Conformation and Aggregation of Biological Molecules: The Latest News

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Organization

1. Ion mobility – background
2. Aβ (Alzheimer’s Disease) – brief summary
3. IAPP (Type 2 Diabetes)
4. Conclusions
Ion Mobility
Ion Mobility Method

ESI Source → Analyzer Region → Detector

- Ion Funnel / MS → MS
- Ion Funnel / MS → Drift Cell → MS

mass spectrum

arrival-time distribution for $M_n^{4n-}$

$n =$ oligomer order
$z =$ charge $= -4n$
$z/n = -4$

closed conformation → fast
large $n$ → fast

open conformation → slow
small $n$ → slow
Arrival Time Distributions & Cross Sections

The velocity of an ion through the drift cell is proportional to the electric field

$$v = K \cdot E$$

$$K = \text{ion mobility}$$

$$K = f(T, p, q, \mu, \sigma)$$

The collision cross section of the ion, $$\sigma$$, depends on its shape

$$\sigma = f(\text{He-ion interaction} \& \text{ion shape})$$

$$t_a = \frac{\ell^2 T_0}{K_0 \rho_0 T} \frac{p}{V} + t_0$$

$$\sigma = \frac{3ze}{16N} \left( \frac{2\pi}{\mu kT} \right)^{\frac{1}{2}} \frac{1}{K_0}$$
Traveling Wave Ion Mobility MS

Prototype built by Waters Corp. (Milford, MA) for their Synapt High Definition MS System

T-Wave Technology:
A new method for separating ions based on their mobility
Amyloid Diseases & Their Worldwide Prevalence

Alzheimer’s Disease: ~27 Million
Type 2 Diabetes: ~160 Million
Parkinson’s Disease: ~4 Million
Human TSEs: < 10,000

IAPP
Aβ
α-Syn
PrP
amyloid fibrils

www.alz.org • www.who.int/diabetes • www.parkinsons.org.uk • www.cdc.gov/ncidod/dvrd/prions
Alzheimer’s Disease: Amyloid β-Protein
Monomer Structures:
Aβ42 Most Abundant Alloform in Plaques

DAEFRHDSGY^10EVHHQKLVFF^20AEDVGSNKGA^30IGLMVGGVV^40IA

- Separate isomers by conformation
- Use modeling to obtain detailed structures

ATD for Aβ42: z/n = -3

Baumketner et al., Protein Sci. 2006, 15, 420-428
Aβ Assembly: Mechanism Depends on Alloform

Separate species by shape and oligomer size to elucidate aggregation mechanisms

Bernstein et al., Nature Chemical Biology, submitted
What Next for Aβ?

- Search for oligomerization inhibitors for Aβ42 C-terminal fragments
  Aβ40

- Look at familial mutants
  E22G Arctic
  E22Q Dutch
  E22K Italian
  D23N Iowa
Mix Aβ40 + Aβ42
1:1

→ Aβ42 is aggregating

• 40/42 ~ 5/1
• 40/42 oligomer observed
  • Expected 1:2:1
Mix Aβ40 + Aβ42
1:1

Aβ40 inhibits aggregation of Aβ42
1:1
$\text{A}\beta\text{40} : 26\text{-AIA}\beta\text{42}$

1:1
$\text{A}\beta\text{40} : \text{A}\beta\text{42}$
Type 2 Diabetes:
IAPP
Role of IAPP in Type II Diabetes

Human IAPP
KCNTATCATQLANFLVHSSNFGAILSSTNVGSNTY-NH₂ → Fibrils

Rat IAPP
KCNTATCATQLANFLVRSSNLLGPVLPPNVGSNTY-NH₂ → No Fibrils

1901 Eugene Opie described degeneration of Islets of Langerhans in patients with hyperglycemia

1986 The primary component of the amyloid deposits was identified as Islet Amyloid Polypeptide (Amylin)

Current Opinion:
Increased demand for insulin → over production of other peptides (IAPP) leading to β-cell death

Hull et al. The Journal of Clinical Endocrinology & Metabolism 88(8):3629-3643
Hoppener et al. The New England Journal of Medicine Vol. 343 No. 6 pg. 411
Challenges & Goals

- Characterize key oligomeric species in the paranuclei regime of the amyloid fibril formation pathway

- Identify structural features of monomers through Ion Mobility Experiments and Molecular Dynamics Simulations

- Investigate possible inhibitors of nuclei and/or fibril formation

Bitan *et al.* PNAS 2003

Luca *et al.* Biochemistry 2007
Effect of Incubation on Human Amylin Fragment

Human Amylin\_(8-37)

20 µM pH 7.4

+3

+1.5 hrs

+4

+5/2

+2

large oligomeric species appear after incubation

+~15 hrs

m/z

700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800 1900 2000 2100 2200 2300 2400 2500 2600 2700 2800 2900 3000 3100 3200 3300 3400 3500
Human vs. Rat Amylin (8-37) Fragments at 100 µM

- Human Amylin$^{(8-37)}$
  - 100 µM pH 4.0
- Rat Amylin$^{(8-37)}$
  - 100 µM pH 4.0

**Higher-order oligomers**

m/z values and charge states:
- +3, +4, +5/2, +2, +7/4, +6/4, +5/3, +7/5, +8/6
Comparison of Human & Rat ATDs at Low Charge States

