

Stabilization of α AG98R mutant protein by a peptide mini-chaperone: A multi-angle light scattering study.

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ABSTRACT

The G98R mutation in α A-crystallin is associated with autosomal dominant cataract in humans. The mutant protein exhibits impaired oligomer assembly with decreased stability. The objective of the present study was to determine the effect of a 'mini-chaperone' on the stability of mutant α A-crystallin (α AG98R) protein. α A-mini-chaperone is α A-crystallin-derived peptide which stabilizes the denaturing substrate proteins and prevents them from aggregation and precipitation. The G98R mutation was generated by site directed mutagenesis method. Mutant and wild-type proteins were expressed and purified by chromatographic methods. Recombinant mutant and wild-type protein (0.5mg/ml) were incubated in presence or absence of mini- α A (10 μ g) at 40°C for 1 hr. The samples were centrifuged to remove any precipitates formed during incubation and the supernatant was injected into TSKG5000PWXL gel filtration column attached to a HPLC system. The HPLC was connected to UV and refractive index detectors and a static multi-angle laser light scattering (DAWN-EOS) and dynamic quasi-elastic light scattering detectors (Wyatt technology). The molar mass (Mw) and hydrodynamic radii (Rh) were determined using ASTRA (5.3.4.14) software. The gel filtration profile showed that α AG98R eluted as two peaks, one at the expected oligomer elution region and a second peak, corresponding to the dissociated subunits. The average molecular mass of α AG98R at the oligomeric peak was 2.3 x10⁶. We recovered only 7.1 μ g mutant protein out of 75 μ g of incubated protein. In presences of mini-chaperone, however, we recovered significantly increased amount (59.2 μ g out of 75 μ g) of mutant protein with molar mass 3.2 x10⁶ indicating that mini-chaperone prevented the aggregation and precipitation of mutant α AG98R protein. Consistent with increase in molar mass the hydrodynamic radius (Rh) of the stabilized α AG98R increased from 15.31nm to 16.46nm. Unlike the mutant protein, the average molar mass of α A- wild-type protein did not change in presence of mini- chaperone at 40°C. Our study shows that the interaction of mini-chaperone with mutant G98R protein prevented the aggregation of unstable α AG98R-crystallin. These data suggest that the mini-chaperone has the potential to become a therapeutic agent for protein aggregation diseases.

METHODOLOGY

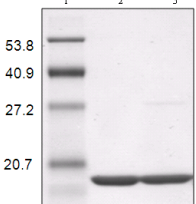
- Mini- α A was synthesized and supplied by Genscript.
- Mutant G98R and wild type α A-crystallin was generated by site directed mutagenesis technique and purified by chromatography.
- Stabilization of mutant protein by mini- α A was investigated by SCE and Multi-angle light scattering.

Human α A-Crystallin / Mini- α A

1 MDVTIQHPWF KRTLGPFYPS RLFDQFFGEG LFEYDLLPFL SSTISPPYYRQ
Mini- α A
51 SLFRTVLDSG ISEVRSDRDK FVIFLDVKHF SPEDLTVKVV DDFVEIHGKH
101 NERQDDHGYI SREFHRRYRL PSNVDQSALS CSLSADGMLT FCGPKIQTGL
151 DATHAERAIP VSREEKPTSA PSS

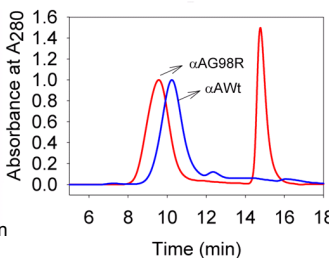
RESULTS

SDS-PAGE



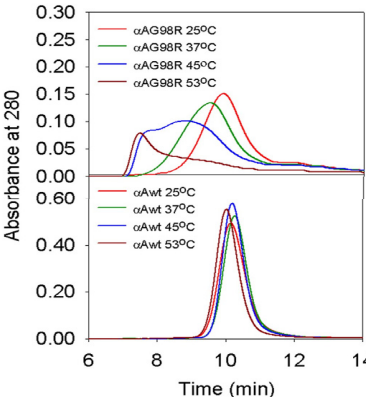
1. Marker; 2. Wild-type α A-crystallin
3. α AG98R-crystallin

Size Exclusion Chromatography of Mutant and Wild-type Protein



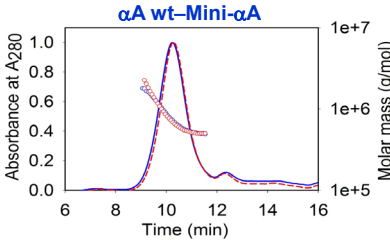
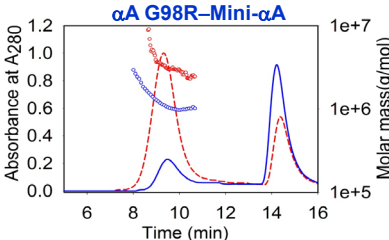
The SEC chromatography of mutant G98R protein at 1 mg/ml concentration gave an elution profile with an oligomer peak, eluting at 9.5 min and a dissociated subunits peak, eluting at 15 min, indicating the dissociation of oligomeric assembly. In contrast, wild-type α A-crystallin eluted as a single peak at 10 min, corresponding to the elution time of α A-crystallin oligomers

Thermal Stability, Molar Mass and Hydrodynamic Radius of Mutant and Wild-type α A-Crystallin



Temperature (°C)	Average Molar Mass (g/mol)	Hydrodynamic radius, Rh(nm)
αA-wt		
25°C	6.71 \pm 0.02e ⁵	8.5 \pm 0.2
37°C	5.68 \pm 0.02e ⁵	7.9 \pm 0.2
45°C	6.32 \pm 0.02e ⁵	8.1 \pm 0.2
53°C	8.67 \pm 0.02e ⁵	9.1 \pm 0.2
αAG98R		
25°C	34.11 \pm 0.02e ⁵	16.0 \pm 0.4
37°C	70.83 \pm 0.02e ⁵	19.4 \pm 0.5
45°C	166.00 \pm 0.02e ⁵	30.2 \pm 0.7
53°C	NA	NA

The wild-type and α AG98R were incubated for 30 min at various temperatures and analyzed by size exclusion chromatography and light-scattering measurements. Wild-type protein remained clear and stable in solution. However, the mutant protein formed light-scattering aggregates with increased molar mass and hydrodynamic radius compared to wild-type α A-crystallin



α AG98R mutant protein or wild-type α A-crystallins (0.5 mg/ml) was incubated in presence or absence of mini- α A peptide for 1 hr at 40°C and were analyzed on a multi-angle light scattering instrument. The average molecular mass of α AG98R at the oligomeric peak was 2.3 x10⁶. We recovered only 7.1 μ g mutant protein out of 75 μ g of incubated protein. In presence of mini-chaperone, we recovered significantly increased amount (59.2 μ g out of 75 μ g) of mutant protein with molar mass 3.2 x10⁶ indicating that mini chaperone prevented the aggregation and precipitation of mutant α AG98R protein.

CONCLUSION:

Mini-chaperone prevents the aggregation of unstable α AG98R protein suggesting that mini-chaperone has the potential to become a therapeutic agent for protein aggregation diseases.

Acknowledgments

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