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STUDY OF MSSoII DNA METHYLTRANSFERASE COMPLEXES WITH LIGANDS BY SYNCHROTRON X-RAY SCATTERING

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Introduction

DNA methyltransferases (DNA-MT) are the important constituent part of gene expression and regulation. They methylate the definite DNA bases regulating site-specific DNA digestion by restriction endonucleases. In procaryotes DNA-MT are involved in the processes of replication initiation and gene expression regulation [1]. The existence of several functions for DNA-MT permits to suppose that these enzymes are structurally labile. The knowledge of their solution structure and that with DNA ligands can shed light on their function. Here we studied the procaryotic 5-methylcytosine DNA methyltransferase ***Sso***II (M.*Sso*II) having two DNA-binding activities: regulation and methylation provided by two different protein domains.

Experimental

M.*Sso*II was isolated from the M15 strain of *E.coli* carried the pREP4 plasmid. The buffer conditions are: 50mM Tris-HCl (pH7.6), 150mM NaCl, 5mM β-mercaptoethanol. Protein concentration was 2.0 mg/ml. Free protein and its complexes with 31-mer DNA-duplex for regulation and 30-mer one for methylation in the presence of corresponding cofactors SAM and SAH have been studied. Synchrotron X-ray measurements were done on a small-angle camera BL-15A (Photon Factory, Tsukuba)

Results

Evaluated radii of gyration (Rg) from Guinier plot were (3.0+/-0.1)nm for free protein, (3.50+/-0.1)nm for M.*Sso*II + 31-mer, (3.4+/-0.1)nm for M.*Sso*II +30-mer. Not linear shape of Guinier plot at the very small scattering angles reflected some association of protein. Evaluated molecular mass for free protein was 45 kD which is close to that from aminoacid sequence.. At the same time evaluated molecular mass for 31-mer and 30-mer complexes (with 1:1 molar ratio) was 2.5 and 3.5 times higher that of free protein, respectively. It means that, at least, two DNA molecules are bound to one protein molecule. Kratky plot (Fig.1) showed globular shape of M.*Sso*II and its comlexes with DNA duplexes. Some tail on the plot at higher scattering angles reflects the binding of DNA molecules to protein one. Large increase of molecular mass of complexes and small change of their R_g values witness the essential compactization of protein upon substrate binding. Further model calculations can present conformational changes in detail.

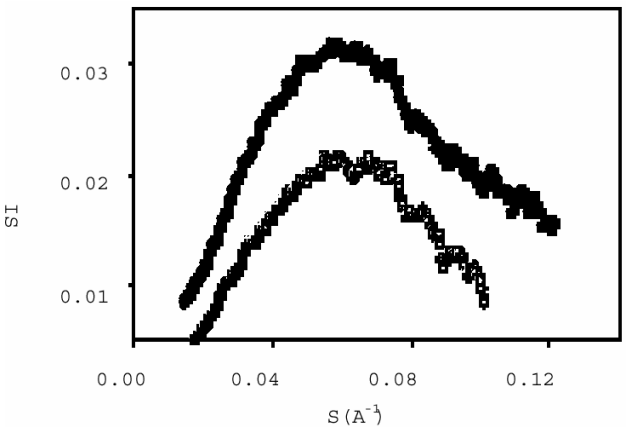

Fig.1 Kratky plot for MSSoII(o), MSSoII+31bp DNA-duplex (•).

References

Heitman, Genetic Engineering. **15**, 57, 1993

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