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Conformation of YB-1 protein at moderate and high ionic strength 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 (2M LiCl) [3]. It was suggested that these fibrils were amyloid-like. It is interesting to understand the initial steps of this process of oligomerization. Here we present SAXS patterns of YB-1 at moderate ionic strength and at high one (1M KCl).

Experimental

The full length YB-1 (M=36kD) was purified as described earlier [2]. The buffer with moderate ionic strength was 500 mM K-phosphate pH 7.4 and protein concentration was 8.8 mg/ml. The buffer with high ionic strength was 20 mM K-phosphate 1M KCl pH 7.4 and protein concentration was 3.8 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

SAXS patterns in the Guinier coordinates (log I versus Q^2) for YB-1 in 0.5 M K-phosphate and in 1 M KCl are presented in Fig. 1. One can see the non-linear character of scattering curves for the protein indicating substantial association of protein molecules. The values of molecular mass (M_w) and a radius of gyration (R_g) of particles estimated from the initial part of scattering curves are given in the legend to Fig. 1. These estimates show the association of the protein in both cases. The analysis of aggregate shape from SAXS data in coordinates log I versus log Q has shown that YB-1 aggregate in 0.5M K-phosphate is strongly elongated consisting of three monomers. The dimensions of this aggregate are given in the legends to Fig. 1. At high ionic strength very large compact aggregates are formed. Thus, one can speculate that at moderate ionic strength the strongly elongated particles consisting of three monomers might be the blocks forming amyloid-like fibrils. At high ionic strength both fibrils (2M LiCl) and compact aggregates (1M KCl) can be formed depending on ion characters.

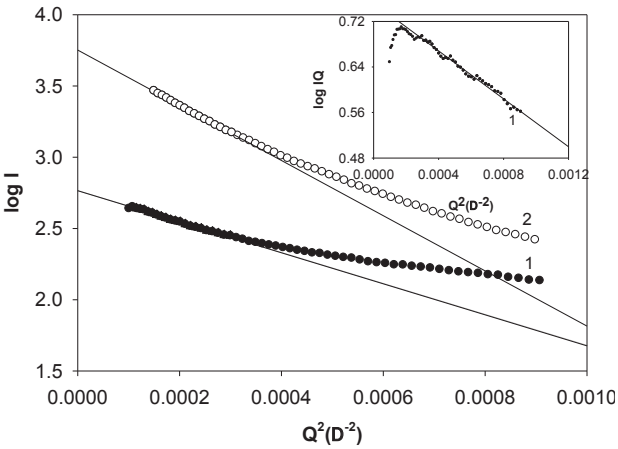

Fig.1 The Guinier plots for YB-1. 1 – at moderate ionic strengh; 2 – at high ionic strength. The corresponding values of molecular mass and radius of gyration evaluated from the initial part of scattering patterns are (M_w= 94 kDa, 1030 kDa; R_g= 60 Å, 116 Å, respectively). *Insert*: Log IQ versus Q^2 plot for the evaluation of R_c value of cross-section. The corresponding R_c value estimated from the slope is 31 Å and, as consequence, the value of length of particle is 285 Å for 1.

References

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