Crystal structure of the *Actinomadura* R39 DD-peptidase (PBP4)

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Actinomadura sp. R39 produces an exocellular DD-peptidase penicillin-binding protein (PBP) whose primary structure is similar to *Escherichia coli* PBP4. It is characterized by a high beta-lactam-binding activity (second order rate constant for the acylation of the active site serine by benzylpenicillin: $k_2/K = 300\ mM^{-1}\ s^{-1}$). The crystal structure of the DD-peptidase from *Actinomadura* R39 was solved at a resolution of 1.8 Å by single anomalous dispersion at the cobalt resonance wavelength. The structure is composed of three domains: a penicillin-binding domain similar to the penicillin-binding domain of *E. coli* PBP5 and two domains of unknown function. In most multimodular PBPs, additional domains are generally located at the C- or N-termini of the penicillin-binding domain. In R39, the other two domains are inserted in the penicillin-binding domain, between the SXXK and SXN motifs, in the way of "Matryoshka dolls". One of these domains is composed of a five-stranded beta-sheet with two helices on one side and the other domain is a double three-stranded beta-sheet inserted in the previous domain. Additionally, the 2.4 Å structure of the acyl-enzyme complex of R39 with nitrocefin reveals the absence of active site conformational change upon binding the beta-lactams.

Keywords: peptidoglycan biosynthesis, carboxypeptidase, penicillin-binding protein