Contributions of Tyr/Phe Pairs to the Folding and Stability of Human γD-Crystallin

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Summary

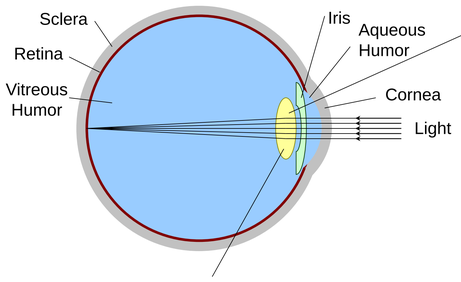
Human γD-crystallin (HγD-Crys), a member of the βγ-crystallin superfamily, is one of the major soluble proteins in the human eye lens. Crystallins are highly stable proteins and remain folded in the human eye lens for the majority of an individual's lifetime. Aggregation of crystallins leads to cataract. The amino acid sequence determinants of the folding, unfolding, and thermodynamic stability of HγD-Crys remain to be identified.

HγD-Crys exhibits two crystallin domains, each containing two Greek key motifs with eight β-strands. 24 aromatic residues are distributed throughout HγD-Crys, including 6 conserved β-hairpin Tyr/Phe pairs. Mutant proteins with these Tyr/Phe residues substituted with Ala all had similar structures as the wildtype (WT) protein by circular dichroism (CD).

Results of equilibrium experiments showed that N-terminal domain (N-td) mutant proteins had the N-td destabilized, but the C-terminal domain (C-td) unaffected, revealing an increased population of the single-domain-folded intermediate. C-td mutant proteins had both the N-td and C-td destabilized, showing a more concerted unfolding/refolding process.

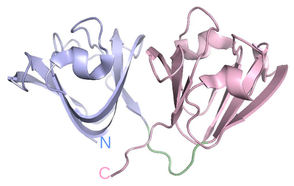
Results of kinetic experiments showed that for unfolding, C-td mutant proteins unfolded significantly faster than the WT, while N-td mutant proteins had no observable difference compared to the WT. For refolding, substitutions of the second Greek key pairs in each crystallin domain slowed down refolding rate compared to the WT, while substitutions of all other pairs had no observable effect.

Background



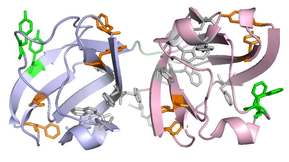
Lens:

- Human eye lens consists of highly organized, elongated fiber cells, which are differentiated from epithelial cells.
- high concentration of crystallins is responsible for the transparency and the refractive index, both essential for functional lens.
- aggregation of crystallins scatters lights, and leads to cataract.



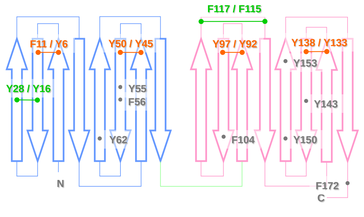
Human γD-Crystallin

Crystal structure of HγD-Crys (1HK0), one of the most abundant proteins in the eye lens. It exhibits two crystallin domains, each containing eight anti-parallel β-strands, organized into two Greek key motifs. Light blue: N-terminal domain (N-td); light pink: C-terminal domain (C-td); light green: linker.



Aromatic Network

- 24 aromatic residues (in sticks), including 14 Tyr, 6 Phe, and 4 Trp, are distributed throughout HγD-Crys.
- 6 β-hairpin Tyr/Phe pairs (in orange or green) are identified by their proximity of partners (distance ~5 Å).



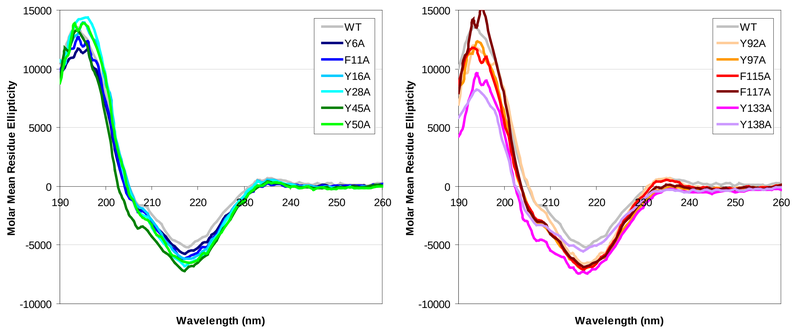
Topology Diagram

- Light blue: N-td; light pink: C-td.
- Orange: 4 Tyr/Phe pairs conserved for each Greek key motifs (Greek key pairs).
- Green: 2 additional Tyr/Phe pairs are at non-conserved positions (non-Greek-key pairs).

