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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C chemical shift assignments of the structured core of the *Pseudomonas* effector protein AvrPto

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Biological context

AvrPto is a unique, 164-residue, 18.3 kDa hydrophilic effector protein of *Pseudomonas sryingae* pv. *tomato*, the pathogenic gram-negative bacteria that causes bacterial speck disease in tomato. Bacterial infection results from the introduction of AvrPto and other *P. sryingae* effector proteins into the cytoplasm of host plant cells through the Hrp (hypersensitive response and pathogenicity) type III secretion system (Ham et al., 1998). Pathogen effector proteins sometimes function by mimicking host cell proteins in order to suppress or alter basic cellular signaling pathways (reviewed in Sessa and Martin, 2000). Virulent infection results in widespread cell death, as the bacteria scavenge cellular components for nutrition and reproduction. While AvrPto has been shown to promote virulence in *P. sryingae*, the molecular basis for this effect remains undefined (Shan et al., 2000a; Chang et al., 2000). Some tomato species have developed resistance to infection by *P. sryingae*, rendering the pathogen avirulent. In a classic 'gene-for-gene' manner, resistance is conferred by the expression of Pto, a small host serine-threonine protein kinase that specifically interacts with AvrPto (Loh and Martin, 1995). The Pto/AvrPto interaction elicits an HR (hypersensitive response) that results in rapid, highly localized cell death and containment of infection (Tang et al., 1999). Truncation studies have identified a minimal region of AvrPto (residues 30–125) that is sufficient to interact with Pto, while mutation studies have shown that residues S94, I96 and G99 are required for this interaction (Shan et al., 2000b). Here we present the backbone and sidechain assignments of a 105-residue truncated construct of AvrPto (TrAvrPto) comprised of residues 29–133. TrAvrPto contains the structured core region of AvrPto and retains Pto binding function (P. Pascuzzi and G. Martin, unpublished results). Knowledge of the TrAvrPto structure will yield insight into its mode of interaction with Pto and the structural features important to its roles in both the virulence and avirulence of *P. sryingae*.

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Methods and experiments

TrAvrPto was expressed as a C-terminal FLAG-tagged fusion protein using the vector pFLAG-CTC (Sigma) in *E. coli* BL21-Gold cells (Stratagene). The construct contained an N-terminal Met, AvrPto residues 29-133, a Val and the FLAG peptide. ^{15}N-labeled samples were grown in M9 media containing ^{15}N NH_4CL (Cambridge Isotope Labs, Andover, MA), and $^{15}N/^{13}C$-labeled samples were purified from cultures grown in M9 media containing ^{15}N NH_4CL and ^{13}C d-glucose (Cambridge Isotope Labs). Cultures were grown at 37 °C with vigorous shaking to an OD_{600} of 0.6, induced with 1 mM IPTG and allowed to grow for 4 h before harvesting. Cell pellets were resuspended in 30 ml TBS (50 mM Tris, 150 mM NaCl, pH 7.4) containing protease inhibitors (1 mM EDTA, 10 μg/ml aprotinin, 1 mM PMSF, 5 μg/ml pepstatin, and 10 μg/ml leupeptin) and lysed by two passes through a French pressure cell (900 PSIG). Tween-20 was added to 0.1% before the cell lysates were cleared by centrifugation, split equally and passed over five 5 ml M2 anti-FLAG columns (Sigma). Each column

