IN VITRO POST-TRANSLATIONAL MODIFICATION MODELS AND THEIR EFFECT ON α-CRYSTALLIN CHAPERONE FUNCTION

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Abstract:

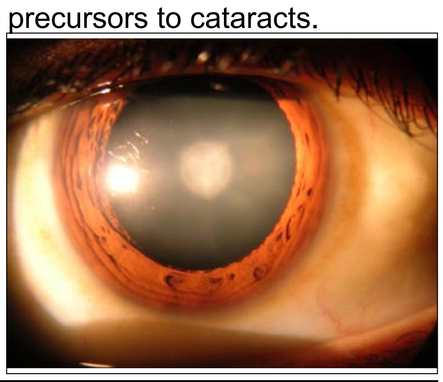
α-Crystallin is the major protein component of the human lens and plays an important role in the prevention of cataracts. α-Crystallin (αX) oligomers consist of two isoforms, αA and αB which share high sequence similarity and define the common α-Crystallin fold found in many small heat shock proteins (sHSPs). αA and αB are hypothesized to play two important roles within the lens. First, αA and αB belong to a group of proteins called Crystallins (α, β, and γ) that are very stable proteins that play a role in preserving a uniform density within the lens, which allows it to focus light. The Crystallin proteins' ability to form diverse and stable oligomers results in a glass-like rather than crystalline organization to the lens protein material, which also aids in the long-term stability of this high-density protein organ. Second, αA and αB both function as sHSPs that bind to misfolded proteins, preventing formation of large, insoluble protein aggregates (the beginning of cataracts). Our lab is investigating the molecular interactions between αA and αB that result in its stability, diverse oligomerization, and chaperone function. To this end we are using a model, inducible misfolding protein (insulin B-chain) to study chaperone function by light scatter under various conditions. We are also using random and targeted modification of αA and αB to simulate long-term protein damage and degradation observed in aged lenses. We hope to identify specific molecular interactions that result in αA and αB's chaperone function, and determine how those interactions relate to stability and self-oligomerization.

The Problem:

- Post-translational modifications (PTMs) of proteins occur via diverse chemical reactions with consequences for protein stability, structure, and activity.
- PTMs may thus serve as the root of pathologies such as cataract formation.
- O'Hara lab is approaching this issue using a number of biochemical techniques in order to learn more about the intimate relationship between protein structure and function.

The Model: Alpha-Crystallin

- It makes up 40% of all protein in the eye's lens and consists of two isoforms, αA and αB.
- αA and αB are capable of forming large, diverse, and stable oligomers that maintain a uniform and high protein density throughout the lens which is necessary to focus light.
- Both isoforms act as small heat shock proteins that bind to denaturing protein and prevent the formation of large insoluble aggregates that are precursors to cataracts.
- Long-term environmental damage to α-crystallin can be modeled via the loss of its chaperone function.
- If the supply of α-crystallin is exhausted, then it will contribute to the aggregation as well, forming cataracts like the nuclear cataract shown right.[1]



