NMR 3D Structure Determination of ApaG/CorD Protein of the Phytopathogen *Xanthomonas axonopodis pv. citri*

A. M. Katsuyama*
Dept. Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo; Centro de Biologia Molecular Estrutural, Laboratório Nacional de Luz Síncrotron (LNLS), Campinas, Brazil
angelamka@yahoo.com.br

C. S. Farah
Centro de Biologia Molecular Estrutural, Laboratório Nacional de Luz Síncrotron (LNLS), Campinas, Brazil

A. Spisni
Dept. Experimental Medicine, University of Parma, Italy; Centro de Biologia Molecular Estrutural, Laboratório Nacional de Luz Síncrotron (LNLS), Campinas, Brazil

T. A. Pertinhez
Centro de Biologia Molecular Estrutural, Laboratório Nacional de Luz Síncrotron (LNLS), Campinas, Brazil

***Keywords*:** protein structure; *Xanthomonas axonopodis* pv. *citri*; phytopathogen.

Abstract: *Xanthomonas axonopodis pv. citri (Xac) is the agent of citrus canker, which causes many economic losses in production of citrus fruit. This impact is severe in Brazil, which produces about one third of the world's citrus fruit crop. The complete sequencing of the Xac genome revealed several conserved proteins of unknown function and structure. In this context, two ORFs (10319 and 2684), located in clusters of genes associated with pathogenicity and/or secretion systems or of unknown function, were selected and the proteins they encode were over-expressed in E. coli and purified. Structural data for these proteins not only could provide information regarding their cellular function but also they might disclose new folds. Here we report on ORF 2684 that codes for the protein ApaG, which is located in a multifunctional operon and which has homology with CorD, a protein of Salmonella typhimurium associated with Co^{2+} and Mg^{2+} control. Circular dichroism spectroscopy has been used to initially characterize the protein fold. Preliminary backbone assignment derived from 3D triple resonance NMR experiments allowed, by means of the analysis of chemical shift index, to derive a preliminary description of the protein secondary structure. Diffusion measurements made it possible to estimate the protein hydrodynamic radius and hydrogen-deuterium exchange permitted the identification of regions with different degrees of exposure to the solvent. We envisage that the knowledge of the protein 3D solution structure will provide clues on its molecular function and will allow us to model ApaG/CorD analogs, including mammalian F-box proteins involved in ubiquitin-linked protein degradation.*

Xanthomonas axonopodis pv. *citri* (*Xac*) is the agent of citrus canker, which causes many economic losses in production of citrus fruit. This impact is severe in Brazil, which produces about one third of the world's citrus fruit crop. The complete sequencing of the *Xac* genome revealed several conserved proteins of unknown function and structure. In this context, from a total of previously selected 20 ORFs located in clusters of genes associated with pathogenicity and/or secretion systems, two (ORF 10319 and 2684) were selected according to the method described in (1), and the proteins they encode were over-expressed in *E. coli* and purified.

Structural data for these proteins could not only provide information regarding their cellular function but might also disclose new folds.

Here we report on ORF 2684, which codes for the protein ApaG, which is located in a multifunctional operon (involved in translational regulation, antibiotic resistance, dinucleotide-polyphosphates hydrolysis and probably in cellular protection against oxidative and thermal stress), and homology with CorD, a protein of *Salmonella typhimurium* associated with Co^{2+} and Mg^{2+} control. Circular dichroism (CD) spectroscopy has been used to initially characterize the protein fold.

Preliminary backbone assignment derived from three dimensional triple resonance NMR experiments, which allowed to analyze the chemical shift index (CSI) results, and other dynamic experiments such as diffusion measurements and hydrogen-deuterium (H-D) exchange are presented. We envisage that the knowledge of the protein 3D solution structure provides clues on its molecular function and allows one to model ApaG/CorD analogs, including mammalian F-box proteins involved in ubiquitin-linked protein degradation.

