Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction analysis of *Saccharomyces cerevisiae* Ygr203p, a homologue of Acr2 arsenate reductase

Jinho Moon,[a]† Young Sil Kim,[a]† Jae Young Lee,[a]† Seung-Je Cho,[a] Hyun Kyu Song,[a] Jong Hyun Cho,[a] B. Moon Kim,[a] Kyeong Kyu Kim[b] and Se Won Suh[a]*

[a]Department of Chemistry, College of Natural Sciences, Division of Chemistry and Molecular Engineering, Seoul National University, Seoul 151-742, South Korea, and [b]Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Chinju 660-701, South Korea

† These authors contributed equally to this work.

Correspondence e-mail: sewonsuh@snu.ac.kr

Received 31 January 2000
Accepted 5 April 2000

Ygr203p, a 148-residue protein encoded by the *ygr203w* gene of *Saccharomyces cerevisiae*, is a homologue of the yeast Acr2 arsenate reductase encoded by the *acr2* (or *ypr200c*) gene. It also shows significant sequence similarity to the human cell-cycle control Cdc25 phosphatase family. It has been overexpressed in soluble form in *Escherichia coli* with a His_6 tag at its C-terminus. The recombinant protein has been crystallized at 296 K using sodium chloride as precipitant. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 40.48, b = 50.95, c = 91.95 Å. The asymmetric unit contains a monomer, giving a crystal volume per protein mass (V_m) of 2.61 Å^3 Da^{-1} and a solvent content of 53.8%. The crystals diffract to better than 1.9 Å resolution with Cu $K\alpha$ X-rays. They are therefore suitable for high-resolution structure determination.

1. Introduction

Arsenical resistance in bacteria is conferred by operons located either on plasmids or on chromosomes (Silver & Phung, 1996). The conjugative R factor R773 isolated from *E. coli* contains one such operon responsible for antimonite, arsenite and arsenate resistance (Chen *et al.*, 1986; San Fransisco *et al.*, 1990). Among the five genes of this *ars* operon, the ArsC protein exhibits arsenate reductase activity by catalyzing the conversion of arsenate to arsenite, which is then excreted from the cells (Gladysheva *et al.*, 1994). Another *ars* operon, containing only the *arsR*, *arsB* and *arsC* genes, was also identified on the *E. coli* chromosome (Carlin *et al.*, 1995; Diorio *et al.*, 1995). The sequences homologous to the *ars* operon are ubiquitous in enterobacterial species (Bobrowicz *et al.*, 1997), indicating that the *ars* operon plays an important role in prokaryotes.

Compared with extensive studies carried out on bacterial systems, considerably less work has been performed on the eukaryotic counterparts. In *S. cerevisiae*, a budding yeast, a cluster of three novel genes *acr1*, *acr2* and *acr3* has been found to confer resistance to arsenic compounds (Bobrowicz *et al.*, 1997). The hypothetical product of the *acr1* gene is similar to the transcriptional regulatory proteins and that of the *acr3* gene is a transmembrane protein. The *acr2* gene encodes a polypeptide of 130 amino-acid residues with a predicted molecular mass of 14.9 kDa. The presence of the *acr2* gene is indispensable for arsenate but not for arsenite resistance (Bobrowicz *et al.*, 1997). Its gene product overexpressed in *E. coli* as a *malE* gene fusion with a C-terminal histidine tag was shown to exhibit arsenate reductase activity (Mukhopadhyay & Rosen, 1998).

