressure from different phases where the BAM image were taken (showed in C). The surface potential or ΔV is the potential across the interface of the film compared to pure subphase that it is taken as cero.
C: BAM imaging from pure DPPC lipid (lower part, pictures 5, 6, 7 and 8) and DPPC/Aβ(1–40) mixture (upper part, pictures 1, 2, 3 and 4). The images correspond for each red dot showed in B. The DPPC lipid and Aβ(1–40) were mixed at (95:05) of PAP where we observed the formation of fibril-like structures.

coverslip glass (Fig. 4A). The images were acquired with an Axioplan Carl Zeiss (Germany) fluorescence microscope equipped with an Olympus video camera (USA). We used a 20× and 40× objectives. The imaging processing and analysis were carried out using both Fiji and Pixinsight v. 1.8.6 software. Both programs allowed us to use some filters and 2D fast Fourier transform to improve imaging resolution.

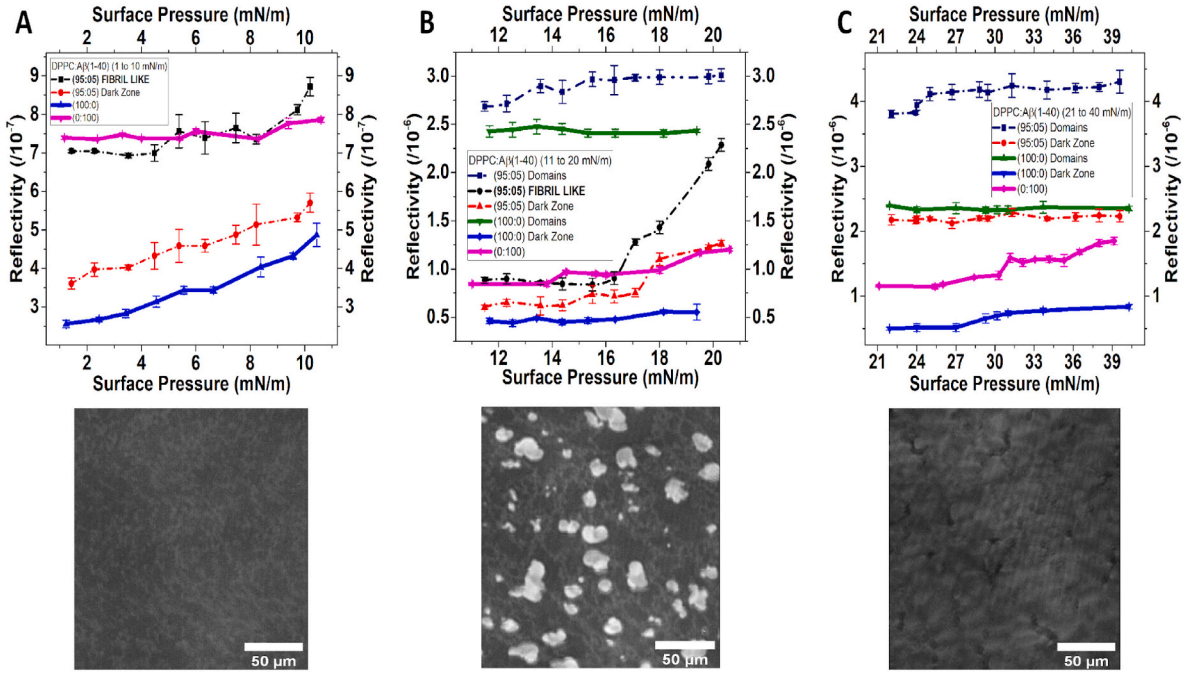

Fig. 2. Reflectivity and BAM imaging analysis of DPPC:Aβ(1–40) film at 25 °C. A: Reflectivity values of liquid-expanded phase showing two measurable regions in BAM imaging intensity from 1 to 10 mN/m. Low reflectivity values are similar to pure DPPC lipid (blue color); high reflectivity range of values correspond to pure film Aβ(1–40) (pink color) B: Reflectivity values corresponding to the gel phase indicating three distinguishable zones in BAM imaging from 11 to 20 mN/m. Lower reflectivity values in blue color represents the dark zone in pure DPPC lipid. The intermediate reflectivity values correspond to pure Aβ(1–40) (pink color). High reflectivity values are close to domains zone in pure DPPC lipid (green color). C: Reflectivity values from 21 to 40 mN/m of solid condensed phase showing the same three different zones in BAM imaging represented in B. The corresponding BAM imaging are showed for each range of lateral packing.

