Acta Cryst. (1997). D54, 436–437

Crystallization and preliminary X-ray studies of hORF6, a novel human antioxidant enzyme

HEE-JEONG CHOI,[a,b] SANG WON KANG,[c] CHUL-HAK YANG,[b] SUE GOO RHEE[c] AND SEONG-EON RYU[a]*
at [a]*Division of Protein Engineering, Korea Research Institute of Bioscience and Biotechnology, KIST, PO Box 115, Yusong, Taejon 305-600, South Korea,* [b]*Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151-742, South Korea, and* [c]*Laboratory of Cell Signaling, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA. E-mail: ryuse@mcrl.kribb.re.kr*

(*Received 24 March 1997; accepted 12 August 1997*)

Abstract

HORF6 is a member of the novel antioxidant enzyme family found in humans. A recombinant form of hORF6 expressed and purified from *E. coli* has been crystallized by the hanging-drop method using various PEG's as precipitating agents. HORF6 crystallizes in two different monoclinic space groups, $P2_1$ and $C2$. The $P2_1$ crystals have unit-cell dimensions of $a = 47.85$, $b = 75.17$, $c = 63.30$ Å and $\beta = 110.21°$ and contain two monomers per asymmetric unit, while the $C2$ crystals have unit-cell dimensions of $a = 165.27$, $b = 95.44$, $c = 166.44$ Å and $\beta = 128.97°$ and contain more than six monomers per asymmetric unit. The $P2_1$ crystals with the smaller unit cell diffract X-rays better and behave well for the X-ray analysis. A native data set from a single crystal of the $P2_1$ space group has been collected to 2.0 Å resolution.

1. Introduction

Reactive oxygen species such as $O_2^{\cdot-}$, H_2O_2 and $HO^{\cdot}$ are produced as a result of the incomplete reduction of oxygen during respiration or by exposure to external agents including UV light and ionizing radiation (Sies, 1993; Fridovich & Freeman, 1986). These highly reactive molecules can damage cellular macromolecules such as DNA, proteins, carbohydrates and lipids. Aerobic organisms developed defense systems against such damage by using various antioxidant enzymes including superoxide dismutases, catalases and peroxidases (Sies, 1993; Fridovich & Freeman, 1986). Recent studies indicate that hydrogen peroxide (H_2O_2), which is one of the reactive oxygen species involved in oxidative stress, is an intracellular secondary messenger in the signal transduction by growth factor receptors (Ignotz & Massague, 1986; Hecht & Zick, 1992; Ohba *et al.*, 1994; Sundaresan *et al.*, 1995) as is nitric oxide ($NO^{\cdot}$)in the regulation of the immune function and blood-vessel dilatation (Lowenstein & Snyder, 1992). Intracellular concentration of H_2O_2 was transiently increased by the stimulation of rat vascular smooth muscle cells (VSMC's) with platelet-derived growth factor (PDGF) (Sundaresan *et al.*, 1995). The magnitude of the rise in the intracellular H_2O_2 concentration may temporarily alter the kinase–phosphatase balance to play a role in the regulation of the signal transduction.

A novel family of thiol-specific antioxidant (TSA) enzymes was identified from various sources ranging from bacteria to humans (Kim *et al.*, 1988; Chae *et al.*, 1993; Chae, Robison *et al.*, 1994; Rhee *et al.*, 1994). The TSA enzymes were shown to possess a peroxidase activity towards H_2O_2 and alkyl hydroperoxides, and thus named peroxiredoxins. These proteins, which have 23–98% sequence identity among the family members, show no sequence homology to previously known antioxidant enzymes, such as superoxide dismutases, catalases and peroxidases (Chae *et al.*, 1993). The peroxiredoxin proteins with a molecular weight of 25 kDa have two highly conserved cysteines at residues 47 and 170, and no redox cofactors. The N-terminal cysteine (Cys47) is conserved in all family members and the C-terminal cysteine (Cys170) is present in most but not all members. Biochemical and mutational studies implicated only Cys47 as the site of oxidation for substrate reduction even though the oxidized form of the peroxiredoxin proteins exists mainly as a dimer linked by disulfide bonds between Cys47 of one molecule and Cys170 of the other molecule (Chae, Uhm *et al.*, 1994). Cys170 is thought to be involved in the ability of the enzyme to use thioredoxin as an electron donor (Netto *et al.*, 1996).

