Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary crystallographic analysis of DJ-1, a protein associated with male fertility and parkinsonism

Kazuya Honbou,[a] Nobuo N. Suzuki,[a,b] Masataka Horiuchi,[a] Takahiro Taira,[b,c] Takeshi Niki,[b] Hiroyoshi Ariga[b,c] and Fuyuhiko Inagaki[a,b]*

[a]Department of Structural Biology, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, [b]CREST, Japan Science and Technology Corporation, Japan, and [c]Department of Molecular Biology, Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

Correspondence e-mail: finagaki@pharm.hokudai.ac.jp

Received 7 March 2003
Accepted 9 June 2003

DJ-1 was identified as a novel oncogene product that transformed mouse NIH3T3 cells in cooperation with activated Ras. DJ-1 was also correlated with male infertility and parkinsonism. DJ-1 was crystallized using sodium citrate and HEPES at pH 7.5. The crystal belongs to space group $P3_1$ or $P3_2$, with unit-cell parameters a = 75.04, c = 74.88 Å and contains two molecules in an asymmetric unit. An intensity data set was collected to 2.00 Å resolution.

1. Introduction

DJ-1 is a novel oncogene product that transforms mouse NIH3T3 cells in cooperation with activated Ras. DJ-1 consists of 189 amino-acid residues and is expressed in various human organs, especially in testis (Nagakubo *et al.*, 1997). DJ-1 was found to promote androgen receptor (AR) dependent transcription. This function arises from binding of DJ-1 to PIASxα, which inhibits AR-dependent transcriptional activity (Takahashi *et al.*, 2001). CAP1/SP22, a rat homologue of human DJ-1, decreased by exposure of rats to sperm toxicants such as ornidazole, which caused male infertility (Klinefelter *et al.*, 1997; Wagenfeld *et al.*, 1998). In addition, treatment of sperm with a DJ-1 antibody significantly inhibited fertility of sperm *in vivo* and *in vitro* (Klinefelter *et al.*, 2002). The above evidence taken together suggests that DJ-1 plays an important role in male fertility. Recently, DJ-1 mutation was found to be associated with PARK7, a monogenic form of human parkinsonism. Parkinsonism is the second most common neurodegenerative disorder (Lang & Lozano, 1998) and PARK7 is one of autosomal recessive early onset parkinsonism. In these patients, the highly conserved leucine at position 166 was mutated to proline (Bonifati *et al.*, 2003).

Here, we report the preliminary crystallographic studies of human DJ-1. The three-dimensional structure of DJ-1 will be helpful to understand the functional role of DJ-1 in fertility and parkinsonism.

2. Materials and methods

2.1. Molecular clonimg, expression and purification of DJ-1

Full-length human DJ-1 (189 amino acids, 20 kDa) was cloned into pGEX6P-1 as a fusion protein with glutathione *S*-transferase (GST) and expressed in *Escherichia coli* BL21 (DE3). The bacterial culture was grown in 2×YT with 0.1 g l^{-1} ampicillin at 310 K until absorbance at 600 nm reached 0.5. Protein expression was induced by addition of isopropyl-1-thio-β-D-galactoside to 0.1 m*M*. Cells were cultured at 298 K and harvested after 7 h. The cells were collected and disrupted by sonication at 277 K in PBS(−) containing 1 m*M* EDTA, 5 m*M* NaN_3. The supernatant was incubated with glutathione Sepharose 4B (Pharmacia Biotech) and GST-fusion protein was eluted with a solution containing 25 m*M* glutathione (reduced form) pH 8.0. The eluate was dialyzed in cleavage buffer (150 m*M* NaCl, 1 m*M* EDTA, 1 m*M* DTT, 50 m*M* Tris–HCl pH 7.0) and the GST tag was cleaved by digestion with 10 units ml^{-1} PreScission Protease (Pharmacia Biotech) for 4 h at 277 K. After digestion, protein solution was again incubated with glutathione Sepharose 4B and both GST and uncleaved GST-fusion protein were removed. Further purification was performed using HiTrap Q (Pharmacia Biotech) followed by HiLoad Superdex 75pg 26/60 (Pharmacia Biotech).