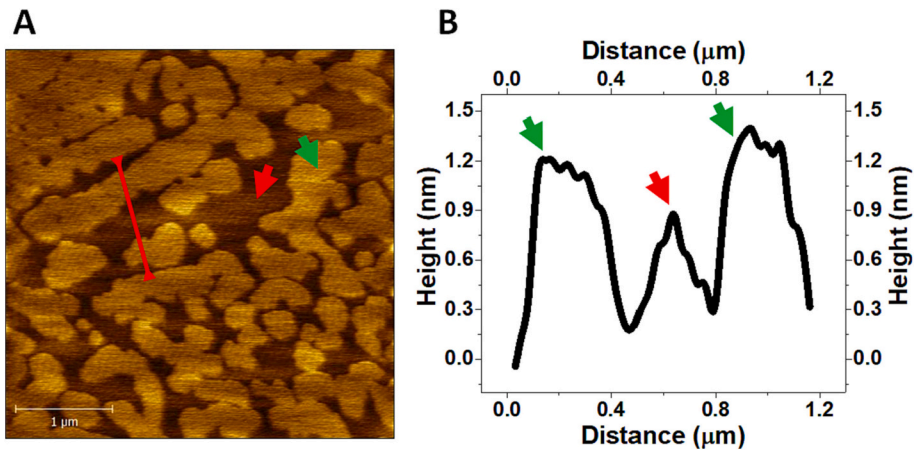

Fig. 3. AFM imaging analysis of DPPC:Aβ(1–40) mixed films. A: AFM imaging topography from mixed DPPC/Aβ(1–40) at 95:05 of PAP compressed up to 20 mN/m and transferred to mica support, at 25 °C. The red line marks the fibril like structures between the domains whose relative heights are then shown in B. Both the red and green arrows mark the fibers and lipid domains, respectively. B: It is shown the height of the fibril-like structures disposed through the domains along the red mark showed in A of 1.2 µm length. The green arrows spot the relative height of the lipid domains (1.2–1.4 nm) compare to height of fibers (~ 0.9 nm). See Fig. S2 for AFM imaging of pure DPPC.

3. Results and discussion

3.1. Surface behavior and BAM imaging of DPPC/Aβ(1–40) mixed films at different temperatures

The Langmuir monolayer technique allows the organization of amphiphilic molecules at the air-water interface forming an insoluble monolayer, which can be compressed by imposing a movable barrier [24]. The compression process generates a surface pressure (π-area) isotherm. The difference between water surface tension (γ_o) and the surface tension of the surface covered by the amphiphilic molecules (γ) is defined as surface pressure, $\pi = \gamma_o - \gamma$ [24–27].

We have reported that pure Aβ peptides form insoluble monolayer at the air-water interface with a molecular area at high packing of 2.5 nm^2/molecule and a lateral stability near to 30 mN/m [8,9]. In this way Aβ (1–40) peptides behave as a typical solid films at the interface with peculiar high rheological property [8]. So, it could be relevant the study of the interfacial behavior when the Aβ amyloid peptide is mixed with a phospholipid that undergoes a remarkable bidimensional phase transition at room temperature such as DPPC lipid (Fig. 1A). DPPC contains two C_{16} palmitic acid chains attached to a phosphatidylcholine head group, with a critical transition point of 41 °C in bulk and in excess of water [28]. Thus, when the temperature is above the 41 °C DPPC is found in liquid-crystalline state whereas below 37 °C the phospholipid is found in the gel phase [28,29]. If a pure DPPC Langmuir film is run below 37 °C, we can appreciate the typical bidimensional first order phase transition from the liquid-expanded state to liquid-condensed state upon lateral compression (see Fig. 1 and S1) as it was earlier reported [30,31].

As expected, BAM imaging of pure DPPC is homogeneous without observable structures at the liquid-expanded phase. Upon compression the forming liquid-condensed phase presents the typical irregular domains (see Fig. 1C, picture 6) [32] and begin to merge as surface pressure increases until the a complete fusion, forming the liquid-condensate phase with a homogeneous appearance in the BAM images (Fig. 1B, C lower part).

