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SAXS study of iron-binding Dps protein interaction with DNA

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Introduction

Multifunctional protein Dps plays an important role in iron assimilation and a crucial role in bacterial genome packaging. Its monomers form dodecameric spherical particles accumulating ~400 molecules of oxidized iron ions within the protein cavity and applying a flexible N-terminal ends of each subunit for interaction with DNA. Deposition of iron is a well-studied process by which cells remove toxic Fe^{2+} ions from the genetic material and store them in an easily accessible form. However, the mode of interaction with linear DNA remained mysterious and binary complexes with Dps have not been characterized so far.. Here we present SAXS patterns of DPS protein from *E.coli* and its complexes with DNA.

Experimental

Recombinant DPS (M_w= 18.7 kDa for monomer) and 420 bp fragment of regulatory region of *dps* gene were applied in SAXS experiments. The buffer 50 mM Tris-HCl pH 8.0, 25mM NaCl, 5% glycerol was used. The protein concentration was 1.36 mg/ml.
The molar ratio of protein-DNA complex was about 27:1. Synchrotron X-ray measurements were done on a small-angle camera BL-6A (Photon Factory, Tsukuba) using PILATUS 100K detector. The range of scattering vectors Q=0.01-0.25 $Å^{-1}$.

Results

SAXS pattern of DPS is presented in Fig.1a. For better accuracy we evaluated radius of gyration (R_g) of protein from the distance distribution function P(r) calculated by GNOM program [1] in the range of Q=0.02-0.25 $Å^{-1}$. It was found that R_g == (35.7±0.2) Å. To exclude the possible uncertainty in concentration value to calculate the molecular mass we compared volumes (V) of particles calculated from Porod invariant. For DPS V=299.5 nm^3 which is closed to the value V=263.5 nm^3 calculated from the SAXS pattern of crystal structure (1JRE.pdb) by CRYSOL program [2] (see Fig. 1b). One can see from Fig.1 the similarity of experimental and calculated curves for DPS. Interaction of DPS with DNA duplex dramatically changes the DPS structure (see Fig. 1c). For complex we have R_g == (42.4±0.2) Å and V=122.6 nm^3. This value is close to half one of the whole DPS. We also calculated SAXS pattern from one hexameric ring of DPS molecule (1JRE.pdb). The calculated SAXS pattern is drawn in Fig. 1d. One can see the similarity of SAXS pattern DPS-DNA and calculated curves for half of DPS. Thus, the interaction of DPS with DNA duplex causes dramatic changes in DPS structure and it needs the further investigation.

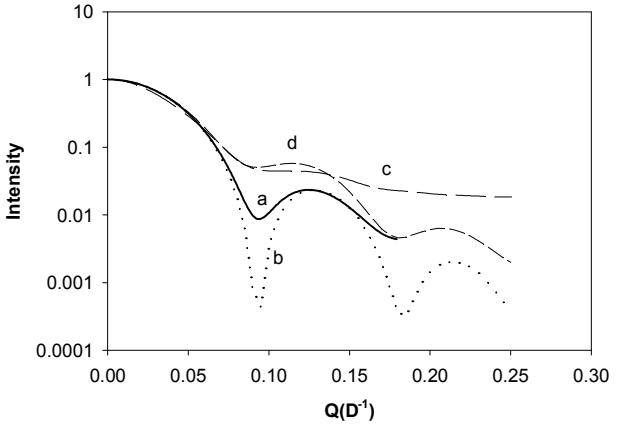

Fig.1 The dependence log I versus Q for DPS (a-DPS, b-DPS crystal structure, c-DPS+DNA, d- half of DPS).

References

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