Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray crystallographic analysis of *Escherichia coli* CyaY, a structural homologue of human frataxin

Myong Gyong Lee, Seung-Je Cho, Jin Kuk Yang, Hyun Kyu Song and Se Won Suh*

Department of Chemistry, College of Natural Sciences, Division of Chemistry and Molecular Engineering, Seoul National University, Seoul 151-742, South Korea

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

Received 25 January 2000
Accepted 17 April 2000

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 $K\alpha$ X-rays. The crystals belong to the trigonal space group $P3_121$ (or $P3_221$), with unit-cell parameters $a = b = 44.66$, $c = 99.87$ Å, $\alpha = \beta = 90.0$, $\gamma = 120.0^\circ$. 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, dysarthria 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, *χ25*, 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 *χ25* 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 dysfuction 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 *Nde*I/*Xho*I-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 m*M* isopropyl β-D-thio-

Table 1
Data-collection statistics.

Number of crystals	1
Temperature (K)	296
Space group	$P3_121$ (or $P3_221$)
Unit-cell parameters (Å, °)	$a = b = 44.66$ (20), $c = 99.87$ (18), $\alpha = \beta = 90.0$, $\gamma = 120.0$
No. of observed reflections	128723
No. of unique reflections	10309
$R_{\rm merge}$† (%)	6.0
Data completeness (%)	92.8 (20.0–1.80 Å), 99.5 (1.86–1.80 Å)

† $R_{\rm merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$, 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.

galactopyranoside (IPTG) at 310 K. Cells were grown in Luria–Bertani medium for 4 h after IPTG induction and were harvested by centrifugation at 4200*g* (Sorvall GS3 rotor) for 7 min at 277 K. The cell pellet was resuspended in ice-cold lysis buffer (20 m*M* Tris–HCl pH 7.9, 0.50 *M* sodium chloride, 50 m*M* imidazole, 1 m*M* phenylmethylsulfonyl fluoride) and was then 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). Next, we performed gel filtration on a HiLoad 16/60 Superdex 200 prep-grade column (Pharmacia), which was previously equilibrated with buffer *A* (50 m*M* Tris–HCl pH 7.5) containing 150 m*M* sodium chloride and 1 m*M* β-mercaptoethanol. We achieved further purification by an ion-exchange chromatographic step on Source 15Q resin packed in a HR 10/10 column (Pharmacia), which was previously equilibrated with buffer *B* [50 m*M* *N*-(2-acetamido)-2-iminodiacetic acid–KOH pH 6.5]. Before loading the protein sample onto this ion-exchange column, we lowered the salt concentration to 50 m*M* by diluting it with buffer *B*. The protein was eluted with a linear gradient of 0–1.0 *M* sodium chloride in buffer *B*. The purified protein was homogeneous as assessed by polyacrylamide gel electrophoresis in the presence of 0.1%(*w*/*v*) sodium dodecyl sulfate (Laemmli, 1970). It was dialyzed against buffer *A* containing 100 m*M* sodium chloride and the protein solution was concentrated using a YM10 membrane (Amicon). The protein concentration was estimated by measuring the absorbance at 280 nm using the calculated molar extinction coefficient 29 730 M^{-1} cm^{-1} (SWISS-PROT; http://www.expasy.ch/).

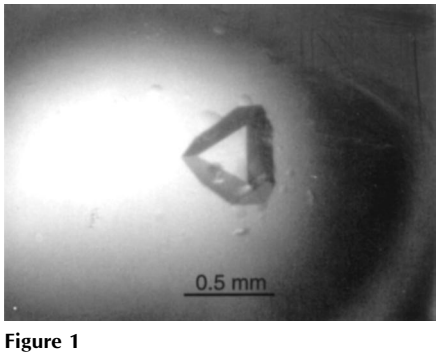

Figure 1
A trigonal crystal of *E. coli* CyaY. Its approximate dimensions are 0.4 × 0.4 × 0.6 mm.

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 crystallization conditions were established by sparse-matrix sampling (Jancarik & Kim, 1991).

X-ray experiments were carried out using Cu $K\alpha$ X-rays produced with double-mirror focusing optics from a rotating-anode generator (Rigaku RU-200BH, running at 50 kV 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_6 tag was overproduced in soluble form, with a yield of ∼13 mg of homogeneous protein from a 1 l culture. Well diffracting crystals were obtained using a reservoir solution consisting of 100 m*M* 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 Cu $K\alpha$ 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 Cu $K\alpha$ radiation (Table 1). The crystals belong to the trigonal space group $P3_121$ (or $P3_221$), with unit-cell parameters $a = b = 44.66$ (20), $c = 99.87$ (18) Å, $\alpha = \beta = 90.0$, $\gamma = 120.0°$, 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_6 tag in the crystallographic asymmetric unit corresponds to a crystal volume per protein mass (V_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 unacceptably low V_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

Bidichandani, S. I., Ashizawa, T. & Patel, P. I. (1997). *Am. J. Hum. Genet.* **60**, 1251–1256.
Campuzano, V., Montermini, L., Lutz, Y., Cova, L., Hindelang, C., Jiralerspong, S., Trottier, Y., Kish, S. J., Fraucheux, B., Trouillas, P., Authier, F. J., Durr, A., Mandel, J. L., Vescovi, A., Pandolfo, M. & Koenig, M. (1997). *Hum. Mol. Genet.* **6**, 1771–1780.
Campuzano, V., Montermini, L., Moltò, M. D., Pianese, L., Cossèe, M., Cavalcanti, F., Monros, A., Rodius, F., Duclos, F., Monticelli, A., Zara, F., Caòizares, J., Koutnikova, H., Bidichandani, S. I., Gellera, C., Brice, A., Trouillas, P., De Michele, G., Filla, A., De Frutos, R., Palau, F., Patel, P. I., Donato, S. D., Mandel, J. L., Cocozza, S., Koenig, M. & Pandolfo, M. (1996). *Science*, **271**, 1423–1427.
Foury, F. & Cazzalini, O. (1997). *FEBS Lett.* **411**, 373–377.
Geoffroy, G., Barbeau, A., Breton, G., Lemieux, B., Aube, M., Leger, C. & Bouchard, J. P. (1976). *Can. J. Neurol. Sci.* **3**, 279–286.
Gibson, T. J., Koonin, E. V., Musco, G., Pastore, A. & Bork, P. (1996). *Trends Neurol. Sci.* **19**, 465–468.
Harding, A. E. (1981). *Brain*, **104**, 589–620.
Harding, A. E. & Hever, R. L. (1983). *Q. J. Med.* **208**, 489–502.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Laemmli, U. K. (1970). *Nature (London)*, **227**, 680–685.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Pentland, B. & Fox, K. A. A. (1983). *J. Neurol. Neurosurg. Psychiatry*, **46**, 1138–1142.
Radisky, D. C., Babcock, M. C. & Kaplan, J. (1999). *J. Biol. Chem.* **274**, 4497–4499.
Romeo, G., Menozzi, P., Ferlini, A., Fadda, S., Di Donato, S., Uziel, G., Lucci, B., Capodaglio, L., Filla, A. & Campanella, G. (1983). *Am. J. Hum. Genet.* **35**, 523–529.
Skre, H. (1975). *Clin. Genet.* **7**, 287–298.