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The crystal structure of the tandem-PAS sensing domain of *Campylobacter jejuni* chemoreceptor Tlp1 suggests indirect mechanism of ligand recognition

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ABSTRACT

Chemotaxis and motility play an important role in the colonisation of avian and human hosts by *Campylobacter jejuni*. Chemotactic recognition of extracellular signals is mediated by the periplasmic sensing domain of methyl-accepting chemotactic proteins (membrane-embedded receptors). In this work, we report a high-resolution structure of the periplasmic sensing domain of transducer-like protein 1 (Tlp1), an aspartate receptor of *C. jejuni*. Crystallographic analysis revealed that it contains two Per-Arnt-Sim (PAS) subdomains. An acetate and chloride ions (both from the crystallisation buffer) were observed bound to the membrane-proximal and membrane-distal PAS subdomains, respectively. Surprisingly, despite being crystallised in the presence of aspartate, the structure did not show any electron density corresponding to this amino acid. Furthermore, no binding between the sensing domain of Tlp1 and aspartate was detected by microcalorimetric experiments. These structural and biophysical data suggest that Tlp1 does not sense aspartate directly; instead, ligand recognition is likely to occur indirectly *via* an as yet unidentified periplasmic binding protein.

1. Introduction

Campylobacter jejuni is a Gram-negative, microaerophilic, flagellated bacterium that colonises the intestines of many wild and domestic animals (Boes et al., 2005; Oporto et al., 2007). *C. jejuni* is a leading cause of bacterial foodborne gastroenteritis in humans (Zilbauer et al., 2008). Human infection occurs by the consumption of contaminated food (especially poultry products) or water (Hepworth et al., 2011). Although this infection is usually self-limiting, it may lead to important post-infection complications such as neuromuscular paralysis (Guillain–Barré syndrome), reactive arthritis, myositis and idiopathic peripheral neuropathy (Friedman et al., 2000; Schmidt-Ott et al., 2006).

The molecular mechanisms of *C. jejuni* pathogenesis are still poorly understood. However, flagellar motility and chemotaxis are known to play a crucial role during the intestinal colonisation of both avian and mammalian hosts (Dasti et al., 2010; Josenhans and Suerbaum, 2002). Non-motile strains of *C. jejuni* could not, for example, colonise the digestive tract of mice (Morooka et al., 1985). Similarly, chemotactic defects such as the loss of chemoreceptor function resulted in reduced motility and infectivity of *C. jejuni* (Hendrixson et al., 2001; Tareen et al., 2010).

Chemotactic movement of *C. jejuni* towards conditions that favour survival has been shown to be directed by various extracellular chemical gradients. These include L-fucose and L-serine (mucin components), citrate, fumarate, α-ketoglutarate and succinate (tricarboxylic acid (TCA) cycle intermediates) and amino acids, such as aspartate and glutamate, that may be deaminated to TCA cycle intermediates (Hugdahl et al., 1988; Sebald and Vernon, 1986). These external stimuli are detected by the sensing domains of methyl-accepting chemotactic proteins (MCPs), or transducer-like proteins (Tlps), which transfer information through their signaling domains, activating signaling cascades that

Abbreviations: MCP, methyl-accepting chemotactic protein; Tlp, transducer-like protein; PAS, Per-Arnt-Sim; PTPSD, periplasmic tandem PAS sensing domain; ITC, isothermal titration calorimetry; CD, circular dichroism.

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control the direction of rotation of flagella (Fernando et al., 2007; Zautner et al., 2012; Zhulin, 2001).

The *C. jejuni* genome encodes at least 11 different putative Tlps, which have been classified into three groups according to their domain organisation: A (Tlp1, Tlp2, Tlp3, Tlp4, Tlp7$_{mc}$, Tlp7$_{m}$, Tlp10 and Tlp11); B (Tlp9); and C (Tlp5, Tlp6, Tlp7$_{c}$ and Tlp8) (Parkhill et al., 2000; Zautner et al., 2012). The group B chemoreceptor is a single-span membrane protein, with no periplasmic domain, whereas group C chemoreceptors are soluble cytoplasmic proteins. As such, group B and C receptors likely sense internal signals such as, for example, changes in redox potential. Group A chemoreceptors have a general membrane topology reminiscent of that of the well-characterised chemoreceptors of *Escherichia coli*. They are predicted to comprise an N-terminal transmembrane helix followed by a periplasmic domain (putative ligand-binding, or sensing domain), a second transmembrane helix and, finally, a C-terminal cytoplasmic signaling domain, which contains a methyl-accepting chemotaxis-like subdomain (chemotaxis sensory transducer) (Fig. 1). The presence of a periplasmic domain in group A Tlps suggests that they sense external signals. The cytoplasmic signaling domain is highly conserved in Tlps from different bacterial species (Marchant et al., 2002; Parkhill et al., 2000). In contrast, the periplasmic sensing domains of *C. jejuni* share no significant sequence similarity with those of *E. coli*, which suggests that the mechanisms by which they recognise ligands are likely distinctly different from *E. coli*.

The periplasmic sensing domains, involved in the recognition of external ligands, are highly diverse across different bacterial species, which reflects evolution to detect a broad spectrum of environmental cues (Wadhams and Armitage, 2004). To date, some of the ligands of five *C. jejuni* Tlps from this group have been identified. Tlp1 has been shown to be involved in sensing aspartate and termed the *Campylobacter* chemoreceptor for aspartate A (CcaA) (Hartley-Tassell et al., 2010). Tlp3 has been shown to directly sense isoleucine and may indirectly recognise several other small molecules (Li et al., 2014; Rahman et al., 2014). Tlp4 has been identified as a sodium deoxycholate receptor, Tlp7 as a formic acid receptor (Tareen et al., 2010), and Tlp11 as a galactose receptor (Day et al., 2014).

