Amyloidogenic Propensities and Conformational Properties of ProIAPP and IAPP in the Presence of Lipid Bilayer Membranes

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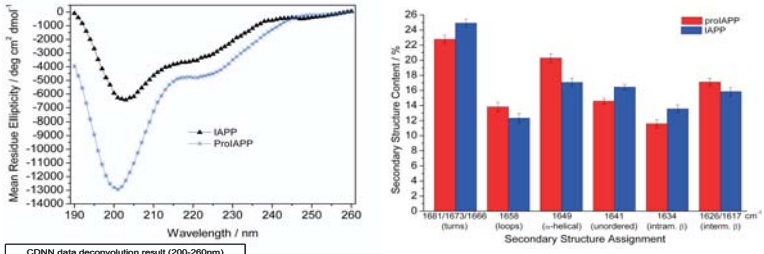
ProIAPP is a precursor protein of IAPP (amylin), which is believed to play a role as an antagonistic hormone to insulin. ProIAPP is stored in secretory granules of pancreatic β-cells and processed by the same enzymes involved in the conversion of proinsulin to insulin. This conversion is affected by numerous factors such as a high level of nonesterified fatty acids (NEFAs) or glucose [1]. In that case, the unprocessed proIAPP interacts with intracellularly present negatively charged surfaces, which might act as a loci of initiation for fibrillation reaction. These fibrillar structures or their prefibrillar forms have cytotoxic propensity [2], thus involved in various diseases like here it is invovled in Type 2 Diabetes Mellitus (T2DM). The conformational and amyloidogenic propensities of human proIAPP (hproIAPP), IAPP (hIAPP) and their molar mixtures in absence and presence of negatively charged membrane surfaces have significant differences. Since the membrane is abundant in cellular environment, our prime goal of the study is to underlie the mechanism of their possible role in onset/prolongation of IAPP/proIAPP aggregation/fibrillation process.

Secondary structure of native hproIAPP and hIAPP

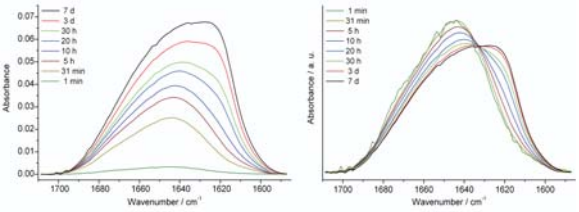


CDNN data deconvolution result (200-260nm)		
Protein 2°structure element	IAPP (value in %)	ProIAPP (value in %)
helix	15	17
anti parallel β-sheets	15	15
parallel β-sheets	12	12
β-turn	18	19
random coil	40	37

Figure 1. CD spectra (top left) of freshly dissolved 10 μM hIAPP and hproIAPP; the table (lower left) shows the secondary structure content of these peptides from the deconvoluted spectra with fitting error of 6-8 %, the bar graph (top right) shows the secondary structure content of the freshly dissolved peptides as obtained by fitting FTIR spectra using Levenberg-Marquardt curve fitting.

Conformational changes in hIAPP and hproIAPP in the presence of lipid membranes as revealed by ATR-FTIR spectroscopy

Figure 2. Left: ATR-FTIR spectra of the amide-I' region after injection of a 40 μM solution of hproIAPP into the ATR flow-cell, containing an anionic DOPC/DOPG (7:3) phospholipid bilayer. Right: selected ATR-FTIR spectra after normalization.



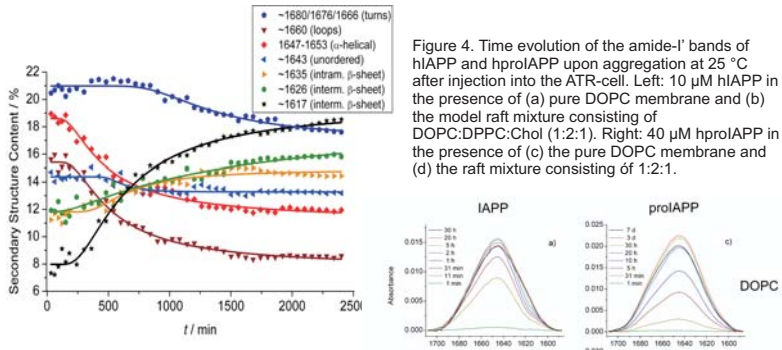
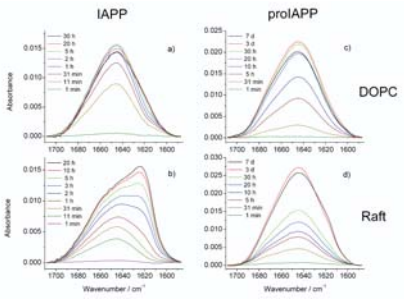


Figure 4. Time evolution of the amide-I' bands of hIAPP and hproIAPP upon aggregation at 25 °C after injection into the ATR-cell. Left: 10 μM hIAPP in the presence of (a) pure DOPC membrane and (b) the model raft mixture consisting of DOPC:DPPC:Chol (1:2:1). Right: 40 μM hproIAPP in the presence of (c) the pure DOPC membrane and (d) the raft mixture consisting of 1:2:1.

