Observation of Liquid-Liquid Phase Separation of FUS-LC using VUV-CD Spectroscopy

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Aggregation of the RNA-binding protein FUS (Fused in Sarcoma) has been implicated in the neurodegenerative diseases such as ALS (amyotrophic lateral sclerosis) and FTD (frontotemporal dementia) [1]. The low-complexity domain of the FUS (FUS-LC) mediated liquid-liquid phase separation (LLPS) [2], but the structural mechanism is not known in detail. To address the revealing the mechanism, several structural analyses such as NMR or x-ray crystallography were examined [2, 3]. Reentry, Murakami and co-authors were performed Raman microscopy to analyze LLPS local structure [4]. They revealed that the FUS LC have extremely high concentrations which could not achieved in vitro experiments. In order to reveal the process to form LLPS such a high concentration, we examined the spectroscopic study using VUV-CD measurement, which can analyze the secondary structure of the proteins.

VUV-CD measurements were performed at BL12 VUV-CD station [5]. To increase S/N ratio of CD intensity around VUV energy region; 180-190nm, we prepared the chloride free buffer of FUS LC sample. The LLPS of our prepared FUS LC sample were observed by changing the sample temperature from room temperature to 5℃ in microfuge tube. 40μL of the sample was encapsulated in a CaF_2 cell (path length, 100μm). The cell was attached to peltier controlled cooling holder to control the temperature of the sample [6]. CD spectra were measured between 185 and 260 nm. The temperature of the samples were controlled from room temperature to 5℃ to obtain the LLPS of the FUS LC.

Obtained CD spectra are shown in Figure 1. The CD spectrum obtained by measuring at room temperature has large peak at nm and small shoulder peak near nm. This shows the major structure is random coil since the spectrum were similar to that of STI which is mainly unordered structure. This result is consistent with that obtained from NMR measurement. The peak intensity around 195 nm decreased by cooling the sample temperature.

We thought that the reasons of the obtained spectral changes as follows; 1) LLPS showed secondary structural changes, 2) LLPS make an effect to decreasing the transmission light intensity by scattering of suspension. We try to examine the measurement by changing the relative distance between the cell and the photo-multiplier detector. This may clarify the contribution of the scattering effect on the obtained spectrum.

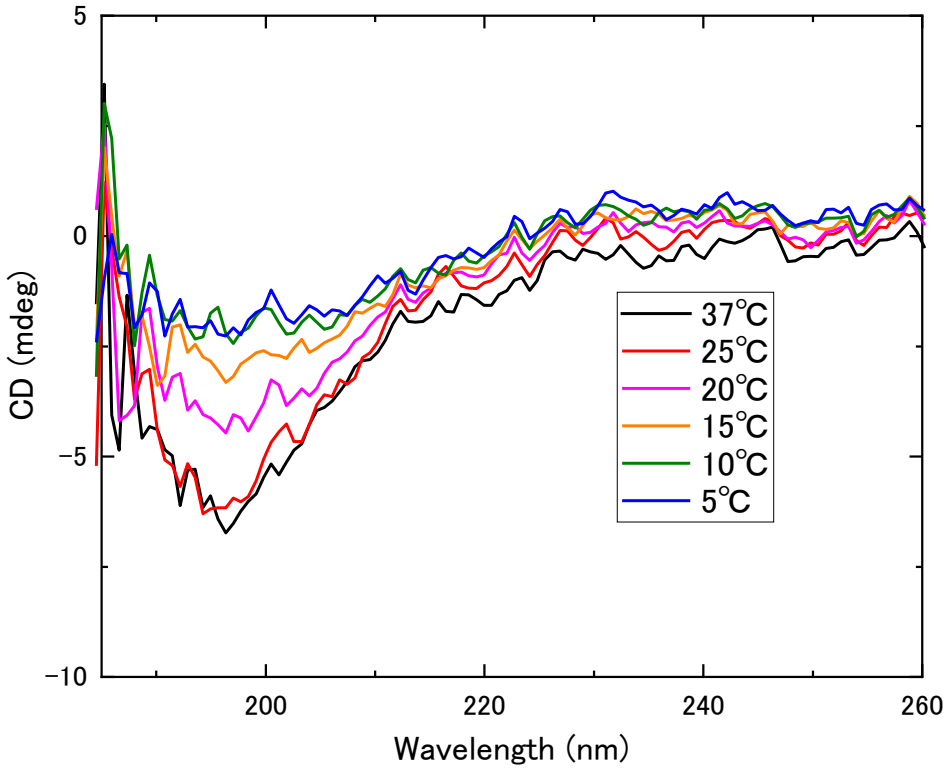

FIGURE 1. The obtained spectra of FUS LC in phosphate buffer pH. 12. By cooling the temperature of the sample, the CD intensity around 195 nm were decreased.

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