Human Amylin (8-37)

Rat Amylin (8-37)

multiple structures

+7/4

+5/3

+3/2

100 μM pH 4.0

Time
human vs rat IAPP (8-37) Fragments

rat Amylin (8-37)

human Amylin (8-37)
human IAPP$_{(8-37)}$ +2 ATD

For a sphere:

$$K \propto q \frac{1}{\sigma} \propto q \frac{1}{m^{2/3}}$$
Summary from (8-37) Fragment Experiments

Rat IAPP at 100μM

Human IAPP at 100μM

(higher-order oligomers) → Fibrils

Rat IAPP at 20μM

Human IAPP at 20μM

(higher-order oligomers) → Fibrils
Need to Look at full length

- “The lag phase was significantly longer for the full-length hA than for hA(8-37).”
  hA lag phase 120 min
  hA(8-37) lag phase 55 min
- This trend was also observed by CD spectroscopy.
- To do this we need a non aggregating system to compare with.

IAPP-GI (Sar$_{24}$-N-Me-Ile$_{26}$ hIAPP )


\[
\text{KCNTATCATQRLANFLVHSSNNFGA}\text{ILSSTNVGSNTYNH}_2\text{(N-Me) (N-Me)}
\]

- Non-amyloidogenic
- Non-cytotoxic
- Reduces IAPP cytotoxicity
IAPP and IAPP-GI Mix

- m/z values:
  - +3: 1311.22
  - +4: 976.64
  - +5/2: 1567.81
  - +4/2: 1986.32
  - +5/2: 1567.81
  - +4/2: 1966.29
  - +7/4
  - +5/3

- % values:
  - 100
  - 0
20μM:20μM IAPP : IAPP-GI Mix
Timecourse

Day 1

[IAPP]+3
1310.92

[IAPP-GI]+3
1351.25

Day 7

[IAPP]+3
1358.50
1398.49
Summary of IAPP and IAPP-GI Oligomer Complexes

IAPP-GI 100µM

IAPP 100µM

IAPP:IAPP-GI 20µM:20µM

IAPP-GI associates with IAPP creating mixed oligos. This reduces the probability that IAPP will form a homomolecular cluster large enough to act as a nucleation center, slowing the kinetics of IAPP fibril formation.
Question:

Does structure play a role in oligomerization process?
$z/n = +4/2$ ATD of IAPP and IAPP-GI Mix

+ 4 dimers

+ 2 monomers
z/n = +5/2 Peak of IAPP and IAPP-GI Dimers and Mixed Dimer

Notes: Mass spectrum of the +5/2 peak the dimers should show up in a 1:2:1 ratio and they appear to do so. The 2IAPP-GI dimer has some underlying adduct peaks making it appear larger than it is.
$z/n = +5/2$ ATD of IAPP and IAPP-GI
Dimers and Mixed Dimer

Notes: The ATD of all the dimer peaks show two main structural features; a compact and an extended structure.
$z/n = +5/2 \text{ ATD of IAPP and IAPP-GI}$

Dimers and Mixed Dimer

+5/2 ATD of all three peaks

Mass Spectrum of all Extended Structures
\[ z/n = +5/2 \text{ ATD of IAPP and IAPP-GI} \]

Dimers and Mixed Dimer

Mass Spectrum of all Compact Structures

+5/2 ATD of all three peaks

Mass Spectrum of all Extended Structures

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2IAPP-GI
IAPP:IAPP-GI Mix
2IAPP
Conclusions

- Both IAPP and IAPP\(_{(8-37)}\) form soluble oligos up to the tetramer before forming fibrils.
- IAPP-GI slows kinetics of aggregation by binding IAPP in mixed oligomers.
- The +5/2 peaks of these two species have compact and extended structures.
- The compact structure appears to be the channel through which the IAPP dimer proceeds to fibrils.
Why Does 8-37 Fragment Aggregate Faster Than Full Length?

- "The lag phase was significantly longer for the full-length hA than for hA(8-37)."
  hA lag phase 120 min
  hA(8-37) lag phase 55 min
- This trend was also observed by CD spectroscopy.

FIG. 4. The kinetics of fibril formation for full-length hA and hA(8–37). (A) Fibril growth measured by the thioflavin-T binding assay indicates a nucleation-dependent mechanism, but is distinct for the two peptides. (B) The peptide conformational change as measured by the development of a CD optimum at 218 nm parallels fibril formation measured by thioflavin-T binding.

\[
[IAPP_{(8-37)}]^+^2 \quad \sigma_{\text{exp}} = 541 \, \text{Å}^2
\]

**FF96 Solution**

\[
\sigma_{\text{calc}} = 638 \, \text{Å}^2
\]

**FF96 Gas Phase**

\[
\sigma_{\text{calc}} = 506, 559, 564 \, \text{Å}^2
\]

- Monomer rearranges to gas-phase structure before IMS
\[ [\text{IAPP}]^{+4} \quad \sigma_{\text{exp}} = 663 \, \text{Å}^2 \]

**FF96 with Implicit Solvent then Dehydrated**

- Full-length IAPP retains solution-like structure
- 2-7 Disulfide bridge stabilizes N-terminal helix
- Stable helix slows down transformation to β-sheet in oligomers
Muchas gracias

Thanks for your attention!