The single-point mutation D59A affects the structural and binding properties of the ModA protein from *Xanthomonas axonopodis* pv. *citri*

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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. Nevertheless, mutation K127S diminished the thermal stability of the holoprotein in 5^{o}C. On the other hand, CD spectra of the ModA D59A mutant revealed an increase in the secondary structure content as well as in the thermal stability of the holoprotein, but this mutant only formed crystals incapable to produce diffraction. The spectroscopic analyses showed that the mutation D59A is important to maintain functional and structural features in the protein although it performs the same function as K127S mutant. 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. Finally, molecular dynamics simulations of the ModA protein were performed in the presence and absence of molybdate and showed that the release mechanism of this ion-binding protein coincides with data already described for another group of periplasmic binding proteins.

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[2] Hollenstein, K., Frei, D.C., Locher, K.P. *Nature*, **446:** 213-216 (2007).

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