was washed with 250 ml TBS before the fusion protein was eluted with five 5 ml aliquots of 100 mM glycine, pH 3.5, into 0.5 ml of 1 M Tris, pH 8.0. Fractions containing fusion protein were pooled and dialyzed against AvP buffer (10 mM Na_2HPO_4, 5 mM KH_2PO_4, 225 mM NaCl, 0.02% NaN_3, pH 6.0) before being concentrated to approximately 1 mM using Centriplus and Centricon 10,000 MWCO concentrators (Millipore). Protein purity was assessed by analytical HPLC and SDS-PAGE and averaged >95%. All NMR experiments were performed at 25 °C on a Varian Inova 600 MHz spectrometer. An ^{15}N TrAvrPto sample was used to collect ^{1}H-^{15}N HSQC, 2D homonuclear NOESY and TOCSY, ^{1}H-^{15}N NOESY-HSQC and ^{1}H-^{15}N TOCSY-HSQC spectra. A ^{13}C/^{15}N double-labeled sample was used to collect HNCA, HNCO, HC(CO)NH, C(CO)NH, CBCANH, CBCA(CO)NH, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C NOESY spectra. The sample was then lyophilized to dryness, re-suspended in 100% D_2O, and used to collect ^{1}H-^{13}C aromatic HSQC, 2D homonuclear NOESY, ^{1}H-^{13}C NOESY and ^{1}H-^{13}C HCCH-TOCSY spectra. Backbone assignments were obtained from ^{1}H-^{15}N NOESY-HSQC, ^{1}H-^{15}N TOCSY-HSQC, HNCA, CBCANH and CBCA(CO)NH spectra. Spin systems were assigned using ^{1}H-^{15}N TOCSY-HSQC, HC(CO)NH, C(CO)NH and ^{1}H-^{13}C HCCH-TOCSY spectra. Aromatic ring systems were assigned using the 2D homonuclear NOESY and aromatic ^{13}C HSQC. Carbonyl resonances were assigned using an HNCO spectrum. Detailed descriptions of these experiments have been reviewed elsewhere (Cavanagh et al., 1996). Spectra were processed with NMRPipe/NMRDraw (Delaglio et al., 1995) and analyzed with PIPP (Garret et al., 1991).

Extent of assignments and data deposition

Backbone assignments are complete with the exception of the carbonyl carbons of L49, A61, N97 and L101 (preceding Proline), and D84. Figure 1 shows the ^{1}H-^{15}N HSQC spectrum and assignments of trAvrPto. Sidechain assignments are complete to the extent of ∼ 82% of the protonated ^{13}C and ∼ 91% of the non-labile ^{1}H resonances. The ^{13}Cδ resonances of L36, and the ^{13}Cγ resonances of I113, L65, L123, P62, P102, Q53, and R120 could not be unambiguously assigned due to spectral overlap. Jpred and CSI predictions of secondary structure (Wishart and Sykes, 1994) indicate a predominantly helical structure for

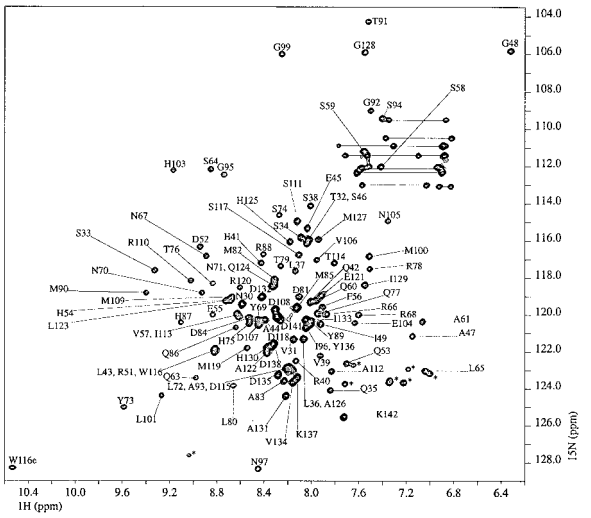

Figure 1. Two-dimensional ^{1}H-^{15}N-HSQC spectrum and assignments of TrAvrPto. Residue numbering is relative to full-length AvrPto. Pairs of peaks connected by horizontal lines are from NH_2 resonances of Asn and Gln side chains. Peaks marked with an asterisk are from Arg and Lys side chains.

TrAvrPto. Short- and medium-range NOE connectivities observed in ^{1}H-^{15}N NOESY-HSQC and ^{1}H-^{13}C NOESY spectra are consistent with the predictions for an α-helical secondary structure (data not shown). In addition, numerous long-range NOE's have also been observed. TrAvrPto assignments have been deposited in the BioMagResBank (accession number 5311).

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