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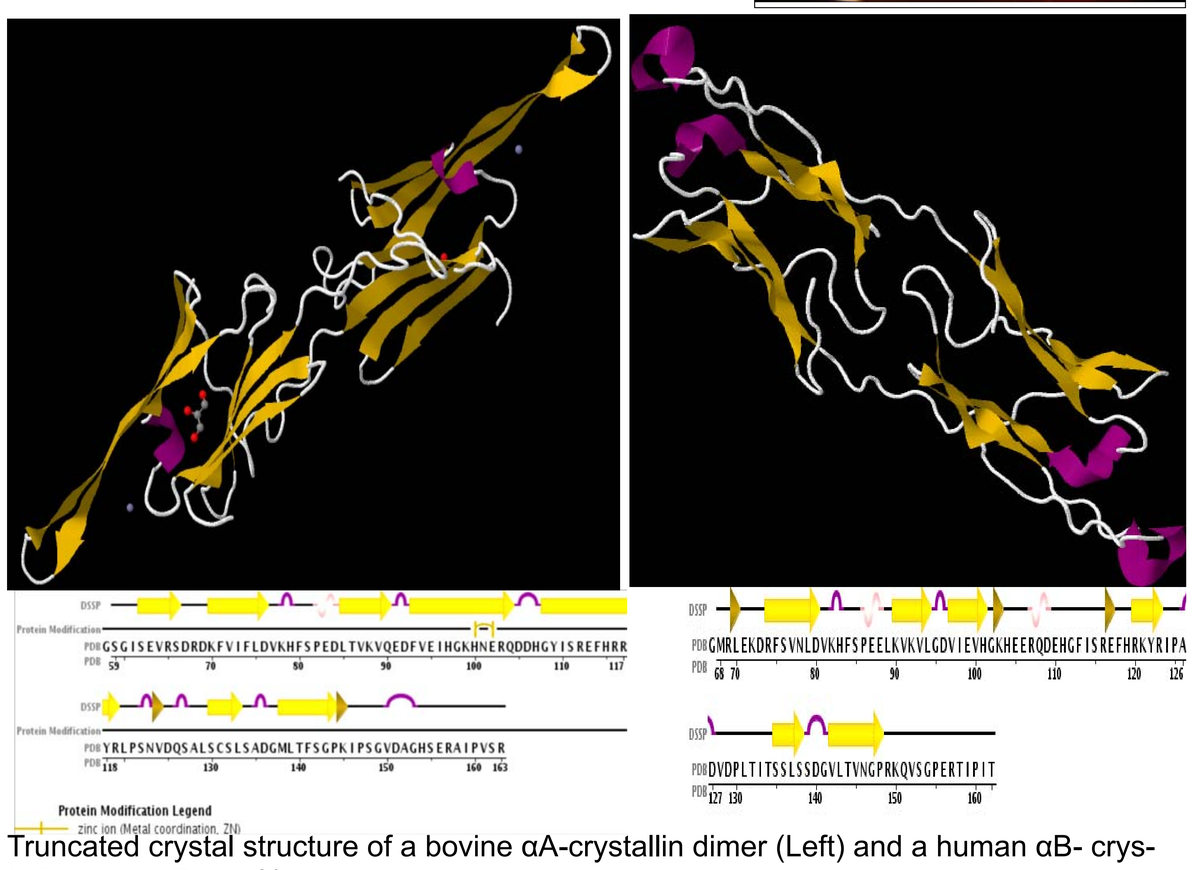
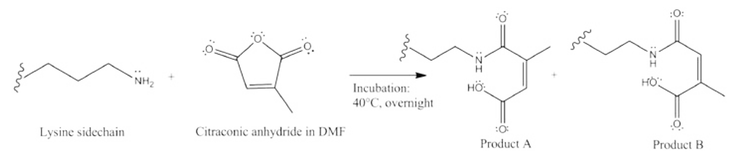


Truncated crystal structure of a bovine αA-crystallin dimer (Left) and a human αB- crystallin dimer (Right).[2,3]

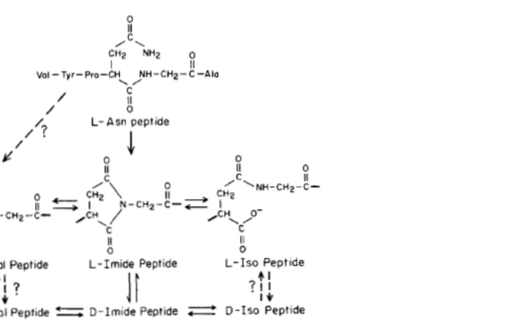
The Approach:

- The induced aggregation of the Insulin B-chain as measured by light scatter at 450nm provides a convenient experimental approach to monitoring a protein chaperone's activity.
- Assays are done in 50 mM Phosphate, 150 mM Sodium Chloride buffer, pH7.
- α-Crystallin is chemically modified and then its effect on chaperone function is determined in an aggregation assay.

