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Crystal structure of capsular polysaccharide synthesizing enzyme CapF

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Introduction

Capsular polysaccharides (CPs) are key virulence factors of *Staphylococcus aureus*. Biosynthesis of type 5 and type 8 CPs (CP5 and CP8), which are produced by most clinical isolates of *S. aureus*, is catalyzed by 16 CP assembling proteins. One of the key enzymes of this biosynthetic pathway is CapF, which catalyzes the synthesis of UDP-2-acetamido-2,6-dideoxy-β-L-talose, a precursor of UDP-N-acetyl-L-fucosamide the major component of both CP5 and CP8.

Methods

Seleno-methionine (SeMet) labelled CapF was cloned, expressed, and purified as described in a previous report [1]. Purified CapF was concentrated to 15 mg ml^{-1} in a solution of 10 mM Tris-HCl at pH 8.0. CapF crystals were obtained by mixing protein with a solution containing 100 mM MES at pH 7.2, 25% (w/v) glycerol, 100 mM $(NH_4)_2SO_4$, 300 mM NaCl, and 3.9 M sodium formate.

Four-wavelengths data-sent was collected at beamline BL17A. Data was processed with HKL2000. Initial phases of SeMet-labelled protein crystals were obtained with program SOLVE/RESOLVE included in the PHENIX suite. Crystal structure was further refined with programs REFMAC5 of the CCP4 suite, and COOT.

Results

Crystal structure of CapF revealed that this enzyme is composed of two domains (Fig. 1). N-terminal domain comprises residues Met-1 to Pro-244, whereas C-terminal domain consisted of residues between Met-254 and Val-369. These two domains are connected by a short linker (Ser255 to Leu253).

CapF forms a tight homodimer (Fig. 2). This topology is consistent with the molecular weight measured by size exclusion chromatography (92 kDa). Buried surface area of each monomer upon dimer formation is very extensive (3,400 A^2).

N-terminal domain contains the characteristic catalytic triad and Rossmann dinucleotide motif found in the family of short-chain reductase/dehydrogenase enzymes. However, neither coenzyme NADPH nor substrate could be seen in the active site of the reductase domain.

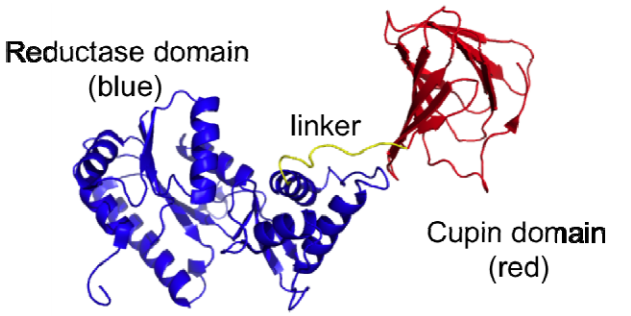

Figure 1. Structure of CapF monomer. N-terminal domain, C-terminal domain, and linker are colored in blue, red and yellow, respectively.

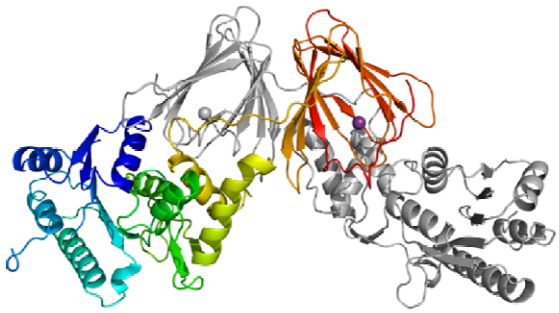

Figure 2. Structure of CapF homodimer. Ribbon colored in blue to red corresponds to one monomer in the asymmetric unit. Monomer in gray was generated by a symmetry operation. Zn^{2+} is shown as sphere in magenta.

C-terminal domain displays a beta-sheet motif characteristic of heterogeneous family of proteins known as cupin. Because of the functional variability among cupins, we could not assign a specific function C-terminal domain solely based on the crystal structure. Cupin domain of CapF features a Zn^{2+} cation at the bottom of a deep pocket that is coordinated by three His residues and one Glu residue, and that we believe bestows a specific function to CapF.

References

[1] T. Miyafusa et al., *Acta Cryst.* F64:512 (2008).

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