Figure 3. Secondary structure analysis of hproIAPP, incubated in presence of the negatively charged DOPC/DOPG (7:3) membrane. Conformational changes within the first 40 h of the experiment are shown.



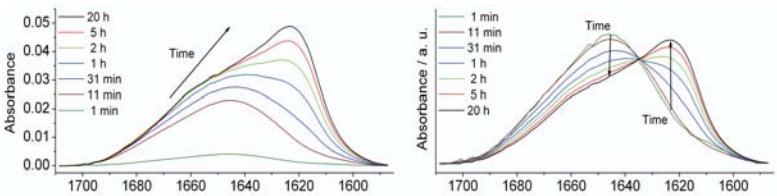
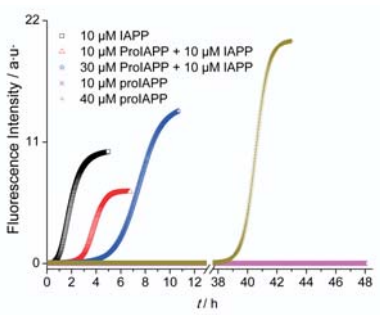


Figure 5. Left: ATR-FTIR spectra of the amide-I' region after injection of a 40 μM solution of hproIAPP in the ATR flow-cell, containing a DOPC/DOPG (7:3) phospholipid bilayer. Right: Same spectra after normalization.

Fibrillation kinetics in the presence of DOPC:DOPG (7:3 w/w) lipid bilayers using the ThT assay



The fibrillation kinetics follow a sigmoid-like curve. The lag phase of the curve is generally used to define the amyloidogenic propensity of a protein. The shorter the lag phase, the more amyloidogenic is the protein. Thus, hIAPP shows a markedly higher amyloidogenic propensity than hProIAPP even at the four-fold lower concentration and in the presence of anionic DOPC:DOPG lipid vesicles. However, the presence of hProIAPP in the same reaction mixture reduces the amyloidogenic propensity of hIAPP.

Figure 6. Thioflavin T dye - monitored amyloid fibril formation kinetics of 10 μM hIAPP (□),10 μM ProIAPP (⋆), 10 μM ProIAPP + 10 μM hIAPP(Δ), 30 μM ProIAPP + 10 μM hIAPP (✲), and 40 μM hproIAPP (+) in the presence of DOPC:DOPG (7:3 w/w) lipid bilayers. ThT was excited at 440 nm and its emission was observed at 482 nm with time in phosphate buffer at 25°C, pH 7.4.

Adsorption and fibrillation kinetics at different membrane interfaces

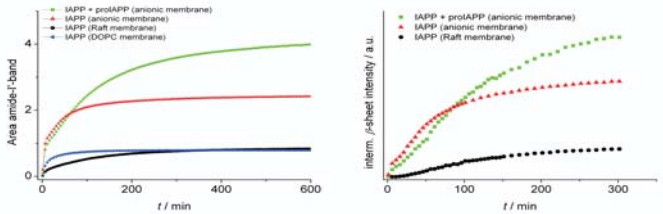
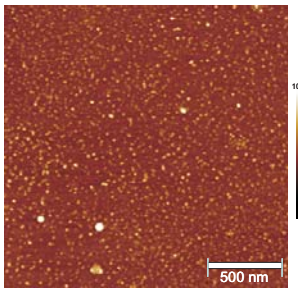


Figure 7. (a) Adsorption kinetics of 10 μM hIAPP and an equimolar mixture of hIAPP and hproIAPP (10 μM each) in presence of an anionic (DOPC:DOPG, 7:3) membrane, the DOPC:DPPC:Chol (1:2:1) raft membrane and of a pure DOPC lipid bilayer membrane (from amide-I' band analysis, 1710-1585 cm^{-1}). (b) The corresponding intermolecular β-sheet content with respect to the whole amide-I' area of hIAPP and the mixture in the presence of the anionic and model raft membrane.

Morphology of hproIAPP aggregates at the lipid interface



Unlike other membrane-induced fibrillating systems, such as hIAPP, hproIAPP was found to form oligomeric or small fibrillar structures, only. Essentially oligomeric structures are found with heights in the range of 2.5 - 3.5 nm. Along with these oligomeric species, a minor population of small fibrillar structures having a length of 50-55 nm was found as well. A similar analysis for 40 μM hproIAPP in bulk solution under the same conditions up to seven days did not show either significant secondary structural changes (by ATR-FTIR) nor changes in the morphology (by AFM).

Figure 8. AFM image of hproIAPP aggregates, aggregated at a concentration of 40 μM in the presence of the DOPC/DOPG (7:3 w/w) membrane at 25°C for 7 days.

Conclusions

Adsorption of predominantly unordered hproIAPP occurs at the membrane interfaces with a relatively slow rate compared to hIAPP.

Structural transitions and aggregation/fibrillation take place after adsorption of the peptides at different membrane interfaces.

Contrary to hIAPP, hproIAPP exhibits only oligomeric and very short fibrillar morphologies.

hProIAPP slow downs the hIAPP adsorption at negatively charged lipid interfaces and hence also the kinetics of hIAPP fibrillation at anionic lipid membranes.

References

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