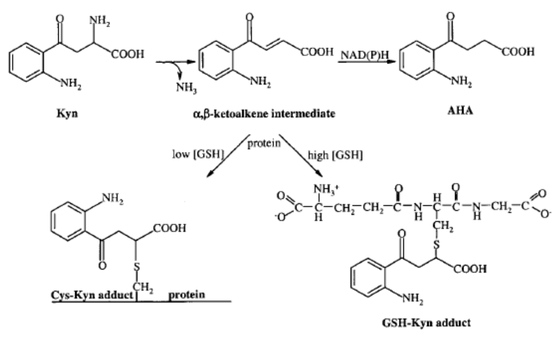
4 Models for PTMs:



1. Charge inversion of lysine residues via the blocking of amino groups with citraconic anhydride.

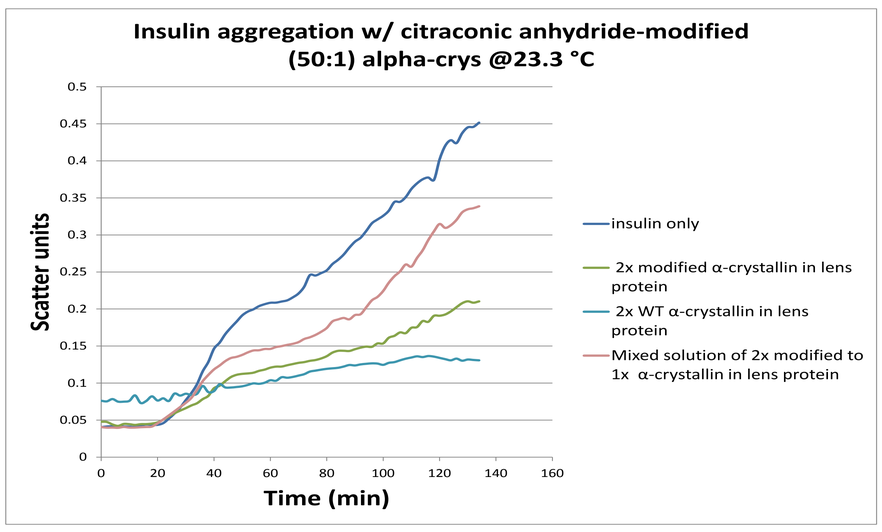


2. Deamidation of asparagine residues to aspartic acid. [4]
3. UV light perturbation of the αB isoform.



4. Photooxidation of the αB isoform by kynurenine-like UV filters. [5]

Methods and Results:



- Aggregation profiles of WT lens protein or citraconic anhydride-blocked α-crystallin and insulin are shown in a 2:1 molar ratio.

Conclusions and Future Studies:

- Citraconic anhydride-modified α-crystallin is a worse chaperone than simply WT crystallin. Mixtures of WT and anhydride-blocked crystallin chaperone less than WT as well.
- Preliminary data on αB-crystallin suggests that deamidation causes extensive αB-crystallin aggregation, rendering about only a seventh of the original protein content as a water soluble fraction.
- Future experiments with kynurenine as an oxidizing agent may introduce fluorescent spectroscopy techniques to this study.
- UV radiation also is an attractive pathway to model environmental damage.

References:

1. wikipedia.org/cataract
2. PDB: 3L1E Source: Laganowsky, A., Benesch, J.L. et al.(2010) Protein Sci 19: 1031-43
3. PDB: 3L1G Source: Laganowsky, A., Benesch, J.L. et al.(2010) Protein Sci 19: 1031-43
4. T. Geiger, S. Clarke J. Biol. Chem., 262 (1987), pp. 785–794
5. L. Taylor et al. (2002) Exp. Eye Research **74**, 503-511.

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