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Crystallization and quaternary structure analysis of an Lrp-like regulatory protein from the hyperthermophile *Pyrococcus furiosus*

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The LrpA transcriptional regulator from *Pyrococcus furiosus*, a member of the leucine-responsive regulatory protein (Lrp) family, has been crystallized by the hanging-drop method of vapour diffusion using ammonium sulfate as the precipitant. The crystals belong to the tetragonal system and are in space group $I4_122$, with unit-cell parameters $a = b = 104.5$, $c = 245.1$ Å. Consideration of the values of V_M and possible packing of the molecules within the cell suggest that the asymmetric unit contains a dimer. Examination of the behaviour of the protein on gel-filtration columns and analysis of the self-rotation function suggests that the molecule is an octamer in solution at around pH 5. Determination of the structure of this protein will provide insights into the mechanisms responsible for DNA–protein recognition at high temperature and into how the regulatory properties of the Lrp family are modified by the presence or absence of effector molecules.

1. Introduction

The leucine-responsive regulatory protein (Lrp) is a global regulator of gene transcription which was first discovered in *Escherichia coli* (Tuang *et al.*, 1990; Willins *et al.*, 1991). It functions both positively and negatively to regulate metabolic pathways at a wide range of operons, mainly in response to the availability of leucine in the external environment. Lrp has also been shown to exhibit autoregulation, resulting in repression of the transcription of the *lrp* gene encoding it. It has been established that Lrp is a global regulator controlling the expression of many genes and operons that are directly or indirectly involved in the amino-acid metabolism of *E. coli* as a response to fluctuating growth conditions (Calvo & Matthews, 1994). Lrp homologues, both global and specific regulators, have been found in many prokaryotes, both Bacteria and Archaea (Charlier *et al.*, 1997; Napoli *et al.*, 1999). The discovery of archaeal Lrp homologues came as a surprise, since the basic transcription machinery of the Archaea closely resembles that of the Eukarya (Bell & Jackson, 1998).

The LrpA protein encoded by the *P. furiosus lrpA* gene is one such homologue which has been isolated and shown to bind to the *lrpA* promoter at a single site from −22 to +24 relative to the transcription start site, as determined by DNaseI and hydroxyl radical footprinting (Brinkman *et al.*, 2000). LrpA is composed of 141 amino acids and has a monomer molecular weight of 15.9 kDa. Previous gel-filtration studies suggested that it is most likely to be a homodimer in solution, with a tendency to form larger species at elevated concentrations. Like its bacterial counterparts, the negative autoregulation exhibited by LrpA has been shown to be independent of all effectors tested thus far (Brinkman *et al.*, 2000 and references therein).

Sequence alignments of a number of Lrp homologues revealed a putative helix–turn–helix motif (Platko & Calvo, 1993) which has been predicted to occur between residues 21 and 40 in *P. furiosus* LrpA (Brinkman *et al.*, 2000). This structural motif has been observed previously in many DNA-binding proteins involved in transcriptional regulation such as the *E. coli* catabolite activator (McKay & Steitz, 1981) and tryptophan repressor (Schevitz *et al.*, 1985) proteins. The presence of the motif in LrpA suggests structural and functional similarities between LrpA and other bacterial repressor proteins.

The function of Lrp as a major transcriptional regulator and its presence in a wide range of prokaryotic organisms make it an extremely important candidate for structural studies. Here, we describe the preliminary results of the crystallization of LrpA from *P. furiosus*.

