Acta Crystallographica Section D
**Biological Crystallography**
ISSN 0907-4449

# Crystallization and secondary-structure determination of a protein of the Lrp/AsnC family from a hyperthermophilic archaeon

**Norio Kudo,[a,b] Mark D. Allen,[a] Hideaki Koike,[a] Yoshio Katsuya[c] and Masashi Suzuki[a,b]***

[a]AIST-NIBHT CREST Centre of Structural Biology, 1-1 Higashi, Tsukuba 305-0046, Japan, [b]Graduate School of Human and Environmental Sciences, University of Tokyo, 3-8-1 Komaba, Meguro, Tokyo 153-8902, Japan, and [c]Hyogo Prefectural Institute of Industrial Research, 3-1-12 Yukihira-cho, Suma, Kobe 654-0037, Japan

Correspondence e-mail: sbcsuzuk@nibh.go.jp

Received 22 November 2000
Accepted 15 December 2000

A protein belonging to the Lrp/AsnC transcription-factor family, pot1216151, from the hyperthermophilic archaeon *Pyrococcus* sp. OT3 was crystallized. In *Escherichia coli*, leucine-responsive protein (Lrp) and AsnC regulate a number of metabolic genes. The crystals of pot1216151 diffracted to 2.3 Å using a conventional X-ray source and to 1.8 Å using a synchrotron-radiation source. The space group was identified to be $P3_121$ or $P3_221$, with unit-cell parameters $a = b = 96.9$, $c = 98.5$ Å. In combination with diffraction data obtained from $K_2[Pt(CN)_6]$ and $K(AuCl_4)$ derivatives, an electron-density map was calculated at a resolution of 3.0 Å. Four monomers were identified in the asymmetric unit, with four $\beta$-strands and two $\alpha$-helices in each monomer.

## 1. Introduction

Until now, no specific transcription factor has been identified experimentally in archaea. The best characterized candidates are archaeal homologues of the eubacterial factors Lrp and AsnC (Kyrpides & Ouzounis, 1995). In *E. coli*, the leucine-responsive regulatory protein (Lrp) activates or represses transcription of a large number of genes, in most cases depending on the concentration of leucine (Calvo & Matthews, 1994). This protein is homologous to AsnC, an activator of the asparagine synthetase A gene (Kolling & Lother, 1985).

In the genome of the hyperthermophilic archaeon *Pyrococcus* sp. OT3 (Kawarabayasi *et al.*, 1998), 14 different genes have been identified as being homologous to *lrp*/*asnC* (Ohfuku *et al.*, 1998). Of these, 11 cover the full length of approximately 160 residues, while the other three genes code only for the C-terminal halves of approximately 80 residues. This suggests that the full-length Lrp/AsnC proteins are composed of at least two domains, but that one of these domains, the head, is missing from the products of the three shorter genes.

In the N-terminal domain of Lrp/AsnC, a helix–turn–helix DNA-binding motif has been predicted (Willins *et al.*, 1991; Charlier *et al.*, 1997), which would leave the C-terminal domain for some other function. In fact, one of the headless Lrp/AsnCs, pot1216151, forms a homodimer as well as a heterodimer with a full-length Lrp/AsnC, pot1613368 (N. Kudo *et al.*, unpublished results); here, the 'pot' code (Koike *et al.*, 1999) is used for identifying these archaeal proteins by the positions of their genes in the genome. This fact indicates that headless Lrp/AsnCs may well regulate transcription of specific genes indirectly *via* its interaction with other Lrp/AsnC proteins. In this paper, the crystallization and preliminary structure of the headless Lrp/AsnC protein pot1216151 are reported.

## 2. Protein purification and crystallization

The *lrp* gene (*pot1216151*) was cloned from the strain *Pyrococcus* sp. OT3 and introduced into a plasmid downstream of the T7 promoter. Transcription of the *lrp* gene in the BL21(DE3)

