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Studies of ribosomal protein S1 from *Thermus thermophilus* at different ionic conditions in solution by X-ray small-angle scattering

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Introduction

Ribosomal protein S1 is the largest protein of eubacterial ribosomes with a molecular mass of 61 kDa and a length of 557 amino acid residues. It is an RNA-binding protein involved in retention of mRNA during initiation of translation and, maybe, during elongation. The most specific feature of the primary structure of *Escherichia coli* protein S1 is the presence of six homologous amino acid repeats of about 70 amino acids long separated by spacers of 10-15 residues**.** Physical studies of isolated protein S1 from *E.coli* showed that the protein in solution manifested a highly extended, non-compact conformation with the longest dimension (23nm) of the whole ribosome. At the same time our recent sedimentation and microcalorimetry data [1] have shown the compact structure of S1 *Th. th.*. at low ionic strength. To study the detailed structural behaviour of this protein in solution at different ionic conditions we used SAXS techniques.

Experimental

Ribosomal protein S1 has been recently identified in *Thermus thermophilus* [1] and its gene has been expressed in *E.coli* overproducing strain BL21(DE3)pET21d-tthS1 and S1 has been successfully purified in the sufficient amounts for physical measurements. The protein was stored at 4°C in the precipitated form in $(NH_4)_2SO_4$. Samples for measurements were prepared by solving the precipitate either in 25 mM HEPES-NaOH buffer, pH 7.5 (buffer A) or in 25 mM HEPES-NaOH (or NaOD) buffer, pH 7.5, with 100 mM NaCl (buffer B) and subsequent dialysis against the same buffers at room temperature overnight. The used protein concentrations were in the range of 1.5-10.0 mg/ml. Synchrotron X-ray measurements were done on the small-angle camera BL-15A (Photon Factory, Tsukuba) using CCD-detector. The range of scattering vectors Q= 0.08-2 nm^{-1}.

Results

Scattering patterns for S1 in both buffers plotted in the Guinier coordinates showed the nonlinear behaviour with the initial ordinate indicating the association of protein molecules. Thus, we can only estimate the value of radius of gyration (Rg). These values are in the range 7-11 nm to be considerably higher the expected one (about 3nm) for globular protein of 61kD. For S1 in the buffer A the essential concentration dependence of scattering pattern is observed, and the associates correspond to trimers and higher order oligomers. For S1 in the buffer B such dependence is weak, and the associates correspond preferentially to dimmers and trimers. The more structural information we can obtain from the scattering patterns plotted in the Kratky coordinates (see Fig.1). One can see the bell-shaped plot for S1 in the buffer B corresponding to compact particles. The value Rg estimated from the position of maximum on the Kratky plot was 2.9 nm which is expected for globular protein of 61kD. Thus, S1 particles at the moderate ionic strength associates in the random order building dimers, trimers with the globular conformation of monomer inside them. Judging the Fig.1, S1 particles at the low ionic strength form the globular-like high-order associates with the globular conformation of monomer inside them. The future model calculations will give the more detailed composition of oligomers.

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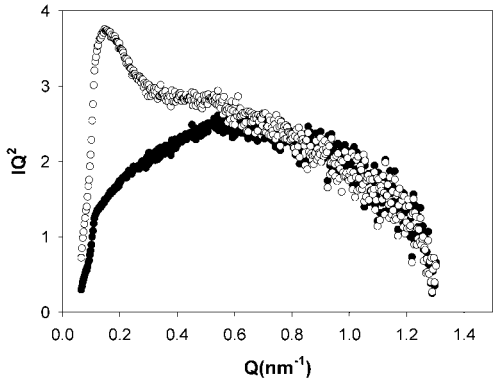

Fig.1 Kratky plot of SAXS data for S1 in buffer B (full circles) and in buffer A (empty circles).

References

1) 1. Selivanova O.M. *et al.* , J.Biol.Chem. , **278**(38):36311-4, 2003.

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