Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization of RusA Holliday junction resolvase from *Escherichia coli*

Tatyana A. Muranova,[a] Svetlana E. Sedelnikova,[a] Philip M. Leonard,[a]† Alessandra Pasquo,[a]‡ Edward L. Bolt,[b] Robert G. Lloyd[b] and John B. Rafferty[a]*

[a]Krebs Institute for Biomolecular Research, Department of Molecular Biology and Biotechnology, University of Sheffield, Western Bank, Sheffield S10 2TN, England, and [b]Institute of Genetics, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH, England

† Present address: Department of Chemistry, University of York, Heslington, York, England.
‡ Present address: Laboratory of Molecular and Cell Biology, IDI-IRCCS, Via dei Monti di Creta 104, 00167 Roma (AP), Italy.

Correspondence e-mail: j.rafferty@sheffield.ac.uk

Received 1 August 2003
Accepted 27 August 2003

Crystals of the *Escherichia coli* Holliday junction resolvase RusA have been obtained using the hanging-drop method and characterized. The crystals have a primitive monoclinic form and belong to space group $P2_1$. The V_M value suggests the presence of two copies of the monomer in the asymmetric unit. A full three-wavelength MAD data collection on a selenomethionine-incorporated form has been undertaken and structure determination is under way using data collected to 2.1 Å resolution.

1. Introduction

The resolution of four-way DNA Holliday junctions is a key step in the process of homologous DNA recombination that occurs to repair or reorganize the genome (West, 1992) and to enable DNA-replication restart upon collapse of the replication fork (McGlynn & Lloyd, 2002). It is catalysed by specific endonucleases known as resolvases that bind and cleave the DNA phosphate backbone at symmetrical sites across the junction point in a cation-dependent reaction (Lilley & White, 2001). The resolvases can display both structural selectivity in binding and sequence specificity in cleavage.

The *Escherichia coli* RusA resolvase is encoded by the cryptic lambdoid prophage DLP12 of *E. coli* K-12 and was identified as a suppressor of mutations in the RuvABC system in *E. coli* (Mahdi *et al.*, 1996; Mandal *et al.*, 1993; Sharples *et al.*, 1994). It has a marked selectivity for four-way Holliday junctions and its binding results in the distortion of the substrate (Chan *et al.*, 1998; Giraud-Panis & Lilley, 1998). RusA catalyses the hydrolysis of the DNA phosphate backbone of Holliday junctions at symmetrical sites across the junction point in a Mg^{2+} cation dependent manner. It displays a clear preference for cleavage on the 5′-side of CC dinucleotide sequences at the point of crossover (Chan *et al.*, 1997, 1998; Sharples *et al.*, 1994).

To date, the structures of the junction resolvases *E. coli* RuvC (Ariyoshi *et al.*, 1994), Hjc resolvase from *Pyrococcus furiosus* (Nishino *et al.*, 2001) and *Sulfolobus solfataricus* (Bond *et al.*, 2001), *Schizosaccharomyces pombe* Ydc2 (Ceschini *et al.*, 2001), bacteriophage T7 endonuclease I (Hadden *et al.*, 2001) and bacteriophage T4 endonuclease VII (Raaijmakers *et al.*, 1999) have been divided into two main classes (nucleases and integrases) with one exception (T4 endonuclease VII). We have undertaken X-ray crystallographic structural studies of the *E. coli* RusA resolvase, which sequence alignment suggests does not belong to either of these two main classes (Sharples *et al.*, 1999, 2002). We report here the purification and crystallization of RusA, plus the preliminary data from a MAD phasing experiment that utilized a selenomethionine-incorporated form.

2. Cloning and overexpression

The *rusA* gene was cloned into a pT7-7 vector and the resulting construct, pEB259, was used to transform *E. coli* strain BL21(DE3)pLysS. The transformed cells were plated onto LB plates containing 50 µg ml^{-1} carbenicillin and 50 µg ml^{-1} chloramphenicol. A single colony was picked and grown overnight at 310 K in 50 ml LB medium containing the same antibiotics as in the LB plates. Cells were collected by centrifugation at 1500*g* for 10 min at 277 K and gently resuspended in 500 ml minimal medium composed of 1 g l^{-1} ammonium sulfate, 10.5 g l^{-1} dipotassium hydrogen orthophosphate, 4.5 g l^{-1} potassium dihydrogen orthophosphate, 0.5 g l^{-1} trisodium citrate.2H_2O, 5 g l^{-1} glycerol, 0.5 g l^{-1} adenine, 0.5 g l^{-1} guanosine, 0.5 g l^{-1} thymine, 0.6 g l^{-1} uracil, 1 ml l^{-1} 1 *M* magnesium sulfate; 50 mg l^{-1} carbenicillin, 50 mg l^{-1} chloramphenicol, 40 mg l^{-1} lysine, phenylalanine, threonine, valine, isoleucine, leucine and selenomethionine, and 2 mg l^{-1} thiamine. The culture was grown at 310 K to an optical density (OD_{600nm}) of 0.6 before IPTG was added to a final concentration of 1 m*M* in order to induce expression. The culture was then incubated for 18 h at 303 K and cell paste was harvested by centrifugation, before being frozen and stored at 253 K.

