Characterizing palindromic strand exchange in alpha-crystallin oligomerization

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Introduction

Alpha-crystallin, a member of the small heat shock protein (sHSP) class, is expressed in eye lens, muscle, and brain tissue where it contributes to homeostasis by chaperoning and thus preventing the pathogenic aggregation of damaged or unfolded proteins. As a major cytoplasmic component in the eye lens, alpha-crystallin is further essential for producing a high index of refraction. Despite its high concentrations, alpha-crystallin does not itself aggregate or crystallize, a property facilitated by its populating dynamic, polydisperse assemblies. Oligomerization in this manner is a common feature of the sHSPs and is likely connected to their chaperone function. Structural studies[1] suggest that one mechanism of polydispersity may be the ability of a palindromic sequence centered on the sHSP IXI motif in the alphaB-crystallin (αB) isoform c-terminus to bind bidirectionally to other monomers. Bidirectional strand exchanges would result in heterogeneous oligomeric structures while maintaining near identical residue interactions.

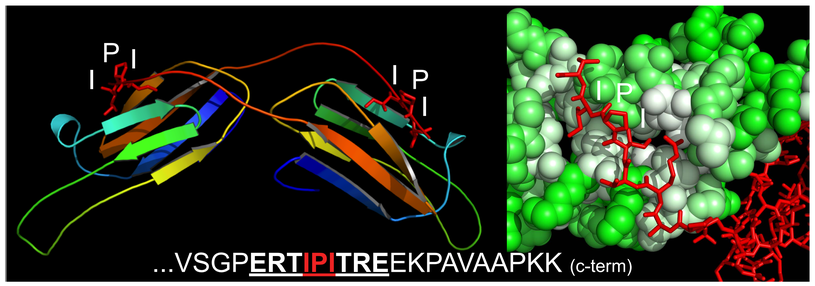

Figure 1: Crystal structure of truncated αB construct showing c-terminal strand exchange between two monomers[1]. At bottom is the palindromic c-terminal sequence with highlighted IXI motif.

Objective and approach

Objective:
- Characterize the role of palindromic strand exchange in αB oligomerization and chaperone function

Approach:
- Measure binding of designed palindromic peptide sequences of αB in solution
- Use single-pair FRET to distinguish binding orientation
- Design and characterize sequences with orientation bias
- Mutate αB with new strand exchange sequences to determine link to oligomerization and function

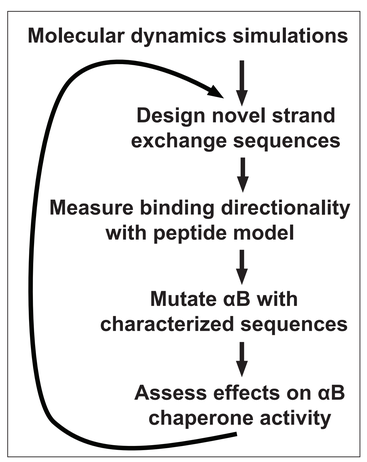

Figure 2: Experimental approach.

Materials and methods

Binding orientation between a peptide model of the c-terminal strand exchange sequence and the αB monomer is measured using single-pair FRET. We have mutated αB to introduce a cysteine residue for site-specific labeling. The protein and peptide are labeled so that binding in opposing directions will be indicated by different observed FRET efficiencies.

Chaperone function is assessed using an insulin model system. Insulin is induced to aggregate and formation of aggregates, in the presence or absence of αB, is monitored over time.

Preliminary results

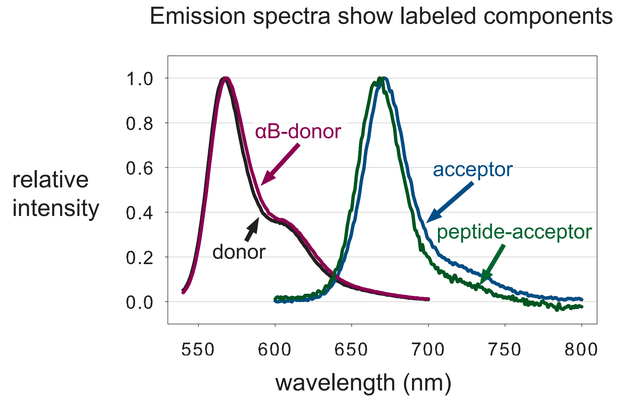

Figure 3: Emission spectra of free donor, free acceptor, donor-labeled αB, and acceptor-labeled strand exchange peptide. The system components are established and suitable for FRET studies.

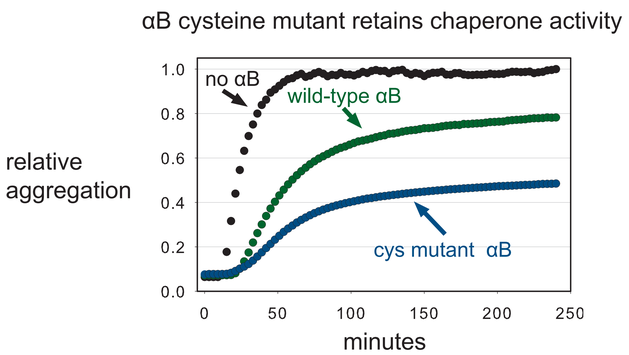

Figure 4: Insulin aggregation assay comparison of wild-type αB and cystein mutant αB. The mutant retains chaperone activity, confirming its relevance for further structural and functional studies.

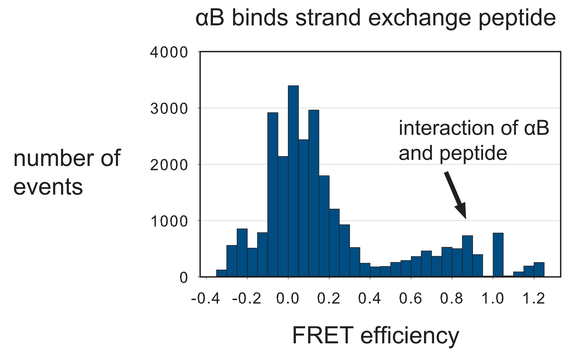

Figure 5: αB protein and the strand exchange peptide bind in solution, as indicated by the detection of high FRET efficiency values. Observed distributions are broader than expected and so do not reveal specific binding orientations. Methods will be tested and refined for greater sensitivity.

Conclusions and future work

Our results show that the experimental system components are established and are amenable to clarifying the importance of a proposed mechanism of alpha-crystallin polydispersity and chaperone activity.

Future work:
- Refine FRET methods for greater sensitivity to enable differentiation of binding orientation
- Employ molecular dynamics to aid in the design of additional strand exchange sequences

Acknowledgements

We thank Raysa Cabrejo and Karen Cheng for related work that was adapted for use in this project and Jonathan Gough for molecular dynamics help. Funding was provided by the Amherst College Department of Chemistry.

Further information

Literature cited:

1. Laganowsky and Eisenberg (2010), Protein Science 19:1031-43.

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