2. Methods

LrpA protein was purified from an overexpressing *E. coli* strain BL21(DE3) which had been transformed with the pLUW604 plasmid encoding the gene for *P. furiosus* LrpA (Brinkman *et al.*, 2000). The pLUW604 plasmid was constructed from the overexpression

plasmid pET9d (Novagen). Cells were grown in a rotary shaker at 310 K in medium containing 100 µg ml^{-1} kanamycin until an OD_{600} of 0.5 was reached. LrpA expression was induced with 0.4 m*M* isopropyl *β*-D-thiogalactopyranoside (IPTG) and cells were left to grow overnight. Induced cells from 1 l cultures were harvested by centrifugation and the cell pellet was resuspended in 30 ml 0.4 *M* NaCl in 40 m*M* Tris–HCl pH 8.0, 2 m*M* ethylenediaminetetraacetic acid (EDTA) (buffer *A*) and disrupted by sonication. The homogenate was heated at 348 K for 20 min, diluted with buffer *A* and centrifuged at 42 000*g* for 10 min. The supernatant fraction was loaded onto a 30 ml heparin-Sepharose column (Pharmacia) equilibrated with buffer *A*. The column was then washed with 60 ml 50 m*M* NaCl in buffer *A* and LrpA was eluted with 0.5 *M* NaCl in buffer *A*. The protein was concentrated using a Vivaspin concentrator (10 000 Da MW cutoff; Viva Science) and applied to a Superdex-200 gel-filtration column (1.6 × 60 cm) equilibrated with 0.1 *M* NaCl in buffer *A*. Under these conditions, LrpA protein elutes from the column as a number of overlapping peaks of molecular mass ranging from 30 to 220 kDa (Fig. 1*a*). Fractions corresponding to a species eluting at approximately 30 kDa were pooled and concentrated as above. Further gel-filtration runs established that at pH 5.3 LrpA runs as a much higher molecular-weight species. Therefore, in order to provide further purification, the pooled fractions from the first gel-filtration step were applied to the same gel-filtration column equilibrated with 20 m*M* sodium acetate pH 5.3, 0.25 *M* NaCl (buffer *B*). The protein eluted from the column as a sharp peak corresponding to 110–120 kDa species (Fig. 1*b*). Fractions containing LrpA were pooled and concentrated to 8–12 mg ml^{-1} as estimated by the Bradford method (Bradford, 1976) using Bio-Rad Protein Dye Reagent. The typical yield of LrpA was about 8 mg from 1 l of cell culture. The purity of preparation, estimated by SDS–PAGE (Laemmli, 1970), was close to 100%.

LrpA protein in buffer *B* was crystallized at a protein concentration of 10 mg ml^{-1}. Samples of the protein (5 µl) were mixed with an equal volume of a precipitant solution consisting of ammonium sulfate in the range 2.4–3.6 *M* in 0.1 *M* sodium citrate buffer pH 6.0 and allowed to equilibrate by vapour diffusion with reservoirs of precipitant solution at 290 K. Crystals with a tetragonal bipyramidal morphology and maximum dimensions 0.7 × 0.5 × 0.5 mm grew overnight. Subsequently, morphologically identical crystals have been grown in the pH range 4.0–9.0.

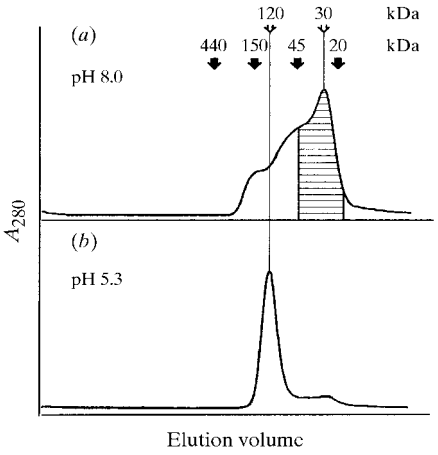

Figure 1
Gel-filtration profile of LrpA on a 1.6 × 60 cm Hi-Load Superdex-200 column (Pharmacia). Flow rate 1 ml min^{-1}. (*a*) Gel filtration was performed in buffer containing 0.1 *M* NaCl, 20 m*M* Tris–HCl pH 8.0 and 1 m*M* EDTA. (*b*) Fractions from the striped area of the gel-filtration run in (*a*) were combined and concentrated and applied on the same column equilibrated with buffer containing 0.25 *M* NaCl and 20 m*M* sodium acetate pH 5.3. Gel filtration was then performed with the same buffer. Filled arrows show the elution positions of proteins used for column calibration: ferritin (440 kDa), alcohol dehydrogenase (150 kDa), egg albumin (45 kDa) and trypsin inhibitor (20 kDa).