Materials and Methods

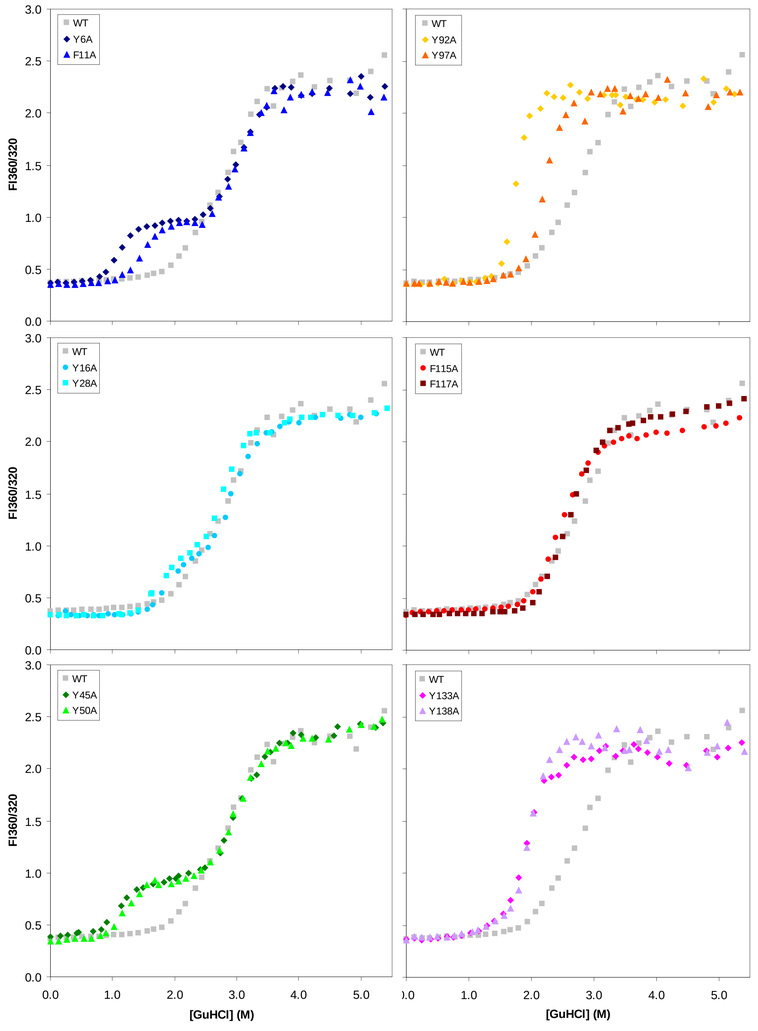
Mutant proteins were constructed by site-directed mutagenesis. Equilibrium and kinetic unfolding/refolding experiments were performed using guanidine hydrochloride (GuHCl) at pH 7.0, 37°C. Tryptophan fluorescence was used to probe the folding states. Equilibrium data were fitted by a two-state or three-state model to calculate the transition mid-point GuHCl concentrations and free energy changes for N-td and C-td. Kinetic data were analyzed qualitatively.

Structural Assessment

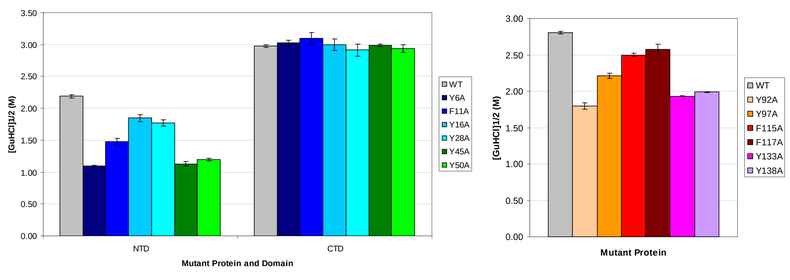


Far-UV CD spectra of the mutant proteins were very similar to that of the WT protein, displaying a major peak at 218 nm with similar intensities. This indicated that the mutant proteins maintained the folded β-sheet structures very similar to the WT protein.

Equilibrium Unfolding/Refolding

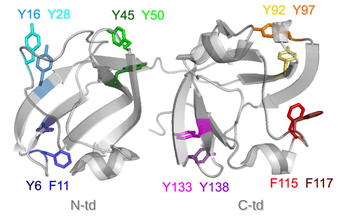


All the mutant proteins were destabilized compared to the WT protein. Left panels: each of the N-td mutant proteins had the N-td transition shifted to lower GuHCl concentration, indicating a destabilized N-td. The C-td transitions were unaffected. Right panels: C-td mutant proteins had both N-td and C-td destabilized. The N-td and C-td transitions were closer and inseparable, showing a more concerted folding process. For clarity, only unfolding data are shown here.



