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Formation of amyloid-like particles by albebetin-thioredoxin fused protein and its H65F, H30F mutant forms studied by SAXS technique

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Introduction

Actual topic at present is the analysis of formation of amyloid fibrils which often cause numerous diseases. This problem is closely related to the protein folding/ misfolding [1]. At the last time the application of artificial protein hybrid constructions (target protein-polypeptide linker-carrier protein) for biochemical assays is widespread one. Very often the aim of such construction is to enhance the protein solubility and decrease its association. However, mutual influence of parts in the fused protein may significantly change their properties. Thus, the artificial protein albebetin (ABB) and its derivatives containing biologically active fragments of natural proteins form fibrils at physiological pH [2]. Recently it has been shown that the fused ABB-thioredoxin protein (ABB-TR) also forms amyloid-like aggregates. Here we study by SAXS this process in detail for ABB-TR and its H65F, H30F mutants.

Experimental

Genes of albebetin with point mutations and gene of thioredoxin were expressed in E.coli BL21 (DE3) as a whole polypeptide chain (M=26kD) with subsequent protein purification [2]. To obtain amyloid-like particles, the protein solutions were kept at 37°C for 24 hours. The used buffer was 20mM Tris-HCl (pH8.0). Protein concentrations were 10.0 mg/ml. 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

Evaluated radii of gyration (Rg) from Guinier plot before heating were 40.1 Å, 45.8 Å, 54.3 Å for ABB-TR, H65F, H30F, respectively. The corresponding values of molecular mass evaluated from I(0) were 23, 38, 46 kD, respectively. It appeared that Guinier plot for all three proteins after incubation at 37°C for 24 hours are not linear (not shown) reflecting essential association of protein. .In this case evaluated radii of gyration (Rg) were 58.6 Å, 69.4 Å, 62.8 Å for ABB-TR, H65F, H30F, respectively. The corresponding evaluated values of molecular mass were 26, 60, 56 kD, respectively.

The above data show that the largest changes in scattering curve are observed for H65F and the smallest ones for H30F. At the same time the Kratky plot in all cases demonstrated bell- like shape of scattering patterns (not shown) indicating the compact conformation of protein molecules inside associates. The essential association of protein molecules permits to elucidate the type of such associates. In Fig.1, log-log dependence of SAXS pattern is presented for H65F. One can see the good linear dependence of SAXS pattern of sample after incubation with slope -0.95 which is close to -1.0 corresponding to highly elongated shape of molecule. For other samples after incubation such dependence is not perfectly linear but slope varies in the range -0.8-0.9 indicating elongated shape of particle. Thus, after incubation at 37°C for 24 hours all three proteins form amyloid-like particles whereas the conformation of protein molecules inside associates is compact.

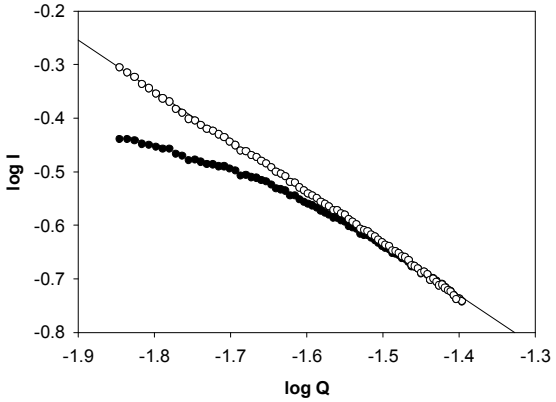

Fig.1 Log-log dependence of SAXS pattern for H65F before (solid circles) and after (open circles) incubation.

References

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