CyaY is a 106-residue protein from *Escherichia coli*. It shows amino-acid sequence similarity to human frataxin and a frataxin homologue in *Saccharomyces cerevisiae*, Yfh1p. The former is associated with the disease Friedreich ataxia and the latter plays a key role in iron homeostasis in mitochondria. CyaY has been overexpressed in soluble form in *E. coli*. The recombinant protein with a His$_6$ tag at its C-terminus has been crystallized at 296 K using polyethylene glycol (PEG) 4000 as a precipitant. Native diffraction data have been collected to 1.8 Å using Cu Kα X-rays. The crystals belong to the trigonal space group $P_3_2_1$ (or $P_{3_1}2_1$) with unit-cell parameters $a = b = 44.66$, $c = 99.87$ Å, $\alpha = \beta = 90.0$, $\gamma = 120.0$. The asymmetric unit contains one molecule of recombinant CyaY, with a corresponding $V_m$ of 2.13 Å$^3$ Da$^{-1}$ and solvent content of 42.3%.

1. Introduction

Friedreich ataxia (FRDA) is the most common hereditary ataxia, with an estimated prevalence of 1 in 50,000 and a deduced carrier frequency of 1 in 120 in European populations (Skre, 1975; Romeo *et al.*, 1983). FRDA is an autosomal recessive neurodegenerative disease characterized by a progressive gait and limb ataxia with lack of tendon reflexes in the leg, dystarthisa and pyramidal weakness of the legs (Geoffroy *et al.*, 1976; Harding, 1981). Hypertrophic cardiomyopathy is found in almost all patients (Harding & Hever, 1983; Pentland & Fox, 1983). A gene, $\chi_{25}$, was identified in the critical region for the FRDA locus on human chromosome 9q13. The majority of FRDA patients were homozygous for an expansion of GAA/TTC triplet repeat inside the first intron of the $\chi_{25}$ gene, but some were found to have point mutations in the frataxin protein-coding region (Campuzano *et al.*, 1996). Human frataxin is localized in the mitochondria (Campuzano *et al.*, 1997), but its biological function has not been clearly established. Frataxin homologues are found in such diverse organisms as *Caenorhabditis elegans*, *Saccharomyces cerevisiae* and Gram-negative bacteria (Campuzano *et al.*, 1996; Gibson *et al.*, 1996). Yfh1p is a frataxin homolog in *S. cerevisiae* and is also localized in mitochondria. It plays a key role in maintaining mitochondrial iron homeostasis at the level of iron efflux (Radisky *et al.*, 1999). The dysfuncion of Yfh1p results in mitochondrial iron overload, leading to an increased production of highly toxic free radicals (Foury & Cazzalini, 1997).

CyaY is an *E. coli* protein with 106 amino-acid residues ($M_r = 12 231$). It shows overall sequence identities of 29.6 and 34.2% with the C-terminal regions of human frataxin (residues 122–192) and Yfh1p (residues 89–164), respectively. The disease-associated point mutations found in human frataxin (Gly130Val, Ile154Phe) are located in the C-terminal region; these residues are highly conserved among the homologous proteins (Campuzano *et al.*, 1996; Bidichandani *et al.*, 1997), including *E. coli* CyaY. Biochemical data on *E. coli* CyaY are not available. Since no three-dimensional structure of frataxin or its homologues has been determined, *E. coli* CyaY is an interesting target for structural studies. Its three-dimensional structure will provide a framework for a detailed understanding of the possible functions of frataxin and its homologues at the molecular level. As a first step toward the structural elucidation of *E. coli* CyaY, well diffracting crystals have been produced. Here, we report preliminary X-ray crystallographic data as well as the crystallization conditions.

