15A/1999G338

STUDY OF URACIL DNA GLYCOSYLASE SOLUTION CONFORMATION BY SYNCHROTRON X-RAY SCATTERING

Alexander TIMCHENKO[1], Elena KUBAREVA[2], Hiroshi KIHARA[*3]

[1] Institute of Protein Research, Pushchino, Russia, 142292;
[2] Institute of Physico-Chemical Biology of Moscow State University, Russia, 117333;
[3] Department of Physics, Kansai Medical University, Uyamahigashi, Hirakata Osaka 573-1136, Japan

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 (SE) from E.coli under 80% of ammonium sulfate was dialyzed against 20mM Tris-HCl (pH8), 0.4mM EDTA, 7mM β-mercaptoethanol and its concentration was 1.7 mg/ml. For the formation of complex with $(pT)_3$ inhibitor the latter was added at 4-fold excess. Unfortunately, the protein concentration was not sufficient for the detailed analysis of enzyme solution structure but the overall dimensions of protein and its molecular mass were estimated. Synchrotron X-ray measurements were done on a small-angle camera BL-15A (Photon Factory, Tsukuba)

Results

Fig.1 shows the Guinier plot for UDG and its complex with $(pT)_3$. The evaluation of molecular mass from the intercept I(0) gives the value (25+/-3) kD which is close to the calculated one of 26kD. The estimated values of radius of gyration were (2.95+/-0.1)nm for the free enzyme and (2.49+/-0.1)nm for its complex with $(pT)_3$. The expected value of radius of gyration for globular protein of 26kD of molecular mass should not exceed 2nm. Thus, our results witness about not compact structure of protein in solution at essential compactization of protein structure upon inhibitor binding. The measurements at higher protein concentration and other ionic conditions have been made and now are under the treatment.

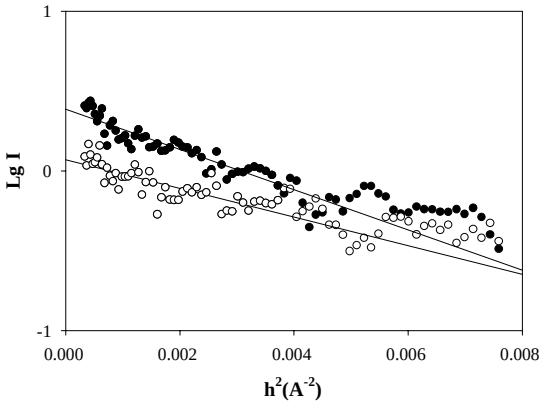

Fig.1 Lg(I) versus h^2 (Guinier plot) for UDG (•) and its complex with $(pT)_3$ (o). $h=4\pi/\lambda\sin(\theta/2)$, where λ-wavelength, θ-scattering angle.

References

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

*kihara@makino.kmu.ac.jp