X-ray Scattering Studies on the Structure of Porcine Pepsin in Various pH Solution

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Introduction

To understand the conformational behavior of a protein, it is necessary to define not only the structure of its native state but also that of various denatured states [1,2]. Recent studies have revealed the biological significance of denatured states in processes such as aggregation [3-5], chaperone binding [6,7], and transport across membrane [8,9]. A variety of denatured states have also been identified, differing in their overall dimensions and the extent of residual secondary and tertiary structures. Pepsin is a particularly good model for the study of conformational behavior under several conditions because detailed information is available on its secondary structure, enzymatic properties, and zymogen activation.

Porcine pepsin is a gastric aspartic proteinase that plays an integral role in the digestive process of vertebrates. The pH optimum of its catalytic activity is less than 2.0. It is derived from its zymogen pepsinogen, by removal of 44 amino acids from its amino terminus, to give a single-chain enzyme with a low pI and three disulfide bridges. From X-ray diffraction analysis, it has been known that the substrate binding cleft is located between two homologous portions of the structure: the N-terminal domain (residues 1-172) and the C-terminal domain (residues 173-326). The secondary structure of both regions consists almost entirely of β-sheets [10]. Spectroscopy studies further found that pepsin undergoes a conformational transition from the native state at the acidic pH condition (pH = 1–5) to the denatured state in a narrow pH range of 6–7[11]. As reviewed above, however, spectroscopy analyses provided information on changes in the local conformations of pepsin in solutions but could not give general features on the overall structure of pepsin at the native state in solution and its structural variations with changing solution condition.

In the present study, in order to obtain detailed information on the overall structure of porcine pepsin at the native state and its structural changes in solutions of various pH values, we carried out solution X-ray scattering (SAXS) experiments by using a synchrotron X-ray source.

Experimental

Materials and sample preparation. Crystallized and lyophilized, highest grade pepsin extracted from porcine stomach mucosa was purchased from Sigma and used without further purification. A 20 mM sodium phosphate, 20mM MOPS, and 20mM potassium phosphate buffer was used, for the X-ray measurements, adjusted to different pH values as required. All of the pH values were adjusted carefully with small amounts of NaOH or HCl. The pH was measured with a Fisher Scientific AB 15 pH meter. Each buffer solution was filtered using a polytetrafluoroethylene (PTFE)-membrane filter of pore size 0.2 ㎛.

Solution SAXS measurements. Solution SAXS experiments were performed at the bending magnet SAXS beamline 4C1[12] of the Pohang Light Source in Korea. The sample-to-detector distance was about 1 m and 50 cm. The scattering vector calibration was done with a precalibrated Silver Behenate of the periodic length of 5.8376 nm as a standard sample. The sample cell was 50 μL in volume with 10 μm thick mica windows, and had a 0.7 mm X-ray path length. Exposure times were typically 10 min for individual measurements. Measurements were carried out at 25 °C. The X-ray wavelength was 1.608 Å and the best resolution was 0.28 $Å^{-1}$ for the 1 m setup and 0.50 $Å^{-1}$ for the 50 cm setup. The final composite scattering curve was obtained by merging the small angle data with the high angle data.

Results and discussion

Solution small angle X-ray scattering (SAXS) is an effective technique for measuring structure and structural difference of protein under various environments. In this study, the structural characteristics of various conformational states of porcine pepsin were studied in terms of size and shape under several pH conditions by solution SAXS. The structural models of the porcine pepsin were reconstructed, which was made inside the search volume of maximum diameter Dmax calculated from the p(r) function. The reconstructed models were obtained without imposing any restrictions on the symmetry and anisometry of pepsin molecule. Under several pH conditions, the reconstructed models reveal various conformational states, when compare to the crystal structure. The structural differences between solution and crystal structure of pepsin can be account for the inherent conformations of the flexible subdomain under carefully controlled specific pH conditions.

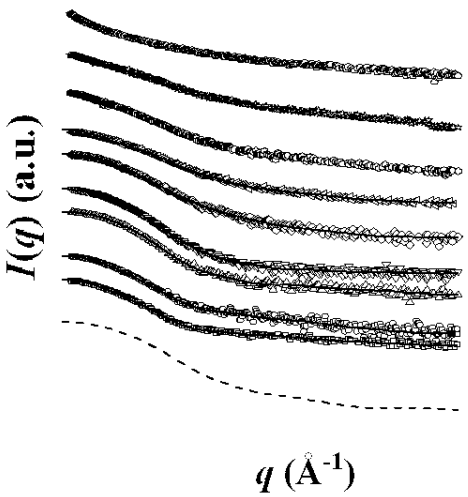

Figure 1. Theoretical SAXS curve calculated from the crystal structure of porcine pepsin (PDB code 3PEP) and experimental SAXS curves from the various conformational states of porcine pepsin in a wide pH range between 1.58 and 12.31.

Conclusions

The fundamental aim of this study was to obtain more detailed information on the structures and structural differences of porcine pepsin in solution under several pH conditions when compared with pepsin crystal structure, and to gain an insight into correlation between the transition of curves in a variety of spectroscopic measurements of previously published article and the structural information about the conformation of pepsin in solution between its enzymatic active acidic and inactive alkaline conditions by small angle X-ray scattering measurements. The structural evidences presented may have important implication in establishing relationship between the structure of porcine pepsin and its enzymatic function.

References

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