Determining the Crystal Structure of Collagenase H S1 Domain and Developing a SAXS-Derived Enveloped Structure of its Binding Domains-Minicollagen Complex

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Collagen is the most abundant protein in mammals forming one-third of its produced proteins. It is present in the extracellular matrix as an intricate network of fibrous proteins for structural support. *Hathewaya histolytica (Clostridium histolyticum)* releases collagenase G and H to degrade the collagen and invade the host. Regardless of its harmful effects, collagenase is relevant in digesting collagen subtypes for medical treatments. This study focuses on collagenase H (Col H), whose segmental structure comprises two polycystic kidney disease (PKD)-like domains, a single collagen-binding domain (CBD), and a catalytic module (S1) for substrate degradation.

The crystal structure of ColH S1 (wildtype) was determined by X-ray diffraction to 2.73 Å resolution at room temperature. The crystals were obtained from a high-throughput screen and optimized. The data collected was good to about 2.73 Å. The phase information was obtained by molecular replacement method. Refinement is in progress. The structure adopts a 'closed' confirmation that has never been seen before and suggests that the enzyme closes its activator domain by 20 Å to unwind triple helical collagen and cleave one peptide at a time.

Small-angle X-ray scattering (SAXS) measurements were used to determine the binding mechanism of the Col H collagen binding segments on minicollagen in a buffer containing 1 mM calcium ions under near-physiological conditions. The reduced scattering profile of the scattering intensity I(Q) versus scattering vector Q of the complex showed a close fit. An indirect Fourier transformation of the I(Q) data was used to generate the pair-distance distribution function P(r) in real space to determine the maximum diameter, D_{max}, and identify the complex as a multidomain. The three-dimensional molecular shape of the Col H PKD2CBD-minicollagen complex was constructed and fitted into the generated envelope to determine the approximate positions and overall structure. There was an asymmetric interaction between the binding segments and minicollagen.

Col H is shown to be an endo-collagenase. PKD2CBD is used to position the S1 domain at the under-twisted region of collagen unidirectionally. Subsequently, the S1 segment undergoes a significant structural change to unwind collagen and position one strand to its zinc-peptidase domain. Mutagenesis of active site residues is underway to elucidate its degradation mechanism.