According to our previous results, Aβ(1–40) amyloid peptide was able to form fibril-like structures when it is mixed with liquid-expanded lipids (either POPC or DMPC) at low peptide proportion [9]. In the present work we studied the surface properties at different temperatures of mixed film DPPC/Aβ(1–40) at 95:05 of percentage of peptide area proportion (PAP, see Section 2.2 of Materials and Methods for definition). We highlight that the fibril-like structures are formed in the liquid-expanded phase interdigitated between the domains of the liquid-condensed phase at a lateral pressure (11–20 mN/m). Where the liquid-expanded phase is dominant (below 10 mN/m at 25 °C) the fibril-like structures is quite manifest, see Fig. 2. These amyloid structures are ThT positive (see below, Fig. 4). Thus, the bidimensional liquid-expanded → liquid-condensed phase transition imposes a new environmental condition to form fibril-like structures. It could be remarked that the formation of Aβ amyloid fibril-like structures is a selective process which markedly depends on the kind of the lipid phase surrounded the peptide. The increase in temperature is proportional to the increase in the initial point of transition pressure in both the pure DPPC and mixed DPPC/Aβ(1–40) film (Fig. 1A), and the fibril-like structures keep the pattern in the liquid-expanded phase (see Fig. S1). The domains corresponding to the liquid-condensed phase in the mixed DPPC/Aβ (1–40) films lost the original patter and typical architecture showing a more irregular shape (Fig. 1C image C2).

3.2. Reflectivity and ThT analysis of the fibril-like structures observed in DPPC/Aβ(1–40) mixtures at the air water/interface

By using BAM imaging analysis, we have achieved a better understanding of the complexity of DPPC/Aβ(1–40) mixed films. In this way, we clearly detected the fibril-like structures in the mixed films from the liquid expanded phase until the bidimensional transition phase takes place (see Fig. 2A). Particularly, the fibrils were organized in between the more irregular DPPC condensed domains (Fig. 2B lower part). A similar pattern in the formation of the fibril-like structures was observed at all temperatures assayed (see Fig. S1). BAM imaging analysis were carefully performed at three distinctives region of surface pressure: low pressure (0–10 mN/m) where the liquid-expanded was the dominant lipid phase, intermediate range (11–20 mN/m) corresponding to the region of phase transition and high lateral packing (21–39 mN/m) corresponding to the dominant liquid-condensed phase (at 25 °C, see Fig. 2). The variation of reflectivity from the BAM imaging is quite useful to determine how the fibers disappear as the more solid lipid domains are merged among them upon film compression. We have defined a specific reflectivity behavior for each observed structure allowing us to follow how the fibril-like structures get into and distort the more condensed lipid domains as the transition process takes place upon compression (Fig. 2C reflectivity values highlighted with different colors). Thus, the lipid phase controls the Aβ amyloid fibrillation at the air/water interface even when there is a coexistence of phases in the same lipid environment as it occurs for DPPC at 25 °C at 10 mN/m. The

