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The Alkanesulfonate-Binding Protein from *Xanthomonas Axonopodis* pv. *Citri.*

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Xanthomonas axonopodis pv. *citri* is the causative agent of the citrus canker, a disease that infects millions of plants in Brazil and in the World. In accordance to the differences in the mechanisms of infection and pathogenesis between this specie and *X. campestris*, the genome of both bacteria revealed the presence of different ABC transporters, including the alkanesulfonate transporter SsuABC, only found in *X. axonopodis* and which function is not known. Here we describe the crystal structure and biochemical characterization of the SsuA periplasmic-binding protein. SsuA shows an α/β sandwich topology with two domains separated by a cleft where the HEPES was bound. Spectroscopic analyses of the protein revealed that it was stable in neutral pH and suffered structural changes in presence of MOPS, CHES and MES. Indeed, in the presence of these ligands, the protein showed an increased thermal stability, as evidenced by thermal shift assays. Molecular modelling of the interactions realized between SsuA and these alkanesulfonates revealed the structural basis for increasing in the thermal stability. The importance of SsuA and SsuABC transporter for *X. axonopodis* is discussed.

Keywords: *xanthomonas axonopodis* pv. *citri*; SsuA; ABC transporter

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The Mutation D59A Affects Molybdate Binding Properties in the *Xac* ModA Protein.

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The *modABC* operon of *Xanthomonas axonopodis* pv. *citri* (*Xac*) encodes for the proteins ModA, ModB and ModC, which couple ATP hydrolysis to the transport of molybdate to the interior of the cell. The *Xac* periplasmic molybdate-binding protein (ModA) determines the affinity of the system and its crystallographic structure was recently solved by our group [1]. Focusing on the analysis of residues that might affect the binding of molybdate, we have obtained and expressed two ModA mutants (K127S and D59A). Spectroscopic analyses by circular dichroism and intrinsic fluorescence of the tryptophans have shown that the mutation K127S, located at the C-terminal domain, did not affect the crystallization and the measured biophysical properties. This was further confirmed by the three-dimensional structure solved at 1.5 Å resolution. On the other hand, the ModA D59A mutant only formed crystals incapable to produce diffraction. The CD spectra revealed a decrease in the secondary structure content, which seems to affect the binding properties as shown by fluorescence assays. The K_d values of the mutant form and of native protein were determined and compared. Additionally, we have modelled the three-dimensional structures of the membrane protein (ModB) and the ATPase-binding protein (ModC) from *Xac* based on the structural coordinates from the *Archaeoglobus fulgidus* (*Afu*) molybdate transporter [2]. These models and the ModA structure were then used to produce the complexes ModA-ModB and ModB-ModC. The analysis of the residues that could be involved in interactions between these complexes in *Xac* revealed differences to the *Afu* complex.

[1] Balan A, Santacruz-Pérez C, Moutran A, Ferreira LC, Neshich G,Gonçalves Barbosa JA. *Biochim Biophys Acta,* **2008**, 1784: 393-399. [2] Hollenstein, K., Frei, D.C., Locher, K.P. *Nature,* **2007**, 446: 213-216.

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