

STRUCTURES OF HEPARIN-bFGF COMPLEXES IN SOLUTIONS OBSERVED BY SMALL ANGLE X-RAY SCATTERING (I)

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Introduction

Basic fibroblast growth factor (bFGF), also called heparin-binding growth factor, is one of the proteins responsible for cell growth. It is known that the sulfated polysaccharide such as heparin or heparan, can form a complex with FGF for promoting its receptor-binding and modulating FGF activities. Understanding this mechanism may expand the application spectrum of bFGF. The structural analyses on bFGF-heparin complexes have hitherto been conducted only in the crystalline state [1].

In this study, the nano-structures of heparin-bFGF complexes were observed by small angle X-ray scattering (SAXS) technique in the aqueous state.

Experimental

The bFGF sample was isolated from silkworms inoculated with recombinant virus Hy-NPV-FGF[2]. Porcine intestinal heparin was obtained from Viobin Corp.

The SAXS experiments were carried out with SAXES installed at BL-10C of Photon Factory.

Molecular models of heparin, bFGF and their complexes were built using "Cerius2" developed by Molecular Simulations Inc. The crystal structures offered the initial atomic coordinates for the modelling.

Results and Discussion

The mixing of heparin and bFGF induce the significant intensity increasing at smaller angle region as shown in Fig.1. This is another confirmation of the complex formation between heparin and bFGF using a scattering technique. The cross-section plots of heparin and the complex show the good linearity at small angle region. According Guinier approximation, both of them can be rod-like particles in solution having the cross-sectional radius of gyration of 0.57nm and 1.51nm, respectively. The mass per unit length, evaluated from the extrapolation of these analytical plots to zero angle, increases around 30% from heparin's one under the assumption of the same electron density of heparin and the complex.

According the above results, the molecular model of the complex can be proposed; where bFGF molecules bind unilaterally to an extended heparin chain. The calculated SAXS intensities from the model are compared with experimental ones in Fig.2. There is the discrepancy at higher angles, which suggests the necessary improvement of the heparin conformation.

References

- [1] S.Faham, D.C.Rees, et al. *Science* **271**,1116-1119, (1996)
- [2] Xiaofeng Wu, Ph.D.Thesis, submitted to *Kyoto Institute of Technology* (2001)

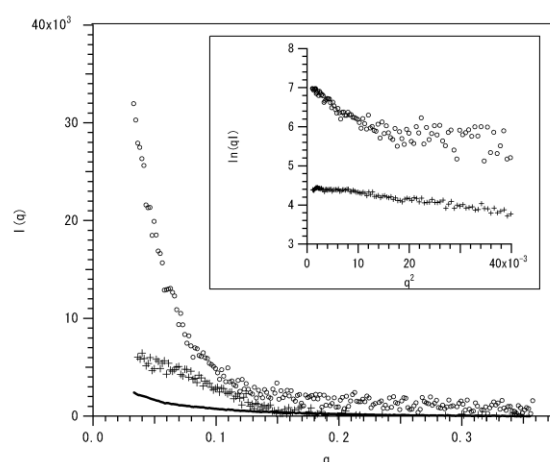


Fig.1 SAXS profiles from bFGF(solid line), heparin(plus) and their mixture(open circle). The inserted figure is the cross-sectional Guinier plot of the same data except bFGF, which does not show rod-like scattering behaviour.

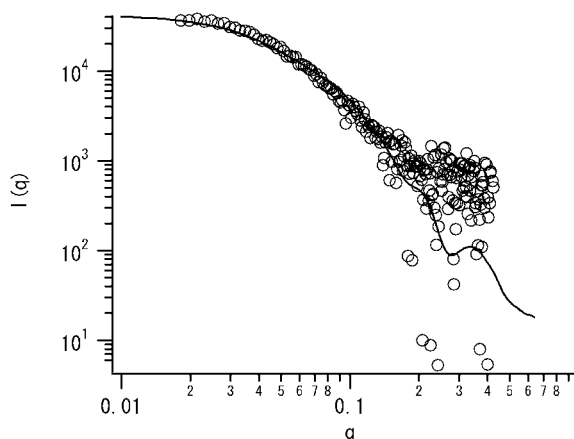


Fig.2 Comparison of SAXS profile obtained from the mixture of bFGF and heparin with one calculated from molecular model

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