The amino-acid sequence of Ygr203p, a 148-residue protein in *S. cerevisiae*, shows a considerable sequence similarity to the yeast Acr2 arsenate reductase (32% sequence identity for residues 30–130; Mukhopadhyay & Rosen, 1998). It also shows significant sequence similarity (28–31% identity for residues 8–128) to the catalytic domain of human cell-cycle control phosphatases, Cdc25A, Cdc25B and Cdc25C, suggesting that this yeast protein may be a close relative of the human Cdc25 phosphatase family (Hofmann *et al.*, 1998). The crystal structure of the catalytic domain of Cdc25A revealed that it resembles that of rhodanese (Fauman *et al.*, 1998). More recently, the three-dimensional fold of ArsC arsenate reductase encoded by the *E. coli* plasmid R773 has been determined by nuclear magnetic resonance (Stevens *et al.*, 1999). However, little overall sequence similarity exists between the yeast Acr2 protein (or its homolog Ygr203p) and the *E. coli* R773 ArsC protein. Therefore, it will be interesting to compare the structures of these two families of arsenate reductase. Since the yeast Acr2 protein formed inclusion bodies when overexpressed in *E. coli*, we have initiated the structure determination of Ygr203p, its homologue in *S. cerevisiae*. As a first step, the protein has been overexpressed in *E. coli* and well diffracting crystals have been obtained. Here, we report preliminary X-ray crystallographic data as well as the crystallization conditions.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell (1.97–1.90 Å).

Resolution range (Å)	20.0–1.90
Number of observations	58139
Unique reflections	14393
Space group	$P2_12_12_1$
Unit cell parameters (Å), e.s.d.s in parentheses	a = 40.48 (2), b = 50.95 (5), c = 91.95 (7)
Data completeness (%)	97.7 (87.2)
Average $I/\sigma(I)$	14.6 (4.1)
$R_{\rm merge}$† (%)	7.0 (37.8)

† $R_{\rm merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| \sum_h \sum_i I(h)_i$, where $I(h)$ is the intensity of reflection h, $\sum_h$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection h.

2. Experimental

2.1. Protein expression and purification

The *ygr203w* gene was amplified by the polymerase chain reaction using the *S. cerevisiae* genomic DNA as a template. The amplified DNA was inserted into the *Nde*I/*Xho*I-digested expression vector pET-21a. This vector construction adds six histidine residues to the C-terminus of the gene product to facilitate protein purification. The enzyme was highly overexpressed in soluble form in B834(DE3) cells upon induction by 0.5 m*M* IPTG at 310 K. Cells were grown in Luria–Bertani medium for 4 h after IPTG induction and were harvested by centrifugation at 7 000 rev min^{-1} (Sorvall GSA rotor) for 7 min at 277 K. The cell pellet was resuspended in an ice-cold lysis buffer (20 m*M* Tris–HCl pH 7.9, 0.50 *M* NaCl, 50 m*M* imidazole and 1 m*M* phenylmethylsulfonyl fluoride) and was homogenized by sonication. The crude lysate was centrifuged at 36 000*g* (18 000 rev min^{-1}, Hanil Supra 21K rotor) for 30 min at 277 K and the recombinant protein in the supernatant fraction was purified by three chromatographic steps. The first step utilized the C-terminal histidine tag by metal-chelate chromatography on Ni–NTA resin (Qiagen, Hilden, Germany). Next, gel filtration was performed on a HiLoad XK16 Superdex 75 prep-grade column (Amersham-Pharmacia, Uppsala, Sweden), which was previously equilibrated with buffer *A* (50 m*M* sodium phosphate at pH 6.0) containing 150 m*M* sodium chloride and 1 m*M* β-mercaptoethanol. Further purification was achieved by an ion-exchange chromatographic step on a Mono-S HR5/5 column (Amersham-Pharmacia), which was previously equilibrated with buffer *A*. Before loading the protein sample onto this column, the salt concentration was lowered to 50 m*M* by diluting it with buffer *A*. The protein was eluted with a linear gradient of 0–1.0 *M* sodium chloride in buffer *A*. The purified protein was homogeneous as judged by polyacrylamide gel electrophoresis in the presence of 0.1%(*w*/*v*) sodium dodecyl sulfate (Laemmli, 1970). This procedure yielded approximately 10 mg of homogeneous Ygr203p from a 1 l culture. Dynamic light-scattering measurements were performed on a Model DynaPro-801 instrument from Protein Solutions (Charlottesville, Virginia, USA) as described previously (Lee *et al.*, 1999).