3. Results and discussion

Preliminary data were collected at room temperature on a crystal stabilized in the crystallization reservoir solution to a resolution of 4 Å on a MAR Research MAR345 image plate mounted on a Rigaku RU-200 X-ray generator. A total of 16 996 measurements were made of 5839 independent reflections and the data were merged to an *R* factor of 9.2% with 98.3% completeness of the data from 20 to 4 Å [in the highest resolution shell 4.14–4 Å, $R_{\rm merge}$ = 34.2%, completeness = 99.3% and $I/\sigma(I)$ = 4.16]. Preliminary analysis of the data showed that the LrpA crystals belong to a body-centred tetragonal system, point group 422, with unit-cell parameters $a = b = 104.5$, $c = 245.1$ Å. Analysis of systematically absent reflections along the *l* axis showed that the space group is most likely to be $I4_122$. Considerations of the unit-cell volume (2.7×10^6 Å^3) and the subunit M_r of 15 900 suggest that the asymmetric unit contains two to four subunits, with corresponding $V_{\rm M}$ values being 5.2, 3.9 and 2.6 Å^3 Da^{-1} for a dimer, trimer and tetramer in the asymmetric unit, respectively. The $V_{\rm M}$ for a dimer in the asymmetric unit is higher than the range observed typically for globular proteins (Matthews, 1968), but such values have been observed previously in hyperthermophilic proteins, where a high solvent content in the crystal lattice is common. Examples of this are the glutamate dehydrogenase from *P. furiosus* (Yip *et al.*, 1995) and the TATA-box binding protein from *P. woesei* (De Decker *et al.*, 1996), which have $V_{\rm M}$ values of 4.3 and 4.1 Å^3 Da^{-1}, respectively.

Table 1
Data-collection statistics.

Native X-ray data set collected at Daresbury station 7.2. The statistics for the highest resolution shell are given in parentheses.

Space group	$I4_122$
Unit-cell parameters (Å)	
a	101.3
b	101.3
c	245.4
Resolution range (Å)	20–2.9
Highest resolution shell (Å)	2.97–2.9
Wavelength (Å)	1.488
Total reflections	30436
No. of unique reflections	13804
Completeness (%)	94.7 (96.2)
$R_{\rm merge}$	0.065 (0.346)
$I/\sigma(I)$	14.7 (2.5)

† $R_{\rm merge} = \sum |I - \langle I \rangle| / \sum \langle I \rangle$.

Previous gel-filtration studies have shown that LrpA elutes from the column as 30, 60 and 120 kDa species, suggesting that it exists as a dimer, tetramer and octamer in solution (Brinkman *et al.*, 2000). We have shown here that at high concentration (8–12 mg ml^{-1}) and at pH 5.3 LrpA elutes as a 120 kDa species possibly corresponding to an octamer (Fig. 1). A self-rotation function calculated with the program *POLARRFN* (Collaborative Computational Project, Number 4, 1994) using the strongest 1666 reflections in the resolution range 15–6 Å

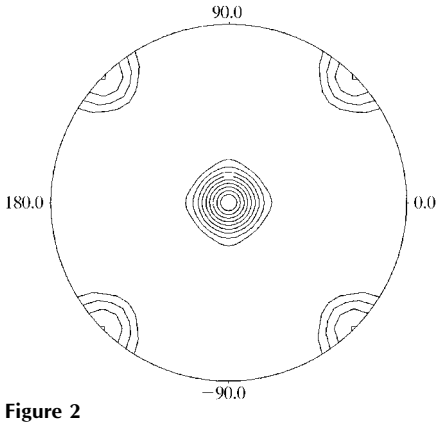

Figure 2
A stereographic projection of the $\kappa = 90^\circ$ section of the self-rotation function of the *P. furiosus* LrpA native data. The putative non-crystallographic four-fold can be seen at the perimeter of the plot at $\omega = 90$, $\varphi = 45^\circ$.

