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Kinetics of amyloid-like particle formation by YB-1 protein and its truncated variants studied by SAXS technique

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Introduction

Amyloid structures were discovered as protein deposits associated with several neurodegenerative diseases. Recent studies have shown that various proteins, even not related to any known amyloid disease, can aggregate into fibrils under the native fold- destabilizing condition [1] and that normal proteins become toxic in this case. YB-1 is a multifunctional RNA and DNA-binding nucleocytoplasmic protein. It was shown that purified YB-1 in solution, as well as YB-1 in association with RNA at a high YB-1/mRNA ratio, formed multimers up to 800 kDa [2]. Recently we reported that YB-1 is capable of forming elongated fibril structures under high ionic strength conditions [3]. It was suggested that these fibrils were amyloid-like. It is generally accepted that initial steps of amyloid structure formation involves oligomerization. It may be proposed that conformational rearrangements could lead YB-1 oligomers to amyloid structure. To study the early stages of amyloid aggregation in detail we used YB-1 protein and its truncated variants. Here we present SAXS patterns of these proteins under conditions of fibril formation at different incubation times.

Experimental

The full length YB-1 (M=36kD) was purified as described earlier [2]. Plasmid pET-3-1-YB-$1_{1\text{-}219}$ was provided by Dr. Alexey Sorokin. YB-$1_{1\text{-}219}$ was purified as YB-1. To construct plasmids for YB-$1_{1\text{-}129}$ and YB-$1_{52\text{-}129}$ corresponding DNA fragments were PCR-amplified and inserted into pET-22b vector. YB-$1_{1\text{-}129}$ and YB-$1_{52\text{-}129}$ were expressed in *E. coli* and isolated by ammonium sulphate fractionation with subsequent chromatography (SP-sepharose, Phenyl-sepharose and MonoS columns). To induce the amyloid formation proteins were transferred to 20 mM Hepes-KOH, 2 M LiCl, pH 7.4. Protein concentrations were 10 μM. Synchrotron X-ray measurements were done on a small-angle camera BL-15A (Photon Factory, Tsukuba) using CCD-detector. The range of scattering vectors Q=0.008-0.2 $Å^{-1}$.

Results

It appeared that Guinier plot for all samples at different time after preparation (1, 3, 6 hours) are not linear (not shown) reflecting essential association of protein. Evaluated radii of gyration (Rg) from Guinier plot after 6 h were 102 Å, 105 Å, 103 Å, 114 Å and 80 Å, 84 Å, 103 Å, (not determined) Å at the beginning for YB-1, YB-$1_{1\text{-}219}$, YB-$1_{1\text{-}129}$, YB-$1_{52\text{-}129}$, respectively. The corresponding values of molecular mass evaluated from I(0) were 235, 425, 788, 491 kD after 6h and 65, 97, 788, (not determined) at the beginning, respectively. The corresponding lag periods of aggregate formation were 3h, 6h, 1h, 6h, respectively. For YB-$1_{52\text{-}129}$ it was impossible on the first stage to obtain SAXS pattern due to small concentration and small particle dimension. One can see from log-log plot in Fig.1 that in the case of YB-$1_{1\text{-}219}$ planar aggregates are formed on the first stage (slope is close to -2) transforming to elongated fibrils (slope is close to -1).Other samples form planar aggregates or more complex ones.

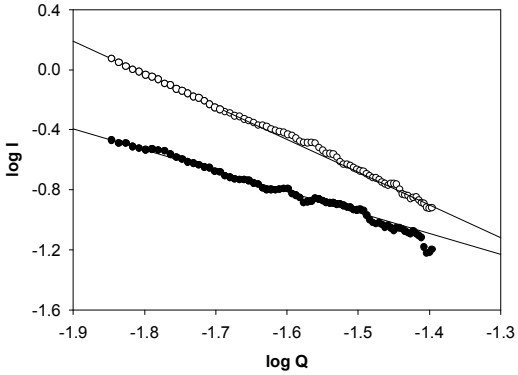

Fig.1 Log-log SAXS pattern for YB-$1_{1\text{-}219}$ 3h (solid circles, slope=-2.2) and 6h after preparation (open circles, slope=-1.3).

References

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