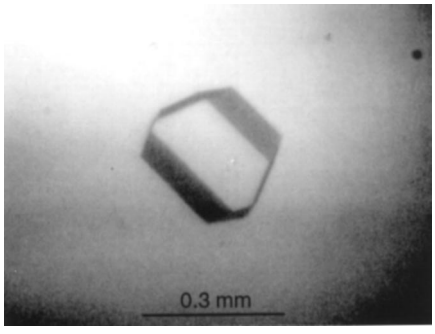

Figure 1
An orthorhombic crystal of *S. cerevisiae* Ygr203p. Its approximate dimensions are 0.4 × 0.3 × 0.15 mm.

2.2. Crystallization and X-ray data collection

The purified protein was concentrated to about 6 mg ml^{-1} using a YM10 ultrafiltration membrane (Amicon, Beverly, Massachusetts, USA) and was then dialyzed against buffer *A* containing 100 m*M* sodium chloride for crystallization. The protein concentration was estimated by measuring the absorbance at 280 nm using the calculated molar extinction coefficient of 38 690 M^{-1} cm^{-1}. Crystallization was performed by the hanging-drop vapour-diffusion method using 24-well tissue-culture plates (Hampton Research, Laguna Niguel, California, USA) at 296 K. Each hanging drop, prepared by mixing 2 µl each of the protein solution and the reservoir solution, was placed over 1.0 ml of reservoir solution. Results from the initial searches for crystallization conditions performed using Crystal Screen I (Jancarik & Kim, 1991) and Crystal Screen II kits (Hampton Research) were optimized.

A crystal was mounted in a thin-walled glass capillary and the capillary was sealed with wax after filling both ends with the mother liquor. X-ray diffraction data were collected at 293 K on an image-plate area-detector system (model 2030b, MacScience, Yokohama, Japan) with double-mirror focused Cu $K\alpha$ X-rays from a rotating-anode generator (MacScience M18XHF) operated at 50 kV and 90 mA. Data were processed and scaled using *DENZO* and *SCALEPACK* from the *HKL* program suite (Otwinowski & Minor, 1997).

3. Results

The native molecular mass of the recombinant Ygr203p from *S. cerevisiae* with the C-terminal His_6 tag was estimated to be 24 kDa, with a polydispersity of 17% from dynamic light-scattering measurements. This indicates that the protein exists as a monomer (calculated monomer M_r 18 071 for the His_6-tagged protein). The optimized reservoir solution for crystallization is 1.0–1.4 *M* sodium chloride without any buffer or additives. Crystals grew reproducibly to maximum dimensions of approximately 0.5 × 0.4 × 0.2 mm within a week (Fig. 1).

The diffraction pattern from the crystals extended beyond 1.9 Å resolution with Cu $K\alpha$ X-rays and the crystals were very stable in the X-ray beam. Diffraction data were collected at 293 K from a single crystal using Cu $K\alpha$ radiation. A total of 14 393 unique reflections were measured with a redundancy of 4.0. The merged data set is 97.7% complete to 1.9 Å resolution and gives an $R_{\rm merge}$ (on intensity) of 7.0%. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 40.48 (2), b = 50.95 (5), c = 91.95 (7) Å, where estimated standard deviations (e.s.d.s) are given in parentheses. The presence of one monomeric molecule in the asymmetric unit gives a crystal packing parameter (V_m) of 2.61 Å^3 Da^{-1} and a solvent content of 53.8%. These values are within the frequently observed ranges for protein crystals (Matthews, 1968). Table 1 summarizes the statistics for data collection. A good mercury derivative has been prepared in order to solve the crystal structure by the multiple isomorphous replacement method.

We thank the Inter-University Center for Natural Science Research Facilities for providing the X-ray equipment. This work was supported by the Center for Molecular Catalysis at Seoul National University and the BK21 program.

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