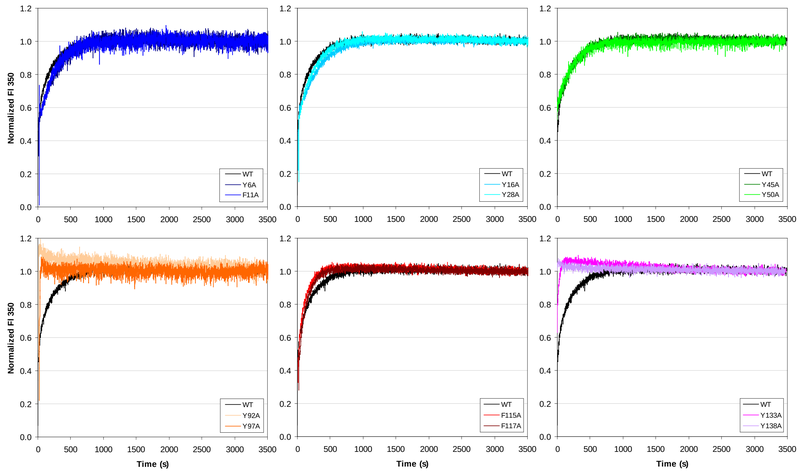
Equilibrium Unfolding/Refolding Transition Midpoints for HγD-Crys WT and Mutant Proteins. The different extents of destabilization depended on the positions of the substitutions. The Greek key pairs (N-td: Y6/F11, Y45/Y50, C-td: Y92/Y97, Y133/Y138) had larger contributions to the thermodynamic stability than the non-Greek-key pairs (N-td: Y16/Y28, C-td: F115/F117). Left: N-td mutant proteins, two transition midpoints corresponding to N-td and C-td stabilities are shown. Right: C-td mutant proteins, the only one transition is shown.

Keeping Track of the Six Pairs



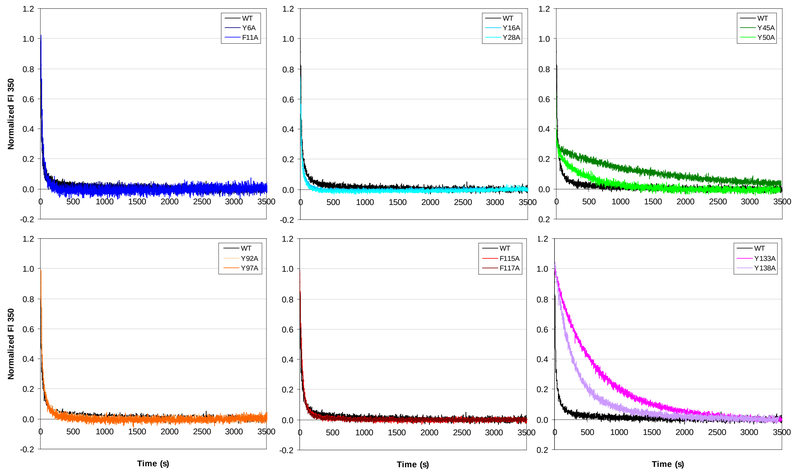
- HγD-Crys is shown in grey and the 6 Tyr/Phe pairs are highlighted in colors.
- 12 single mutant proteins with these Tyr/Phe residues substituted with alanines were constructed.
- This color scheme is used throughout the presentation of data in this poster.

Kinetic Unfolding



Upper panels: N-td mutant proteins had no observable difference compared to the WT. Lower panels: C-td mutant proteins unfolded significantly faster than the WT. In particular, mutant proteins of the Greek key pairs (Y92A, Y97A, Y133A, Y138A) unfolded extremely fast. Mutant proteins of the non-Greek-key pair (F115A, F117A) unfolded slightly faster than WT.

Kinetic Refolding



Right panels: Mutant proteins of the second Greek key pair in each crystallin domain refolded significant slower than WT (N-td: Y45A, Y50A; C-td: Y133A, Y138A), but in different manners. For Y45A, Y50A proteins, the initial 70% of the fluorescence change occurred at similar rate as the WT, but the later 30% occurred at slower rate. The refolding of Y133A, Y138A proteins were slower than the WT from the beginning of the reaction. Left and mid panels: all other mutant proteins had no observable difference compared to the WT.

Conclusion

- All 6 β-hairpin Tyr/Phe pairs were clearly important in the thermodynamic stability of HγD-Crys.
- The Greek key pairs had larger contributions than the non-Greek-key pair to the thermodynamic stability of each crystallin domain.
- Specific subsets of these Tyr/Phe pairs contributed to the unfolding or refolding kinetics, suggesting a role in defining unfolding/refolding pathways of HγD-Crys.

Acknowledgements

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