Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization of full-length CysB of *Klebsiella aerogenes*, a LysR-type transcriptional regulator

Koen H. G. Verschueren,* Christine Addy,† Eleanor J. Dodson and Anthony J. Wilkinson

York Structural Biology Laboratory, Department of Chemistry, University of York, Heslington, York YO1 5DD, England

† Present address: Research Institute for Innovative Technology for the Earth, 9-2 Kizugawadai, Kizu-cho, Soraku-gun, Kyoto 619-0292, Japan.

Correspondence e-mail: koen@ysbl.york.ac.uk

Received 15 September 2000
Accepted 9 November 2000

CysB is a positive regulator of transcription of genes involved in cysteine biosynthesis in Gram-negative bacteria and belongs to the large family of LysR-type transcriptional regulators. The full-length protein from *Klebsiella aerogenes* has been crystallized from solutions containing PEG 8000 in the presence and in the absence of the inducer *N*-acetylserine by the method of vapour diffusion in hanging drops. For the complexed protein different crystal forms appear in the same drops.

1. Introduction

The LysR-type transcriptional regulators (LTTRs) form one of the largest families of bacterial coinducer-responsive gene-activator proteins. They control the expression of genes associated with a multitude of highly diverse processes (Schell, 1993). They share sequence similarities spanning 280 residues, with the highest homology in the 66 N-terminal residues which constitute the DNA-binding domain. CysB is a typical LTTR; it is a tetrameric DNA-binding protein and an activator and a repressor of transcription whose activity is responsive to the presence of a small-molecule inducer, in this case *N*-acetylserine (Schell, 1993). We are investigating the mechanism of transcriptional regulation in LTTRs through crystallographic studies of CysB, which regulates the genes involved in cysteine biosynthesis in Gram-negative bacteria (Kredich, 1992, 1996).

Crystallization experiments with intact CysB protein have been hampered by the low solubility of the protein. As a result, we have hitherto focused on fragments of CysB which constitute stable structural and functional domains. We have solved the structure of a C-terminal chymotryptic fragment, CysB(88–324), which constitutes the cofactor-binding domain, by multiple isomorphous replacement techniques (Tyrrell *et al.*, 1997). It consists of two domains which enclose a cavity in which the inducer can easily fit. A similar fold is found in the *lac* repressor (Lewis *et al.*, 1996).

CysB(88–324) is a dimer in solution as well as in the crystal structure. This dimer, however, has an organization profoundly different from that in the dimers which make up the *lac* repressor tetramer. The twofold symmetry axis relating the subunits of the CysB(88–324) dimer is perpendicular to the long axis of the cofactor-binding domain, whereas in the *lac*

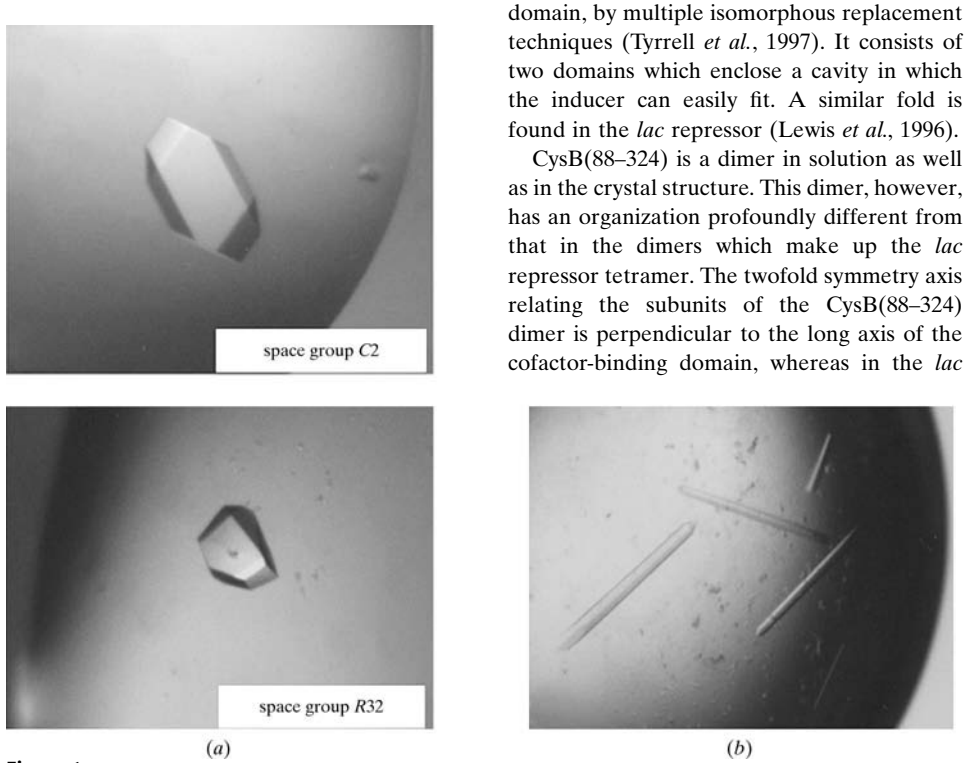

Figure 1
Crystals of full-length CysB grown (*a*) in the presence and (*b*) in the absence of *N*-acetylserine. The two crystal forms grow under the same crystallization conditions.

