- 3P052 β 2-ミクログロブリンのアミロイド線維形成に対する還元剤の効果
○山本 香織¹、小澤 大作¹、八木 寿梓¹、内木 宏延^{2,3}、後藤 祐児^{1,3}
¹阪大・蛋白研、²福井大・医学部、³CREST/JST
The effects of reductant on the amyloid fibril formation of β 2-microglobulin
Kaori Yamamoto (1), Daisaku Ozawa (1), Hisashi Yagi (1), Hironobu Naiki (2,3) and Yuji Goto (1,3). (1: Inst. Protein Res., Osaka Univ.; 2: Fac. Med. Sci., Univ. of Fukui; 3: CREST/JST)

The deposition of amyloid fibrils is associated with over 20 degenerative diseases. Dialysis-related amyloidosis is one of such diseases, which is commonly developed in patients undergoing long-term haemodialysis. The major component of the fibrils is β 2-microglobulin (β 2-m). In the patients, β 2-m is not degraded and forms amyloid fibrils, depositing in joints. To prevent such diseases, inhibition of the fibril formation is important. β 2-m has one disulfide bridge which contributes to the stability of native proteins. In this study, we examined the effects of reduction of the disulfide bridge by addition of reductants, dithiothreitol (DTT) and cysteine, at neutral pH.

Three types of fibril formation, seed-controlled, ultrasonication-induced, and heat and salt-induced fibrillation were investigated by thioflavin T (ThT) fluorescence, electron micrograph and CD. As for seed- and ultrasonication-induced fibrillations, the increase of ThT fluorescence was significantly suppressed and no mature fibril was observed in the presence of reductants. In contrast, as for heat and salt-induced fibrillation, the amyloid-like fibrils were observed even in the presence of reductants. However, the increase of ThT fluorescence was suppressed and the CD spectrum did not correspond with that of the fibrils formed from intact β 2-m, indicating that the amounts of the fibrils formed in the presence of reductants was a little. Thus, our results suggest that DTT and cysteine were effective to inhibit various types of the amyloid fibril formation of β 2-m.

- 3P054 固体NMRによるアミロイド形成ペプチドのアミロイド線維形成阻害機構の解明
○内藤 晶¹、大道寺 謙吾¹、伊藤 ひかり¹、山根 衣寿美¹、川村 出¹
¹横浜国大・院工
Amyloid fibril inhibition mechanism of amyloidogenic peptides as studied by solid state NMR spectroscopy
Akira Naito, Kengo Daidoji, Hikari Itoh, Izumi Yamane and Izuru Kawamura, (Graduate School of Engineering, Yokohama National University)

Amyloid fibril formation is now seen in biochemically diverse conditions and are related to the misfolding of proteins leading to severe diseases such as Alzheimer's disease. To understand the molecular mechanism of fibril formation, it is important to elucidate the fibril forming mechanism of amyloid forming peptides under various conditions. Solid state NMR spectroscopy has been realized to be a valuable method to gain insight into the structure of amyloid fibril. Inhibition of amyloid fibril formation is an important target for the study of amyloid fibril. We have previously determine the structure of a fragment Asp15-Phe19 of human calcitonin (hCT). It turned out that Phe16 residue aligns to stabilize the antiparallel β -sheet by π - π interaction. By knowing the structure, T12L/P16L/P19L-hCT (L3-hCT) was prepared. The kinetics were analyzed as a two-step autocatalytic reaction mechanism and the rate of fibril growth at pH 7.5, was markedly slower compared with that of hCT. Because π - π interaction between the aromatic side chains may stabilize the fibril structure, lack of this interaction may lead to the reduction of the rate constant of the fibril elongation. We have also realized that charged amino acids such as Arg can inhibit the fibril formation of hCT. In case of the addition of Arg, rate constant of nucleation process significantly reduced, while fibril elongation rate was not slower. It is, therefore, explained that Arg may associate with hCT in the solution, leading to prevent aggregation of hCT.

