doi:10.1017/S1431927619007323
Microsc. Microanal. 25 (Suppl 2), 2019
© Microscopy Society of America 2019

Structural Organization of the Guinea Pig αA-Crystallin and $\alpha A^{66\text{-}80}$ Peptide Complex

Pramod K. Yadav[1], Min Su[1], Sivakumar Jeyarajan[2], Frank J.Giblin[2] and Melanie D. Ohi[1*]

[1.] Life Sciences Institute, University of Michigan, Ann Arbor, USA.
[2.] Eye Research Institute, Oakland University, Rochester, MI, USA.
* Corresponding author: mohi@umich.edu

The eye is a vital sensory organ for vertebrates critical for vision and systematic locomotion. The eye lens focusses light rays onto the retina to create clear images of objects that we see. As an organism ages the proteins in the eye lens undergo various post translational modifications leading to protein aggregation and cloudiness in the lens. This leads to the formation of cataracts that cause blurred vision and difficulty with night vision. The mechanism of protein aggregation in the eye lens is not well understood even though the phenomenon has been studied for decades. One proposed model arises from mass spectrometric studies of isolated cataract from eye lenses of a guinea pig model, suggesting that low molecular weight peptides are formed due to age related degradation of the major lens protein αA-crystallin [1]. These low molecular weight peptides in turn accelerate the aggregation of remaining intact α-crystallin protein which leads to protein aggregation and ultimately result in cataract formation in the lens nucleus [2]. Specifically a peptide named αA(66-80) with the sequence SDRDKFVIFLDVKHF cleaved from the region of 66-80 within the α-crystallin was found to form amyloid fibrils and accelerate the aggregation of recombinant αA-crystallin proteins *in vitro*. To study whether this *in vitro* generated model can occur *in vivo*, we used a guinea pig model treated with hyperbaric oxygen to induce age-related cataract formation. The aggregating peptide αA(66-80) was also detected in the guinea pig model treated with hyperbaric oxygen.

In order to study the mechanism of protein aggregation, we incubated recombinant guinea pig αA-crystallin and its splice variant α^{ins}-crystallin with the αA(66-80) peptide. This splice variant is called alpha A insert crystallin because it contains additional 23 amino acids arising from the intron and is present at 10% of the total α-crystallin in guinea pig lenses. This αA^{ins}-crystallin when incubated *in vitro* with the αA(66-80) peptide , forms amyloid like aggregates, whereas the αA-crystallin formed aggregates by clustering of αA-crystallin oligomers. The amyloid like aggregates when analyzed by electron microscopy, have a repeating structure that is induced by addition of the αA(66-80) peptide. These amyloid-like aggregates were not formed when the α^{ins}-crystallin was incubated in the presence of the reducing agent dithiothreitol (DTT). From these results we hypothesize that the presence of two cysteine (-SH) groups along with the 23 amino acid sequence in α^{ins}-crystallin may contribute to form amyloid-like aggregates induced by αA(66-80) peptide.

In the present study, we have used cryo-electron microscopy (cryo-EM) to characterize the structural organization of the filaments formed due to interaction of guinea pig αA^{ins}-crystallin and $\alpha A^{66\text{-}80}$ peptide. Guinea pig αA^{ins}-crystallin and peptide mixture ~0.25 mg/ml in 1 X PBS [1] was applied to a glow discharged holy carbon grid (Electron Microscopy Sciences) in the chamber of a Vitrobot (Thermo Fisher) set at 4°C and 100% relative humidity. The grid was then blotted for 3.5 s (Blot force 1) and plunged into liquid ethane. Micrographs (movie stacks) were collected on a Thermo Fisher Titan Krios electron microscope operated at 300 kV equipped with Gatan K2 direct electron detector (DED) camera. A total of 1290 micrographs were collected with a nominal magnification of 29,000x giving a final pixel size of 1.0 Å per pixel. Fourty frames of 200 ms each were collected with a defocus range from 1.3 to 3.5 µm at

https://doi.org/10.1017/S1431927619007323 Published online by Cambridge University Press

a total dose of 40.0 $e^- / Å^2$. Micrographs were motion corrected using MotionCor2 [3] resulting in global motion-corrected frame stacks and summed micrographs (Fig. 1A). Contrast transfer function parameters were calculated using CTFFIND4 [4]. Filaments were manually selected using Relion [5] with box size of 500 pixels and extracted with an interbox distance of ~25 Å. The particle stack was further processed for 2D classification and refinement in cryoSPARC [6] (Fig. 1B) as single particle to generate 3D model. Initial Model was generated using class averages in EMAN2 [7,8].