Purified protein contains an additional 15 amino acids at the N-terminus derived from the linker connecting DJ-1 and GST. In order to remove these N-terminal residues, trypsin digestion was performed by incubating 0.5 mg ml^{-1} protein with 10 pmol ml^{-1} trypsin (sequencing grade, Roche Molecular Biochemicals) with a solution containing 0.1 *M* ammonium bicarbonate for 1.5 h at 310 K. After digestion, protein was purified by two passes of HiLoad Superdex 75 pg 26/60 (Pharmacia Biotech). MALDI–TOF MS and N-terminal sequence analyses were performed, which revealed that 15 amino acids were removed from the N-terminus. Thus, intact DJ-1 (1–189) was obtained. A MALDI–TOF MS spectrum of purified DJ-1 is shown in Fig. 1.

2.2. Crystallization

The crystallization conditions for DJ-1 were established by sparse-matrix screening

© 2003 International Union of Crystallography
Printed in Denmark – all rights reserved

(Jancarik & Kim, 1991) using Crystal Screen I (Hampton Research) and sitting-drop vapor diffusion. In each trial, a sitting drop of 2 µl of purified protein solution (10 mg ml^{-1} in 5 m*M* NaN_3 solution) was mixed with 0.5 µl of reservoir solution. A single-crystal was grown at 293 K in precipitant solution containing 1.4 *M* sodium citrate, 0.1 *M* HEPES pH 7.5 in 1 d. The crystals reached maximal dimensions of 0.3 × 0.3 × 0.1 mm.

2.3. Data collection and processing

All diffraction data were collected from crystals cooled at 100 K in a cold nitrogen stream using an R-AXIS IV imaging-plate detector on an FR-C X-ray generator (Rigaku). The data collection was performed with 2.0° steps over a total oscillation range of 142°, with an exposure time of 60 min for each frame. The camera distance was 130 mm. The crystal was found to diffract to 2.00 Å resolution and belongs to space group $P3_1$ or $P3_2$, with unit-cell parameters a = 75.04, c = 74.88 Å. All data were processed using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The crystallographic parameters and data-collection statistics are shown in Table 1. The total number of observed reflections was 124 947, which gave 29 240 unique reflections. The resulting data gave an $R_{\rm merge}$ of 9.76 (29.4% for the outer shell, 2.07–2.00 Å) with a completeness of 97.6% (92.6% for the outer shell). The crystal mosaicity was estimated to be 0.446°. The present crystals contain two molecules in the asymmetric unit, with a $V_{\rm M}$ value (Matthews, 1968) of 3.07 Å^3 Da^{-1}, corresponding to a solvent content of 59%. The crystal data were used for calculating a self-rotation function using *POLARRFN* (Collaborative Computational Project, Number 4, 1994), as shown in Fig. 2. In the κ = 180° section, the self-rotation map shows strong peaks at (ω, φ) = (90, 0), (90, 60), (90, 120), (90, 180), (90, 240) and (90, 300), indicating that there are three local twofold axes related by the crystallographic threefold symmetry. This result fits well with the estimated value of two DJ-1 molecules in the asymmetric unit.

Sequence alignment using *FASTA* (Pearson & Lipman, 1988) has shown that DJ-1 has no significant homology with other proteins of known tertiary structure. Therefore, a heavy-atom multiple isomorphous replacement method for the structure analysis was applied. An extensive search for heavy-atom derivatives is currently under way.

Table 1
Crystallographic parameters and data-collection statistics.

Values in parentheses refer to the highest resolution shell (2.07–2.00 Å).

Space group	$P3_1$ or $P3_2$
Unit-cell parameters (Å)	$a = b$ = 75.04, c = 74.88
Mathews coefficient (Å^3 Da^{-1})	3.07 (2 mols per AU)
Solvent content (%)	59
Resolution range (Å)	50–2.0
Observed reflections	124947
Unique reflections	29240
$R_{\rm merge}$† (%)	9.76 (29.4)
Completeness (%)	97.6 (92.6)
Mosaicity (°)	0.446

† $R_{\rm merge} = \sum |I_i - \langle I \rangle| / \sum I_i$, where I_i is the observed intensity and $\langle I \rangle$ is the averaged intensity obtained from multiple observation of symmetry-related reflections.

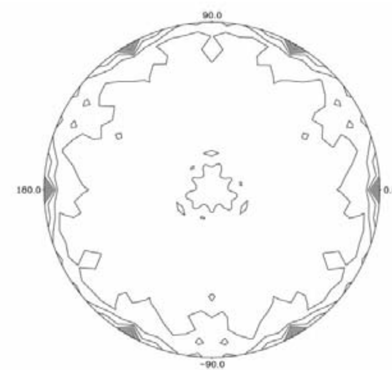

Figure 2
A stereographic projection of the self-rotation function from the DJ-1 data set at κ = 180°. The self-rotation function was calculated using a 29 Å radius of integration and data in the resolution range 7–4 Å. The obvious peaks corresponding to twofold non-crystallographic symmetry can be seen on this section.

