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Conformational analysis of the unfolding intermediate of aspergillopepsin II

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Introduction

Aspergillopepsin II is a non-pepsin type acid proteinase. It 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. The pH-dependent unfolding study of the enzyme was performed in terms of equilibrium [1] and kinetics [2]. It revealed that the heavy chain just after the dissociation still kept molecular compactness despite the loss of the native-like secondary and tertiary structures, and gradually increased its dimensions in the higher pH regions. The noncoincidence of the two phenomena (the chain dissociation and the swelling) strongly suggested the existence of an intermediate species, whose features are very similar to those of molten globule except for the loss of the native-like secondary structure. In order to characterize this intermediate state in more detail, we prepared a single-chain recombinant to compare its unfolding profile with that of the authentic enzyme.

Experimental

All the 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, and 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 single-chain recombinant, in which the two chains are bound covalenty through the intervening sequence, was expressed in *E.coli* in a similar manner as reported [4] unless otherwise noted. Since the intervening sequence was processed auto-catalytically, we designed the recombinant with no catalytic activity. Sample solutions were prepared at a protein concentration of 0.5 – 2.0 mg/ml.

Results and Discussion

The recombinant enzyme was expressed as inclusion bodies, and submitted to solubilization, column chromatography and refolding. Since the conformation of the refolded enzyme was not identical with that of the authentic one, we checked the solublization conditions extensively with various kinds of solvents such as urea, DMSO, and GdnHCl. The sample was first solubilized in 8M urea in the usual manner [4], and the solvent was exchanged with the appropriate one by dialysis. Then SAXS measurements were performed under these conditions. The results showed that the enzyme was not solubilized completely and that there was a large amount of aggregates with high molecular weight and large radius of gyration, except at high concentration of GdnHCl. The Kratky plots under these conditions also showed that the molecule was not fully coiled. At 6 M GdnHCl, the sample was solubilized and coiled relatively well although there was some extent of aggregations. At present, we are investigating the solublization and refolding conditions using Gdn-HCl to obtain an adequately folded single-chain enzyme, and will perform unfolding experiments with the sample.

References

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