Tlp1 is ubiquitous and the most conserved among *C. jejuni* strains (Day et al., 2014; Korolik and Ketley, 2008). Previous studies showed that the *tlp1* gene was strongly upregulated in the *C. jejuni* strains passaged through chicken (King et al., 2013), and its insertional inactivation resulted in significantly decreased colonisation of the chicken intestinal tract (Hartley-Tassell et al., 2010). Furthermore, the *tlp1* mutant strain of *C. jejuni* showed drastically reduced ability to invade human epithelial cells and chicken embryo (Vegge et al., 2009). Tlp1 is therefore involved in the commensal colonisation of the chicken intestine by *C. jejuni* and may play an important role in the infection and colonisation of the human host.

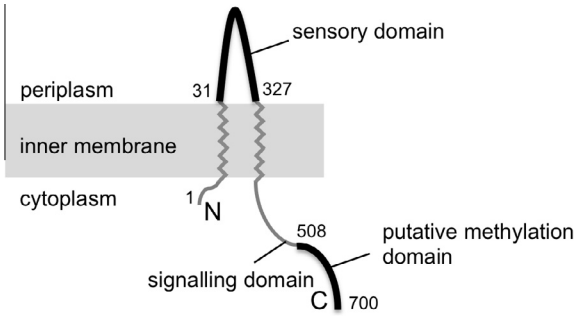

Fig. 1. The predicted membrane topology of *C. jejuni* Tlp1 and the boundaries (amino acid residue numbers) of the periplasmic sensing domain and the putative methylation domain.

The *tlp1*- isogenic mutant of *C. jejuni* showed a significant decrease in chemotaxis towards aspartate (Hartley-Tassell et al., 2010). Migration towards aspartate could be restored to this mutant by complementation with an intact copy of the *tlp1* gene. These findings suggested that Tlp1 plays an important role in sensing aspartate. The periplasmic sensing domain of Tlp1 has no sequence homology with that of Tar, the well-characterised aspartate receptor of *E. coli* (Hartley-Tassell et al., 2010), or any of the chemoreceptors described to date. Therefore, the mechanism involved in the aspartate recognition by *C. jejuni* Tlp1 may be unique to this bacterial genus.

Recently, we determined the crystal structure of the sensing domain of *C. jejuni* Tlp3 and its complex with isoleucine (Liu et al., 2015), and analysis of its ligand-binding pocket provided a molecular explanation for the specificity of that chemoreceptor. Here, we report the 1.4-Å resolution crystal structure of the periplasmic region of *C. jejuni* Tlp1 comprising amino acid residues 31–327. The structure revealed that, like Tlp3, Tlp1 contains a periplasmic tandem-Per-Arnt-Sim (PAS) sensing domain (PTPSD). Our structural and biophysical analysis suggests that, in contrast to Tlp3, the mechanism of amino acid recognition by Tlp1 does not involve direct ligand-receptor interaction; aspartate is likely sensed by Tlp1 indirectly, *via* an as yet unidentified periplasmic binding protein.

2. Materials and methods

2.1. Crystallisation and data collection

Tlp1-PTPSD (residues 31–327 plus GIDPFT sequence at the N-terminus as a cloning artifact) was crystallised in the presence of 10 mM L-aspartic acid as described (Machuca et al., 2015). The crystals belong to space group *P*1, with unit cell parameters $a = 39.3$ Å, $b = 43.3$ Å, $c = 50.9$ Å, $\alpha = 92.5°$, $\beta = 111.4°$, $\gamma = 114.7°$ and a monomer in the asymmetric unit. Although we were unable to crystallise Tlp1-PTPSD in the absence of aspartate under similar conditions, we noted that addition of 10 mM aspartic acid to the crystallisation buffer reduced pH from 5.5 to 5.2. To test if it was lower pH, rather than the presence of aspartic acid, that was important for the crystal formation, we prepared a crystallisation mix that had the same components (minus aspartic acid), except the pH of the buffer was 5.2 (23% PEG 3350, 200 mM ammonium acetate and 100 mM Bis-Tris pH 5.2). Tlp1-PTPSD did crystallise under these conditions, and the crystals had similar morphology to those grown in the presence of aspartic acid (unit cell parameters $a = 39.0$ Å, $b = 43.7$ Å, $c = 50.4$ Å, $\alpha = 92.7°$, $\beta = 110.6°$, $\gamma = 115.0°$). Crystals obtained in the presence of aspartate (the putative Tlp1 ligand) were selected for the subsequent analysis.

To perform data collection at cryogenic temperatures, the crystals were briefly soaked in a cryoprotectant solution consisting of 220 mM ammonium acetate, 26% (*w/v*) PEG 3350, 100 mM Bis-Tris pH 5.5, 10 mM aspartic acid and 10% (*v/v*) glycerol, and flash-cooled by plunging into liquid nitrogen. X-ray diffraction data were collected to 1.4 Å using the MX1 beamline of the Australian Synchrotron (AS). The diffraction data were processed and scaled using *iMOSFLM* (Battye et al., 2011) and AIMLESS (Evans and Murshudov, 2013) from the CCP4 software suite (Winn et al., 2011). Data collection statistics are summarised in Table 1.

2.2. Structure determination

The structure of Tlp1-PTPSD was determined using PHASER (McCoy et al., 2005) with coordinates of *Helicobacter pylori*