学 位 論 文 の 要 約

Summary of Doctoral Dissertation

博士の専攻分野の名称　博士（**生命科学**）　氏 名　Rajesh Kumar Sharma

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学 位 論 文 題 名

Title of Doctoral Dissertation

Investigation on the Effect of Flow Field on the Amyloid Fibril Formation
(アミロイド線維形成に及ぼす流動場の効果に関する研究)

Several studies have been done on the fibrillogenesis of β-Lactoglobulin (βLG) because of its importance in food industries to change the textural properties of the food products and also its similar secondary structure to those obtained in several amyloidogenic diseases. Heat-induced fibrillogenesis of βLG has been studied mainly because long and fine structured fibrils can achieve by heating than other process. A nucleation-elongation growth mechanism is responsible for the fibril formation of βLG and the rate of this nucleation-growth mechanism influenced by several factors like shearing, stirring, seeding etc. have been studied earlier by using different techniques. These studies have revealed that the rate of the fibrillogenesis βLG or WPI (whey protein isolate) was enhanced over all factors (shearing, stirring and seeding).

In this dissertation, we investigated the effect of stirring on the heat-induced fibrillogenesis of βLG to gain more knowledge about the effect of stirring. We characterized heat-induced βLG fibrils using an unique elongational flow birefringence method by using Taylor's Four-Roller Mill (FRM) system. The elongational flow birefringence method has advantage over the traditional shear flow experiments to characterize the polymer molecules in the solution. In the simple shear flow like Couette flow, the extensional and rotational components of the strain rate are equal. Therefore, complete extension or alignment is not achieved specially for the flexible chains. Whereas, in elongational flow birefringence method, we achieve a coil-stretch transition for the flexible molecules.

In this dissertation, A general introduction and aim of this thesis has been described in Chapter 1.

The effect of stirring on the heat-induced fibrillogenesis of β-Lactoglobulin at 80°C and pH 2 has been described in chapter 2. Samples were prepared under the stirred (250 and 474 rpm) and unstirred conditions. All incubated samples containing fibrils were characterized quantitatively by using flow-induced birefringence method and observed a non-localized birefringence pointed out the rigid-rod like molecules in all samples. Rotational diffusion coefficient (D_r) was calculated to obtain the fibril lengths by measuring the relaxation data during the birefringence measurements. Atomic force microscopy (AFM) was used for the morphology of the fibrils which follows the birefringence

studies. From this study, we investigated that the fibrillogenesis of the βLG was enhanced over stirring and the order of the expected length of the resultant fibrils in both longer and shorter length distributions was those stirred at 250 rpm≈474 rpm >0 rpm. Fibrils in the sample which was stirred at 474 rpm were slightly smaller in length to those stirred at 250 rpm due to the fragmentation of the fibrils at higher stirring rate.

In chapter 3, we have also described the effect of stirring on the heat-induced fibril formation of the βLG in the presence of reducing sugar (Glucose) at 80°C and pH 7. Recently, it has been studied by researchers that the glucose inhibited the heat-induced fibrillogenesis of the βLG. In this study, we incubated the βLG solution with and without glucose under stirred (250 and 474 rpm) and unstirred conditions same like as those incubated in chapter 2. X-ray diffraction (XRD) and Thioflavin T (ThT) fluorescence data clearly demonstrated the presence of fibril molecules in all samples. Flow-induced birefringence measurements of all samples show the localized birefringence with coil-stretch transition indicating most probable the presence of 'worm' like flexible molecule in all samples. Atomic force microscopy (AFM) techniques were employed as morphology of the fibrils formed. This study revealed that the glucose inhibited the fibrillogenesis of βLG and also fibril lengths decreased with increasing stirring rate. In this study, we observed the 'worm' like flexible fibrils at pH 7 whereas at pH 2 we obtained the rigid-rod like fibrils (Chapter 2). This is due to the weak hydrophobic interaction at pH 7 than at pH 2 which is mainly responsible for the fibril formation. Therefore, due to the weak hydrophobic interaction, fibrils fragmented easily over increasing stirring rate at pH 7.

In the final chapter 4, we have been concluded the overall dissertation.