Table 1
Data-collection statistics for selenomethionine-incorporated RusA MAD data.

Values in parentheses refer to the outer shell.

Data set	Inflection	Peak	Remote
Wavelength (Å)	0.98048	0.98013	0.88560
Resolution (Å)	48.3–2.1 (2.15–2.10)	50–2.1 (2.15–2.10)	44.3–2.1 (2.15–2.10)
No. of observations	58134	58049	58911
Unique reflections	12725 (835)	12721 (833)	12719 (834)
Data completeness (%)	99.7 (98.5)	99.6 (98.2)	99.7 (99.8)
Mean $I/\sigma(I)$	14.6 (4.8)	13.9 (4.2)	9.3 (2.0)
$I/\sigma(I) > 3$ (%)	88 (69)	85 (60)	72 (37)
$R_{\rm merge}$† (%)	4.4 (20.1)	4.6 (24.1)	7.1 (51.6)

† $R_{\rm merge} = \sum_{hkl} |I_i - I_m| / \sum_{hkl} I_m$, where I_m is the mean intensity of the reflection.

3. Purification

To purify RusA, cell paste was defrosted and suspended in buffer *A* (1 *M* NaCl, 50 m*M* Tris–HCl pH 8.0, 2 m*M* EDTA). Cells were disrupted by sonication on an ice bath at 16 µm amplitude for four cycles of 20 s and debris was removed by centrifugation at 40 000*g* for 20 min at 277 K. The supernatant was diluted with 50 m*M* Tris–HCl pH 8.0 to 0.2 *M* NaCl and applied onto a heparin Sepharose column (Pharmacia) that had been equilibrated with 0.2 *M* NaCl in 50 m*M* Tris–HCl pH 8.0. Proteins were eluted from the column using a 0.2–2.0 *M* NaCl gradient in 50 m*M* Tris–HCl pH 8.0. Fractions were analysed by SDS–PAGE (4–12% bis-tris gel, Novex) and those containing a major protein sample of the expected molecular weight of RusA (~14 kDa) were collected and diluted with 50 m*M* Tris–HCl pH 8.0 to about 0.2 *M* NaCl (conductivity of 25–30 mS). Further purification was carried out by chromatography with a phosphate cellulose P11 column (Whatman) using a 0.2–1.25 *M* NaCl gradient in 50 m*M* Tris–HCl pH 8.0. Fractions that showed the presence of a 14 kDa protein by SDS–PAGE were collected, concentrated on a VivaSpin concentrator (Viva Science) to 9.3 mg ml^{-1} as estimated by the method of Bradford (1976) and dialysed against 0.3 *M* NaCl in 50 m*M* Tris–HCl pH 8.0. The purity of the RusA was estimated by SDS–PAGE to be approximately 97%. The yield of the RusA selenomethionine derivative was 1 mg from 1 l of cell culture. The level of selenomethionine incorporation into the protein was estimated using a Q-Tof mass spectrometer (Micromass UK Ltd) and was about 92%.

4. Crystallization and data collection

Crystallization of RusA was achieved using the hanging-drop vapour-diffusion technique by mixing 1–2 µl of the protein solution with an equal volume of precipitant, followed by equilibration at 290 K. Initially, crystals were obtained with the Crystal Screen I crystallization kit (Hampton Research) conditions Nos. 22 [30%(*w*/*v*) PEG 4000, 0.2 *M* sodium acetate trihydrate, 0.1 *M* Tris–HCl pH 8.5] and 43 [30%(*w*/*v*) PEG 1500]. After optimization of the crystallization conditions, trapezoid crystals of RusA with a maximum dimension of 0.3 mm were grown at a protein concentration of 5 mg ml^{-1} in 30%(*w*/*v*) PEG 4000 with 0.3 *M* sodium acetate and 0.1 *M* Tris–HCl pH 8.0.

For flash-cooling prior to data collection, a crystal was transferred into a precipitant solution identical to that used for optimal crystallization but containing 20%(*v*/*v*) glycerol before being positioned in a nitrogen-gas stream at 100 K. Data were collected from crystals of RusA at the European Synchrotron Radiation Facility (ESRF) on stations ID14-4 and BM30 and on BM14 as part of a three-wavelength MAD phasing experiment.

