Biological Science

15A/2003G317

Molecular properties for irreversible unfolding of aspergillopepsin II

Takashi UCHIDA[1], Masaki KOJIMA*[1], Hideshi INOUE[1], Hiroshi KIHARA[2], Kenji TAKAHASHI[1]
[1]Tokyo Univ. of Pharm. and Life Sci., Hachioji, Tokyo 192-0392, Japan
[2]Kansai Med. Univ., Hirakata, Osaka 573-1136, Japan

Introduction

Irreversible conformational change plays an essential role for controlling the physiological activity of proteins. The study for the unfolding not only provides the information of molecular architecture, but is also important to understand the control mechanism described above.

Aspergillopepsin II (aspergilloglutamic peptidase) consists of two polypeptide chains, a light chain of 39 residues and a heavy chain of 173 residues, which are bound non-covalently to each other. This enzyme acts under acidic condition, while it is unfolded irreversibly above neutral pH accompanied with the dissociation of the two polypeptide chains [1, 2]. It was suggested from the previous SAXS analyses that the dissociated heavy chain is not completely coiled in the completely unfolded state (pH 8.0 and above), and that the molecule may have a core or residual structure. In the present study, we further investigate the details of the structure in the alkaline-induced unfolded state by *ab initio* modeling calculation.

Experimental

All experiments were performed at a sample-to-detector length of 1.3 m and at room temperature with a CCD-based X-ray detector. The exposure time was 30 s in one measurement. The sample in the cuvette was exchanged every three times. The data were corrected for distortion of images, non-uniformity of sensitivity, and contrast reduction for an X-ray image intensifier before analyses [3].

The heavy chain was dissociated from the light chain at pH 8.4, and purified by gel filtration. Sample solutions were prepared at a protein concentration of 0.5 – 2.0 mg/ml.

ab inito modeling was performed with the program DAMMIN [4] and GASBOR [5].

Results and Discussion

From the scattering profile, we first estimated the various parameters such as R_g and $I(0)$. These parameters were independent of sample concentration. Molecular weight deduced from $I(0)$ value was consistent with that of the heavy chain. R_g was estimated to be about 30 A.

Then we restored the low-resolution structure in the unfolded state by *ab initio* modeling. The restored structure is expressed as an ensemble of small beads, and satisfied the experimental scattering pattern. Fig. 1 shows the result for the unfolded structure of the heavy chain. Unlike the random coil, the molecule had an unisotropic shape, and bore a core inside itself. And the resultant shape was almost the same irrespective of used algorithm. We are trying to map this beads model into the atomic-level high resolution structure.

References

[1] M. Kojima et al., Biochemistry 39, 1364 (2000).
[2] M. Maeda et al., Biochem. Biophys. Res. Commun. 301, 745 (2003).
[3] K. Ito et al., PF Activity Report 18, 275 (2001).
[4] D. I. Svergun, Biophys. J. 76, 2879 (1999).
[5] D. I. Svergun et al. Biophys. J. 80, 2946 (2001).

* mkojima@ls.toyaku.ac.jp

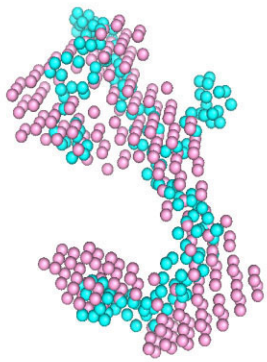

Fig. 1. Restored structure of the heavy chain in the unfolded state. Beads in cyan and magenta correspond to the structure restored by DAMMIN and GASBOR, respectively.