2. Experimental

2.1. Protein expression and purification

The gene encoding CyaY was amplified by polymerase chain reaction using *E. coli* BL21(DE3) genomic DNA as template. The amplified DNA was inserted into the Ndel/Xhol-digested expression vector pET-22b. This vector construction, designated as pET-22b-CyaY, adds six histidine residues to the C-terminus of the gene product to facilitate protein purification. The complete nucleotide sequence of the insert was confirmed by dideoxy-DNA sequencing. We overexpressed the protein in soluble form in BL21(DE3) cells by induction with 0.5 mM isopropyl $\beta$-d-thio-
Table 1

<table>
<thead>
<tr>
<th>Data-collection statistics</th>
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<tbody>
<tr>
<td>Number of crystals</td>
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<td>Temperature (K)</td>
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<td>Space group</td>
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<td>Unit-cell parameters (Å, °)</td>
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<td>No. of observed reflections</td>
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<td>(R_{\text{merge}}) (%)</td>
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<td>Data completeness (%)</td>
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\(R_{\text{merge}} = \sum_{i} \sum_{h} (I(h)) - (\langle I(h) \rangle) / \sum_{i} \sum_{h} I(h), \) where \(I(h)\) is the intensity of reflection \(h\), \(\sum_{i}\) is the sum over all reflections and \(\sum_{h}\) is the sum over \(i\) measurements of reflection \(h\).

2.2. Crystallization and X-ray diffraction experiment

Crystallization was achieved using the hanging-drop vapour-diffusion method at 296 K using 24-well tissue-culture plates (Hampton Research). Each hanging drop was prepared by mixing 2 µl of the reservoir solution, 2 µl of the protein solution (17 mg ml\(^{-1}\)) and 0.4 µl of 2.0 M magnesium chloride. Each hanging drop was placed over 0.9 ml of the reservoir solution. Initial crystal growth was established by sparse-matrix sampling (Jancarik & Kim, 1991).

X-ray experiments were carried out using C\(_{\text{x}}\)Ka X-rays produced with double-mirror focusing optics from a rotating-anode generator (Rigaku RU-200BH, running at 50 kW and 90 mA). A set of X-ray diffraction data was collected at 296 K on the MacScience DIP2030 image-plate area-detector system. A total of 120 1.0° oscillation frames were recorded at a crystal-to-detector distance of 120 mm. The data were processed and scaled using the programs DENZO and SCALEPACK (Otwinowski & Minor, 1997).

3. Results

The recombinant CyaY from \(E.\) coli with a C-terminal His\(_{\text{6}}\) tag was overproduced in soluble form, with a yield of \(\sim 13\) mg of homogeneous protein from a \(1\) culture. Well diffracting crystals were obtained using a reservoir solution consisting of 100 mM sodium acetate and 34% (w/v) PEG 4000 (final pH 5.12). The crystals grew to maximum dimensions of 0.4 × 0.4 × 0.6 mm within 2 d (Fig. 1). The native crystals diffracted to 1.8 Å resolution with CuKa X-rays from a rotating-anode source and were very stable in the X-ray beam. Diffraction data were collected from a native crystal of approximate dimensions 0.4 × 0.4 × 0.6 mm using CuKa radiation (Table 1). The crystals belong to the trigonal space group P3\(_2\)1 (or P3\(_3\)1), with unit-cell parameters \(a = b = 4.466\) (20), \(c = 9.987\) (18), \(a = \beta = 90.0, \gamma = 120.0\)°, where the estimated standard deviations of the unit-cell parameters are given in parentheses. The presence of a single copy of the recombinant CyaY with a C-terminal His\(_{\text{6}}\) tag in the crystallographic asymmetric unit corresponds to a crystal volume per protein mass \((V_{\text{m}})\) of 2.13 Å\(^3\) Da\(^{-1}\) and a solvent content of 42.3%. More than one copy is not possible, as it leads to unacceptable low \(V_{\text{m}}\) values for protein crystals (Matthews, 1968). A search for heavy-atom derivatives in order to solve the structure by the multiple isomorphous replacement method is in progress.

This work was supported by the Center for Molecular Catalysis at Seoul National University and the BK21 program.

References


Figure 1

A trigonal crystal of \(E.\) coli CyaY. Its approximate dimensions are 0.4 × 0.4 × 0.6 mm.