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Crystals of *trp* repressor suitable for high-resolution neutron Laue diffraction studies

Brenda V. Daniels,[a] Dean A. A. Myles,[b] V. Trevor Forsyth[b,c] and Catherine L. Lawson[d]*

[a]Biology Department, Brookhaven National Laboratory, Upton, NY 11973, USA, [b]European Molecular Biology Laboratory Grenoble Outstation, Institut Laue–Langevin, 6 Rue Jules Horowitz, 38042 Grenoble, France, [c]School of Chemistry and Physics, Keele University, Staffordshire ST5 5BG, England, and [d]Department of Chemistry and Chemical Biology, Rutgers University, 610 Taylor Road, Piscataway, NJ 08854, USA

Correspondence e-mail: lawson@rutchem.rutgers.edu

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Crystallization and preliminary neutron-diffraction measurements of wild-type variant Val58→Ile of the *Escherichia coli trp* repressor are reported. A vapor-diffusion chamber suitable for initial protein-solution volumes in the range 0.2–0.5 ml was used to grow cube-shaped crystals with edge dimensions in the range 0.8–1.4 mm. Neutron Laue measurements to a nominal resolution of 2.1 Å were recorded from a D_2O-exchanged crystal using the LADI instrument at ILL. These results demonstrate that it will be possible for the first time to obtain a full-atom neutron structural model of a DNA-binding protein plus its associated solvent. Direct observation of hydrogen bonding between protein and solvent should enhance understanding of the role of solvent in protein–DNA recognition.

1. Introduction

H-atom positions of crystallized macromolecules and their associated solvent can be identified by measurement of high-resolution neutron-diffraction data (Knott & Schoenborn, 1996; Korszun, 1997; Kossiakoff, 2001; Niimura, 1999). Widespread application of neutron diffraction to the study of solvent structure in biological systems has historically been hindered by the requirement for well ordered crystal specimens significantly larger than those needed for X-ray analyses. However, several recent improvements in the method have lowered the minimum crystal volume needed to about 1 mm^3 (Gutberlet *et al.*, 2001; Helliwell, 1997; Niimura *et al.*, 1997; Shu *et al.*, 2000; Tanaka *et al.*, 1997). Employing a neutron source with a moderate energy bandpass greatly increases data-collection efficiency, since multiwavelength Laue patterns contain many more reflections than corresponding patterns from a monochromatic source (Cruickshank *et al.*, 1987, 1991). This approach has been successfully developed for neutron protein crystallography at the Institute Laue–Langevin (ILL; Cipriani *et al.*, 1996; Myles *et al.*, 1998) and has now been utilized for neutron measurements in a number of different crystal systems (Bon *et al.*, 1999; Coates *et al.*, 2001; Gilboa *et al.*, 2001; Habash *et al.*, 1997).

The *E. coli trp* repressor (trpR) is a small dimeric ligand-activated DNA-binding protein of the helix–turn–helix motif type (12 500 Da per subunit). Because solvent plays a critical role in the high-affinity interaction between trpR and its cognate operator DNA sequences (Joachimiak *et al.*, 1994; Lawson & Carey, 1993; Otwinowski *et al.*, 1988), a complete description of the interaction chemistry awaits the elucidation of the H-atom positions. While it is not currently feasible to prepare large crystals of trpR–operator DNA complexes, X-ray analyses indicate that the solvent structure of trpR crystallized by itself resembles that of trpR bound to DNA (Otwinowski *et al.*, 1988). Neutron-diffraction studies of trpR by itself can thus provide new insight into the role of solvent in complex formation. A variant trpR bearing the single mutation Val58→Ile has structure and activity indistinguishable from the wild type, but fortuitously forms large orthorhombic crystals (up to 0.5 mm^3) in hanging drops (Arvidson *et al.*, 1994; Lawson, 1996*a*,*b*). We report here the production of crystals with volume $\geq$ 1 mm^3 in scaled-up vapor-diffusion chambers as well as the statistics of preliminary neutron Laue diffraction from one of these crystals.