5. Data analysis

Autoindexing of the RusA data using the program *DENZO* (Otwinowski & Minor, 1997) indicated that the crystals have a primitive monoclinic lattice with unit-cell parameters a = 45.4, b = 50.0, c = 49.6 Å, β = 101.4° and therefore a unit-cell volume of approximately 1.10 × 10^5 Å^3. Processing and scaling of the data in the *HKL* suite of programs resulted in the statistics given in Table 1. Inspection of the diffraction pattern using the program *HKLVIEW* (Collaborative Computational Project, Number 4, 1994) revealed that those reflections on the 0*k*0 axis with *k* odd were systematically absent, thus suggesting the space group to be $P2_1$. Self-rotation functions were calculated on the data at different combinations of resolution ranging from 25 to 3.5 Å and radii of integration varying from 30 to 15 Å using the program *MOLREP* (Collaborative Computational Project, Number 4, 1994). The results of this self-rotation function did not provide convincing evidence for the presence of any symmetry axis other than a twofold corresponding to the crystallographic axis. Calculations of $V_{\rm M}$ indicate two copies of the RusA monomer in the asymmetric unit ($V_{\rm M}$ = 2.0 Å^3 Da^{-1}). A native Patterson synthesis showed no significant indications of pseudo-translation and we envisage that RusA has crystallized as a dimer. This would be consistent with RusA gel-filtration data (Chan *et al.*, 1997) and with the dimeric forms reported to date for other resolvases (Lilley & White, 2001). Thus, the absence of any extra twofold symmetry in the self-rotation function could reflect either the presence of a non-crystallographic twofold dimer axis parallel to the crystallographic axis or the breakdown of the twofold dimer symmetry under the conditions of the crystallization experiment such that it can no longer be observed in the self-rotation function.

Determination of the structure of RusA by MAD phasing is under way and the selenium substructure is currently being sought. The resulting model of RusA will be used to predict possible modes of interaction of RusA with its Holliday junction substrate.

We thank Dr Alison Ashcroft for the mass-spectrometry analysis and Drs Martin Walsh and Bill Shephard and staff on BM14, ID14-4 and BM30 (ESRF) for assistance with data collection. This work was supported by funding from BBSRC, NEDO, MRC, EMBL and the Royal Society. The Krebs Institute is a designated BBSRC Biomolecular Sciences Centre and a member of the North of England Structural Biology Centre. JBR is a Royal Society Olga Kennard Fellow.

References

Ariyoshi, M., Vassylyev, D. G., Iwasaki, H., Nakamura, H., Shinagawa, H. & Morikawa, K. (1994). *Cell*, **78**, 1063–1072.
Bond, C. S., Kvaratskhelia, M., Richard, D., White, M. F. & Hunter, W. N. (2001). *Proc. Natl Acad. Sci. USA*, **98**, 5509–5514.
Bradford, M. (1976). *Anal. Biochem.* **72**, 248–254.
Ceschini, S., Keeley, A., McAlister, M. S., Oram, M., Phelan, J., Pearl, L. H., Tsaneva, I. R. & Barrett, T. E. (2001). *EMBO J.* **20**, 6601–6611.
Chan, S. N., Harris, L., Bolt, E. L., Whitby, M. C. & Lloyd, R. G. (1997). *J. Biol. Chem.* **272**, 14873–14882.
Chan, S. N., Vincent, S. D. & Lloyd, R. G. (1998). *Nucleic Acids Res.* **26**, 1560–1566.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Giraud-Panis, M. J. & Lilley, D. M. (1998). *J. Mol. Biol.* **278**, 117–133.

Hadden, J. M., Convery, M. A., Declais, A. C., Lilley, D. M. & Phillips, S. E. (2001). *Nature Struct. Biol.* **8**, 62–67.
Lilley, D. M. & White, M. F. (2001). *Nature Rev. Mol. Cell Biol.* **2**, 433–443.
McGlynn, P. & Lloyd, R. G. (2002). *Nature Rev. Mol. Cell Biol.* **3**, 859–870.
Mahdi, A. A., Sharples, G. J., Mandal, T. N. & Lloyd, R. G. (1996). *J. Mol. Biol.* **257**, 561–573.
Mandal, T. N., Mahdi, A. A., Sharples, G. J. & Lloyd, R. G. (1993). *J. Bacteriol.* **175**, 4325–4334.
Nishino, T., Komori, K., Tsuchiya, D., Ishino, Y. & Morikawa, K. (2001). *Structure*, **9**, 197–204.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Raaijmakers, H., Vix, O. T., Golz, S., Kemper, B. & Suck, D. (1999). *EMBO J.* **18**, 1447–1458.
Sharples, G. J., Bolt, E. L. & Lloyd, R. G. (2002). *Mol. Microbiol.* **44**, 549–559.
Sharples, G. J., Chan, S. C., Mahdi, A. A., Whitby, M. C. & Lloyd, R. G. (1994). *EMBO J.* **13**, 6133–6142.
Sharples, G. J., Ingleston, S. M. & Lloyd, R. G. (1999). *J. Bacteriol.* **181**, 5543–5550.
West, S. C. (1992). *Annu. Rev. Biochem.* **61**, 603–640.