

Role Of Oligomeric Size In Chaperone Function Of α B-Crystallin

Oligomerization of α B-crystallin is essential for its function as a molecular chaperone. The objective of this study was to delete residues $_{54}\text{FLRAPSWF}_{61}$ in the N-terminal domain in α B-crystallin and see the effect of deletion on oligomerization and chaperone like function of the protein.

The oligomeric size and hydrodynamic radius of purified recombinant wt- α B and α B Δ_{54-61} proteins were determined using a multi-angle dynamic light scattering instrument (DAWN EOS + QELS, Wyatt Technology) coupled to an HPLC fitted with TSKG5000PWXL gel filtration column. The data were acquired and analyzed using ASTRA software. The chaperone activity of the wt- α B and deletion mutant was compared at 37°C using alcohol dehydrogenase (ADH) substrate.

Both wt- α B and α B Δ_{54-61} eluted from the column as polydisperse aggregates (Fig 1). The aggregates of α B Δ_{54-61} were much smaller when compared to the aggregates of wt- α B (Fig 1 table). The light scattering data suggests that α B Δ_{54-61} oligomers are made of 17 – 31 subunits in contrast to the 25 – 40 subunit oligomers of wt- α B.

Comparison of the chaperone activity showed that 46.31 mg of wt- α B is required to suppress the aggregation of ADH (250 μ g) by 50%, in contrast only 4.52 μ g of α B Δ_{54-61} is sufficient to provide the same level of protection (data not shown). The complexes formed during chaperone assay were analyzed using the DAWN (Fig 2).

Upon interaction with ADH, the average mass of the deletion mutant (complex peak) increases by 2-fold, while the average mass of α B-ADH peak increases by 1.16 fold (Fig 2 Table). Deletion of residues 54-61 in α B-crystallin *increases* its ability to sequester denatured ADH and the complexes formed by the deletion mutants are more stable than those of native α B-ADH complexes.

We conclude that residues $_{54}\text{FLRAPSWF}_{61}$ in α B-crystallin are *not* essential to exchange subunits but the region contributes to higher-order organization of subunits. Smaller and more stable oligomers of α B-crystallin seem to function better as molecular chaperones.

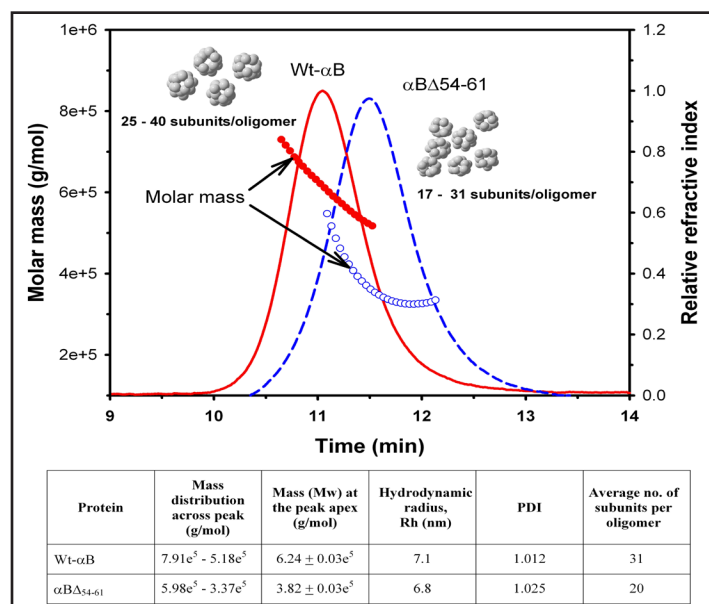


Figure 1. Molar mass distribution across wt- α B (red) and α B Δ_{54-61} (blue) peaks.

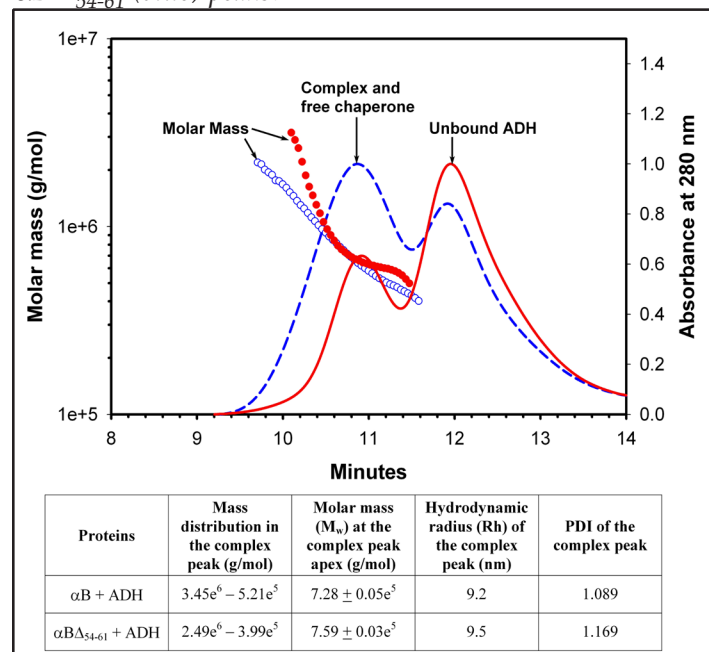
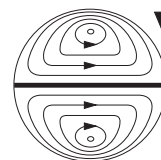


Figure 2. Dynamic light scattering (from WyattQELS) analysis of chaperone assay samples. Red-ADH + wt- α B; Blue-ADH + α B Δ_{54-61} .

This note graciously submitted by Puttur Santhoshkumar and K. Krishna Sharma, Department of Ophthalmology University of Missouri, Columbia, MO 65212



Wyatt Technology
CORPORATION

6300 Hollister Avenue • Santa Barbara, CA 93117
TEL (805) 681-9009 • FAX (805) 681-0123
E-mail: info@wyatt.com • URL <http://www.wyatt.com>