2. Methods

2.1. Purification

I58trpR was expressed in *E. coli* strain BL21DE3 transformed with a T7 expression system plasmid bearing the full coding sequence (Studier *et al.*, 1990). Initial extraction and fractionation of the protein from 2 l of cell culture followed the procedure of Joachimiak *et al.* (1983). Subsequent HQ and HS ion-exchange chromatography was performed using a BioCad SPRINT perfusion workstation. Purity was estimated by SDS–PAGE. Peak fractions were pooled conservatively, concentrated and exchanged into 0.1 m*M* sodium phosphate, 250 m*M* NaCl pH 7.0. The

final yield was 17 mg, stored frozen (253 K) at a concentration of 86 mg ml^{-1}.

2.2. Crystallization

Preliminary screening was by standard hanging-drop vapor diffusion in Linbro plates, with 1 ml reservoirs of 0.6 *M* ammonium chloride and 1.9–2.4 *M* sodium phosphate pH 5.0 (sodium phosphate stock is prepared to 4.0 *M*, with a monobasic/dibasic ratio yielding a pH of 5.0 upon 15-fold dilution). Hanging drops with 20 µl initial volume were composed of protein solution (10.0 mg ml^{-1} I58trpR, 6.25 m*M* L-tryptophan) mixed with an equal volume of reservoir. Sitting-drop vapor-diffusion trials with 30–100 µl initial drop volume were conducted in standard depression plates sealed with 10 ml reservoir solution inside sandwich boxes. Vapor-diffusion chambers suitable for scaling up were constructed from modified prototype microgravity crystallization units placed into 2 dram vials (Fig. 1; details of construction provided in legend). Initial crystallization volume was 200 µl; reservoir volume was 3.0 ml. Chambers were incubated at 291–295 K.

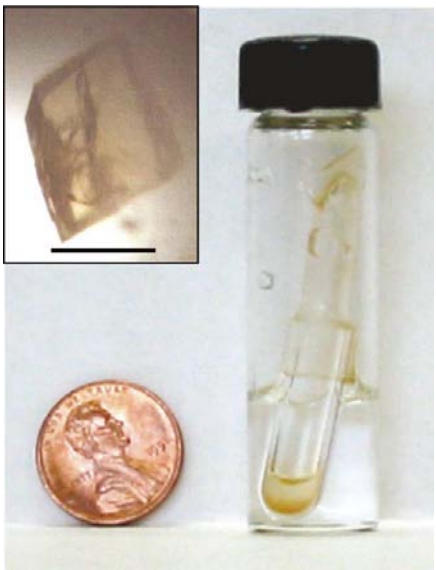

Figure 1
Vapor-diffusion chamber. The inner unit containing the protein sample (example shown here contains precipitate only) is a prototype microgravity crystallization device that was obtained as a complimentary sample (Intospace). The unit consists of a small round-bottom polystyrene test tube, 6 mm inner diameter × 35 mm height, capped with a 30 mm length of Tygon tubing that is crimped at one end. We cut a small hole near the top of the tubing to permit vapor exchange with the reservoir solution. The crimped end provides a convenient handle for loading the inner unit into the 2 dram vial. Inset: I58trpR crystal grown using the chamber method, scale bar = 1.0 mm. Approximate crystal dimensions: 1.4 mm height, 1.0 mm width, 0.8 mm depth.

2.3. Neutron diffraction

To minimize incoherent background scatter of neutrons from ^{1}H atoms, selected crystals were transferred in a series of conservative steps to deuterated solution (2.5 *M* sodium deuterium phosphate, 0.5 *M* sodium chloride, 2.0 m*M* L-tryptophan in D_2O, prepared with ≥99.8% D solutions of D_3PO_4, NaOD and D_2O purchased from Aldrich). Approximately one month later they were transported from Rutgers University to the EMBL Grenoble Outstation. Neutron Laue diffraction data were collected at 293 K using the Laue diffractometer (LADI) neutron-sensitive image-plate detector installed on end-station T17 of cold neutron guide H142 at ILL. An Ni/Ti multilayer filter selected a $\Delta\lambda/\lambda$ = 25% bandpass centered at 3.4 Å. Four circular LiF apertures were adjusted to collimate the beam to a diameter of 2.2 mm. One crystal (∼1 mm^3 volume) was mounted in a 2 mm diameter quartz capillary tube and aligned with *b** parallel to the spindle axis and *a** parallel to the neutron beam. A total of 14 still diffraction images with 23 h exposure times were collected in 7.5° steps between spindle settings of 0 and 105°. Diffraction patterns were indexed using a version of *LAUEGEN* (Campbell *et al.*, 1998) modified to account for the cylindrical geometry of the detector. Data from all images were scaled together and wavelength-normalized using the program *LAUENORM*. (Note: crystals were produced at BNL in 1996 and had originally been intended for experiments at the BNL High Flux Beam Reactor. After this facility closed, they were transported to Rutgers and stored in their original crystallization vials until time on the LADI instrument at ILL was obtained in 2001.)

