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Distribution of the αC Region of Fibrinogen Molecule

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1 Introduction

Fibrinogen is a rod-shaped plasma protein that plays an essential role in the blood coagulation process. Fibrinogen is a dimer consisting of three peptide chains, Aα, Bβ and γ, and those peptides chains form unique functional domains that conduct in the coagulation process. Among them, the carboxyl terminal region of Aα peptide chain, αC region, has been taken much notice about its role in the lateral aggregation of protofibrils, which are the half-staggered double stranded axial association of fibrin converted from fibrinogen by the enzymatic action of thrombin. αC region contains b-sheet structure and is likely to form aggregates. Synthesized recombinant αC chains forms inclusion body easily, and forms amyloid structure in some case. It is useful to understand the spatial distribution of αC region in the fibrin polymerization process.

Fragment X is one kind of derivatives of fibrinogen by the enzyme plasmin, and the αC regions of intact fibrinogen molecule are cleaved. Therefore, fragment X is a useful material for the study on the functional roles of the αC regions. Additionally, it has been known the αC regions dissociate from the central region at acidic condition as pH 3. Then, scattering measurements of SAXS as well as light scattering will give important information about the spatial distribution.

2 Experiment

Bovine fibrinogen purchased from Sigma-Aldrich Co. was used. Fibrinogen dissolved in PBS was treated by plasmin (5 mU/1 mg fibrinogen) at 25°C. The reaction was stopped at the incubation time of 70 min by the addition AEBSF (protease inhibitor), and the digestion was passed through lysine-sepharose 4B column to remove plasmin. The elution was then analysed by GPC (HiLoad Sephadex 200) using tris-buffer with 0.3 M NaCl. Thus purified fragment X solution was stocked in the deep-freezer until use. By the SDS-PAGE results of those samples it was ascertained that the αC region was cleaved thoroughly and a decent amount of amino terminal region (Bβ1-49) was deleted additionally.

Measurements were carried out for the intact fibrinogen in PBS (pH 7.4), fragment X in tris-buffer (pH 7.4), and intact fibrinogen in 1 mM HCl (pH 3.0). SAXS measurements were achieved at KEK-PF (BL 10C) at Tsukuba. The obtained scattered intensity as a function of scattering vector Q was analysed by the cross-sectional Guinier plots to determine the average diameter of fibrinogen molecules. Dynamic light scattering measurements were also carried out to determine the Stokes diameter. Stokes diameter was obtained by the Stokes-Einstein equation from the characteristic decay time of the correlation function.

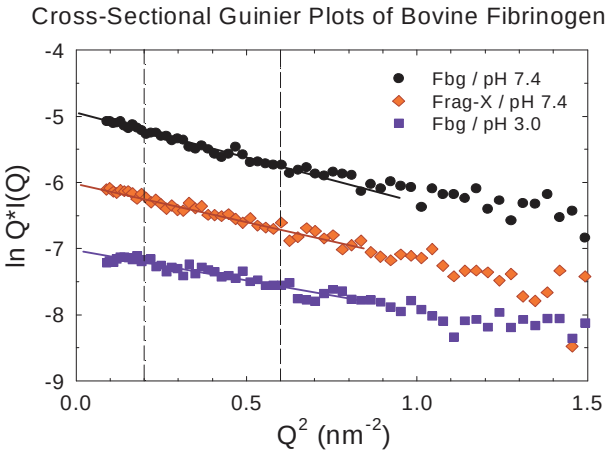

Fig. 1: Cross-sectional Guinier plots of bovine fibrinogen as a function of Q^2.

3 Results and Discussion

Cross-sectional diameter D_c of fragment X (4.2 nm) was significantly less than that of the intact fibrinogen (4.7 nm), and that of fibrinogen at pH 3.0 (4.0 nm) was further less than that of fragment X. On the other hand, Stokes diameter D_s of fragment X (15.8 nm) was significantly less than that of intact fibrinogen at pH 7.4 (22.8 nm). Stokes diameter of fibrinogen at pH 3.0 was a little larger than that at pH 7.4. When NaCl was added to the solution at pH 3.0 and the ionic strength was augmented up to 150 mM, a remarkable increase of diameter was observed (ca. 120 nm) suggesting the occurrence of aggregation.

The decrease of D_c as well as D_s in fragment X is attributable essentially to the deletion of the αC region, and this result suggests that the αC region snuggles up to the backbone of fibrinogen molecule. The decrease of D_c and increase of D_s at pH 3.0 means that the αC region dissociates from the central region of fibrinogen and is extended. Therefore, the contour of fibrinogen molecule becomes thinner. Cancellation between the elongation of molecular length and the decrease of cross-sectional diameter results in a slight increase of D_s.

In the fibrin polymerization, especially in the lateral aggregation of protofibrils, the αC regions dissociate from the central region and can interact with each other. At 150 mM NaCl the αC-αC interaction might result in the aggregates, where the electrostatic interaction is weakened. That interaction should be the origin of formation of aggregation in variant fibrinogen lacking N terminal region of Bβ chain, which might play the central role in the lateral aggregation.

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