The Effect of Protein-Protein Interactions on the Pre-amyloid Structural Change of β-2-microglobulin as Measured by Covalent Labeling Mass Spectrometry

University of Massachusetts Amherst

*Blaise G. Arden and Richard W. Vachet**

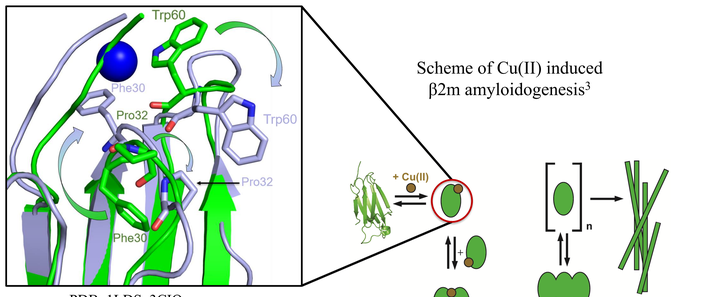
Department of Chemistry, University of Massachusetts Amherst

OVERVIEW

- Amyloid formation in β-2 microglobulin (β2m) can be induced by protein-protein interactions with amyloidogenic monomers (ΔN6) or oligomeric seeds
- Covalent labeling-mass spectrometry (CL-MS) is used to measure the rate of a known pre-amyloid structural change that leads to amyloid formation
- Changes in pH have a strong effect on amyloid formation
- Oligomeric seeds induce amyloids significantly faster than ΔN6

β-2 MICROGLOBULIN AMYLOID FORMATION

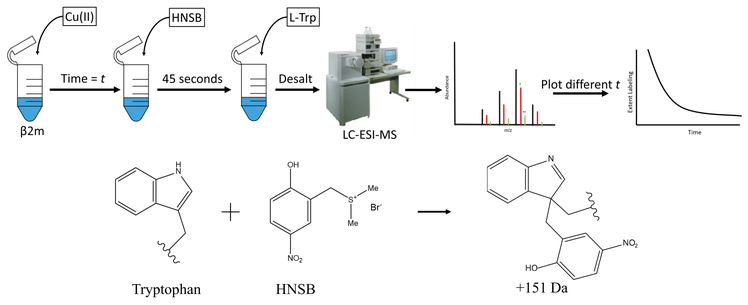
- Fibrils of β2m (a protein found in all human nucleated cells) cause dialysis-related amyloidosis (DRA)[1]
- β2m undergoes structural conversion from native to amyloid competent state, involving *cis-trans* isomerization of Pro32, several structural rearrangements including burial of previously exposed Trp60[2]
- Amyloid formation can be induced by protein-protein interaction between wild-type β2m and amyloidogenic variant ΔN6 or preformed oligomeric seeds



- Covalent labeling using HNSB to probe, by proxy, *cis-trans* isomerization of Pro32 by monitoring burial of Trp60
- Can elucidate rates of pre-amyloid structural change of wild-type β2m induced by ΔN6 or preformed oligomeric seeds

COVALENT LABELING TO MONITOR STRUCTURAL CHANGE

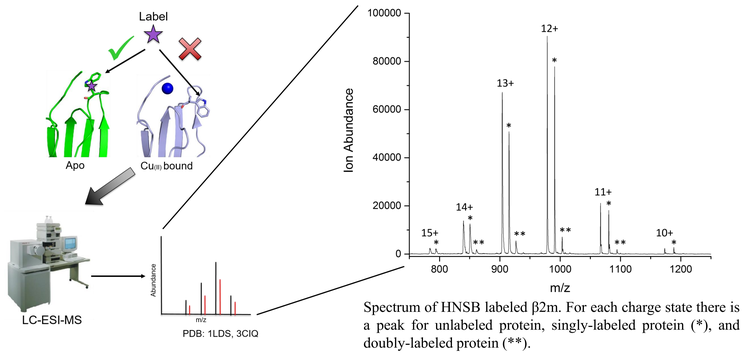
- β2m introduced to: (1) the amyloidogenic truncated variant ΔN6[4] or (2) preformed oligomeric seeds



Reaction of dimethyl(2-hydroxy-5-nitrobenzyl)sulfonium bromide (HNSB) with solvent exposed tryptophan[5]

SOLVENT EXPOSED TRYPTOPHAN LABELING

- Proteolytic digestion, LC/MS/MS illustrates ~ 90% of Trp labeling by HNSB at Trp60 and 10% at Trp95
- Trp95 largely buried, shows no change in extent of labeling over time
  - Total protein labeling can be used as an indicator of Trp60 burial



Spectrum of HNSB labeled β2m. For each charge state there is a peak for unlabeled protein, singly-labeled protein (*), and doubly-labeled protein (**).

