

Subunit exchange study on alpha-crystallin hetero-oligomer

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α -crystallin exists as oligomers consisting of a total of about 20–40 subunits of two homologues: A and B. The most fascinating and interesting feature of α -crystallin is its chaperone activity, suppressing the onset of abnormal aggregation of various target proteins. However, the detailed mechanism of chaperone activity in α -crystallin is still a matter of discussion. α -crystallin does not possess fixed quaternary structure rather “dynamic quaternary structure” induced by reorganization of constituting subunits in α -crystallin. One of the candidates for elementary step for dynamic quaternary structure must be subunit exchange between α -crystallin oligomers. We applied deuteration-assisted small angle neutron scattering (DA-SANS) method on recombinant α -crystallin. Especially, we have prepared partially deuterated (75% deuterated) α -crystallin. From preliminary calculation, it was estimated that 75% deuterated is nearly contrast matched in 100% D₂O. We focused on subunit exchange between hydrogenated A-crystallin and 75% deuterated B-crystallin under the mixing ratio of 3:1, mimicking the native eye lens environment. DA-SANS experiment was performed with Quokka at 37 C at the time interval of 15 min. Fig. 1 (A) shows the scattering profile from 75% deuterated B-crystallin in 0%, 60% and 100% D₂O buffer. It can be clearly seen that 75% deuterated B-crystallin was matched out in 100% D₂O buffer, certifying the proper deuteration level of partially deuterated B-crystallin. Fig. 1(B) shows the time dependence of forward scattering ($I(0)$) after mixing 75% deuterated B-crystallin and hydrogenated A-crystallin. Decrease of $I(0)$ was observed, implying the existence of subunit exchange between B-crystallin and hydrogenated S-crystallin. Interestingly, it was found

that about half of the constituting subunits in oligomeric A-crystallin was exchangeable from the fit to the time dependence of $I(0)$. At present, biological interpretation for the difference of subunit exchange between homo-oligomeric B-crystallin and hetero-oligomeric is not obtainable. The complementary studies with mass spectrometry and small angle X-ray scattering (SAXS) are on going.

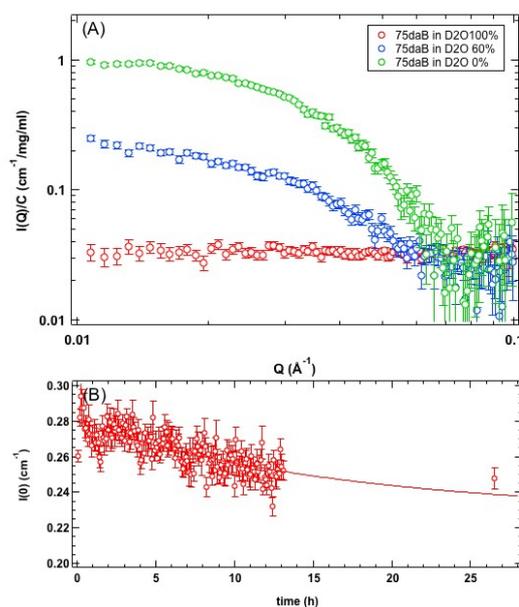


Fig. 1. (A) 75% α pgB-crystallin in 0%, 60% and 100% D₂O buffer. (B) Time evolution of $I(0)$ of 75% deuterated B-crystallin and A-crystallin with the mixing ratio of 1:3.