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# Crystallization and preliminary X-ray studies of the *Rhizobium meliloti* DctD two-component receiver domain

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**Abstract**

The *Rhizobium meliloti* DctD two-component receiver domain was expressed in *Escherichia coli* and purified to homogeneity. Crystals were obtained using the hanging-drop vapor-diffusion geometry with ammonium phosphate as the precipitant. The crystals diffract to 2.3 Å and exhibit the symmetry of space group $I222$ or $I2_12_12_1$. The unit-cell dimensions are $a$ = 59.0, $b$ = 58.6 and $c$ = 169.8 Å. The asymmetric unit contains a dimer and the crystals have a $V_m$ of 2.16 Å$^3$ Da$^{-1}$.

## 1. Introduction

The two-component signal-transduction system is widespread in bacteria and regulates important and diverse functions such as nitrogen fixation, virulence and chemotaxis. Recently, examples of two-component signal-transduction proteins have been found in eukaryotes, in which they contribute to the regulation of development and responses to changes in osmolarity (Maeda *et al.*, 1995; Schuster *et al.*, 1996; Wang *et al.*, 1996; Alex *et al.*, 1996; Chang & Meyerowitz, 1994; Loomis *et al.*, 1997). Although the functions regulated by two-component systems are diverse, structural studies of three response regulators (CheY, NtrC and NarL) in the CheY superfamily (Volz, 1993) indicate that these receiver domains exhibit a similar overall fold and suggest that the final step in signal transduction involves a conformational change that may occur by a common mechanism in response to phosphorylation (Stock *et al.*, 1989, 1993; Volz & Matsumura, 1991; Bruix *et al.*, 1993; Bellsolell *et al.*, 1994, 1996; Volkman *et al.*, 1995; Baikalov *et al.*, 1996). However, the exact nature of this conformational change has not been determined since the phosphorylated versions of these proteins are typically not sufficiently stable for high-resolution structural studies.

DctD is a two-component $\sigma$54-dependent transcriptional activator that is used by *Rhizobium meliloti* and *Rhizobium leguminosarum* to control the expression of a C4-dicarboxylate transport gene (see Scholl & Nixon, 1996, and references cited therein). These bacteria are typically found closely associated with plant roots and are capable of symbiotic nitrogen fixation in the presence of their respective host plants, alfalfa and pea. The correct expression of this C4-dicarboxylate uptake system is required for symbiotic nitrogen fixation.

An exciting feature of the DctD system is that single point mutations have been isolated which mimic phospho-DctD, causing the protein to constitutively activate transcription in the absence of phosphorylation (Nixon, unpublished data). Based on sequence homology of DctD with the three known structures in the CheY superfamily, these mutations map to the receiver domain and tend to cluster near the C-terminal end of $\alpha$-helix 5. Unlike similar mutant proteins that have been studied for CheY, in which the amino-acid substitutions are within or near the site of phosphorylation (Volz, 1993, Zhu *et al.*, 1997), these substitutions in DctD are likely to be far from the site of phosphorylation. A comparison between structures of the wild-type N-terminal receiver domain of DctD with various constitutively active mutants that mimic phospho-DctD may further elucidate the mechanism for activation.

In this paper, we describe an efficient overexpression system for the two-component receiver domain of DctD and describe the protocol for the purification of this domain. Crystals of the DctD protein have been obtained and diffract to high resolution.

## 2. Materials and methods

### 2.1. *Cloning of the gene and expression of protein*

PCR primers were designed to amplify codons 1 to 143 of *R. meliloti dct*D (Jiang *et al.*, 1989). The primers introduced an *Nde*I restriction site at codon 1 and a *Hin*dIII restriction site immediately following codon 143. The *Nde*I/*Hin*dIII fragment was cloned into the ampicillin-resistant plasmid pET21a (Novagen) to create pT143WT, and the new construct was verified by dideoxy sequencing. Insertion into the pET21a vector resulted in the addition of the following sequence to the end of residue 143: KLAAALEHHHHHH. In intact DctD, this sequence would be PLIGQTPVMERLR.