PROTEINS ALONE SHOW NO STRUCTURAL CHANGE

Control Samples of WT and ΔN6 β2m exhibit no structural change over time

- ΔN6 is a naturally occurring genetic variant of β2m discovered in fibrils of amyloidosis patients[4]
- ΔN6 contains a *trans* Pro32 and more buried Trp60[6], has overall lower extent of Trp labeling
- On its own, it is naturally amyloidogenic[7]
- No change in extent of labeling indicates no change in Trp burial over time

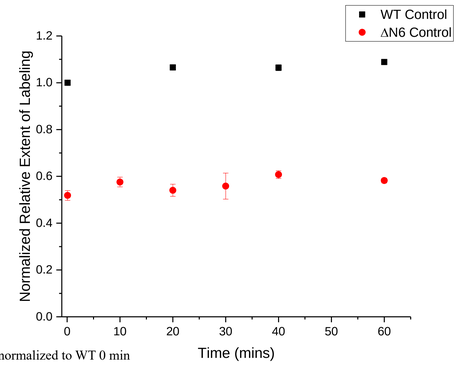
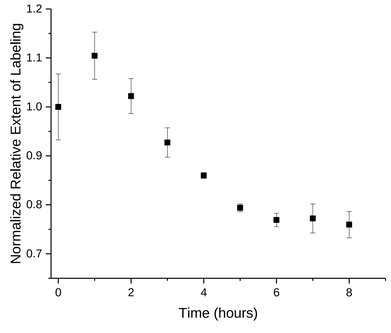


Figure normalized to WT 0 min

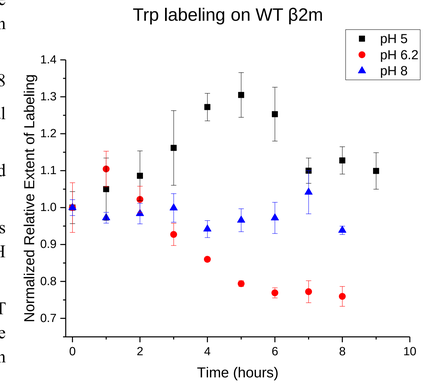
ΔN6 INDUCED STRUCTURAL CHANGE

- When co-incubated with wild-type β2m, ΔN6 can induce fibril formation of the wild-type protein
- When co-incubated at pH 6.2 with 100 mM NaCl structural change is slow
- Co-incubation at 3:1 ΔN6:WT results in amyloid formation within 7 days
- A temperature dependent increase in Trp labeling is seen prior to Trp labeling decrease
  - May indicate another structural change occurring before Pro32 isomerization
- Trp labeling from 1 hr to 8 hr decreases at a rate of $0.33 \pm 0.08\ hr^{-1}$



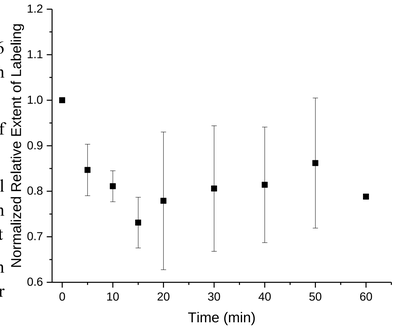
PH EFFECTS

- ΔN6 and WT β2m were incubated at different pH at 40° C to determine effect of varying charge on interaction between the proteins
- Amyloid formation is observed at pH < 8
- At pH 6.2 the pre-amyloid structural change occurs at a rate of $0.33\ hr^{-1}$
- At pH 8 no structural change is observed as expected
- At pH 5 an increase in Trp labeling is seen before the decrease, similar to pH 6.2, but over a longer time period
- Changing the charge on ΔN6 and WT β2m changes the interaction between the two and can slow down or even eliminate amyloid formation



PREFORMED OLIGOMERIC SEEDS

- Amyloidogenesis can be induced through interaction of WT β2m with preformed oligomeric seeds
- Seeds produced by sonicating preformed ΔN6 fibrils grown at pH 6.2 and incubated with WT β2m at pH 7.4
- Decrease in Trp labeling observed at a rate of $0.29\ min^{-1}$
- Later time points indicate structural heterogeneity develops as seeding reaction progresses, could be due to seeds falling apart
- Structural change occurs faster than when induced by Cu(II) ($0.16\ min^{-1}$), but slower than when induced by acid ($0.49\ min^{-1}$)
- Much quicker than when induced by interaction with monomeric ΔN6 (hours)



CONCLUSIONS

- Co-incubation of wild-type β2m and ΔN6 results in pre-amyloid structural change slower than other methods of inducing amyloidogenesis *in vitro*
- Varying charge has a significant effect on the interaction between ΔN6 and WT β2m and pre-amyloid structural change and can slow down or even stop amyloid formation
- Seeding with preformed oligomeric seeds causes structural change significantly quicker than monomeric ΔN6
- Protein-protein interactions that induce amyloid formation in β2m are strongly influenced by form (monomeric or oligomeric) and by solution conditions (pH)

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