Experimental report

02/02/2016

Proposal: 8-02-742 **Council:** 4/2015

Title: Interaction between amyloid peptides and lipid bilayers.

Research area: Biology

This proposal is a continuation of 8-02-679

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Samples: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine
Islet Amyloid Polypeptide

Instrument	Requested days	Allocated days	From	To
FIGARO	4	4	16/10/2015	20/10/2015

Abstract:

IAPP is an amyloid peptide which causes diabetes by killing beta-pancreatic cells. However, its mechanism of cell destruction is still under debate. It is not clear which structural species is responsible of cell disruption, whether it is the final amyloid fibril, a fibrillation-intermediary polymer or a stable oligomer. Here, we intend to characterize the mechanism of IAPP interaction with a model membrane by studying how different fragments of this peptide adsorbs, inserts and possibly desorbs, once in an oligomeric form, in order to map IAPP sequence for membrane disruption capabilities. The results of this functional study will complement those obtained on a previous ILL experiment and bring direct information about the role of each part of IAPP in the bilayer disruption mechanism that will have tremendous implication in the development of therapeutics against amyloid diseases.

Experimental report for proposals

TEST2358, TEST2335, 8-02-742 and 8-02-688

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These beam times were part of a large study including Neutron reflectometry (NR), SANS and laboratory techniques to investigate the interaction of IAPP fragments with POPC bilayers. IAPP is the peptide involved in β-cell depletion leading to the development of Diabetes Mellitus type 2. In order to understand the interaction of the peptide with cell membrane and its consequences, we used SANS an NR. The peptides we choose are depicted table 1: IAPP human peptide ($hIAPP_{1-37}$) has but splitted in two halves: the N-terminal half ($hIAPP_{1-20}$) is suspected to interact with lipids and the C-terminal half ($hIAPP_{23-37}$) is very aggregation-prone and has been shown to fold into a beta-sipper and form the spine of the amyloid fibers. The rat homologs ($rIAPP_{1-37}$, $rIAPP_{1-20}$ and $rIAPP_{23-37}$) have been used as non-amyloidogenic and non-toxic controls. Human and rat peptides differ by 6 positions which only one is in the C-terminal half. The chimeric peptide $rIAPP_{1-37}$R18H associating the human N-terminal half and the rat C-terminal half has also been analysed.

Table 1- Sequence of the peptides used in this study: the $hIAPP_{1-37}$: the human peptide (top) and the $rIAPP_{1-37}$, the rat peptide (bottom) are split in two fragments: the N-terminal from amino acid 1 to 20 ($hIAPP_{1-20}$ and $rIAPP_{1-20}$) and the C-terminal from aminoacid 23 to 37 $hIAPP_{23-37}$ and $rIAPP_{23-37}$). The mismatches between the human and rat sequences are marked in grey. The sequence of the chimeric peptide $rIAPP_{1-37}$R18H is in the middle.

The ability of each peptide to permeate a POPC bilayer has been measured on the PSCM set up for Electrophysiology on DIB. Figure 1 shows the results of these measurements. They show that $hIAPP_{1-37}$ is able to permeate the model membrane but $hIAPP_{1-20}$ and $rIAPP_{1-37}$R18H are much more efficient than the full length human peptide. An interpretation of these results is that the N-terminal half of the human peptide is responsible for lipid bilayer disturbance and that the amyloidogenicity of the C-terminal half inhibits it. The other peptides: the 3 rat peptides and the human C-terminal half, does not have any effect on the POPC bilayer conductance.

