

表題：Crowding 環境下におけるアルファクリスタリンのサブユニット動態

Subunit dynamics of alpha-crystallin under crowding condition

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In-vivo environment is highly crowded due to the presence of proteins, nuclear acids, and lipids, constituting the conditions of so-called "crowding". One of the representative organs attaining highly concentrated system is eye lens. Eye lens are composed of three different types of crystallins, α , β -crystallin and γ -crystallin is the dominant crystallin among them. α -crystallin exists as oligomers consisting of approximately 20~40 subunits. At present, the quaternary structure of α -crystallin has not been determined due to the unavailability of its crystal structure. It is considered that the failure of availability of its crystal structure might be originated from dynamical aspect of quaternary structure: "dynamic quaternary structure" induced by subunit exchange between α -crystallin oligomers. In order to reveal the mechanism of subunit exchange in α -crystallin, we applied deuteration-assisted small angle neutron scattering (DA-SANS) on α -crystallin at the concentration of ~1 mg/ml. On the other hand, the crowding environment is expected to be totally different from dilute system, in which the excluded volume effect or osmotic pressure is negligible. It is then postulated that the presence of close contact neighboring oligomers would alter the subunit exchange in α -crystallin. In order to mimic the crowding, we have prepared 75% deuterated α -crystallin, of which scattering length density was estimated to be equal to 100% D₂O. Fig. 1(a) shows the scattering profiles from hydrogenated

α -crystallin at the concentration of 0.45 mg/ml and 75% deuterated α -crystallin at the concentration of 28 mg/ml in 100% D₂O buffer. It is clearly seen that the scattering contribution from highly concentrated 75% deuterated α -crystallin is drastically suppressed in 100% D₂O buffer.

使用施設：JRR-3M，装置：C1-2:SANS-U
分野：Biology

order to find out the possible effect of crowding on resulting subunit exchange in α -crystallin, we have prepared two systems. One is consisted of the combination of hydrogenated α -crystallin at the concentration of 0.45 mg/ml and 75% deuterated α -crystallin at the concentration of 0.45 mg/ml in 100% D₂O buffer (dilute system). The other is comprised of the combination of hydrogenated α -crystallin at the concentration of 0.45 mg/ml and 75% deuterated α -crystallin at the concentration of 28 mg/ml in 100% D₂O buffer (crowding system). Both of kinetics study was performed with Quokka installed at the Australia nuclear science and technology (ANSTO) at 37 C. at the time interval of 20 min. Fig. 1 (B) and (C) shows the time evolution of forward scattering intensity ($I(0)$) from dilute system and crowding system, respectively. After mixing 75% deuterated α -crystallin and hydrogenated α -crystallin decrease of $I(0)$ was observed, implying the existence of subunit exchange in α -crystallin. It was found that both system reached equilibrium state after 12 h after mixing. Interestingly, the exchange rate evaluated from crowding system is the same as that from dilute system within experimental error. This experimental observation reinforces our idea that subunit exchange progresses through not the collision between α -crystalline oligomers but the librated subunits. The supporting works with analytical ultra centrifuge and small angle X-ray scattering (SAXS) are on going.

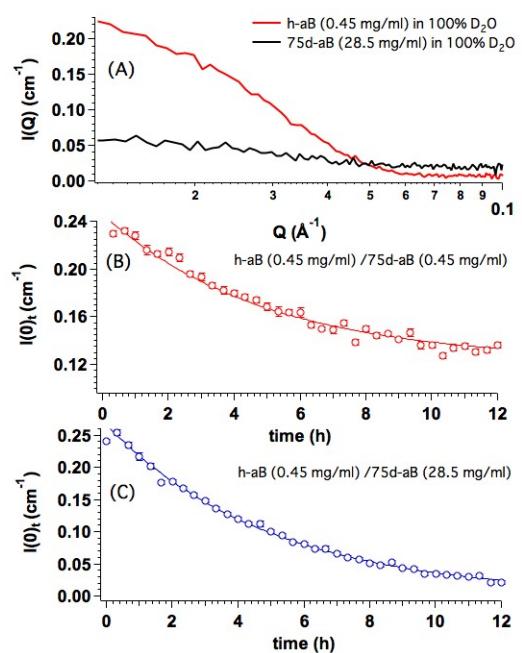


Fig. 1. (a) scattering profiles from crystallin. (b)(c) Subunit dynamics under dilute and crowding system.