Experimental report

10/04/2016

Proposal: 8-03-854 **Council:** 4/2015

Title: Kinetic studies on subunit exchange in alpha-crystallin under external stress

Research area: Biology

This proposal is a new proposal

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Samples: D2O
hydrogenated alphaA-crystallin
deuterated alphaB-crystallin

Instrument	Requested days	Allocated days	From	To
D11	0	0		
D22	3	2	01/12/2015	03/12/2015

Abstract:

Alpha-crystallin, which is the major protein in the eye lens, exists as huge molecule consisting of approximately 40 subunits and comprised of two highly homologue: alpha-A and alpha-B. Most interesting feature in alpha-crystallin is its chaperone activity, preventing the aggregation of various target proteins under external stresses such as heat and UV. One of the proposed ideas for the mechanism of its chaperone activity of alpha-crystallin is the hybridization of alpha-crystallin with target proteins and it was also found that reorganization of quaternary structure of alpha-crystallin was essential for realizing complexation. Hence it is strongly expected that subunit exchange between alpha-A and alpha-B crystallin in alpha-crystallin must be an elementary step for commencing the reorganization of quaternary structure, leading to onset its chaperone activity.

We then try to study the subunit exchange between alpha-A and alpha-B crystallin under external stress:UV irradiation and heat to unveil the mechanism of its chaperone activity.

α-crystllin, which is the dominant structural protein in the eye lens, exists as oligomers consisting of a total of about 20~40 subunits of two homologues: αA and αB. The most fascinating property of α-crystllin is its chaperone activity, which suppresses the onset of abnormal aggregation of various target proteins under external stresses such as heat and UV. At present the detailed mechanism of α-crystllin is still unresolved.

One of the proposed ideas is the reorganization of its quaternary structure, realizing preferred complexation with target protein. In other words, α-crystallin is a dynamic entity which quaternary structure is submitted to a constant reorganization following a certain kinetic (subunit exchange). In the previous works, the exchange rate was increased with temperature qualitatively, along with the enhancement of chaperone activity. It is strongly expected that subunit exchange between αA and αB crystallin is an elementary step essential to trigger α-crystallin reorganization, leading to onset its chaperone activity. We then studied the subunit exchange between αA and αB crystallin under physiological condition (310K) and heat stress (321K) and compared the results from two conditions. In order to monitor the subunit exchange, we utilized deuteration-assisted small-angle neutron scattering (DA-SANS).

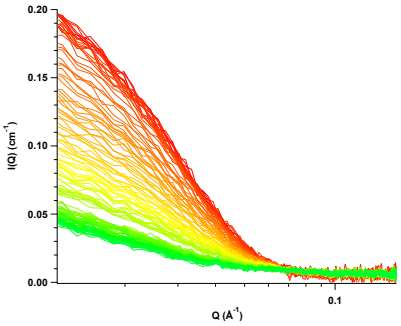

Fig.1 Time evolution of SANS profile upon mixing h-αA and d-αB in 82% D_2O buffer at 310K. Change of red from green curve means the lapse of time.

Before introducing the result under heat stress, we would like to introduce results from the subunit exchange between hydrogenated αA (h-αA) and deuterated αB crystallin (d-αB) at 310K. Fig. 1 shows the time evolution of SANS profile upon mixing h-αA and d-αB in 82% D_2O buffer, at which he scattering contrasts from h-αA and d-αB have the same absolute value but opposite in signs. The gradual decrease of scattering intensity was clearly seen with the lapse of time, certificating the existence of subunit exchange between h-αA and d-αB.

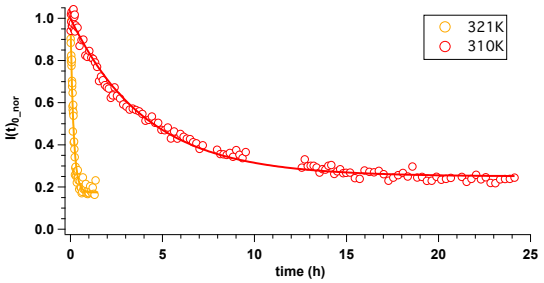

Fig.2 $I_{0\_nor}(t)$ at 321K and 310K.

Time evolution of $I_0(t)$ normalized by $I_0(t=0)$ ($I_{0\_nor}(t)$) is given in Fig. 2 and $I_{0\_nor}(t)$ is well described by following equation.

$$I_{0\_nor}(t)=[A+(1-A)\exp(-\tau/t)], \quad \text{eq. (1)}$$

where τ and A correspond to the decay time constant and the ratio of decay to the initial intensity at the isotopic equilibrium state, respectively. τ and A were evaluated at 0.24 h^{-1} and 0.25, respectively. Based on our previous work, A is directly related to the number of exchangeable subunits in an oligomer. It was preliminary found that both oligomers of h-αA and d-αB were consisting of 26-mer. From simple calculation, it was revealed that 13~14 subunits among 26-mer were exchangeable. It implies that only half of constituting subunits are exchangeable for h-αA and d-αB.

As a next step, we studied the subunit exchange between h-αA and d-αB at 321K and $I_{0\_nor}(t)$ at 321K is also included in Fig. 2. τ and A at 321K were evaluated at 6.1 h^{-1} and 0.17, respectively. Namely, drastic acceleration of exchange rate was observed at 321K. As described above, the enhancement of chaperone activity was reported under external stress. Then it is expected that the acceleration of subunit exchange would enhance the encountering probability between subunit of α-crystllin and target protein, contributing the suppression of abnormal aggregation of target protein. In other words, subunit exchange in α-crystllin must be regulator for chaperone activity. To verify such an idea, the other supporting measurements such as native mass spectrometry are on going.