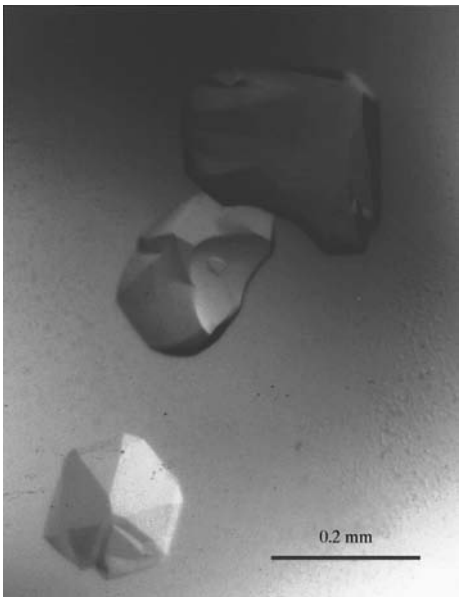


**Figure 1**
A single crystal of the protein pot1216151 grown at 278 K using PEG 6000 and LiCl as precipitant.

strain of *E. coli* (Novagen) was induced by 2 m*M* isopropyl-*β*-D(−)-thiogalactopyranoside. The *E. coli* cells were treated with a French press (SLM) in 100 m*M* Tris–HCl pH 7.0 containing 1 m*M* ethylenediaminetetraacetic acid. After centrifugation at 25 000 rev min$^{-1}$ for 30 min at 277 K, the supernatant was incubated for 1 h at 348 K, leaving most contaminating *E. coli* proteins denatured and aggregated. After centrifugation at 25 000 rev min$^{-1}$ for 30 min at 277 K, the supernatant was subjected to successive ion-exchange chromatography and gel filtration, repeating the whole process twice. A Resource Q (Pharmacia) column eluted with a 0–2 *M* gradient of NaCl in 30 m*M* Tris–HCl buffer pH 7.0 and a Superdex 75 (Pharmacia) column were used. A single band of pot1216151 was observed using SDS–gel electrophoresis.

Crystals were grown at 278 K by the vapour-diffusion method using the sitting-drop technique. The initial droplets contained 4 µl protein solution (20 mg ml$^{-1}$ in 10 m*M* Tris–HCl pH 7.0) and 4 µl precipitant solution [10%(*w*/*v*) PEG 6000 and 1.0 *M* LiCl in 100 m*M* citrate buffer pH 4.0] and were equilibrated against a reservoir containing 800 µl of the precipitant solution. In approximately one week, hexagonal form crystals were obtained; they were approximately 0.2 mm in each dimension (Fig. 1). Two isomorphous derivatives were obtained by soaking these crystals in 100 m*M* citrate buffer pH 4.0 containing 12%(*w*/*v*) PEG 6000, 1.0 *M* LiCl and 10 m*M* $K_2[Pt(CN)_6]$ or 0.1 m*M* $K[AuCl_4]$ for approximately 2 d.

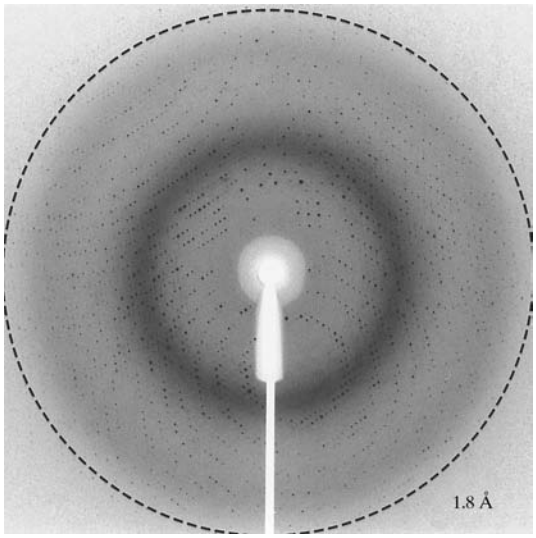


**Figure 2**
A diffraction image of a crystal of the protein pot1216151 exposed at the SPring-8 BL24A facility, showing diffraction beyond 1.8 Å.

## 3. Secondary-structure determination

Diffraction data from native crystals (to 2.3 Å) and the two derivatives (to 2.5 Å) were recorded at 291 K (Table 1) using an area detector (Rigaku R-AXIS IV). The X-ray source used was a rotating-anode generator (Rigaku, UltraX 18) focused by a double-mirror system. The space group of the crystals was determined to be trigonal: $P3_121$ or the enantiomorphic $P3_221$.

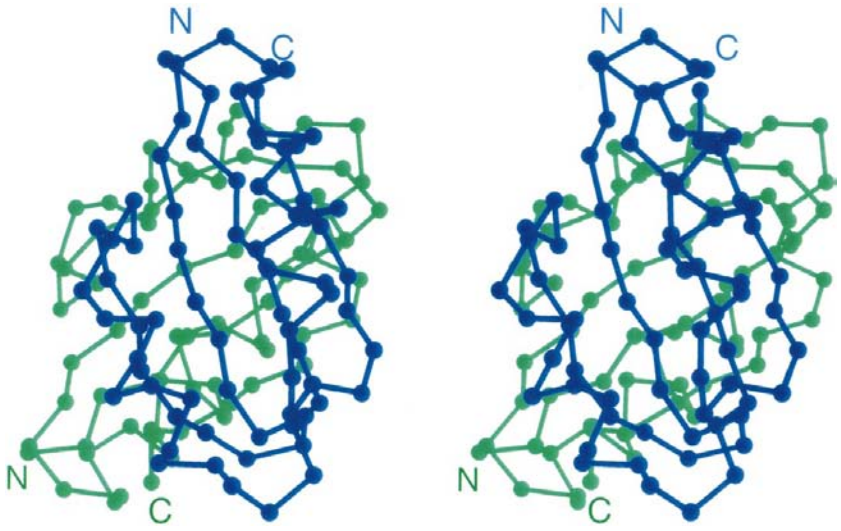


**Figure 3**
A trace of $C^{\alpha}$ atoms in the dimer shown in stereo. The two monomers are differentiated by different colours.

