Fibrillation of human calcitonin as studied by solution x-ray scattering

Tadashi TOMIZAWA [1], Yasutaka SEKI [2], Yuzuru HIRAGI [3], Kunitsugu SODA [*4]
[1] GSC, RIKEN, Yokohama, Kanagawa 230-0045; [2] Div. Biol. Sci., Grad. Sch. Sci., Nagoya Univ., Nagoya, Aichi 464-8602; [3] Inst. Chem. Res., Kyoto Univ., Kyoto, Kyoto 611-0011;
[4] Dept. Bioeng., Nagaoka Univ. Technol., Nagaoka, Niigata 940-2188, Japan

Introduction

Human calcitonin (hCT) is a peptide hormone with 32 amino acid residues and is known to form fibrous aggregates as β -amyloid. In this study, fibrillation of hCT at neutral pH and with high protein concentration has been analyzed by solution x-ray scattering (SXS) method, where time dependence of the average degree of association and the global shape of aggregates were measured. Combined with results of other experiments and molecular modeling, SXS study would contribute greatly to elucidate the fibrillation mechanism of proteins.

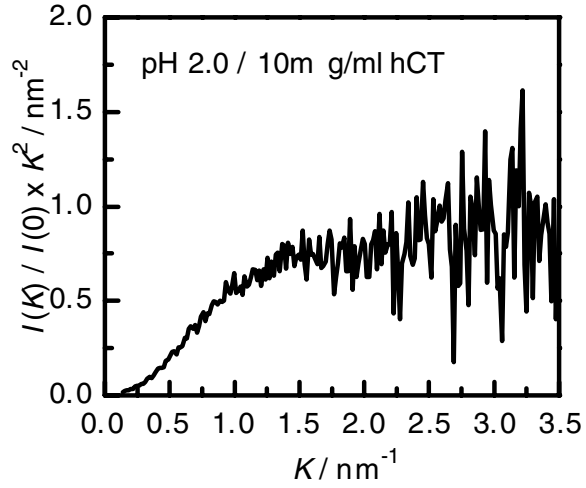
Method

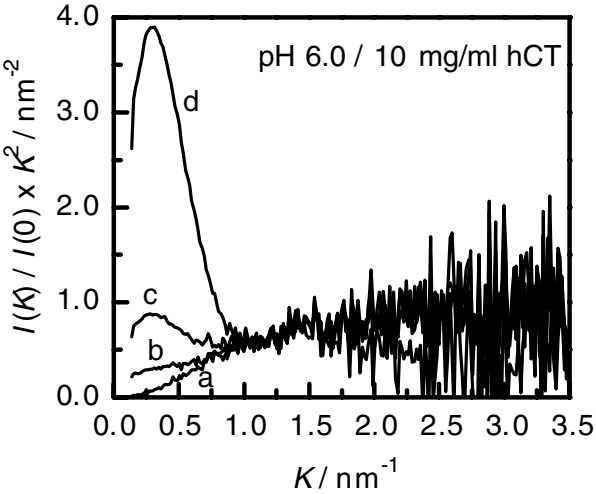
SXS experiment has been done using the PF beam line BL10C. Time dependence of the SXS profile of hCT at a concentration of 10 mg/ml has been measured at acidic pH 2.0 (10mM HCl) and neutral pH 6.0 (10mM HEPES). To minimize the damages of sample protein molecules by x-ray irradiation, SXS measurement has been made flowing continuously the sample solution through a sample cell. The actual x-ray irradiation time of each hCT molecule is 0.5 s. SXS data were accumulated for 5 minutes. The SXS profile of the protein in each sample was obtained by subtracting the SXS profile of solvent from that of solution.

Result

The upper figure shows the Kratky plot for an acidic hCT solution of pH2.0. The mean square radius R_{sq} of hCT was found to be 1.3 nm from Guinier analysis. Comparison of the forward scattering intensity between hCT and myoglobin confirmed the monomeric form of hCT. Under this solution condition, no change in the SXS profile was observed within 90 minutes after sample preparation. As seen from the upper figure, hCT in acidic solution has a random-coil structure, which is consistent with the result of CD-spectrum measurement.

Kratky plots of the SXS profile at neutral pH 6.0 are presented in the lower figure. The profiles in the figure are normalized with the forward scattering intensity of monomeric hCT. The plots of a, b, c and d are respective profiles at 5, 80, 95 and 165 minutes after the sample preparation. No change in the SXS profile was observed within 80 minutes after the dissolution of hCT into solvent. As the forward scattering intensity and the value of R_{sq} in this range of time coincide with those observed under the acidic condition, hCT just after dissolving into neutral solvent is found to have monomeric, random coil





conformation. The scattering intensity suddenly increases at 80 minutes after dissolution to yield finally the profile as shown in the plot d. It was found after the SXS measurement that the sample solution geled and resonance Raman scattering measurements showed that hCT molecules have conformations rich in β -strand. Combined with results of CD measurement, these SXS data shows that the fibrillation of hCT proceeds with the nucleation of some hCT oligomer followed by growth of the fibril. Detailed analysis of the SXS profiles is now in progress.

References

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* soda@vos.nagaokaut.ac.jp