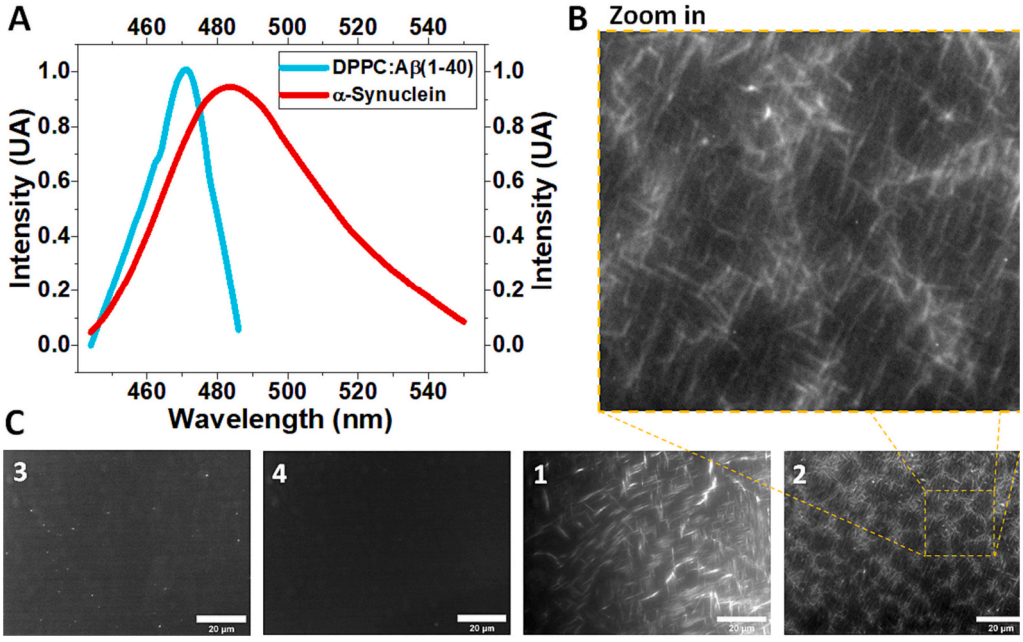

Fig. 4. ThT spectra confirmation and fluorescence microscopy imaging of DPPC/Aβ(1–40) mixed films. A: Fluorescence emission spectra of the fibril-like structures from DPPC/Aβ(1–40) mixed films (95:05 of PAP), λ_{em} 472 nm. α-synuclein has been used as positive control, λ_{em} = 482 nm. B: *Zoom in* of fluorescence microscopy imaging from picture 2 of DPPC/Aβ(1–40) transferred to mica support at ~12 mN/m. The dark zones correspond to the more enriched lipid domains which they are not labeled by ThT. C: Fluorescence microscopy imaging of DPPC/Aβ(1–40) mixture transferred to mica support taken at different surface pressures corresponding to the numbers indicated in Fig. 1B.: 1 = 5 mN/m (dominant liquid-expanded state); 2 = 12 mN/m (transition region); 3 = 30 mN/m (dominant liquid-condensed stated) and 4 = 48 mN/m (near collapse pressure). Experiments were done at 25 °C.

above results are in keeping with our previous studies. We have demonstrated a similar behavior for Aβ(1–40) amyloid peptide forming fibril-like structures with only mixed with POPC (liquid expanded film) but not with DSPC with it behaves a liquid-condensed behavior [5]. We are claiming that the selective formation of fibril-like structures is triggered by an confined liquid-expanded lipid phase arrangement provided by DPPC at the interface when it is mixed with Aβ(1–40) peptide at low PAP (Fig. 2).

We have used the ThT probe to visualize, by fluorescence microscopy, the formation of interfacial amyloid fibers in mixed lipid-peptide films as previously reported [9,33]. As the compression process takes place in the mixed film, the forming lipid solid phase imposes a new lateral environment forcing to the fibril-like structures, formed in the previous homogeneous liquid-expanded phase, to remain in the coexistent more liquid phase regions laterally deforming the solid domains. Progressively, the fibrils disassemble until the film becomes homogeneous above 20 mN/m. At above this lateral pressure, the fibril-like structures are no longer detected judging by a negative ThT staining together with a homogeneous reflectivity observation in BAM (Figs. 1 and 2B-C and Fig. 4B-C). The complete compression process of the mixed film from 0 to 45 mN/m, this is: the formation of fibrils in the liquid-expanded phase (ThT positive coming from the fibril-like structures), the two-dimensional transition of DPPC and the transformation into a homogeneous film (above 20 mN/m) is carried out without desorption of the protein from the surface, since the mean molecular areas of the mixed film is preserved throughout the isotherm until the collapse point near to 45 mN/m (see the larger molecular area of the mixed film with respect to pure DPPC in Fig. 1B). By using the ThT probe, Fig. 4 clearly shows a strong labeling of the fibril-like structures in the liquid-expanded regions and a negligible dyed into the dark zones which they correspond to the DPPC irregular domains of the condensed phase. As Aβ-amyloid fibril-like structures are formed inside the liquid-expanded phase upon compression, the fibrils begin to laterally constrict the condensed lipid phase imposing an additional interfacial restriction in the lipid transition. This may be explaining the more irregular shape of lipid domains when compared to the condition of pure DPPC films (compare picture C2 with C6 in Fig. 1). However, in a *zoom in* picture (see Fig. 4B) it can be appreciated the sharp fiber bundles in between lateral interface of the condensed domains. In Fig. 3 we show a AFM imaging analysis of DPPC/Aβ(1–40) mixed film highlighting the relative height of both fibrils and domains which were ~9 nm and ~14 nm respectively (Fig. 3B, see Fig. S2 for a AFM imaging of pure DPPC in similar condition). According to the image, fibrils (red arrow) get into the irregular domains enriched with DPPC lipids (green arrow) which goes along with the ThT fluorescence analysis (see Fig. 4B). However, it should be kept in mind that BAM and AFM provide information on the observed structures with much difference in the resolution.