Expression of His-tagged DctD from pT143WT is under the control of an IPTG-inducible T7 phage promoter (Dubendorff & Studier, 1991). Except where noted, all cultures were grown in LB medium containing ampicillin (100 mg ml$^{-1}$) and chloramphenicol (34 mg ml$^{-1}$) at 310 K with shaking (250 rev min$^{-1}$) and cell densities were measured at 600 nm. A frozen stock of *Escherichia coli* BL21(DE3) pLysS carrying pT143WT was used to inoculate 5 ml of LB and incubated overnight. Cells from 500 ml of the culture were recovered, washed free of $\beta$-lactamase and placed in 50 ml of fresh LB in a 250 ml flask. The culture was grown to an absorbance of 0.8; cells were recovered by centrifugation, washed twice and used to seed a 5 l fermentation in a BioFlow 3000 fermenter (New Brunswick Scientific; medium: 100 g tryptone, 200 g yeast extract, 150 g glycerol, 35 g $K_2HPO_4$, 40 g $KH_2PO_4$, 500 mg ampicillin and 170 mg chloramphenicol). Phosphoric acid and NaOH were used to maintain the pH at 7.0 and dissolved oxygen was maintained at 30% of the maximum possible. At an absorbance of 10, the cells were induced with 1 m*M* IPTG. At an absorbance of 28, the cells were harvested by centrifugation and placed at 193 K. For small-scale preparations, a 500 ml shaking-flask culture is induced with 1 m*M* IPTG at an absorbance of 0.6 and cells are harvested after 5 h further incubation.




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Table 1. *Summary of diffraction data from native DctD two-component receiver-domain crystals*

All data were taken from the output of *SCALEPACK*. $R_{merge}$ is reported on intensities. Observations list the number of times that a reflection was observed.

| Lower resolution | Upper resolution | Average intensity | Average error | $R_{merge}$ | Observations | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 0 | 1 | 2 | 3 | 4 | 5+ | Total |
| 100.00 | 4.96 | 18490.9 | 362.1 | 0.043 | 259 | 169 | 277 | 178 | 178 | 385 | 1187 |
| 4.96 | 3.93 | 25833.7 | 481.7 | 0.051 | 106 | 87 | 230 | 197 | 179 | 554 | 1247 |
| 3.93 | 3.44 | 13177.1 | 340.5 | 0.064 | 57 | 81 | 247 | 214 | 159 | 599 | 1300 |
| 3.44 | 3.12 | 6852.9 | 247.5 | 0.078 | 30 | 56 | 239 | 228 | 152 | 632 | 1307 |
| 3.12 | 2.90 | 3739.5 | 209.9 | 0.097 | 32 | 58 | 241 | 231 | 152 | 620 | 1302 |
| 2.90 | 2.73 | 2127.8 | 199.1 | 0.129 | 28 | 54 | 229 | 213 | 158 | 640 | 1294 |
| 2.73 | 2.59 | 1464.9 | 187.7 | 0.151 | 34 | 42 | 247 | 221 | 143 | 643 | 1296 |
| 2.59 | 2.48 | 1033.7 | 179.3 | 0.194 | 35 | 38 | 227 | 213 | 152 | 644 | 1274 |
| 2.48 | 2.38 | 759.7 | 178.0 | 0.253 | 39 | 38 | 234 | 213 | 162 | 632 | 1279 |
| 2.38 | 2.30 | 643.0 | 176.8 | 0.285 | 43 | 37 | 213 | 194 | 181 | 631 | 1256 |
| All reflections | | 7288.3 | 255.0 | 0.069 | 663 | 660 | 2384 | 2102 | 1616 | 5980 | 12742 |

### 2.2. *Protein purification*

Typically, 3 g of frozen cell paste was added to 10 ml of lysis buffer (20 m*M* Tris pH 8.0, 0.5 *M* NaCl) and set on ice for 30 min. Cells lyse upon thawing because the host strain also expresses T7 lysozyme (Studier, 1991); however, the lysates were sonicated for 30 s and then set on ice for another 10 min. The lysate was centrifuged at 100 000*g* for 30 min at 277 K and the supernatant was collected. The pellet was resuspended in 10 ml of lysis buffer, centrifuged a second time and the supernatant collected. The two 10 ml fractions were pooled and loaded (0.02 ml $min^{-1}$; 277 K) onto a 1 ml nickel column previously equilibrated with 20 ml of lysis buffer. After washing the column with 30 ml of lysis buffer (0.5 ml $min^{-1}$) followed by 20 m*M* Tris (pH 8.0; 0.5 ml $min^{-1}$), it was equilibrated with 50 m*M* sodium phosphate (pH 8.0) and then subjected to a 20 ml linear gradient of imidazole (0 to 200 m*M* imidazole, 50 m*M* sodium phosphate buffer, pH 8.0; 0.5 ml $min^{-1}$). Purified protein was concentrated to 8 mg $ml^{-1}$ in collodion membranes (Schleicher & Schuell, molecular-weight cut-off 10 kDa) and was simultaneously dialyzed against 20 m*M* Tris (pH 8.0), 2 m*M* $MgCl_2$ and 2 m*M* $NH_4H_2PO_4$. The BCA assay (Pierce) was used to quantify protein concentration. The protein was immediately used for crystallization, or was frozen in small aliquots at 193 K for future use. Material which was frozen will also crystallize.