The three sets of diffraction data were merged and processed using the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). The phases of the reflections were calculated by the multiple isomorphous replacement method at a resolution of 3.0 Å. Using difference Patterson maps, two sites were identified as being occupied by Au and three sites by Pt. The numbers and positions of these sites and their occupancies by the metals were refined and an electron-density map was calculated. The map was improved by the use of solvent flattening and histogram mapping. Only by assuming four independent molecules in the asymmetric unit (unit-cell parameters $a = b = 96.9$, $c = 98.5$ Å) and $P3_221$ symmetry instead of $P3_121$ could right-handed helix moieties be clearly identified in the corresponding map.

An atomic model was built using the program *O* (Jones *et al.*, 1991) by folding a chain composed of 72 alanine residues so that the model would best follow the density continuum in the map. The model was refined using the *X-PLOR* program (Brünger *et al.*, 1987; Brünger, 1992) and the parameter set of Engh & Huber (1991). Further refinement was carried out manually using the program *O*, monitoring an SA-omit map (Brünger, 1992) or a $\sigma_A$ map (Read, 1986). In these processes the three-dimensional models of the four independent molecules were restrained to be the same.

The resultant model of a monomer is composed of a *β*-sheet composed of four strands and two *α*-helices (Fig. 3), the *R* factor being 39.1%. Two monomers interact tightly with each other and the resultant dimers associate further *via* weaker interactions. In each dimer, the two *β*-sheets face each other in an orthogonal geometry (Chothia & Janin, 1981), with the four helices being outside this interface. The topology of these secondary elements in the monomer and in the dimer resembles that in the DNA-binding domain of transcription regulator E2 from papillomavirus (Hegde *et al.*, 1992).

Diffraction at higher resolution (to 1.8 Å) was observed with the original type of crystals using a synchrotron-radiation source (Fig. 2, Table 1) at the Hyogo beamline (BL24XU) in SPring-8. The wavelength of the source was monochromated to 0.834 Å. Using liquid nitrogen, crystals were frozen at 77 K in 100 m*M* citrate buffer pH 4.0 containing 14%(*w*/*v*) PEG 6000, 1.0 *M* LiCl and 20%(*v*/*v*) glycerol; they were kept cool during the measurements using nitrogen gas maintained at 100 K. Refinement of the model is continuing using this data.

**Table 1**
Crystal parameters and data reduction of pot1216151.

Values in parentheses refer to the highest resolution shell.

| | Native, 100 K | Native, 291 K | $K[Au(Cl_4)]$, 291 K | $K_2[Pt(CN_6)]$, 291 K |
|---|---|---|---|---|
| Data collection | | | | |
| Unit-cell parameters (Å) | | | | |
| *a*, *b* (Å) | 96.3 | 96.9 | 96.8 | 96.8 |
| *c* (Å) | 97.1 | 98.5 | 98.4 | 98.3 |
| X-ray source | Synchrotron | Conventional | Conventional | Conventional |
| Resolution range (Å) | 38.3–1.8 | 83.9–2.3 | 48.4–2.5 | 63.8–2.5 |
| Highest resolution shell (Å) | 2.0–1.8 | 2.4–2.3 | 2.6–2.5 | 2.6–2.5 |
| Total No. of reflections | 145218 | 116983 | 45230 | 39208 |
| No. of independent reflections | 48151 | 18994 | 15204 | 16776 |
| Completeness (%) | 98.0 (98.0) | 96.1 (90.9) | 94.1 (95.0) | 94.1 (95.5) |
| $I/\sigma(I)$ | 8.5 (3.0) | 11.2 (3.0) | 10.1 (3.3) | 9.6 (3.3) |
| $R_{merge}$ (%) | 7.8 (17.4) | 7.5 (19.2) | 8.7 (15.5) | 5.3 (13.9) |
| Phasing statistics | | | | |
| $R_{iso}$ (%) | | | 7.3 | 6.9 |
| No. of sites occupied by heavy atoms | | | 2 | 3 |
| Phasing power | | | 0.84 | 0.69 |
| $R_{Cullis}$ (%) | | | 0.70 | 0.77 |
| Figure of merit | | 0.43 | | |

We thank Dr Lester Clowney for his critical reading of the manuscript. This work was supported by the Core Research for Evolutional Science and Technology (CREST) program of the Japan Science and Technology Corporation (JST). NK is on a research fellowship sponsored by Japan Society for the Promotion of Science (JSPS).

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