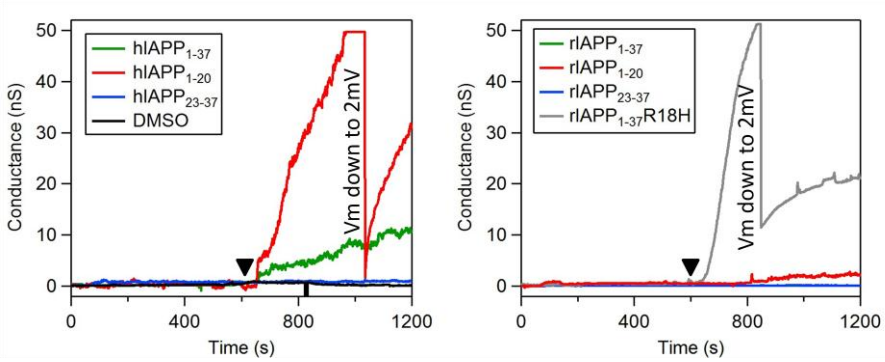

Figure 1- Conductance of a POPC bilayer plotted as a function of time. The black arrow figures the time point of injection of the peptides: full length (green), fragment 1-20 (red) and fragment 23-37 (blue). The left plot concernes human-derived peptides and the rigth plot, rat-derived peptides, including the chimeris $rIAPP_{1-37}R18H$ (grey). When the amplifier saturated, the tension was decreased from 10mV to 2 mV to pursue the recording.

Structural characterization of the POPC bilayer disturbed by the peptides has been performed by NR with the POPC membrane deposited on the surface of a Silicon crystal on top of the buffer chamber for eventual aggregates to be discarded from the interface by gravity. Buffers with various D_2O content were used for contrast variation and the curves were simultaneously fitted using Aurora software. The SLD profiles resulting from these fittings are shown by figure 2. We observed a dramatic lipid depletion following the injection of $hIAPP_{1-20}$ nearby the bilayer. A less pronounced effect was observed with $hIAPP_{1-37}$ which apparently inserts through the first leaflet of the bilayer, mainly amongst the headgroups, as well as $rIAPP_{1-37}R18H$ which insertion in the first leaflet is accompanied with lipid depletion. These results confirm that the N-terminal half, in particular the hitidine18, is crucial for the interaction of the peptide with a POPC bilayer. They also strongly suggest that lipid depletion could be the mechanism of bilayer permeation.