### 2.3. *Crystal growth*

Initial crystallization trials were performed using the sparse-matrix method of Jancarik & Kim (1991). Crystals were grown by hanging-drop vapor-diffusion geometry (294 K) where a drop contained 5 ml of 5–7 mg $ml^{-1}$ protein and 5 ml of precipitant. Initially, 18 conditions yielded crystals. Crystal-growth conditions were further refined and subsequent crystals were grown using a well solution of 50 m*M* succinate (pH 5.6) and 75 m*M* $NH_4H_2PO_4$. For a typical crystallization, 1 ml of precipitant solution is placed in the well of a Falcon 3047 24-well tissue-culture plate. Glass cover slips were treated with Sigmacote and then with chloroform. The protein solution is filtered through a 0.1 µm filter immediately prior to setting up crystallizations. Crystallization drops which are set up with unfiltered protein typically give scores of small crystals. After filtration, only 1–4 crystals appear in each drop and grow to the size of 600 × 400 × 300 µm. Using 42 mg $ml^{-1}$ protein produced even larger crystals which were 1 × 1 × 2 mm in size.

### 2.4. *Crystal characterization*

Prior to diffraction, suitable crystals were transferred to 50 m*M* succinate (pH 5.6) and 300 m*M* $Na_2SO_4$ and were soaked for at least 24 h. Crystals were then mounted in thin-walled quartz capillaries. A native data set was collected at room temperature to 2.3 Å resolution using a Rigaku R-AXIS IV imaging-plate detector on a Rigaku RU200 rotating-anode X-ray generator operating at 50 kV and 100 mA and equipped with a graphite monochromator. Data were collected with 1.5° oscillation frames, processed with *DENZO* and frames were scaled with *SCALEPACK* (Otwinowski & Minor, 1997). The space group was confirmed using precession photographs taken on a Charles Supper precession camera using Ni-filtered Cu $K\alpha$ radiation from an Enraf–Nonius X-ray generator operating at 40 kV, 30 mA.

## 3. Results and discussion

The nature of the conformational change which activates DctD is the focus of our structural work. Ideally, we would like to obtain structures of the entire DctD protein and of constitutively active mutants of this protein. The intact protein is thought to have three domains: the CheY-like receiver domain, an ATPase domain and a DNA-binding domain. However, the full-length DctD protein has not been amenable to crystallographic studies. In this paper, we report the characterization and crystallization of a fragment of DctD which contains the receiver domain (residues 1–125) plus a substantial portion of the linker region between the receiver domain and the ATPase domain. The fragment contains residues 1–143 from DctD and an additional 13 amino acids. The last six of these 13 additional amino acids are all histidine. The other seven amino acids link the DctD sequence to the poly His tag. We hope that structural studies of this fragment of DctD and the constitutively active mutants will give some insight into the conformational change which occurs after phosphorylation of a two-component receiver domain.

A typical fermentation yielded 250–300 g of cell paste, and approximately 2 mg of DctD(1–143) protein could be purified from each gram of cell paste. The His tag made purification of the protein relatively easy using a nickel column. About 4 mg of the protein could be bound to 1 ml of the nickel resin (Qiagen). The protein eluted from the column between 60 and 100 m*M* imidazole and was greater than 99% pure as judged by Coomassie Blue staining. Although the molecular weight of

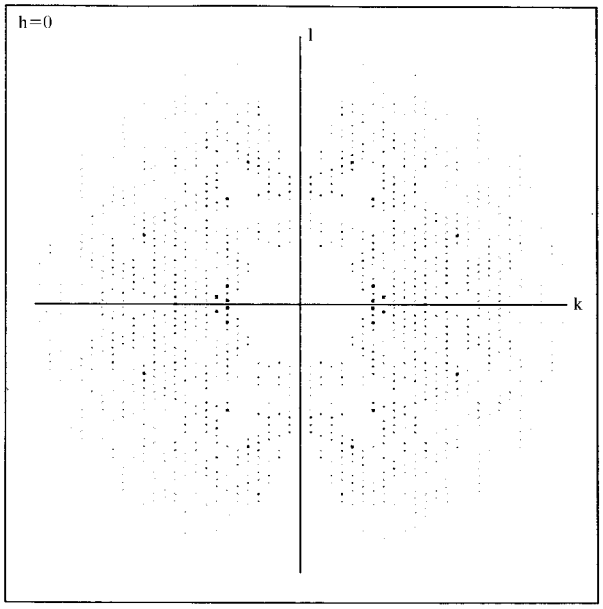


Fig. 1. Simulated diffraction pattern of the 0*kl* zone. All of the zero-level zones from the DctD1-143 crystals exhibit *mm* symmetry. This figure was generated using *HKLVIEW* in the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994)

the purified protein is 17 kDa, it ran at 11 kDa on a 15% SDS–PAGE gel. However, amino-terminal peptide sequencing showed that only the initial methionine was removed from the recombinant protein.