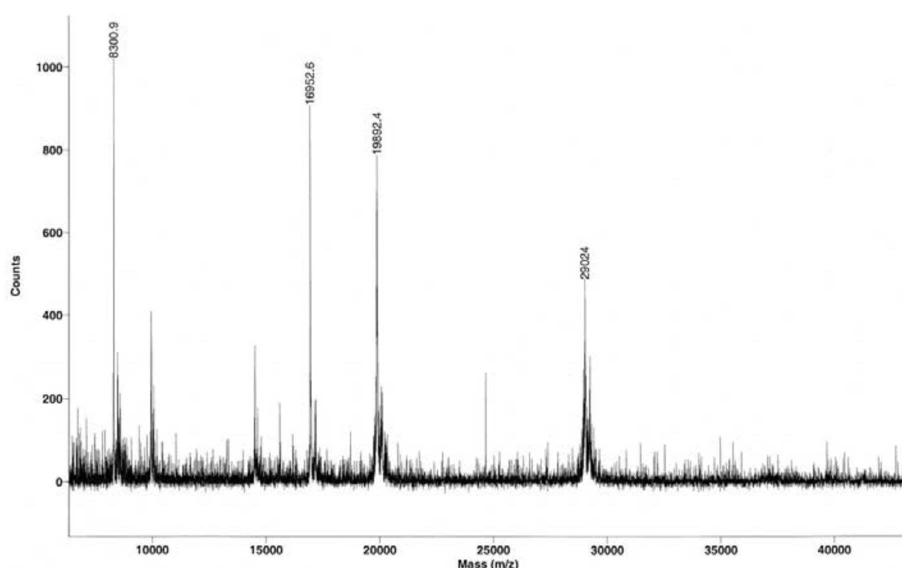

Figure 1
MALDI-TOF/MS spectra of purified DJ-1 using 3,5-dimethoxy-4-hydroxycinnamic acid as a matrix. Myoglobin (Sigma, M-0630) and carbonic anhydrase (Sigma, C-7025) were used as the internal standard. The observed peak species of (m/z) = 29 024, 169 52.6 and 8300.9 correspond to carbonic anhydrase, myoglobin and doubly charged species of myoglobin, respectively. After calibration, the DJ-1 (m/z) peak was estimated as 19 892.4, in good agreement with full-length DJ-1 (calculated mass 19 891.2).

This work was supported by CREST of Japan Science and Technology (JST) and by a Grant-in-Aid for Scientific Research on Priority Areas and National Project on Protein Structural and Functional Analyses from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References

Bonifati, V., Rizzu, P., van Baren, M. J., Schaap, O., Breedveld, G. J., Krieger, E., Dekker, M. C., Squitieri, F., Ibanez, P., Joosse, M., van Dongen, J. W., Vanacore, N., van Swieten, J. C., Brice, A., Meco, G., van Duijn, C. M., Oostra, B. A. & Heutink, P. (2003). *Science*, **299**, 256–259.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Pearson, W. R. & Lipman, D. J. (1988). *Proc. Natl Acad. Sci. USA*, **85**, 2444–2448.

Jancarik, J. & Kim, S. H. (1991). *J. Appl. Cryst.* **24**, 409–411.

Klinefelter, G. R., Laskey, J. W., Ferrell, J., Suarez, J. D. & Roberts, N. L. (1997). *J. Androl.* **18**, 139–150.

Klinefelter, G. R., Welch, J. E., Perreault, S. D., Moore, H. D., Zucker, R. M., Suarez, J. D., Roberts, N. L., Bobseine, K. & Jeffay, S. (2002). *J. Androl.* **23**, 48–63.

Lang, A. E., & Lozano, A. M. (1998). *N. Engl. J. Med.* **339**, 1044–1053.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Nagakubo, D., Taira, T., Kitaura, H., Ikeda, M., Tamai, K., Iguchi-Ariga, S. M. & Ariga, H. (1997). *Biochem. Biophys. Res. Commun.* **231**, 509–513.

Otwinowski, W. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Takahashi, K., Taira, T., Niki, T., Seino, C., Iguchi-Ariga, S. M. & Ariga, H. (2001). *J. Biol. Chem.* **276**, 37556–37563.

Wagenfeld, A., Gromoll, J. & Cooper, T. G. (1998). *Biochem. Biophys. Res. Commun.* **251**, 545–549.