Interaction between Fengycin Synthetases FenA and FenB

Yu-Chieh Cheng¹, Shih-Tung Liu¹
¹Molecular Genetics Laboratory, Graduate Institute of Biomedical Sciences, Chang-Gung University

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Contact information: Yu-Chieh Cheng (d9401203@stmail.cgu.edu.tw)

Abstract

Background: Fengycin is a cyclic antifungal lipopeptidic antibiotic produced by Bacillus subtilis F29-3. This antibiotic is synthesized nonribosomally on an enzyme complex that contains five fengycin synthetases -- FenC, FenD, FenE, FenA, and FenB. These synthetases contain a 26-amino acids COM-donor domain in the C-terminal region and a 16-amino acid COM-acceptor domain in the N-terminal region, which are critical to peptide synthesis. This study investigates the interaction between FenA and FenB to elucidate how fengycin synthetases interact to form a complex. Methods: Interaction between different regions in FenA and FenB was examined by protein pull down with glutathione-Sepharose and Ni-NTA agarose beads. Results: This study demonstrates that FenB interacts specifically with the 20-amino acid COM-donor domain in FenA. This study also finds that the COM-acceptor domain alone insufficiently supports the interaction between FenA and FenB; a region significantly larger than the COM-acceptor domain, the N-terminal 227-amino acid region, is required for the interaction. The other fengycin synthetases in the enzyme complex appear to interact through a similar fashion. Conclusion: The COM-donor domain and the N-terminal 227-amino acid region between two partner fengycin synthetases are required for specific binding in the fengycin synthetase complex.
Introduction

Nonribosomal peptide synthetases (NRPSs) are multifunctional enzymes, which interact in a specific manner to form a complex for peptide synthesis (1). In these enzymes, the COM-domains, which are located at the C-terminus of donor enzymes and the N-terminus of acceptor enzymes, are important to the recognition between two enzymes (2-5). Fengycin is synthesized nonribosomally by fengycin synthetases, including FenC, FenD, FenE, FenA and FenB (6-11). Our previous studies demonstrated that fengycin peptide is synthesized in an orderly fashion on an enzyme complex formed by these five enzymes (1). This study investigates the interaction between FenA and FenB to elucidate the mechanism by which fengycin synthetases interlock in the enzyme complex.

Fig. 1. Modules and domains in fengycin synthetases. Fengycin synthetases are encoded by the *fen* operon. The five enzymes encoded by the operon contain one to three modules; each activates a specific acid. A module typically contains a condensation domain (C), an adenylation domain (A), and a thiolation domain (T). An epimerase domain (E) is presence in the C-terminal region of each fengycin synthetase, except for FenB. A thioesterase domain (TE) is present in the C-terminal region of FenB, which terminates fengycin synthesis.
Result

The C-terminal region in FenA interacts with FenB.

AN1058, which contains the N-terminal 1058-amino acid region and a histidine tag, does not interact with FenB (Fig. 2B), indicating that FenB does not interact with the N-terminal region in FenA.

A GST-pull down study also showed that AC2403-, AC2714-, AC3284-, and AC3449- Glutathione-Sepharose beads pull down FenB (Fig. 2C), indicating that FenB interacts with the C-terminal 3449-3596 amino acid region in FenA.
Fig. 2. The C-terminal in FenA interacts with FenB. (A) FenA is deleted to determine the region that interacts with FenB. H: histidine tag; G, GST. These proteins bound to Ni-NTA agarose beads (B) or glutathione-Sepharose beads (C) were mixed with FenB. Proteins were finally detected by immunoblotting (IB).
The COM-donor domain in fengycin synthetase is important to the specific binding of partner enzymes.

Delineating the region in FenB that interacts with FenA

BC507, which contains FenB 507-1274 amino acids region fused with histidine tag, does not interact with C-terminal of FenA (Fig. 5B, lane 6). On the other hand, BN657, which contains FenB N-terminal 657 amino acids region fused with histidine tag, interacts with C-terminal of FenA (Fig. 5B, lane 3).

Fig. 5. The N-terminal region in FenB interacts with FenA. (A) FenB is deleted to determine the region in FenB that interacts with FenA. H: histidine tag. After mixing bacterial lysates that contained BN657, BC507, and AC2404, proteins were pulled down by glutathione-Sepharose beads (B). Proteins were detected by immunoblotting (IB).
Conclusion

The N-terminal region of FenB interacts with the C-terminus of FenA.

The COM-donor domain in FenA interacts with FenB.

The epimerase domain in FenA is not involved in the interaction with FenB.

FenB does not interact with FenA after the COM-acceptor domain in FenB is deleted.

FenB COM-acceptor domain interacts with both FenA and FenC, showing that the domain does not confer the specificity of the interaction between FenA and FenB.

The N-terminal 227-amino acid region in FenB interacts with FenA but not FenC, indicating that a sequence other than the COM-acceptor domain is required for FenB to recognize the COM-donor domain in FenA.

Reference