Recently molecular simulations (MS) studies reported that Aβ amyloid forms stable complex with DPPC in 1:3 peptide/lipid ratio [34]. Although, the main structure adopted by Aβ(1–40) peptide is the β-sheet conformation, others studies of MS have reported that this peptide get predominantly into an α-helix conformation by interacting with DPPC lipid [35]. Based on our results found with ThT fluorescence experiments of transferred films we have to firmly conclude that the more probably secondary structure adopted by Aβ(1–40) amyloid mixed with DPPC is β-sheet (Fig. 4A).

In previous work we have observed that both Aβ(1–40) and Aβ(1–42) have similar surfaces properties and behavior at the air water interface [8]. Following amyloid/lipid studies, Hane et al., by using AFM, have shown that Aβ(1–42) cannot be inserted into DPPC bilayer at room temperature, but the peptide is able to bind throughout the lipid polar head groups [11,36]. However, previously Chi et al. highlighted that the incorporation of Aβ amyloid into DPPC lipid can occur below 23 mN/m [15]. In keeping with this idea, our AFM and fluorescence microscopy results have allowed to visualize, for the first time, a reduced height of the fibers respect to the domains is justifying the merging of Aβ amyloid fibrils into the lipid condensed domains (Fig. 4B). In this way, extrapolating to biological membranes we could suggest that Aβ amyloid fibrillation is a selective process towards the lipid phase specifically the liquid-expanded state where the confined lipids keep in a less ordered

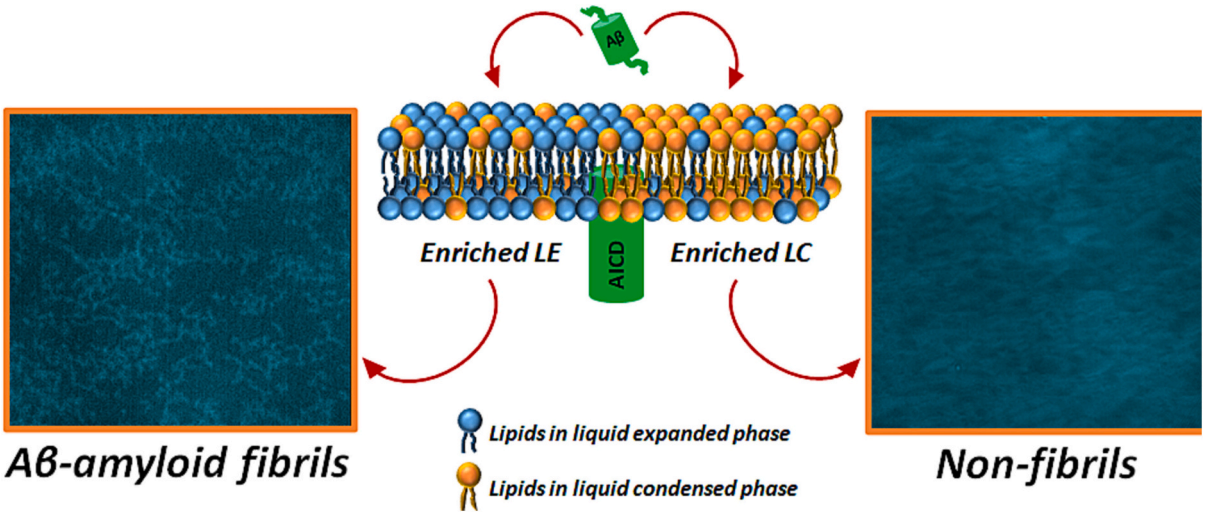

Fig. 5. Schematic visualization of the physical state of the phospholipid as an inductor of amyloid fibrils. Representation of a membrane with coexistent domains in liquid-expanded/liquid-condensed phases (or its equivalence liquid-crystalline/gel phases for bilayers) differentially enriched in the lateral plane of the membrane (middle part). Confined association of Aβ peptide molecules in the more disordered lipid domain triggers the formation of fibril-like structure observable by BAM (left part indicated with brilliant green) in contrast with the no reactivity of the phospholipid phase in the liquid-condensed ordered state. Aβ represents the amyloid fragment. AICD is the Intra-Cellular Domain C-terminal fragment after β-secretase cleavage [38].

arrangement than lipids in a liquid-condensed state and, not least, with a different hydration state [18,37].