- 3P053 ¹³C 固体 NMR によるグルカゴンの脂質二重膜存在下におけるアミロイド線維構造と線維形成機構の解明
○山根 衣寿美¹、藤田 英樹¹、吉本 瑛梨¹、内藤 晶¹
¹横国大・大学院
Amyloidogenic fibrils and the structure of glucagon in the presence and absence of phospholipids as studied by ¹³C solid-state NMR
Izumi Yamane, Hideki Fujita, Eri Yoshimoto, Akira Naito. Graduate School of Engineering, Yokohama National University

Glucagon is a 29 amino acid peptide hormone secreted by pancreatic islet. Glucagon tends to form amyloidogenic fibrillar aggregates which is cytotoxic through the activation of apoptotic signaling pathways. To understand the fibrillation mechanism and the structure under aqueous acetic acid solution (pH 3.3) in the presence and absence of lipids, we have observed ¹³C solid-state NMR spectra of glucagon fibril. In the absence of lipids, glucagon fibril shows β -sheet structures around Gly⁴, Ala¹⁹, whereas it shows random coil around Phe⁶, Leu¹⁴. However, in the presence of lipids, the fibril shows α -helical structures around Gly⁴, whereas β -sheet structures around Gly⁴, Ala¹⁹, and random coil around Phe⁶. In the presence of lipids, we found that the fibril structure around Gly⁴ was different significantly from that in the absence of lipids. ¹³C resonances of fibril grew after a certain delay time suggesting that the fibrillation could be explained by a two step reaction mechanism. The first step is a homogeneous nuclear formation (rate constant of k_1), and the second step is a homogeneous fibrillation (rate constant of k_2). In the absence of lipids, k_1 and k_2 values were determined to be 10^{-8} - 10^{-7} [s⁻¹] and 10^{-3} - 10^{-2} [s⁻¹ M⁻¹], respectively. On the other hand, k_1 and k_2 values were 10^{-6} - 10^{-5} [s⁻¹] and 10^{-5} - 10^{-3} [s⁻¹ M⁻¹] respectively, in the presence of lipids. It is, therefore, important to point out that in the presence of lipids, nuclear formation can be faster and fibrillation can be slower than the case where the lipids are absent.

- 3P055 超音波破碎をもちいたアミロイド線維の分子量均質化
○茶谷 絵理¹、足達 瑠美¹、酒井 美世¹、内木 宏延²、後藤 祐児¹
¹阪大・蛋白研、CREST、²福井大・医、CREST
3aF01 Uniforming the Molecular Weight of Amyloid Fibrils by Ultrasonication
Eri Chatani(1), Rumi Adachi (1), Miyo Sakai (1), Hironobu Naiki (2) and Yuji Goto (1). (1: Institute for Protein Research, Osaka Univ. and CREST; 2: Faculty of Medical Sciences, Univ. of Fukui and CREST)

Amyloid fibrils are assemblies of proteins often associated with serious diseases called amyloidosis. Because of their polymeric properties with large and heterogeneous molecular size, detailed physicochemical analyses have often been hampered. Here, we present our recent results suggesting that the fragmentation of amyloid fibrils by ultrasonication is an effective method for decreasing and uniforming their molecular size.

We adopted amyloid fibrils formed by β 2-microglobulin (β 2-m) and those formed by a Ser20-Lys41 peptide fragment of β 2-m. When ultrasonication pulses were applied, the fibrils were fragmented into short fibrils with apparently similar lengths, as indicated by AFM and EM images. To characterize their size distribution, analytical ultracentrifugation measurements were performed. Sedimentation velocity experiments showed that, although the original amyloid fibrils have a broad distribution of the sedimentation coefficient, the fragmented fibrils have a remarkably sharp distribution, demonstrating the uniformity of molecular weight after the ultrasonication treatment. Together with sedimentation equilibration experiments, molecular weight, shape, and packing density of the amyloid fibrils have been proposed. The present results suggest the usefulness of ultrasonication, by which amyloid fibrils can be treated like a soluble homogeneous protein solution.