References:

[1] A Kumarasamy et al., Experimental Eye Research **179** (2019), p. 193.
[2] M Raju et al., Experimental Eye Research **132** (2015), p. 151.
[3] SQ Zheng et al., Nature Methods **14** (2017), p. 331.
[4] A Rohou and N Grigorieff, Journal of Structural Biology **192** (2015), p. 216.
[5] SH Scheres, J Struct Biol **180** (2012), p. 519.
[6] A Punjani et al., Nature Methods **14** (2017), p. 290.
[7] G Tang et al., Journal of Structural Biology **157** (2007), p. 38.
[8] We thank Prof. Justin Kollman (University of Washington) and Drs. Chung and Porta for helpful discussion and Dr. Bondy for maintenance of the electron microscopes and acknowledge financial support for the cryo-EM facility from the Life Sciences Institute, University of Michigan.

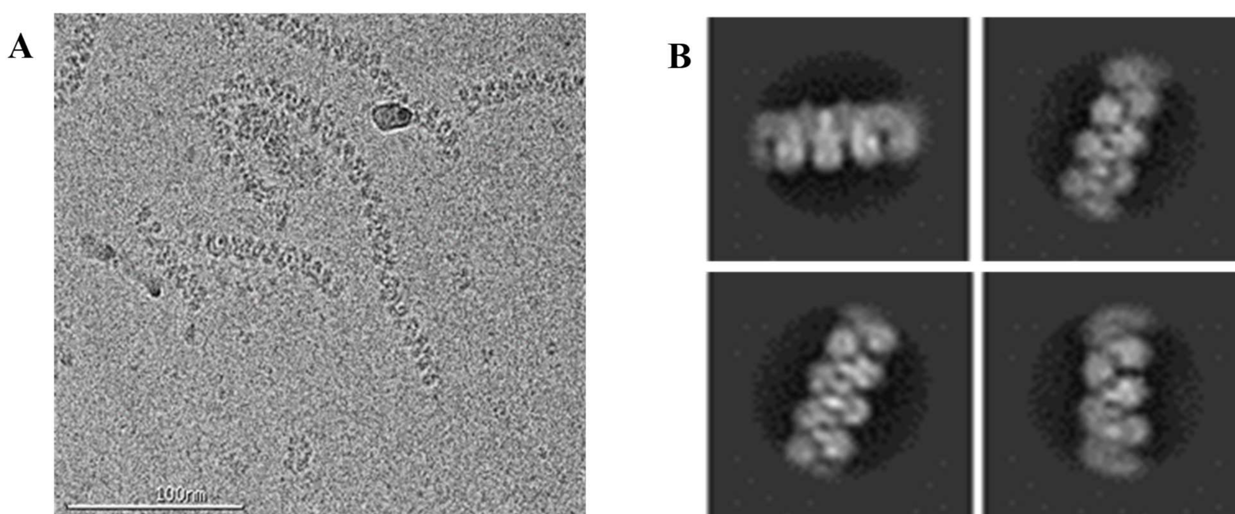

Figure 1. Cryo-electron microscopy analysis of guinea pig $αA^{ins}$-crystallin and $αA^{66\text{-}80}$ peptide complex. (A) Representative motion-corrected micrograph of guinea pig $αA^{ins}$-crystallin and $αA^{66\text{-}80}$ peptide complex. Scale bar, 100 nm. (B) Representative 2D class averages generated using cryoSPARC2 [6]. Box dimension, 50 x 50 nm.

https://doi.org/10.1017/S1431927619007323 Published online by Cambridge University Press