

Systematic study on protection of Chitinase A1 from X-ray irradiation at the solution x-ray scattering measurement

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Introduction

Strong x-ray beam generated by the synchrotron have brought about the generalization of rapid measurement of solution Small-Angle X-ray Scattering (SAXS). With the increase of beam intensity, however, radiation effect of X-rays on the protein has become not at all negligible and an experimenter required always being careful of the damage on a protein. Although protection effect on protein radiation damage by ethylene glycol (EGOH) is practically known [1] & (Narayanan personal communication), no systematic survey has yet been done despite experimental importance. We have been systematically studying the effect of X-ray irradiation and protection of EGOH on proteins Here we report the results of Chitinase A (ChiA) a typical domain protein.

Results and Discussion

As seen in Fig. (1), change in the Guinier plot profile without EGOH (blue lines; measuring time 5, 10, 15 and 20 min from top to the lower, respectively) is large compared with the solution contained EGOH (red lines; 1000 times of protein concentration). It is quite clear that the radius of gyration of increased with the increase of measured (irradiated) time.

Looking over the Kratky profiles in Fig. (2), no obvious difference in the profile is seen. Because the Kratky profile of a monomeric protein mostly reflects the folding configuration as typically known in folding & unfolding studies, change in third structure (unfolding), due to X-ray irradiation seems not occurred.

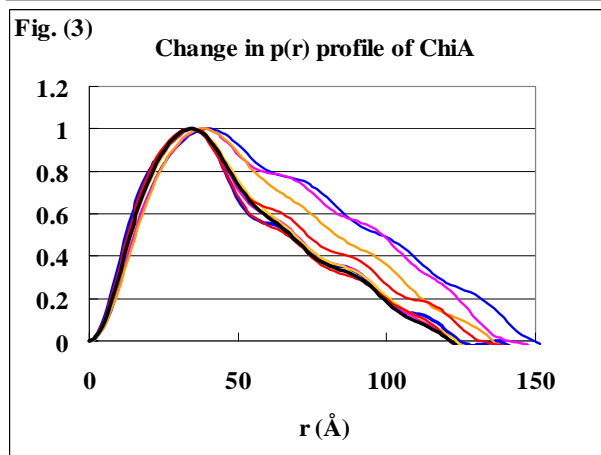
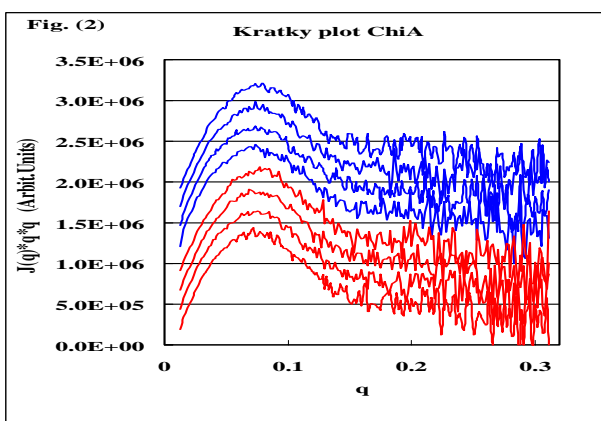
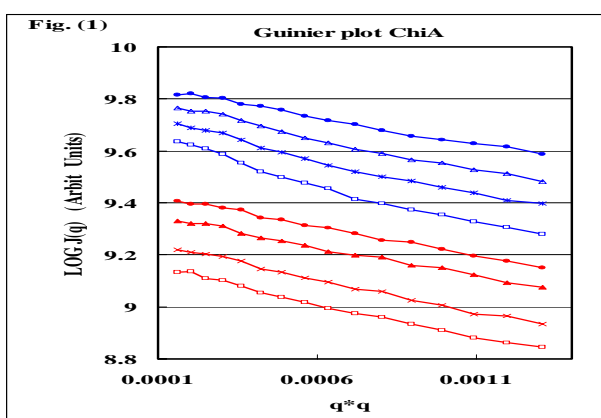
Considering from the aspect of distance distribution function, $p(r)$, peak position corresponding the highest particle density is kept unchanged while maximum distance D_{max} , size of ChiA, increased as seen in Fig. (3).

Conclusion

Protection of the ChiA was most achieved by the use of a flow cell we devised. Changes in any molecular parameters as of R_g , volume, spherical radii and D_{max} were minimum by the flow rate of $1\mu l / \text{sec}$ and 5minute of measured time.

Reference

[1] Kihara, H., Barman, T.E., Jones, P.T., and Moody, M.F. (1984) *J. Mol. Biol.*, **176**, 523-534.



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