Structural Studies of the Disulfide Oxidoreductases DsbA from Xylella fastidiosa

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The first member of the disulfide oxidoreductases (DsbA) family was identified and characterized in Escherichia coli as a periplasmic protein involved in disulfide bond formation. It was also shown that the DsbA protein assists the correct folding of exported proteins containing disulfide bonds and in Vibrio cholerae a member of this family is required for the functional maturation of secreted virulence factors. Xylella fastidiosa is a phytopathogenic bacterium that causes serious diseases in a wide range of economically important crops. X. fastidiosa genome analysis revealed the presence of two members of the DsbA family (from now named DsbA1 and DsbA2). Furthermore, a sequence alignment showed that the active site regions of DsbA1 and DsbA2 differ from each other by one residue, usually considered important for the enzymatic activity. The purified proteins DsbA1 and DsbA2 were submitted to crystallization trials. Crystals of DsbA1 were obtained and X-ray diffraction data were collected at the Brazilian Synchrotron Light Laboratory. Best crystals diffracted to 2.2 Å resolution and belong to space group C2 with unit cell parameters a= 200.06 Å, b= 41.24 Å, c= 79.97 Å and β = 96.17°. DsbA1 crystals were also obtained after protein incubation with a reducing agent and diffracted up to 1.9 Å. The quick cryo-soaking technique was applied and some data sets were collected from heavy atom-derivative crystals. Attempts to solve the structure using the SIRAS and MIRAS methods are in progress. Circular Dichroism and Fluorescence experiments are being performed in order to obtain complementary structural data.

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