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# Molecular mechanism of amyloidogenesis of α-synuclein

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## Introduction

α-synuclein is the major component of the filamentous Lewy bodies and Lewy-related neurites, neuropathological hallmarks of Parkinson's disease. In spite of numerous studies reported previously, the molecular mechanisms of aggregation and fibrillation at the initial stage are still unclear.

In the present study, structural properties and propensities to form fibrils of a-synuclein at the initial stage were investigated using small angle X-ray scattering (SAXS) as well as CD spectroscopy [1]. Oligomerization comprising heptamer was successfully monitored at the initial stage using the time-dependent SAXS measurements [2].

## Experimental

All experiments were performed at a sample-to-detector length of 1 m with a CCD-based X-ray detector, and the data were corrected for distortion of images, non-uniformity of sensitivity, and contrast reduction for an X-ray image intensifier. The exposure time was 24 s in one measurement, and the sample in a cuvette was exchanged every three times. The sample solutions were incubated at 0, 6, 12, and 18 h at 50°C before measurement. The final concentration of the protein incubated for various intervals was adjusted to be 1.5 mg/ml.

Singular value decomposition (SVD) analysis was performed in a similar manner as previously reported [2].

## Results and Discussion

Fig. 1 shows the time course of Kratky plots for α-synuclein fibrillation. The pattern of the plot revealed that the molecule was coiled in the beginning and that its shape became more compact during the incubation. The molecular weight estimated from $I(0)$ value at 0 h incubation time corresponded to that of monomer, and the values gradually increased in the course of incubation owing to polymerization. There was an isoscattering point observed around $h$ = 0.1 $A^{-1}$, which indicated that the fibrillation process could be a two-state transition in the observed time range of 0 - 18 h. The SVD analysis also confirmed this result. Since there were no fibrils formed up to 18 h in electron microscopy [2], the two states can correspond to natively unfolded state ($N$) and transient intermediate state ($I$). The reconstructed SAXS profile from SVD bases showed that the molecule consisted of coiled monomer in $N$ state, and that it was more globular forming partially folded structure in $I$ state. The $I(0)$ value of $I$ state was 7.0 times as large as that of $N$ state, suggesting the molecule formed heptamer in $I$ state. The rate constant of oligomerization was estimated to be 0.13 $h^{-1}$, that is, the half-life for that process was 5.6 h. The reconstructed SAXS profiles at each time interval were mostly consistent with the raw profile except for the late times (Fig. 1). This suggested that the third base might be able to contribute to the SAXS profile at those times, when a sign of phenomena deviated from the two-state transition, e.g. degradation, might occur.

## References

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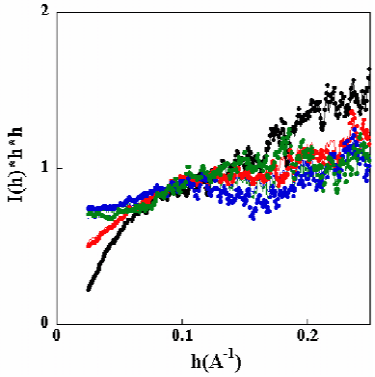


Fig. 1. Kratky plots of SAXS profiles at incubation times of 0 (black), 6 (red), 12 (blue), and 18 h (green). Circles and lines represent raw experimental data, and those reconstructed from SVD results, respectively.