Three-Dimensional Structure and Orientation of Rat Islet Amyloid Polypeptide Protein in a Membrane Environment by Solution NMR Spectroscopy

Nanga et al. University of Michigan, Ann Arbor May 2009. JACS.

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Background

- Islet amyloid polypeptide (IAPP)
  - 37-residue peptide hormone associated with glucose metabolism (highly co-secreted with insulin)
  - Human variant is highly amyloidogenic, associated with the death of β-cells in the early stages of type II diabetes
- Transient membrane-bound α-helical structures are precursors to the formation of these amyloid deposits
  - But mechanism is not well established
Research Approach

• Determine high-resolution structure of full length rat IAPP associated with dodecylphosphocholine (DPC) micelles by NMR
  – Human IAPP structure resolved on SDS micelles
  – rIAPP is stable in solution on micelles for months by CD (doesn’t easily form amyloids)
  – Differences in the initial helical state may affect formation of later aggregates and the process of membrane disruption
Structure Workflow

• Combination of 2D $^1$H-$^1$H TOCSY and 2D $^1$H-$^1$H NOESY for backbone and sidechain resonance assignments
  – Numerous well-resolved NOE crosspeaks -> well-folded
• X-PLOR-NIH program for structure calculations
  – Simulated annealing from 4000K for 100 initial structures
  – Further refinement with energy minimization and gradual introduction of van der Waals radii
• 485 NOEs (262 intraresidue and 223 inter-residue)
• 10 lowest energy structures selected for further analysis
  – No NOE violations greater than 0.5Å
  – No bond angle violations higher than 5°
  – No bond length violations higher than 0.05Å
Sequential and medium range NOE connectivities

Histogram of intraresidue, sequential (i-j=1), and medium-range (i-j=2,3,4) NOEs

Greatest amount of secondary structure

Chemical shift index calculated by subtracting the values measured for the peptide from random coil shifts
Fingerprint region of the 2D $^1$H-$^1$H NOESY showing the α-proton connectivities
Ensembles of conformers

From the density of sequential residue NOEs, they identify three distinct regions:
- Ala5-Val17 N-terminal helix
- Short, weaker helix from Ser20-Leu23
- Long, flexible loop region consisting of residues 24-37
Paramagnetic quenching with 2D $^1$H-$^1$H TOCSY indicates solvent accessibility in micelles

- Shift and broadening of peaks at even low Mn$^{2+}$ conc shows peptide is well exposed on micelle surface
- Lack of significant decrease in the N-terminus α-helix residues indicated that the helix is bound to the surface of the micelles

% Decrease of peak intensity after 0.8mM MnCl$_2$ exposure and TOCSY peak assignment
**Differential scanning calorimetry**

- Addition of the full length rIAPP reduces:
  - Phase transition temperature
  - Enthalpy change
  - Transition cooperativity
- The asymmetric transition at higher concentrations may indicate formation of peptide-rich domains
- The significant, but small degree of reduction relative to fully inserted peptides supports the binding of rIAPP to the surface of the micelles
Molecular dynamics simulation of rIAPP with 54 DPC molecules

- Surface-bound N-terminal helix and a relatively disordered C-terminus
- N-terminus doesn’t have an NMR signal but is inferred to curve towards the micelle due to the hydrophobic nature of the amino acids
- Lack of long-range NOEs indicate a lack of tertiary structure
Important regions

- R18 prevents membrane insertion accounting for the decreased membrane permeabilization of rlAPP\textsubscript{1-19} relative to the hIAPP\textsubscript{1-19}
  - However proline-substituted hIAPP is still able to aggregate
- Major differences in the C-terminal region
  - hIAPP C-terminal region forms a loose helical structure with a hinge which may stabilize membrane binding and subsequent binding cooperativity
  - rlAPP proline substitutions greatly slow the formation of amyloid β-sheet structures (as well as intermolecular interactions in general)
N-terminal loop may account for differences in self-association

- C2-C7 disulfide restricts the N-terminus in both rIAPP and hIAPP
  - Yet the hIAPP is the significantly more toxic form (even the truncated variants)
- Differential binding to the surface affects how the N-terminal loop is oriented (as mediated by R18 mutation and C-terminus stability)
  - The full length rIAPP has several NOEs within the disulfide bridge indicating rigidity that bends the loop towards the hydrophobic face of the helix, protecting it from inter-IAPP interactions
  - The hIAPP has less constraints within the loop allowing greater flexibility, exposing the hydrophobic side of the loop which may favor inter-IAPP interactions
Conclusion

• Solved the first structure of rat IAPP in a membrane environment
• Identified multiple structural features that correlate with the toxicity of the peptide relative to human IAPP and other CGRP family peptides

Questions?