Structural and functional characterization of Mycobacterium tuberculosis Suf machinery

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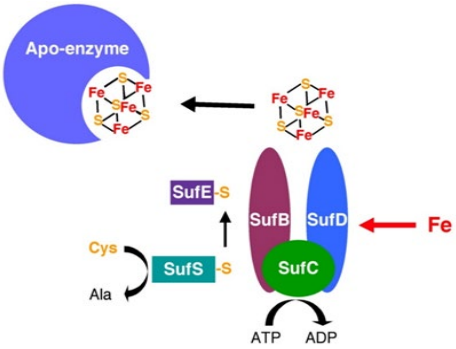
The worldwide recrudescence of tuberculosis and widespread antibiotic resistance have strengthened the need for the rapid development of new antituberculous drugs targeting essential functions of its etiologic agent, Mycobacterium tuberculosis (Mtb). The recently identified mycobacterial SUF machinery constitutes a new potential target as being implicated in the pathogen's survival. The Suf pathway is one of the three main pathways for the biosynthesis of Fe-S clusters in bacteria. While utilized under iron limitation and oxidative stress in E. coli, it is the unique way to assembly and repair the [Fe-S] clusters in M. tuberculosis. The mycobacterial Suf locus encodes SufB, SufC, SufD, SufU, and SufS ortholog proteins, which present high homology with the E. coli proteins. In contrast, no ORF coding for orthologs of SufA and SufE from E. coli was found in the mycobacterial locus. Generally, in bacteria SufE (in gram negative) or SufU (in gram positive) accelerates the cysteine desulphurase activity of SufS.

So the first aim of this thesis was to identify a new ortholog sequence that shares high homology (60%) with E. Coli SufE. That was not part of the suf operon but it was expressed in the same growth dependent pattern of the other suf proteins. The next step was to go deeply and understand why a microorganism such Mtb possesses both SufU and SufE while every microorganism has SufE or SufU. Therefore, we decomposed the Suf system in its basic components: SufS, SufU and SufE. Each component was cloned in an expression vector and expressed as phusion-protein in E. coli heterologous system. Protein purification was performed through different chromatrographic techniques (affinity, ion exchange and size exclusion chromatrography). Once we obtained the purified proteins we were able to characterize them structurally and test their stability by circular dicroism. Afterwards, we studied SufE/SufU function by enzymatic assays in presence and absence of SufS to test if they were able or not to enhance its desulfurase activity.



References

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