Single crystals are stable and diffract to 2.3 Å. A native data set collected at 298 K with three crystals had an overall merging *R* factor (on intensities) of 6.9% to 2.3 Å. The data set is 95% complete, contains a total of 12742 reflections and is highly redundant (Table 1). Autoindexing with *DENZO* indicated an orthorhombic space group with cell dimensions of $a = 59.0$, $b = 58.6$ and $c = 169.8$ Å. Precession photos of principal axes confirmed *mm* symmetry consistent with orthorhombic space groups *I*222 or $I2_12_12_1$ with the *a* dimension coincidentally similar to *b* (Fig. 1). The results from both *SCALEPACK* and the precession photos were not consistent with the tetragonal space groups *I*4 or *I*422. Based on the Matthews $V_m$, it is most likely that there is a dimer in the asymmetric unit. Assuming a dimer, the $V_m$ is 2.16 Å$^3$ Da$^{-1}$. This corresponds to 43% solvent content and is consistent with that expected for proteins (Matthews, 1968). Both the monomer and trimer models lead to unreasonable packing densities.

Attempts to solve the structure of the DctD receiver domain by molecular replacement using the *E. coli* CheY model (Volz & Matsumura, 1991) with *AMoRe* (Navaza & Saludjian, 1997) failed in both the *I*222 and $I2_12_12_1$ space groups. *Salmonella typhimurium* CheY (Stock *et al.*, 1993), *S. typhimurium* NtrC (Volkman *et al.*, 1995) and *E. coli* NarL (Baikalov *et al.*, 1996) were also used as models with no success. Heavy-atom screens are now under way.

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## References

Alex, L. A., Borkovich, K. A. & Simon, M. I. (1996). *Proc. Natl Acad. Sci. USA*, **93**, 3416–3421.
Baikalov, I., Schroder, I., Kaczor-Grzeskowiak, M., Gunsalus, R. P. & Dickerson, R. E. (1996). *Biochemistry*, **35**, 11053–11061.
Bellsolell, L., Cronet, P., Majolero, M., Serrano, L. & Collect, M. (1996). *J. Mol. Biol.* **257**, 116–128.
Bellsolell, L., Prieto, J., Serrano, L. & Collect, M. (1994). *J. Mol. Biol.* **238**, 489–495.
Bruix, M., Pascual, J., Santoro, J., Prieto, J., Serrano, L. & Rico, M. (1993). *Eur. J. Biochem.* **215**, 573–585.
Chang, C. & Meyerowitz, E. M. (1994). *Res. Microbiol.* **145**, 481–486.
Collaborative Computational Project, Number 4. (1994). *Acta Cryst.* D**50**, 760–763.
Dubendorff, J. W. & Studier, F. W. (1991). *J. Mol. Biol.* **219**, 45–59.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Jiang, J., Gu, B. H., Albright, L. M. & Nixon, B. T. (1989). *J. Bacteriol.* **171**, 5244–53.
Loomis, W. F., Shaulsky, G. & Wang, N. (1997). *J. Cell Sci.* **110**, 1141–1145.
Maeda, T., Takekawa, M. & Saito, H. (1995). *Science*, **269**, 554–558.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Navaza, J. & Saludjian, P. (1997). *Methods Enzymol.* **276**, 581–594.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Scholl, D. & Nixon, B. T. (1996). *J. Biol. Chem.* **271**, 26435–26442.
Schuster, S. C., Noegel, A. A., Oehme, F., Gerisch, G. & Simon, M. I. (1996). *EMBO J.* **15**, 3880–3889.
Stock, A. M., Marinez-Hackert, E., Rasmussen, B. F., West, A. H., Stock, J. B., Ringe, D. & Petsko, G. A. (1993). *Biochemistry*, **32**, 13375–13380.
Stock, A. M., Mottonen, J. M., Stock, J. B. & Schutt, C. E. (1989). *Nature (London)* **327**, 745–749.
Studier, F. W. (1991). *J. Mol. Biol.* **219**, 37–44.
Volkman, B. F., Nohaile, M. J., Amy, N. K., Kustu, S. & Wemmer, D. (1995). *Biochemistry*, **34**, 1413–1424.
Volz, K. (1993). *Biochemistry*, **32**, 11741–11753.
Volz, K. & Matsumura, P. (1991). *J. Biol. Chem.* **266**, 15511–15519.
Wang, N., Shaulsky, G., Escalante, R. & Loomis, W. F. (1996). *EMBO J.* **15**, 3890–3898.
Zhu, X., Rebello, J., Matsmura, P. & Volz, K. (1997). *J. Biol. Chem.* **272**, 5000–5006.