Study of uracil DNA glycosylase complexes with ligands by synchrotron X-ray scattering

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Introduction

Uracil DNA glycosylase (UDG) is a key enzyme in the DNA repairing system. The last X-ray analysis data [1] of UDG from different origins elucidated the high specificity of this enzyme to uracil base produced by cytosine deamination in DNA. The large changes of DNA conformation are observed at UDG action. We decided to test large-scale conformational changes of UDG in solution upon ligand binding by synchrotron X-ray scattering.

Experimental

UDG was isolated from the M15 strain (REP4) of *E.coli* carried the pR632 plasmid. The buffer conditions are: 20mM Tris-HCl (pH7.6), 60mM NaCl, 1mM EDTA. Protein concentration was 5.5 mg/ml (0.21mM). Free protein and its complexes with $(pT)_3$ inhibitor (c=0.87mM), with 18-mer non-hydrolyzable oligonucleotide (c=0.36mM), with 14-mer oligonucleotide (c=0.36mM) and their duplex (c=0.36mM) have been studied Synchrotron X-ray measurements were done on a small-angle camera BL-15A (Photon Factory, Tsukuba)

Results

Guinier plot were not linear reflecting the protein association preferably in dimer form. Evaluated radii of gyration (Rg) were (2.27+/–0.1)nm for free protein, (2.10+/–0.1)nm for UDG+$(pT)_3$, (2.34+/–0.1)nm for UDG+18-mer, (2.35+/–0.1)nm for UDG+14-mer, (2.68+/–0.1)nm for UDG+duplex. For globular protein of 26 kD molecular mass the expected Rg doesn't exceed 2nm. Fig.1 shows the distance distribution function P(R) for all studied samples. One can see the essential compactization of protein upon $(pT)_3$ binding and partial distruction of protein associates what correlates with our previous data on His-tag UDG. Such destruction is also observed for oligonucleotides in contrary to duplex action. Evaluation of molecular masses shows that in all cases the complexes are formed and their dimensions increase with the molecular mass increase what is evident from the shift of maximum on P(R). In contrast to His-tag UDG UDG with native sequence is globular. Further analysis of data will permit to elucidate the conformational changes in UDG upon ligand binding.

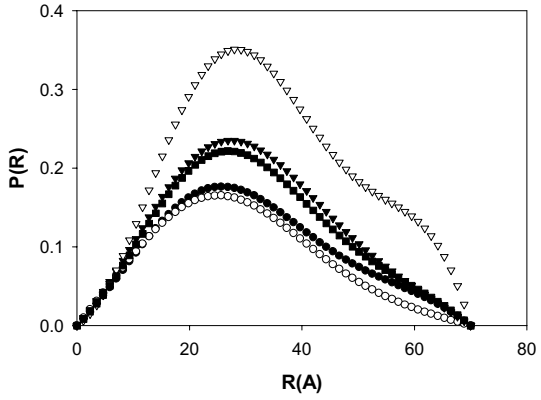

Fig.1 P(R) for UDG (●), UDG+$(pT)_3$ (○), UDG+18-mer (▼), UDG+14-mer (■), UDG+duplex (▽).

References

G.Slupphaug et al., Nature **384**, 87, 1996

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