3. Results and discussion

A major challenge in the preparation of large crystals with volume ≥ 1 mm^3 for neutron-diffraction studies is optimizing the use of limited starting material. 1 mm^3 of crystalline I58trpR contains roughly 0.7 mg protein, a sizable fraction (1/24th) of the total material that was purified for the experiment. Since variations in stock solutions can alter nucleation behavior, we used hanging-drop experiments to initially identify the precipitant level supporting growth of large crystals but permitting few (0–2) spontaneous nucleations per drop. The optimized recipe was first employed in a sitting-drop apparatus commonly used for scaling up, but crystal size was not significantly improved and the crystal shape tended towards plates. We subsequently designed a simple vapor-equilibration chamber (Fig. 1) that produced larger more cube-shaped crystals than could be attained with either hanging or sitting drops. The chamber design permits a larger starting volume for the protein sample, as well as a reduced surface-to-volume ratio. Of the total of six vapor-diffusion chamber trials, two resulted in production of large single approximately cube-shaped crystals with edge lengths in the range 0.8–1.4 mm and one produced 8–9 smaller crystals (edge lengths ≤ 0.6 mm); the remaining vials contained only precipitate.

Table 1
Processing statistics for I58trpR neutron Laue data.

d_{min} (Å)	R_{merge}	R_{cum}	$\langle I/\sigma(I)\rangle$	Completeness (%)	Multiplicity
6.40	0.069	0.069	7.8	88.7	2.3
5.07	0.077	0.073	8.3	90.8	2.8
4.33	0.091	0.080	7.2	94.7	3.0
3.84	0.093	0.084	6.4	89.7	3.0
3.49	0.100	0.087	6.0	89.7	2.9
3.22	0.109	0.090	5.7	90.7	2.7
3.00	0.117	0.092	5.9	83.0	2.8
2.82	0.156	0.096	4.6	79.6	2.5
2.67	0.149	0.099	4.8	67.7	2.5
2.54	0.156	0.102	4.7	75.0	2.5
2.43	0.167	0.105	4.5	67.2	2.4
2.34	0.185	0.108	3.8	67.6	2.3
2.25	0.178	0.110	4.1	61.8	2.1
2.17	0.175	0.112	4.1	54.7	1.8
2.10	0.205	0.114	3.5	54.3	1.9
Total	0.114	0.114	5.5	72.6	2.5

Reflections resulting from neutron Laue diffraction measurements of one crystal exchanged with deuterated soaking solution were indexed as primitive orthorhombic. Refined unit-cell parameters of a = 53.6, b = 53.3, c = 32.7 Å and systematic absences corresponding to space group $P2_12_12$ are consistent with prior X-ray studies (Lawson, 1996*a*; Lawson & Sigler, 1988; Lawson *et al.*, 1988). The neutron wavelength range yielding measurable reflections was 2.8–3.8 Å. Statistics of the integrated merged reflection data are reported in Table 1 and a representative diffraction image is shown in Fig. 2. Although measurement redundancy is low, completeness is better than 90% for reflections in the resolution range 50–3.0 Å. Better completeness at high resolution (3.0–2.1 Å) would have been attained with collection of additional images around a second oscillation axis to fill the blind cone region (Cooper & Myles, 2000). Structure factors calculated using neutron-scattering lengths for non-H atoms of X-ray model 1jhg (Lawson, 1996*a*) have an initial R value of 36% against all 4057 unique reflections of the merged neutron data set. Model statis-