and a 20 Å radius of integration gave a peak with 51% of the origin in the $\kappa = 90^\circ$ section at $\omega = 90^\circ$, $\varphi = 45^\circ$ corresponding to a non-crystallographic fourfold symmetry axis lying in the *ab* plane, orthogonal to *c* and bisecting the *a* and *b* axes (Fig. 2). These results together with the gel-filtration studies would suggest that LrpA forms an octameric species with *D*4 symmetry under the conditions of crystallization.

For test exposures using synchrotron radiation, crystals were cryoprotected in mineral oil by removing them from the hanging drops with a cryoloop, removing excess precipitant using absorbent dental points, dragging the loop through an oil reservoir and placing it in the Cryostream. Experiments on crystals at 100 K on station 7.2 on the synchrotron-radiation source at CLRC Daresbury Laboratory showed that the LrpA crystals diffract to beyond 3 Å and a complete data set was collected to 2.9 Å resolution (Table 1). An attempt to solve the structure of LrpA by multiple isomorphous replacement is now under way. The results of this analysis will provide insights into how LrpA functions to regulate transcription in *P. furiosus* and will enhance our understanding of homologous proteins from other organisms.

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References

Bell, S. D. & Jackson, S. P. (1998). *Trends Microbiol.* **6**, 222–228.
Bradford, M. M. (1976). *Anal. Biochem.* **72**, 248–254.
Brinkman, A. B., Dahlke, I., Tuininga, J. E., Lammers, T., Dumay, V., de Heus, E., Lebbink, J. H. G., Thomm, M., de Vos, W. M. & van der Oost, J. (2000). *J. Biol. Chem.* **275**, 38160–38169.
Calvo, J. M. & Matthews, R. G. (1994). *Microbiol. Rev.* **58**, 466–49.
Charlier, D., Roovers, M., Thia-Toong, T.-L., Durbecq, V. & Glansddorff, N. (1997). *Gene*, **201**, 63–68.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
De Decker, B. S., O'Brien, R., Fleming, P. J., Geiger, J. H., Jackson, S. P. & Sigler, P. B. (1996). *J. Mol. Biol.* **264**, 1072–1084.
Laemmli, U. K. (1970). *Nature (London)*, **227**, 680–685.
McKay, D. B. & Steitz, T. A. (1981). *Nature (London)*, **290**, 744–749.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Napoli, A., van der Oost, J., Sensen, C. W., Charlebois, R. L., Rossi, M. & Ciaramella, M. (1999). *J. Bacteriol.* **181**, 1474–1480.
Platko, J. V. & Calvo, J. M. (1993). *J. Bacteriol.* **175**, 1110–1117.
Schevitz, R. W., Otwinowski, Z., Joachimiak, A., Lawson, C. L. & Sigler, P. B., (1985). *Nature (London)*, **317**, 782–786.
Tuang, L. R., D'Ari, R. & Newman, E. B. (1990). *J. Bacteriol.* **172**, 4529–4535.
Willins, D. A., Ryan, C. W., Platko, J. V. & Calvo, J. M. (1991). *J. Biol. Chem.* **266**, 10768–10774.
Yip, K. S. P., Stillman, T. J., Britton, K. L., Artymiuk, P. J., Baker, P. J., Sedelnikova, S. E., Engel, P. C., Pasquo, A., Chiaraluce, R., Consalvi, V., Scandurra, R. & Rice, D. W. (1995). *Structure*, **3**, 1147–1158.