Crystallography

NW12A/2005G259

Crystal structure of a clip-domain serine protease, prophenoloxidase-activating factor (PPAF)-II

Shunfu Piao[1], Young-Lan Song[2], Jung Hyun Kim[1], Sam Young Park[3], Ji Won Park, Bok Leul Lee*[1], Byung-Ha Oh*[2] and Nam-Chul Ha*[1]

[1]Pusan National Univ., Jangjeon Dong, Geumjeong Gu, Busan, Korea
[2]Pohang Univ. of Science and Technology, Pohang, Kyungbuk, Korea
[3]Yokohama City Univ., Suehiro, Tsurumi-ku, Yokohama, Japan

Introduction

Serine protease (SP) cascades amplify signals from physiological or pathological responses in the extracellular region of vertebrates and invertebrates. SP cascades drive various biological processes, especially in embryonic development and the innate immune responses of invertebrates. SPs consist of a chymotrypsin-like SP domain and one or two clip domains at the N-terminus. Prophenoloxidase-activating factor (PPAF)-II, which belongs to the noncatalytic clip-domain SP family, is indispensable for the generation of the active phenoloxidase leading to melanization, a major defense mechanism of insects. Here, the crystal structure of PPAF-II reveals that the clip domain adopts a novel fold containing a central cleft, which is distinct from the structures of defensins with a similar arrangement of cysteine residues.

Experiments

Crystallization of PPAF-II was reported [1]. Data collection and processed procedures were reported [2].

Results and Discussion

Overall structure of PPAF-II

The *PPAF-II* gene encodes a protein with 415 amino acids. The N-terminal 24-amino-acid segment of the protein functions as a signal peptide for secretion. The mature protein (392 amino acids) is made up of two parts: the clip domain including the flanking sequence composed of 114 amino acids, and the SP-like (SPL) domain composed of 278 amino acids. The final 2.0 Å resolution model contains residues 22–42 with an *N*-glycosylated residue, 47–116, 129–173, and 180–415, which include most of the clip and the SPL domains (Figure1). The missing residues constitute the loop segments flanking the clip domain and a flexible loop in the SPL domain. The small clip domain is bound to the larger SPL domain via hydrogen bonds, hydrophobic interactions, and a salt bridge. While the structure of the SPL domain of PPAF-II is similar to chymotrypsin-like proteases that of the clip domain is a new fold according to a database search using the program DALI.

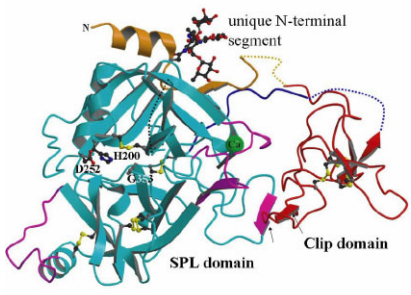

Figure1. Overall structure of PPAF-II. A ribbon representation of PPAF-II structure. PPAF-II family-specific N-terminal segment is in yellow. The four signature sequences are in magenta. The nonfunctional catalytic triad residues (Gly-His-Asp) are shown in the ball-and-stick representation.

Structure feature of clip domain

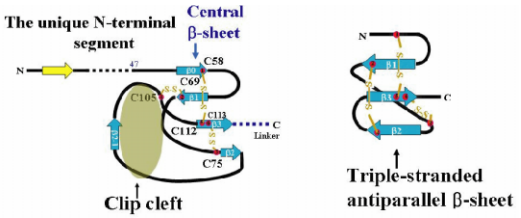

Figure 2. Comparison of the clip domain with human β-defensins 1. Left and right are a schematic drawing of the clip domain and a schematic drawing of β-defensins, respectively.

The clip domain is composed of a high portion of loops and a central, four-stranded, irregular β-sheet. The conserved three disulfide bonds knotting the loops and the β-strands together appear critical for the structural integrity of the central β-sheet that serves as the main framework of the clip domain structure. It has been suggested that clip domains may be structurally similar to antimicrobial proteins, β-defensins, based on the identical arrangement of the cysteine residues. We show here that the clip domain structure is distinctively different from those of β-defensins. First, β-defensins contain three, instead of four, β-strands forming the central antiparallel β-sheet (Figure2). Second, while β2 and β3 are antiparallel with each other in the structure of β-defensins, they are parallel in the structure of the clip domain (Figure2).

References

[1] S. Piao et al., Biochim Biophys Acta 1752: 103–106 (2005).
[2] S. Piao et al., EMBO 24, 4404-4414(2005).
*bhoh@postech.ac.kr