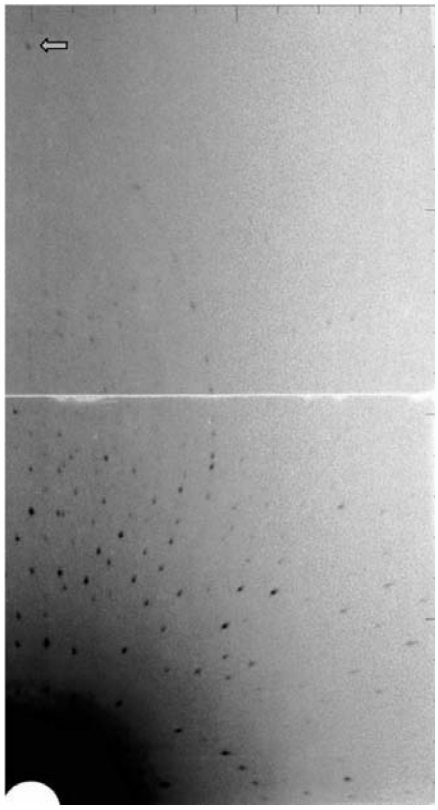

Figure 2
Representative neutron Laue diffraction image from an I58trpR crystal collected on the LADI instrument at ILL. A quarter of the full image from the cylindrical image-plate detector is shown. The arrow indicates a 2.1 Å reflection.

tics are expected to improve with inclusion of well predicted H/D atoms (Kossiakoff, 2001). However, standard crystallographic refinement of ~2000 atom positions (H plus non-H), thermal factors and H/D occupancies in the crystal asymmetric unit must await collection of more complete higher resolution data with at least twice as many reflections.

We anticipate additional improvement to data quality, resolution and map interpretation by repeating the neutron-diffraction experiment with fully deuterated protein. This procedure removes incoherent scattering from ^{1}H that contributes to background noise and results in positive density for all atoms (Gamble *et al.*, 1994; Shu *et al.*, 1996, 2000). We have been able to purify 9 mg of I58trpR produced in 5 l of deuterated minimal media (BVD, unpublished work). Since the yield of deuterium-substituted protein is limited, we plan further optimization of scaled-up vapor-diffusion trials. In the current study, nucleation occurred several months to a year after setup and in only one-half of the experiments. One of a number of techniques might be employed to induce nucleation, *e.g.* introduction of prepared macroseeds or alteration of the incubation temperature. Increasing the protein mass by adjustment of either the concentration or volume may permit production of even larger crystals.

Neutron-diffraction analysis of I58trpR will provide for the first time a complete structural model of a DNA-binding protein including all H-atom positions of the protein and associated solvent. We note the recent report of a DNA dodecamer crystal suitable for neutron-diffraction studies (Arai *et al.*, 2002). Direct study of protein–DNA complexes by high-resolution neutron diffraction is desirable because of the highly polar nature of the corresponding interfaces (Jones *et al.*, 1999). Such analyses should be achievable with planned improvements in detector and source design at current and future neutron facilities (Gutberlet *et al.*, 2001).

