Structure of a bacterial full-length type 2 IleRS reveals the C-terminal tRNA binding domain insertion dispensable for aminoacylation

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Isoleucyl-tRNA-synthetases (IleRS) are universally conserved enzymes that covalently couple isoleucine to its cognate $tRNA^{Ile}$ in a two-step aminoacylation reaction. These multidomain proteins consist of an aminoacylation domain, a proofreading domain and a C-terminal anticodon-binding domain involved in tRNA recognition. IleRSs cluster into two clades, IleRS1 and IleRS2, which differ in antibiotic resistance and the architecture of their C-terminal domain. The structure of the C-terminal anticodon-binding domain of IleRS1 is already known and entails three subdomains (SD): SD1 with helical bundle topology, SD2 consisting of four antiparallel β-sheets and SD3 that is a αβ-fold with a zinc-binding motif. [1] At the same time, the structure of the C-terminal domain of IleRS2 remained unknown as only structures of truncated enzymes were reported. Here, for the first time, we present the structure of full-length *Bacillus megaterium* IleRS2 with a completely resolved C-terminal domain at 2.3 Å resolution. The structure unveils that the C-terminal domain of IleRS2 consists of three subdomains analogously to IleRS1. SD1 and SD2 in IleRS2 align structurally well with the corresponding subdomains in IleRS1. In contrast, SD3 lacks the zinc-binding motif of IleRS1 SD3 and surprisingly, topologically resembles the SD2. Finally, the structure visualized a novel 75 amino acid long SD2 insertion, which is absent in IleRS1. We prepared a *B. megaterium* IleRS2 mutant with the SD2 insertion exchanged to a $[Gly_4Ser]_2$ loop. The mutant has relatively modest 5-fold decrease in aminoacylation rate as compared to the wild-type enzyme, which indicates that the SD2 insertion is important, but not essential for IleRS2 aminoacylation. The results thus open an intriguing question whether SD2 of IleRS2 has a role outside of translation.

References:
[1] F. L. Silvian, J. Wang, A. T. Steitz, Science. 285 (1999) 1074–1077.