Small-angle X-ray scattering study of amyloid fibril formation of hen egg white lysozyme

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Introduction

Hen egg white lysozyme (HEWL) has been known to form amyloid fibrils [1,2]. Since HEWL is one of the proteins that have been studied most extensively and closely related to human lysozyme, the variants of which form the amyloid fibrils [3,4], this protein is an ideal model to study the mechanism of the amyloid fibril formation. HEWL was shown to form the amyloid fibrils in highly concentrated ethanol solutions [1]. In order to gain an insight into the mechanism of the amyloid fibril formation, structural states of HEWL under solutions of various HEWL concentrations in various ethanol concentrations were investigated with small-angle X-ray scattering.

Materials and Methods

Hen egg white lysozyme, purchased from Seikagaku Kogyo, was purified as described [1]. Concentrated solution of this purified HEWL was mixed with H_2O and ethanol to obtain the solutions of the desired protein and ethanol concentration. These sample solutions were used in X-ray scattering experiments. Small-angle X-ray scattering experiments were performed at the BL-15A, using monochromatic X-ray beam of the wavelength 0.15 nm, with the sample-to-detector length of 2.33 m. The obtained scattering curves were analyzed to yield the structural parameters, based on the Guinier approximation [5]. The radius of gyration and I(0)/c which is proportional to molecular weight of the particle, and, if the particles were filamentous, the cross-sectional radius of gyration and $I_x(0)/c$ which is proportional to the mass per unit length of the particle, were estimated from the analysis.

Results

We measured small-angle X-ray scattering patterns of HEWL solutions in the concentration ranges between 2 and 10 mg/ml, under various conditions where the ethanol concentration in the solution was between 0% and 90% (v/v). Each scattering pattern was analyzed to yield the structural parameters as mentioned in *Materials and Methods*. From the results of this analysis, the structural states of HEWL were distinguished as the monomer state, the state of the formation of the dimers, the states of the protofilament formation, the protofilament state, and the states towards the formation of the amyloid fibrils. These structural states changed as a function of both the protein concentration and ethanol concentration. It was found that HEWL under the monomer state had an overall shape similar to that without ethanol, and the dimers, formed by the association at the end of the long axis of HEWL, had an elongated shape. The structure of the protofilament was characterized by its radius of gyration of the cross-section of 2.4 nm. Comparison of the mass per unit length of the protofilament with that of f-actin, one of standard filamentous protein complexes, yielded the mass per unit length of the protofilament 16000 ± 2300 daltons/nm. It was shown that these protofilaments were formed in a manner consistent with nucleation dependent polymerization mechanism [6]. It was also shown that the changes of the structural states towards the amyloid fibril formation occured via lateral association of the protofilaments. Thus, the amyloid fibril formation of HEWL occurs with two steps, the formation of the protofilament by a nucleation dependent polymerization mechanism, followed by the formation of the amyloid fibril via lateral association of the protofilaments.

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References

[1] S. Goda et al., Protein Sci. 9, 369 (2000).
[2] M.R.H. Krebs et al., J. Mol. Biol. 300, 541(2000).
[3] M. Pepys et al., Nature, 362, 553 (1993).
[4] J. Funahashi et al., J. Biochem., 120**,** 1216 (1996).
[5] A. Guinier and G. Fournet, *Small-angle scattering of X-rays*, John Wiley & Sons, New York, (1955).
[6] T.R. Serio et al., Science, 289**,** 1317 (2000).

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