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References

Arai, S., Chatake, T., Minezaki, Y. & Niimura, N. (2002). *Acta Cryst.* D**58**, 151–153.
Arvidson, D. N., Arvidson, C. G., Lawson, C. L., Miner, J., Adams, C. & Youderian, P. (1994). *Nucleic Acids Res.* **22**, 1821–1829.
Bon, C., Lehmann, M. S. & Wilkinson, C. (1999). *Acta Cryst.* D**55**, 978–987.
Campbell, J. W., Hao, Q., Harding, M. M., Nguti, N. D. & Wilkinson, C. (1998). *J. Appl. Cryst.* **31**, 496–502.
Cipriani, F., Castagna, J.-C., Wilkinson, C., Oleinek, P. & Lehmann, M. S. (1996). *J. Neutron Res.* **4**, 79–85.
Coates, L., Erskine, P. T., Wood, S. P., Myles, D. A. A. & Cooper, J. B. (2001). *Biochemistry*, **40**, 13149–13157.
Cooper, J. B. & Myles, D. A. (2000). *Acta Cryst.* D**56**, 246–248.
Cruickshank, D. W. J., Helliwell, J. R. & Moffat, K. (1987). *Acta Cryst.* A**43**, 656–674.
Cruickshank, D. W. J., Helliwell, J. R. & Moffat, K. (1991). *Acta Cryst.* A**47**, 352–373.
Gamble, T. R., Clauser, K. R. & Kossiakoff, A. A. (1994). *Biophys. Chem.* **53**, 15–25.
Gilboa, A. J. K., Myles, D. A. A., Habash, J., Raftery, J. & Helliwell, J. R. (2001). *J. Appl. Cryst.* **34**, 454–457.
Gutberlet, T., Heinemann, U. & Steiner, M. (2001). *Acta Cryst.* D**57**, 349–354.
Habash, J., Raftery, J., Weisgerber, S., Cassetta, A., Lehmann, M. S., Hoghoj, P., Wilkinson, C., Campbell, J. W. & Helliwell, J. R. (1997). *J. Chem. Soc. Faraday Trans.* **93**, 4313–4317.
Helliwell, J. R. (1997). *Nature Struct. Biol.* **4**, 874–876.
Joachimiak, A., Haran, T. E. & Sigler, P. B. (1994). *EMBO J.* **13**, 367–372.
Joachimiak, A., Kelley, R. L., Gunsalus, R. P., Yanofsky, C. & Sigler, P. B. (1983). *Proc. Natl Acad. Sci. USA*, **80**, 668–672.
Jones, S., van Heyningen, P., Berman, H. M. & Thornton, J. M. (1999). *J. Mol. Biol.* **287**, 877–896.
Knott, R. B. & Schoenborn, B. P. (1996). *Basic Life Sci.* **64**, 1–15.
Korszun, Z. R. (1997). *Methods Enzymol.* **276**, 218–232.
Kossiakoff, A. A. (2001). *International Tables for Crystallography*, Vol. F, edited by M. G. Rossmann & E. Arnold, pp. 419–422. Dordrecht, The Netherlands: Kluwer Academic Publishers.
Lawson, C. L. (1996*a*). *Nature Struct. Biol.* **3**, 986–987.
Lawson, C. L. (1996*b*). *Biological Structure and Dynamics*, Vol. 1, edited by R. H. Sarma & M. H. Sarma, pp. 83–90. Schenectady, New York: Adenine Press.
Lawson, C. L. & Carey, J. (1993). *Nature (London)*, **366**, 178–182.
Lawson, C. L. & Sigler, P. B. (1988). *Nature (London)*, **333**, 869–871.
Lawson, C. L., Zhang, R. G., Schevitz, R. W., Otwinowski, Z., Joachimiak, A. & Sigler, P. B. (1988). *Proteins*, **3**, 18–31.
Myles, D. A. A., Bon, C., Langan, P., Cipriani, F., Castagna, J.-C., Lehmann, M. S. & Wilkinson, C. (1997). *Physica B*, **241**, 1122–1130.
Niimura, N. (1999). *Curr. Opin. Struct. Biol.* **9**, 602–608.
Niimura, N., Minezaki, Y., Nonaka, T., Castagna, J. C., Cipriani, F., Hoghoj, P., Lehmann, M. S. & Wilkinson, C. (1997). *Nature Struct. Biol.* **4**, 909–914.
Otwinowski, Z., Schevitz, R. W., Zhang, R. G., Lawson, C. L., Joachimiak, A., Marmorstein, R. Q., Luisi, B. F. & Sigler, P. B. (1988). *Nature (London)*, **335**, 321–329.
Shu, F., Ramakrishnan, V. & Schoenborn, B. P. (1996). *Basic Life Sci.* **64**, 309–323.
Shu, F., Ramakrishnan, V. & Schoenborn, B. P. (2000). *Proc. Natl Acad. Sci. USA*, **97**, 3872–3877.
Studier, F. W., Rosenberg, A. H., Dunn, J. J. & Dubendorff, J. W. (1990). *Methods Enzymol.* **185**, 60–89.
Tanaka, I., Minezaki, Y., Harada, K. & Niimura, N. (1997). *Physica B*, **241**, 227–230.