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Conformation of ribosomal protein S1 from *Thermus thermophilus* and its 49 kDa fragment in the presence of nucleotides studied by SAXS technique

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Introduction

Ribosomal protein S1 T.*therm*. is the largest protein of ribosome with a molecular mass of 60 kDa and a length of 536 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 this protein is the presence of five homologous amino acid repeats of about 70 amino acids long separated by spacers of 10-15 residues and sixth degenerate one on C-terminal **.** Up to now the tertiary structure of S1 is unknown. Possible step to elucidate its structure is a crystallization of some fragments. Here we present SAXS data for S1 and its 49 kDa fragment (without C-terminal repeat) in free state and at the presence of GDP

Experimental

The gene of S1 from *Thermus thermophilus* has been expressed in *E.coli* overproducing strain BL21(DE3)pET28a-tthS1) and that of fragment in BL21(DE3)pET28a-tthS1(49) [1]. Both proteins were successfully purified. The proteins were stored at 4°C in the precipitated form in $(NH_4)_2SO_4$. Samples for measurements were prepared by solving the precipitate and making dialysis against 25 mM HEPES-NaOH buffer, pH 7.5, with 100 mM NaCl. The concentration of GDP was 100 μM. 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= 0.008-0.12 nm^{-1}.

Results

Scattering patterns for S1 and its fragment plotted in the Guinier coordinates showed the nonlinear behaviour with the initial ordinate indicating the association of protein molecules in free state and in the presence of GDP. The analysis of protein conformation in these associates from the scattering patterns plotted in the Kratky coordinates showed in both cases the bell-shaped plot corresponding to compact particles. At the same time the arrangement of monomers inside of associates differs for both proteins in free state and in the presence of GDP. In Fig.1, the plot for the evaluation of cross-section of particles is presented for protein S1. One can see the elongated conformation of associates in free state (R_c=30Å) and some branched conformation upon interaction with GDP. For 49 kDa fragment of S1 one can see in Fig.2 that the addition of GDP stimulates the growth of elongated particles (R_c=13Å) Thus, GDP strongly influences on the conformation of protein associates and nucleotides may be considered as potential tools for protein crystallization.

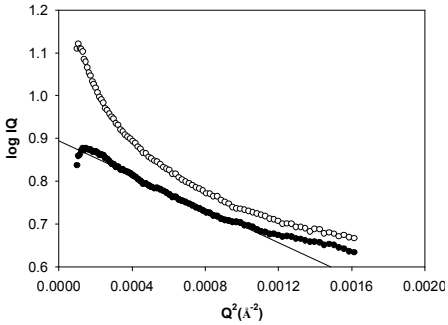

Fig.1 Cross-section plot of S1 with (o) and without GDP(●).Incline corresponds to R_c=30Å.

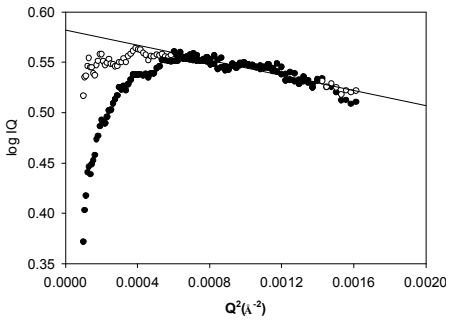

Fig.2 . Cross-section plot of 49 kDa fragment with (o) and without GDP(●).Incline corresponds to $_c$=13Å.

References

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

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