DATE: 22 May, 2017

SUMMARY of
2016 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		Real-time monitoring of κ-casein fibril formation by total internal reflection fluorescence microscopy
Applicant	**Name**	Carver, John Adrian
	Affiliation	Research School of Chemistry, The Australian National University, Canberra, Australia
	Present Title	Professor and Director of the Research School of Chemistry
Research Collaborator (Host PI)		Goto, Yuji

Summary

Alzheimer's, Parkinson's, and Huntington's diseases are age-related, neurodegenerative diseases which have at least one feature in common. Sufferers of these diseases generally show the presence of deposits in the brain comprising highly ordered protein aggregates termed amyloid fibrils. Despite this clear connection, it has been difficult to establish a causative link between the formation of amyloid fibril deposits in neural tissue and the onset and progression of neural decline. κ-Casein, a major milk protein, rapidly forms amyloid fibrils at physiological pH and temperature following chemical modification. Because κ-casein is inexpensive, readily available, and forms amyloid fibrils in a timely and highly reproducible fashion, κ-casein has served as a very useful system. Carver and coworkers have proposed that the rate of κ-casein fibril formation is limited by the release of monomeric subunits from the micelle, with the monomers being highly prone to fibril assembly.

In collaboration with Prof. Goto, IPR, we used total internal reflection fluorescence (TIRF) microscopy to monitor aggregation and fibrillation of κ-casein at a single molecule level. Additionally, the effect of ultrasonication on fibril formation was examined.

We confirmed fibril formation of κ-casein by standard assays with thioflavin T binding and light scattering. Thereafter, we verified fibril formation by TIRFM. However, the images were less clear than those of Aβ fibrils. We then examined the effects of ultrasonication on fibrillation of κ-casein. No clear effects were observed, in contrast to several other systems where ultrasonication has been reported to accelerate fibril formation. The absence of an effect on κ-casein may stem from its non-nucleation dependent mechanism of fibril formation, where the rate-limiting step is dissociation from the protein's oligomeric state. We tested this hypothesis by examining fibril formation by monomeric κ-casein, which was prepared by dissolution of reduced and carboxymethylated κ-casein in 6 M Gdn-HCl. Against our predictions, monomerization of κ-casein appeared to slow down fibril formation. Further studies are required to understand these intriguing results.

We will continue this international collaboration to further improve our molecular understanding of aggregation of proteins, in particularly the mechanisms underlying the formation of amyloid fibrils.

***Deadline: May 19, 2017**
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