A Synchrotron X-ray Scattering Study on the Structure of Pepsin in Solution

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ABSTRACT

Solution small angle X-ray scattering (SAXS) is an effective technique for measuring structure and structural difference of protein under various environments, quantitatively. Structural characteristics of various conformational states of porcine pepsin were studied in terms of size and shape under several pH conditions by solution SAXS. Under nearly physiologically enzymatic active conditions, the reconstructed models exhibit a more extended C-terminal domain, when compare to the crystal structure. The structural differences between solution and crystal structure of pepsin can be accounted for the inherent conformations of the flexible subdomain in the C-terminal domain in solution under carefully controlled specific pH conditions. Furthermore, this flexibility may provide a clue that lead to the solution of enzymatic inactivity of pepsin under mild acidic conditions. The structural evidences presented may have important implication in establishing relationship between the structure of porcine pepsin and its enzymatic function.

Keywords: porcine pepsin, pH, small angle X-ray scattering

1 RESULTS AND DISCUSSION

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]. The catalytic site is formed by two aspartate residues, Asp32 and Asp 215, one of which has to be protonated, and the other deprotonated, for the protein to be active [11]. 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 D_{max} 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. The structural evidences presented provide important implication in establishing relationship between the structure of porcine pepsin and its enzymatic function.

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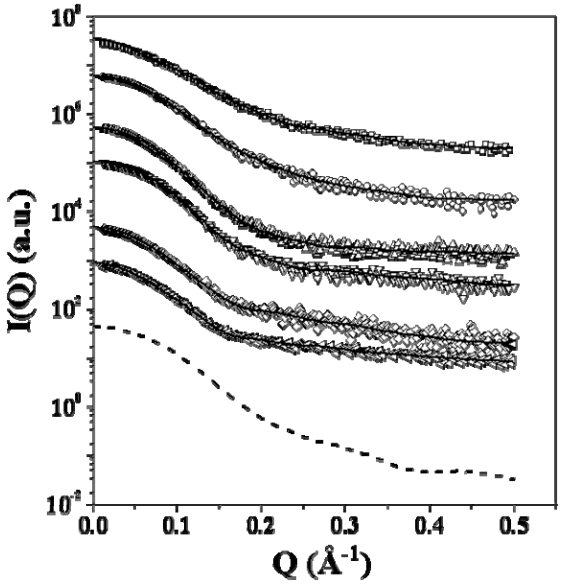

Figure 1 : The theoretical SAXS curve calculated from the crystal structure and the experimental SAXS curves from porcine pepsin in solution in a wide pH range between 1.58 and 7.93.