NMR Structure of the complex formed by VirB9 and VirB7 from the Type IV Secretion System of Xanthomonas citri and studies of intermolecular interactions.

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Gram-negative bacteria use specialized supramolecular 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 docks on the outer membrane. It consists of fourteen repetitions of a heterotrimer formed by VirB7 and the C-terminal domains of VirB9 (VirB9CT) and VirB10. We showed previously that the VirB7 of the phytopathogen Xanthomonas citri (Xac) has an extra C-terminal globular domain (residues 52-133) that is absent in the VirB7 of other organisms. VirB7 is bound via its N-terminal region to VirB9 in the outer layer complex. While the two domains are only partially structured in the free state, measurements of 15N relaxation (R1, R2 and (1H)-15N NOE) and of the apparent Kd (1 μM) by isothermal titration calorimetry (ITC) at 37°C indicate that VirB9CT and VirB7NT form a tight and rigid complex. In order to obtain further high-resolution structural information on Xac’s T4SS, we solved the NMR structure of the complex formed by Xac-VirB9CT and Xac-VirB7NT. For this purpose a 1:1 (molar ratio) sample of Xac-VirB9CT labeled with 13C and 15N, and a non-labeled VirB724-46 peptide derived from the VirB7 N-terminal tail (VirB7NT) was prepared. Multidimensional tripleresonance NMR experiments for backbone and side chain resonance assignments of VirB9CT were recorded and analyzed. Homonuclear 2D-NOE and TOCSY filtered experiments were collected in order to assign Xac-VirB7NT. Intra- and intermolecular NOE peaks from 15N-NOESY-HSQC, 13C-NOESY-HSQC and 2D-NOE spectra were collected in order to obtain distance restraints. The structure was calculated by semi-automated NOE assignment using Cyana, followed by recalculation and water refinement in CNS. The calculated conformers reveal that VirB9CT consists of two β-sheets forming a β-sandwich. VirB7NT forms a short β-strand that binds almost perpendicularly across the VirB9CT β-sandwich via specific interactions involving hydrophobic side chains. This orientation is consistent with the homologous structure found in the pKM101 T4S conjugation system (PDB 3JQO and 2OFQ). We prepared mutant complexes where some of the amino acids in VirB7NT were replaced by alanine or glycine, and evaluated the intermolecular binding affinity by ITC. These experiments showed that a valine at position 37 and a tryptophan at position 34 of VirB7NT are absolutely essential for complex formation. At the moment we are performing a functional assay in order to evaluate in vivo the importance of the B9:B7 intermolecular contacts to the stability of the channel and the function of Xac’s T4SS.

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