Structural analysis of insulin B-chain protofibril complexed with αB-crystallin by small-angle neutron scattering

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Amyloid fibrils are aberrant protein aggregates that are implicated in numerous amyloidoses and neurodegenerative diseases. Elucidating the mechanism of their formation and regulation is essential for establishing the molecular basis for the development of diagnostic and therapeutic strategies. Amyloid formation sometimes proceeds through the growth of transient protofibrillar intermediates, and in our previous SAXS and DLS studies, we found that αB-crystallin (αBC) suppresses the elongation of insulin B-chain protofibrils through complex formation. Interestingly, the resulting complexes became shorter while exhibiting a larger cross-sectional radius than protofibrils alone; however, the spatial organization of αBC within the complex remained unclear.

To clarify the binding mode of αBC within the complex, we performed small-angle neutron scattering (SANS) combined with protein deuteration and solvent contrast variation. Two types of complexes were prepared using insulin B chain together with hydrogenated and partially (~75%) deuterated αBC, and SANS measurements were carried out in 100% D_2O buffer. This experimental design enabled selective visualization of either the entire complex or the B-chain component within the complex.

SANS measurements of the two complexes in 100% D_2O buffer yielded profiles exhibiting an $I(q) \sim q^{-1}$ relationship suggesting a rod-like shape of the complex. However, distinct cross-sectional radii were obtained, and this result indicated that αBC is distributed around the outer surface of the protofibrils forming a core–shell structure. This structural interpretation was further supported by αBC-selective vialization in 40% D_2O buffer, which yielded a larger R_p than that obtained from the entire complex. Furthermore, the B-chain core within the complex was thinner than that of aBC-free protofibrils. This indicates that αBC suppresses the lateral growth of protofibrils through its association with the protofibril surface. These findings provide structural basis for the molecular arrangement of αBC in the protofibril–αBC complex and offer new insights into the molecuar mechanism by which small heat shock proteins inhibit amyloid protofibril growth.

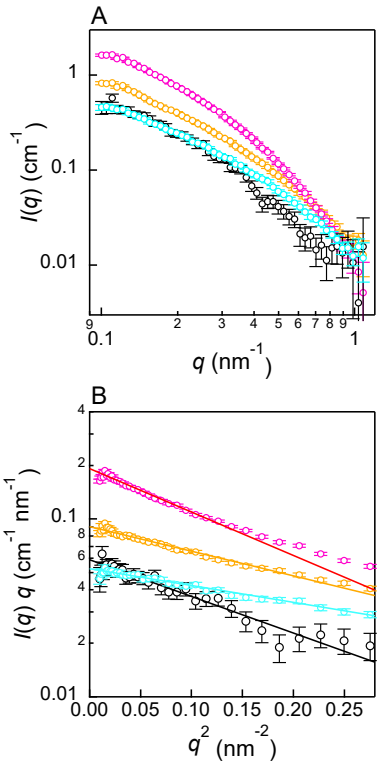

Fig. 1. SANS profiles of the complex comprising hydrogenated insulin B chain and hydrogenated αBC in 100% D_2O buffer (magenta), the complex comprising hydrogenated insulin B chain and partially deuterated αBC in 100% (cyan) and 40% D_2O buffer (black), and insulin B chain protofibrils formed in the absence of αBC in 100% D_2O buffer (orange). (A) Double-logarithmic plots. (B) Cross-sectional Guinier plots.