© 2001 International Union of Crystallography
Printed in Denmark – all rights reserved

repressor it is parallel to this axis. This implies a quite different mode of DNA binding. A structure of the intact CysB tetramer is urgently sought in order to (i) reveal the structure of the DNA-binding domain, which is unknown, and (ii) define the quaternary organization of the protein and the contribution of the DNA-binding domains to the assembly of the tetramer.

2. Experimental procedures

2.1. Protein purification

Difficulties in reproducibly obtaining pure CysB protein have led us to revise our purification procedures. The full-length protein is overexpressed in *Escherichia coli* JM101 cells harbouring *pKKCysB* (Lynch *et al.*, 1994) and purified from solutions buffered at pH 8.5 instead of pH 7.5. After ammonium sulfate fractionation, CysB was resolved successively on Q-Sepharose and heparin Sepharose columns. In both chromatography steps, the protein was eluted with a NaCl gradient giving rise to a rather broad CysB peak at approximately 230 m*M* NaCl for the Q-Sepharose column and a sharp peak at 500 m*M* NaCl for the heparin Sepharose column. At this stage CysB was judged to be >95% pure by gel electrophoresis criteria. The purified CysB was stored at <1 mg ml^{-1} in 50 m*M* Tris–HCl pH 8.5, 1 m*M* EDTA buffer.

Table 1
Data-collection statistics for the four CysB–NAS space groups.

Space group	*C*2 (form I)	*R*3	*C*2 (form II)	*R*32
Resolution range (Å)	25–4.2	25–2.8	25–2.9	25–2.3
Unit-cell parameters (Å,°)	183.2, 185.9, 130.6, 90.0, 90.89, 90.0	187.4, 187.4, 224.7, 90.0, 90.0, 120.0	130.9, 185.5, 112.7, 90.0, 124.95, 90.0	185.9, 185.9, 113.0, 90.0, 90.0, 120.0
Completeness (%)	82.6 (84.8)	99.9 (99.4)	99.4 (89.3)	99.9 (100)
Unique reflections	26129	72415	47867	33205
Mean redundancy	2.5	5.7	3.2	11.0
$R_{\rm merge}$ (%)	15.3 (22.6)	8.9 (35.6)	8.4 (38.3)	6.7 (57.2)
$I/\sigma(I)$	5.5	5.9	12.5	13.6
Unit-cell volume (Å^3)	4447925	6835436	2244834	3381895
Molecules per asymmetric unit	12	8	6	2
$V_{\rm M}$ (Å^3 Da^{-1})	2.58	2.64	2.60	2.61
Solvent content (%)	51.91	53.06	52.35	52.56
Comments	Reindex to *R*3 (185.6, 185.9, 223.4, 90.00, 89.88 120.04); Hamburg X11	Pseudo-twofold along *b* axis; Daresbury 9.6	Reindex to *R*32 (185.8, 185.5, 112.7, 90.0,0 89.94, 119.95); home source	Daresbury 9.6

2.2. Crystallization of full-length CysB

The addition of 10 m*M* non-detergent sulfobetaines (NDSB195) facilitates the concentration of CysB to levels suitable for crystallization. The first crystals of intact CysB were grown by the method of vapour diffusion from hanging drops. Crystals grow from 12–17% PEG 8000, 0.2 *M* sodium tartrate and 0.1 *M* Tris–HCl pH 8.5 over a period of 3 d, their form being determined by the presence or absence of the inducer *N*-acetylserine (NAS). The size and stability of the CysB–NAS crystals were subsequently improved by adding 0.2 *M* KSCN to the crystallization buffer.

Diffraction data (Table 1) were processed using *DENZO* and scaled using *SCALEPACK* (Otwinowski & Minor, 1997).

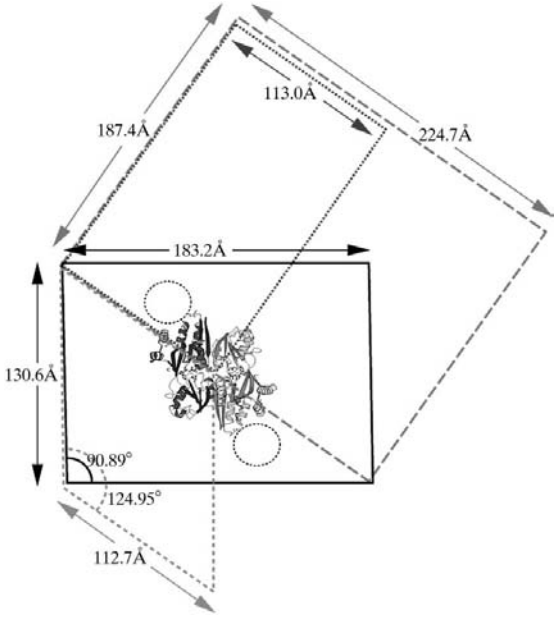

Figure 2
Relationship between the unit cells of the four different space groups. The *ac* planes are shown with the twofold or pseudo-twofold *b* axis perpendicular to the plane of the page. The threefold (in *R*3 and *R*32) or pseudo-threefold (in *C*2) is along the 224.7 Å axis. The CysB(88–324) dimer and the DNA-binding domains represented as dashed circles are shown in a putative position on the crystallographic twofold axis in the *R*32 space group. Full lines, *C*2 form I; short-dashed lines, *C*2 form II; long-dashed lines, *R*3; dotted lines, *R*32.

