

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

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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 His₆ 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 *P*2₁2₁2₁, 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 Å³ Da⁻¹ and a solvent content of 53.8%. The crystals diffract to better than 1.9 Å resolution with Cu Kα X-rays. They are therefore suitable for high-resolution structure determination.

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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 Francisco *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_{merge}^\dagger (%)	7.0 (37.8)

$^\dagger R_{\text{merge}} = \frac{\sum_h \sum_i |I(h)_i - \langle I(h) \rangle|}{\sum_h \sum_i I(h)_i}$, 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 *NdeI/XhoI*-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 mM 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 mM Tris–HCl pH 7.9, 0.50 M NaCl, 50 mM imidazole and 1 mM phenylmethylsulfonyl fluoride) and was homogenized by sonication. The crude lysate was centrifuged at 36 000g (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 mM sodium phosphate at pH 6.0) containing 150 mM sodium chloride and 1 mM 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 mM 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 11 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).

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 mM 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 μ l 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 His₆ 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 His₆-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 \times 0.4 \times 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_{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 Å³ 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.

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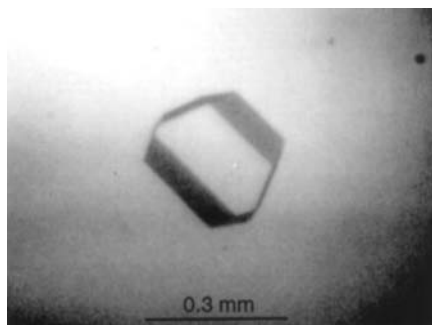


Figure 1
An orthorhombic crystal of *S. cerevisiae* Ygr203p. Its approximate dimensions are 0.4 \times 0.3 \times 0.15 mm.

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