Analysis of unfolding and refolding of HIV protease

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Introduction

HIV-1 protease is an acid proteinase, and is essential for the maturation of the infectious virions. It consists of two identical subunits with 99 residues, which are bound non-covalently (Fig. 1). Like other acid proteinases such as pepsin and aspergillopepsin II, the enzyme is unfolded at higher pH. In order to elucidate the unfolding profiles of HIV-1 protease, small angle X-ray scattering (SAXS) experiments were performed.

Experimental

All the measurements were performed at BL-15A with a CCD-based X-ray detector [1] at 20℃.

HIV-1 protease was expressed in *E.coli* and purified by the method reported previously [2]. Sample solutions were prepared by diluting 2-fold the enzyme solution (7.3 mg/ml in 10 mM Na acetate, pH 3.5) with 10mM Na MES (pH 5.5) or 10 mM Na CAPS (pH 10.0). All sample solutions contained 1 mM dithiothreitol.

The data were corrected for distortion of images, non-uniformity of sensitivity, and contrast reduction of an X-ray image intensifier [3] before analyses.

Results and Discussion

Fig. 2 shows the SAXS pattern of HIV-1 protease in the native state (pH 3.5). Radius of gyration (R_g) was estimated to be 19.1 A from the Guinier approximation, and the Kratky plot indicated that the molecule was globular in the native state. On the other hand, the SAXS pattern in the unfolded state suggested that there were some aggregates in the sample solution. This may be because the two subunits dissociated at alkaline pH could easily form aggregates. We are now investigating the relationship between unfolding of HIV-1 protease and its aggregation.

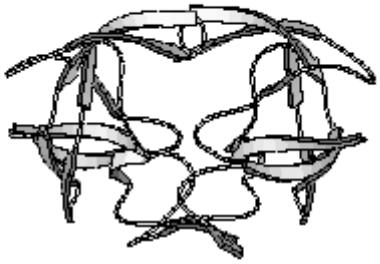

Fig. 1. Schematic representation of the structure of HIV-1 protease [4].

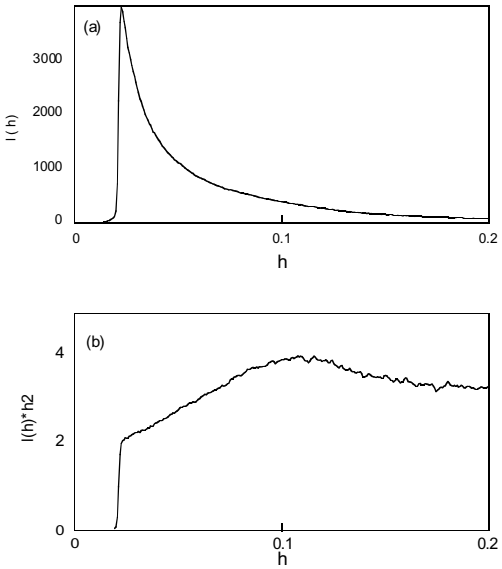

Fig. 2. SAXS profile (a) and Kratky plot (b) of HIV-1 protease at pH 3.5

References

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