3. Results and discussion

Previous attempts to crystallize intact CysB were thwarted by its low solubility, a feature common among LysR family proteins. CysB precipitates from dilute aqueous solution at 1–1.5 mg ml^{-1}. This problem has been overcome by including 10 m*M* non-detergent sulfobetaines (NDSB) during the protein-concentration step. NDSBs are a class of mild solubilizing agents originally used in protein renaturing and refolding experiments (Goldberg *et al.*, 1995). In the presence of NDSB195, CysB can be concentrated to 4–5 mg ml^{-1}, suitable for crystallization trials.

A number of different crystal forms have been obtained from a narrow range of PEG 8000 solutions. In the presence of 10 m*M* NAS, CysB forms chunky crystals, while in the absence of cofactor needles are obtained (Fig. 1). The first CysB–NAS crystals were generally weakly diffracting and belong either to space group *C*2, with a possible 12 molecules per asymmetric unit, or to space group *R*3, with a possible eight molecules per asymmetric unit (Table 1). The *R*3 crystals are often severely twinned. The two crystal forms appear in the same drops and are indistinguishable. The needle-like crystals of CysB grown in the absence of NAS diffract very weakly; the limited data available suggest a hexagonal space group.

The addition of 0.2 *M* KSCN to the crystallization buffer improves the quality of the CysB–NAS crystals considerably, both in size and the quality of diffraction. Although the morphology of the crystals is similar, there are variations in unit-cell parameters. A proportion of the crystals are in a second smaller *C*2 unit cell which could contain between six and eight molecules in the asymmetric unit. Most, however, are in space group *R*32 with just two molecules per asymmetric unit. Table 1 provides a summary of the different crystal forms and some of the data-collection statistics.

Interestingly, close examination of the four different unit cells reveals how each may be related to the others. As can be seen from Table 1, the asymmetric unit volumes have ratios which are almost exactly 6:4:3:1 and the four unit cells have at least one axis (the *b* axis) in common. Fig. 2 shows the relationship of all four space groups to each

other. It is possible to reindex the *C*2 data sets in *R*3 and *R*32 with unit-cell parameters deviating only slightly from those of the trigonal crystals and with nearly exact threefold symmetry (Table 1).

The *R*32 crystals, which have just two molecules in the asymmetric unit, are the most promising for molecular-replacement calculations using the structure of the cofactor-binding fragment as a search model. There is no evidence for a non-crystallographic twofold axis in the self-rotation search in *R*32. As the CysB(88–324) dimer has dyad symmetry (Tyrrell *et al.*, 1997), it seems most likely that the *R*32 crystallographic twofold will coincide with a corresponding twofold axis in the full-length protein, as shown in Fig. 2. Unfortunately, we have so far found no straightforward molecular-replacement solutions. We are therefore preparing selenomethionine derivatives for multiwavelength anomalous dispersion experiments.

KHGV is supported by grant BO4808 from the BBSRC, CA by a BBSRC studentship and EJD by the MRC. We are grateful to Axel Müller for technical assistance and staff at the SRS Daresbury and the EMBL Hamburg Outstation for assistance with data collection. Data collection at EMBL was facilitated by the European Union HCMP Large Installation programme (CHGE-CT93-0040).

References

Goldberg, M. E., Expert-Bezançon, N., Vuillard, L. & Rabilloud, T. (1995). *Fold. Des.* **1**, 21–27.

Kredich, N. M. (1992). *Mol. Microbiol.* **6**, 2747–2753.

Kredich, N. M. (1996). *Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology*, edited by F. C. Neidhart, R. Curtiss, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter & H. F. Umbarger, pp. 514–527. Washington DC: American Society for Microbiology.

Lewis, M., Chang, G., Horton, N. C., Kercher, M. A., Pace, H. C., Schumacher, M. A., Brennan, R. G. & Lu, P (1996). *Science*, **271**, 1247–1254.

Lynch, A. S., Tyrrell, R., Smerdon, S. J., Briggs, G. S. & Wilkinson, A. J. (1994). *Biochem. J.* **299**, 129–136.

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Schell, M. A. (1993). *Annu. Rev. Microbiol.* **47**, 597–626.

Tyrrell, R., Verschueren, K. H. G., Dodson, E. J., Murshudov, G. N., Addy, C. & Wilkinson, A. J. (1997). *Structure*, **5**, 1017–1032.