In our experimental setting we have transferred the DPPC/Aβ amyloid mixed films to mica support at ~5 mN/m, ~12 mN/m, ~30 mN/m and ~48 mN/m. These surface pressures correspond to the following the liquid-expanded state, transition region, liquid-condensed state and film collapse, respectively. At region of surface pressure of neither predominant liquid-condensed state nor film collapse we have detected fibril-like structures by using ThT probe in fluorescence microscopy (Fig. 4C images 1 and 2). Thus, these results are in contrast with those obtained by Ege et al. claiming that Aβ amyloid does not insert into DPPC lipid monolayer at high packing. So, the liquid-condensed state creates a lipid arrangement leading to an inhibitory effect or an inappropriate environment to trigger the formation of fibril-like structures of Aβ amyloid peptides. In this connection, it was reported the importance of lipid phase on Aβ(1–42) amyloid peptide binding [19,20].

However, in the liquid-expanded phase even in the transition region, the interfacial properties of the lipid are more appropriate to induce Aβ amyloid fibril-like structure. For this reason, in the transition region of the DPPC/Aβ mixed film the domains become into irregular morphology (losing the original shape) with respect to the domains observed in pure DPPC. In turn, the formation of fibrils alters the expanded-condensed equilibrium by sequestering lipids from the lateral interfacial contact between the coexisting phases leading to the deformation of the condensed domains.

4. Conclusions

The DPPC is a zwitterionic phospholipid which undergoes bidimensional transition from the liquid-expanded to liquid-condensed state depending on temperature and degree of compactness (lateral pressure). Thus, this unique property of DPPC allows us to study in a dynamic way how the lipid phase can influence amyloid formation by the variation of lateral packing and working temperature in a same lipid environment.

We have demonstrated that Aβ fibrillation can occur from 15 °C to 40 °C specifically in the liquid-expanded state. However, we have evidenced that the fibril-like structures keep stable in the transition coexisting region. The DPPC lipid domains which get an irregular morphology reducing the lateral contact surface probably because the Aβ amyloid fibrils compete with lipids from the liquid-expanded phase that surround the more condensed lipid domains as monolayer compression takes place.

We have detected the fibril-like structures in both the liquid-expanded and in the phase transition region by using BAM. Those structures were ThT positive according to fluorescence microscopy. These results are in concordance with previous studies in which we have shown that the fibril-like structures are formed in POPC/Aβ amyloid peptide mixed films but not in DSPC [9]. A graphical summary of our results is shown in Fig. 5.

Thus, we can conclude that the fibril-like structure formation is a protein disorder → order (monomer → fibrils) process induced by the liquid-expanded character of the lipid phase and does not necessary depend on the temperature of the confined interfacial region, without using any extreme non-physiological conditions for fibrillation, such as acidic pH, long-time heating, strong agitation or co-solvents. We postulate that if a similar behavior is concomitantly occurring at the neuron membrane, the system would self-trigger the formation of amyloid fibers, that in turn, it would produce cell dysfunction and death long-time before the familiar amyloid plaques can be seen in postmortem brains. From our results we are emphasizing why the lipid phase stated of the neuron membranes matters in AD and should be taken with greater consideration.

We conclude an important molecular aspect of Aβ amyloid interfacial fibrillation with DPPC lipid monolayers. The interfacial fibrillation process from monomer to fibrils it is a selective process since it discriminates between two distinctive lipid phases, liquid-expanded character from the liquid-condensed phase one. What it is emerging is that amyloid fibrillation is a process that not only involves the molecular properties of the peptide, but that the interfacial conditions of the confined membrane environment appear as a necessary triggering factor.

Declaration of competing interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

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