NMR analyses of hypothetical secretion chaperones: XACb0033 and XAC0419

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Xanthomonas axonopodis pv. *citri* (*Xac*) causes citrus canker utilizing secretion systems to transfer virulence factors into eukaryotic cells. Although is known that secretion processes are assisted by secretion chaperones, the virulence mechanisms aren't fully understood. With the aim to understand better these mechanisms we have investigated structurally two chaperones that are possibly involved in *Xac* virulence. Two chaperones, type III (XAC0419) and type IV (XACb0033), earlier cloned in pET23a, were expressed using *BL21(DE3)plysS E. coli* strains, and purified applying the ion exchange and size exclusion chromatography techniques. Gel electrophoreses, size exclusion chromatography, circular dichroism (CD), and emittion fluorescence (in the case of XACb0033) data indicate folded structures of these proteins and dimeric forms, probably due to formation of disulfide bonds between monomers. To confirm our data, the more reliable experiments such as NMR DOSY are proposed, where lower diffusion coefficients are expected for the higher molecular weight proteins. The ^{1}H NMR and DOSY spectra were collected on a VARIAN INOVA 500 MHz spectrometer. NMR samples were prepared into 600 μL of 99.9% D_2O and water suppression was achieved by WATERGATE pulse. Two types of protein samples were analyzed: 1) native, by dialysis to water, and lyophilization (CD experiments showed no alteration in secondary structure features upon this treatment), and 2) samples treated with 4-fold excess of β-mercaptoethanol and iodacetamide, then dialyzed to water and lyophilized. This way, native and dimeric (1) and monomeric proteins (2) were obtained. Standard DOSY experiments were executed, and bovine serum albumine (BSA, 66.4 kDa) and lysozyme (14.3 kDa) with measured diffusion coefficients of 0.46×10^{-10} m^2/s and 0.98×10^{-10} m^2/s, respectively, were utilized to fit the data.

Keywords: Secretion chaperones, protein NMR and DOSY.
Acknowledgements: FAPESP.