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Analysis of Unfolding and Refolding of HIV-1 Protease

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Introduction

HIV-1 protease (HIV-1 PR) is an acid proteinase, essential for the maturation of the infectious virions. It consists of two identical subunits with 99 residues, which are bound non-covalently. The enzyme is unfolded at the high concentration of denaturants or under alkaline condition [1]. In the present report, we analyze the urea-induced unfolding of HIV-1 PR by the small angle X-ray scattering (SAXS) method.

Experimental

All measurements were performed at BL-15A with a CCD-based X-ray detector [2] at 20 °C. The exposure time was 24 s in one measurement, and the sample in the cuvette was exchanged every three times.

HIV-1 PR was expressed in *E.coli* and purified according to the method reported previously [3] with slight modifications. Sample solutions were prepared by mixing the enzyme solution (1 mg/ml in 10 mM Na acetate, pH 3.5) with the urea solution of each concentration. 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 [4] before analyses.

Results and Discussion

From the SAXS data, the radius of gyration (R_g) and the zero-angle scattering intensity ($I(0)$) at each concentration of urea were determined from the Guinier approximation. Fig. 1 shows the dependences of these parameters upon the urea concentration. From the figure, the midpoint concentration of urea were 3.3 M for R_g (Fig. 1(a)) and 3.6 M for $I(0)$ (Fig. 1(b)), which is nearly the same as the values obtained by other experiments [1]. On the other hand, ΔG^{H2O} values were estimated to be 9.83 kcal/mol for R_g and 8.94 kcal/mol for $I(0)$, and were slightly lower than that in the reference [1].

The R_g value in the native state was estimated to be 21.2 A, and almost consistent with that calculated from the crystal structure. At high concentration of urea, the R_g and $I(0)$ values were respectively 1.5 and 3 times as large as those in the native state. The increase of $I(0)$ upon unfolding suggested a molecular association. Considering this effect, the R_g value of each subunit was estimated to increase slightly (by about 10 %) during unfolding. The structural features in this associable state are being characterized further by other experimental methods such as CD spectroscopy.

References

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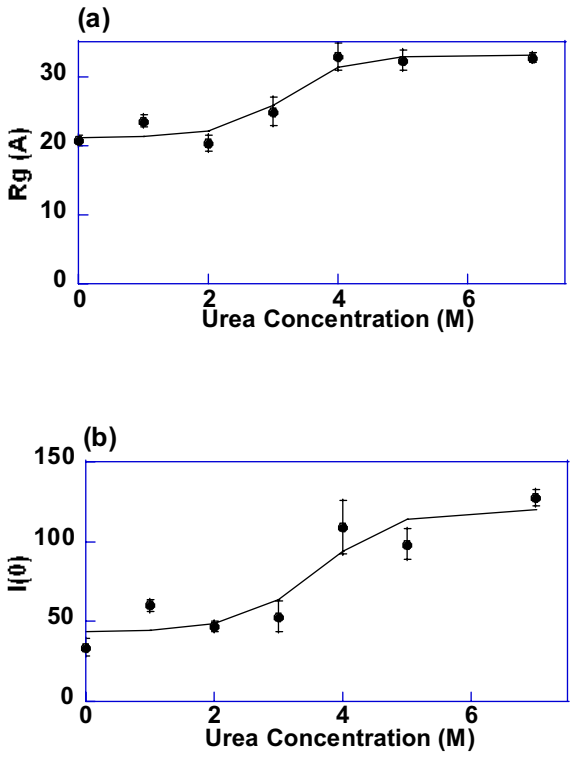

Fig. 1. Dependence of R_g (a) and $I(0)$ (b) upon urea concentration for the unfolding of HIV-1 PR.