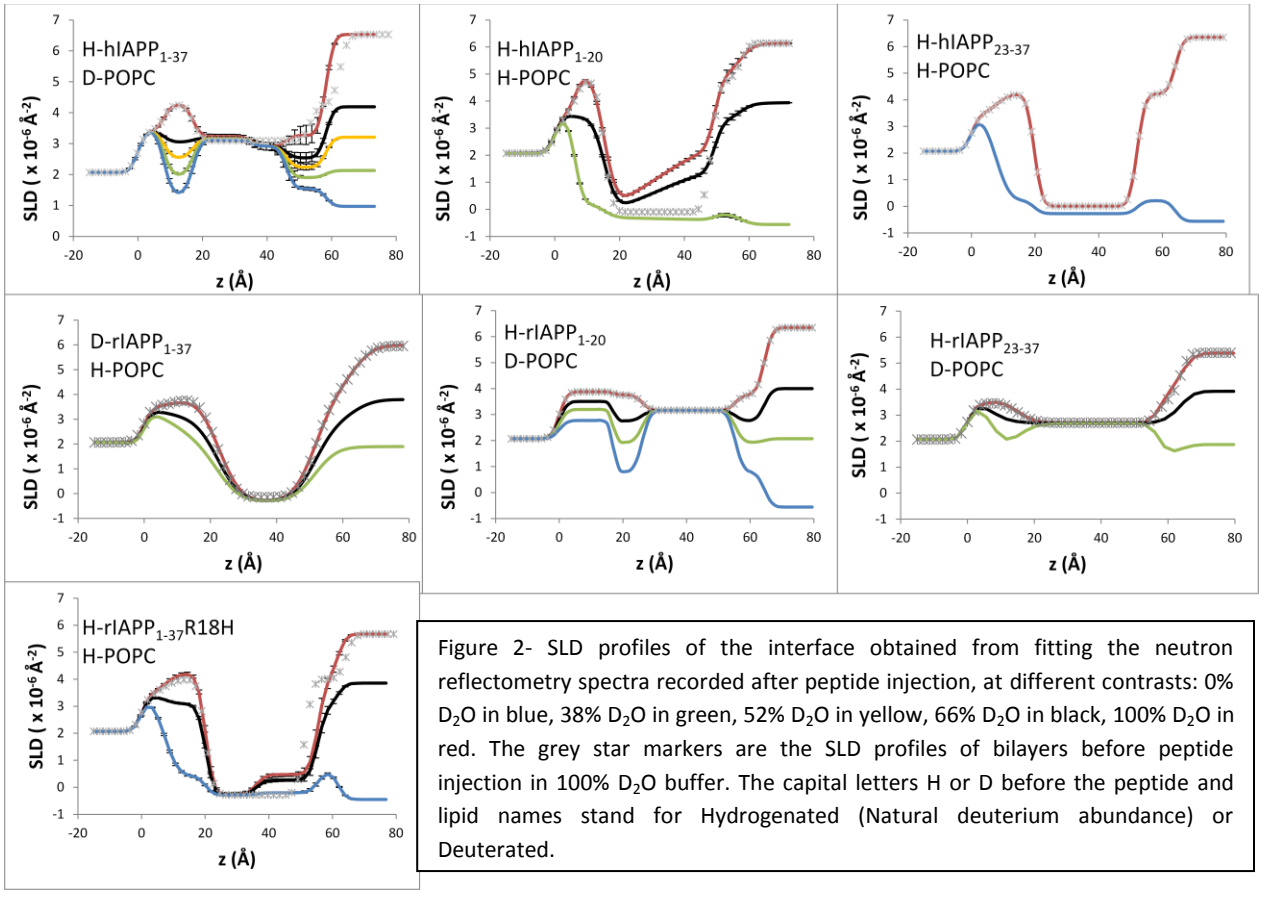

Figure 2- SLD profiles of the interface obtained from fitting the neutron reflectometry spectra recorded after peptide injection, at different contrasts: 0% D_2O in blue, 38% D_2O in green, 52% D_2O in yellow, 66% D_2O in black, 100% D_2O in red. The grey star markers are the SLD profiles of bilayers before peptide injection in 100% D_2O buffer. The capital letters H or D before the peptide and lipid names stand for Hydrogenated (Natural deuterium abundance) or Deuterated.

This hypothesis raises the question of the fate of the lipids removed from the bilayer. The structural analysis of aggregates of $hIAPP_{1-37}$ formed in presence or absence of POPC vesicles have been carried out by contrast-variation SANS (D22). The results are presented on figure 3 as the square root of the intensity extrapolated at Q=0 angle as a function of D_2O content of the buffer. This analysis shows that the presence of POPC vesicles during $hIAPP_{1-37}$ aggregation changes the aggregates match point from 39% to 26% D2O, which would correspond to an incorporation of three lipid molecules per peptide within the aggregates. Furthermore, the Intensity scattered by the lipid-peptides co-aggregates never reaches 0, which suggest that within the aggregates, the lipids and the peptides segregate in two phases rather than mixing intimately.

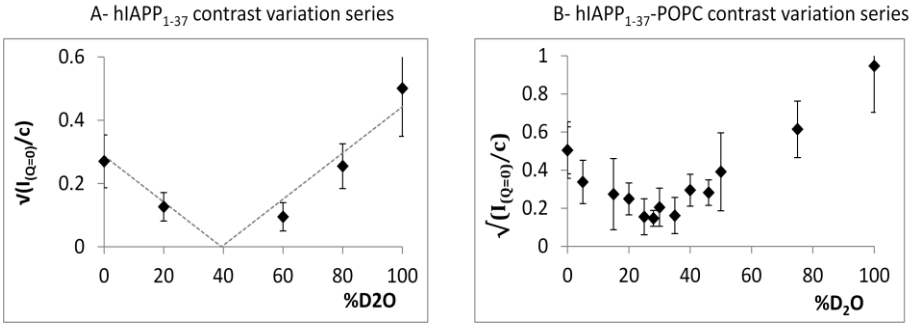

Figure 3- Matchout plots from SANS measurements of $hIAPP_{1-37}$ aggregates formed in absence (A) or presence (B) of POPC. The square root of the neutron scattering intensity at Q = 0, normalized by the sample concentration, is plotted as a function of the D_2O content of the solvent.

All together, these results enable us to propose a cytotoxicity mechanism in which the N-terminal half of $hIAPP_{1-37}$ would insert in the lipid bilayer and extract a large amount of lipids from it leading to membrane permeation. The amyloid aggregation could be an inhibitory mechanism selected as a rescue system to delay the onset of the disease.

A publication presenting these results and interpretation is in preparation.