Initially, CD spectra were acquired at 20ºC, with the protein dissolved in 10 mM NaH_2PO_4 buffer, pH 6.8. The far UV spectrum suggests that the protein possesses some β-sheet secondary structure, and the optical activity observed in the near-UV spectrum indicates structural organization of the aromatic side chains.

The hydrodynamic radius of ApaG was obtained carrying out DOSY experiments and applying the equation: $R_H^{protein} = D_{ref} / D_{protein} \times R_H^{ref}$ (2). The protein and dioxane (used as internal reference) were dissolved in 100% D_2O, 40 mM NaH_2PO_4 buffer, 50 mM NaCl, 0,5% NaN_3, pH 6.8. These experiments, due to the similarity of experimental and theoretical hydrodynamic radii values, indicate that the protein is globular, thus ruling out the possibility of this protein forming dimmers. The preliminary backbone assignment was executed using 3D triple resonance data, which were acquired on a Varian Inova 600 AS spectrometer operating at 14.1 Tesla. The well resolved ^{15}N-HSQC spectrum (Figure 1), combined with these other multinuclear, multidimensional experiments, allowed us to assign the ^{15}N, ^{13}C and ^{1}H resonances of the protein backbone. On the basis of the backbone assignment, we performed the CSI analysis method described by (3) and we could derive a preliminary description of the protein secondary structure. The global chemical shift analysis evidences the existence of several stretches forming β-strands, thus supporting both the CD data and the computational secondary structure prediction obtained through the homepage http://www.embl-heidelberg.de/predictprotein/.

For H-D exchange experiment, the lyophilized protein was dissolved in a volume of deuteron water, and sequential ^{15}N-HSQCs were collected at various time intervals. In 120 hours the total disappearing of the resonance peaks could be observed. We have currently been able to identify regions with different degrees of exposure to the solvent. The knowledge of 3D fold will provide a more detailed analysis of the data.

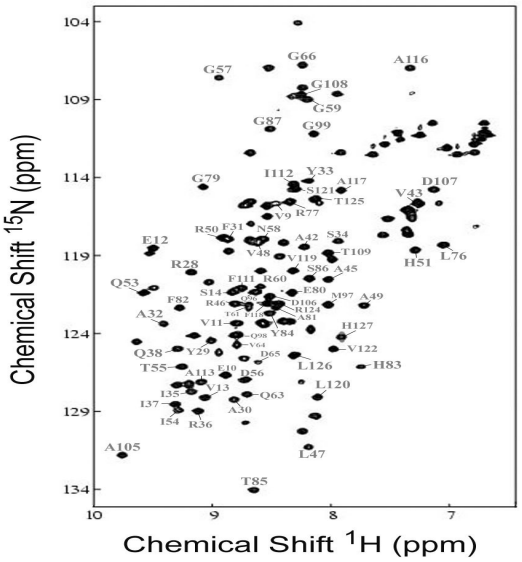

Figure 1. ^{15}N-HSQC of ApaG/CorD protein showing the assigned amino acids.

The work is ongoing and the following activities are underway: CD experiments to better characterize the protein folding pattern and its stability, side-chain assignment, NOEs collection to obtain distance constraints and analysis of the $J_{NH\alpha}$ to determine dihedral angles, residual dipolar coupling determination to complement NOEs data. When all this information is available molecular modeling calculations will be carried out to obtain the final refined three-dimensional structure of ApaG/CorD protein.

Acknowledgements

The authors acknowledge FAPESP and Varian Brasil.

References

1. L.M.P. Galvão-Botton, A.M. Katsuyama, C.R. Guzzo, F.C.L. Almeida, C.S. Farah, A.P. Valente, *Biochemistry* (2003) submitted.
2. D.K. Wilkins, S.B. Grimshaw, V. Receveur, C.M. Dobson, J.A. Jones, L.J. Smith, *Biochemistry* **38** (1999) 16424.
3. D.S. Wishart, B.D. Sykes, F.M. Richards, *Biochemistry* **31** (1992) 1647.