

Structural Studies of Periplasmic Proteins From The Type IV Secretion System of *Xanthomonas citri* (Xac)

Luciana Coutinho de Oliveira¹, Diorge Paulo de Souza¹, Hans Wienk², Hugo van Ingen², Denize Favaro¹, Rolf Boelens², Chuck S. Farah¹, Roberto Kopke Salinas¹

¹ Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo-USP, Brazil

² NMR Spectroscopy, Bijvoet Center for Biomolecular Research, Utrecht University, The Netherlands

Introduction Gram-negative bacteria use specialized complexes to translocate macromolecules across the bacterial cell envelope. One of such complexes is the Type IV Secretion System (T4SS). T4SSs are generally composed of 12 proteins, VirB1 to VirB11 and VirD4. The channel is organized in two layers. The upper layer consists of fourteen repetitions of an heterotrimer formed by VirB7 and the C-terminal domains of VirB9 and VirB10^{1,2}. We showed previously that the VirB7 of *Xanthomonas citri* (Xac) has an extra C-terminal globular domain that is absent in the VirB7 of other organisms³. This finding suggests a structural variation in the Xac's T4SS compared to other Gram-negative organisms. **Goals** VirB7 is bound, via its N-terminal region to VirB9 in the outer layer complex. In order to obtain further high-resolution structural information on Xac's T4SS, we initiated a study of the three-dimensional structure of the complex formed by Xac-VirB9^{CT} and Xac-VirB7^{NT}. **Material and Methods** For this purpose, a 1:1 complex of Xac-VirB9^{CT}, isotopically labeled with ¹³C and ¹⁵N, and a non-labeled VirB7₂₄₋₄₆ peptide derived from the VirB7 N-terminal tail (VirB7^{NT}), was prepared. Multidimensional triple-resonance NMR experiments for backbone and side chain assignments of VirB9^{CT} were recorded and analyzed. Homonuclear filtered NOESY and TOCSY experiments were collected in order to assign Xac-VirB7^{NT}. Intra- and intermolecular NOEs from 3D-¹⁵N-NOESY-HSQC, 3D-¹³C-NOESY-HSQC and 2D-NOE spectra were collected in order to calculate the complex structure. **Results and Discussion** The structure was calculated by Cyana⁴, followed by water refinement in CNS⁵. Dihedral-angle restraints predicted by Talos+⁶, intermolecular NOE-derived distance restraints and hydrogen bonds were used as input. **Conclusions** The calculated conformers reveal that VirB9^{CT} shows two β -sheets forming a β -sandwich. VirB7^{NT} forms a β -strand and binds almost perpendicularly across the VirB9^{CT} β -sandwich via specific interactions involving hydrophobic side chains. This orientation is consistent with the homologous structure of the complex from the pKM101 T4S conjugation system (PDB 3JQO, 2OFQ).

Keywords: Protein structure, T4SS, Protein NMR, *Xanthomonas*

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