NMR solution structure of CdnL, a vital *Myxococcus xanthus* protein that belongs to a large family of bacterial proteins of unknown function

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Protein family PF02559 (http://pfam.sanger.ac.uk/)[1] is defined by the N-terminal domain of CarD, a global regulator found in the Gram negative bacterium *Myxococcus xanthus*[2]. Members of this family are present in many bacterial species but, other than for CarD, their functions are unknown. *M. xanthus* contains a second, distinct PF02559 member, a 164-residue protein of unknown function that we have named CdnL (for CarD N-terminus-Like). We have found that despite their sequence similarities, CdnL and the N-terminal domain of CarD are not functionally equivalent, and that CdnL is essential for *M. xanthus* viability. Although CdnL lacks any inherent DNA-binding activity, subcellular localization studies indicated that it is associated to the nucleoid, suggesting that CdnL participates in some essential DNA transaction. In order to gain functional insights into CdnL, and since no high-resolution structural information is available for any member of the PF02559 protein family, we are examining the structure of CdnL by NMR. At protein concentrations of 0.5-1 mM, NMR spectra of CdnL were characterized by peak-broadening effects consistent with a monomer-dimer exchange, as confirmed by analytical ultracentrifugation experiments. Limited proteolysis of CdnL revealed a stable, 110-residue C-terminal domain, CdnL-Cter, whose NMR spectra showed excellent spectral dispersion and overall quality.

In this communication we report the determination of the three-dimensional structure of CdnL-Cter. A complete assignment of backbone atoms was performed by analysing a series of 3D NMR spectra (CBCANH, CBCAcoNH, HBHANH, HBHAcoNH, HNCO, HNCA, HNcoCA, HAcaNH) acquired using a $^{15}N,^{13}C$-CdnL-Cter sample in aqueous solution. This assignment was extended to side chain atoms, ^{1}H and ^{13}C, by analysis of 3D HCCH-TOCSY spectra recorded in D_2O. Structure calculation was performed by using an iterative protocol with an automatic NOE assignment and incorporating dihedral angle restraints derived from the $^{1}H_{\alpha}$, $^{13}C_{\alpha}$, $^{13}C_{\beta}$, and $^{13}C'$ chemical shifts. The resulting all-helical structure contains five well-packed α-helices extending over residues 14-26, 34-48, 52-67, 73-96 and 98-110. Further structural details and their potential functional implications will be discussed.

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[2] Cayuela, M.L., Elías-Arnanz, M., Peñalver-Mellado, M., Padmanabhan, S., and Murillo, F.J. (2003) *J Bacteriol*, 185**,** 3527-3537.