The information from the three dimensional structure of this new class of antioxidant proteins should shed light on the mechanism of H_2O_2 reduction by the thiol groups of the peroxiredoxin proteins. However, previous crystallization attempts with the native proteins of this family were not successful mainly because of the heterogeneous oligomerization probably arising from the random oxidation of the reactive thiol groups. HORF6 is a novel human member of the peroxiredoxin family and has one conserved cysteine (Cys47) and one non-conserved cysteine (Cys91) (Chae, Robison *et al.*, 1994). The mutation of Cys91 into Ser preserves the enzyme activity (Kang & Rhee, 1998). We report here a preliminary X-ray crystallographic analysis of the crystals obtained from the hORF6 with the C91S mutation.

2. Protein expression and purification

The mutant hORF6 protein was overexpressed in the *E. coli* strain BL21(DE3) as a soluble form. The protein was purified from the *E. coli* cells employing ammonium sulfate fractionation and column chromatographies using TSK phenyl-5PW and Mono-Q HR 10/10 columns as described (Kang & Rhee, 1998). The purified protein was dialyzed against 20 m*M* sodium Hepes (pH 7.0) and concentrated to 16 mg ml^{-1} for the crystallization experiments.

3. Crystallization and data analysis

Crystallization of the C91S hORF6 protein was achieved by the hanging-drop method in Linbro tissue-culture plates, sealed with transparent plastic tape. The 4 µl droplet containing 2 µl of the concentrated protein solution and 2 µl of the reservoir solution was equilibrated against 1 ml of the reservoir solution. Two kinds of crystals were obtained from

© 1998 International Union of Crystallography
Printed in Great Britain – all rights reserved

Acta Crystallographica Section D
ISSN 0907-4449 © 1998

the initial crystallization experiments using the Crystal Screen macromolecular crystallization reagent kit (Hampton Research, Riverside, CA). The first type appeared in the droplets that were equilibrated for 1–3 d at 298 K against the reservoir solutions containing polyethylene glycol (PEG) 4000 or 8000 as precipitating agents. These crystals usually grew as twinned blocks that had to be separated using microtools before analysis. After a series of refinements in the crystallization conditions we were able to obtain good crystals whose size often reached 0.7 × 0.7 × 0.5 mm. In refinement careful adjustments of the incubation temperature was critical. The best crystals were obtained from 25%(*w*/*v*) PEG 4000, 0.2 *M* magnesium acetate and 0.1 *M* sodium cacodylate, pH 6.5 at 295 K. They diffract X-rays to beyond 2.0 Å resolution and behave well for the X-ray analysis. These were characterized to be in the monoclinic space group $P2_1$. The unit-cell dimensions were $a = 47.85$, $b = 75.17$, $c = 63.30$ Å and $\beta = 110.21^\circ$. The systematic absences were evident along $0k0$ for $k \neq 2n+1$ in diffraction data sets collected from the crystals. Judged from the cell parameters and the molecular weight of the protein, the asymmetric unit is likely to contain two 25 kDa monomers of the protein, and the Matthews coefficient (Matthews, 1968) and the solvent content were estimated to be 2.14 $Å^3\,Da^{-1}$ and 43%, respectively.

Another kind of crystals with a morphology of rectangular prisms were grown from 2%(*w*/*v*) PEG 400, 2.0 *M* ammonium sulfate and 0.1 *M* sodium Hepes, pH 7.5 at 298 K. These crystals grow slowly and appear in the droplets only after 2 weeks of incubation, but eventually reach the large size of 1 × 1 × 0.7 mm in 3–4 weeks. Even though these large crystals have a good morphology the diffraction is limited to approximately 2.7 Å resolution. These crystals belong to another monoclinic space group, $C2$ with unit-cell dimensions of $a = 165.27$, $b = 95.44$, $c = 166.44$ Å and $\beta = 128.97^\circ$. The relatively large unit cell explains the relatively weak diffraction of this type of crystal. Six to 12 monomers of the protein can be accommodated in the asymmetric unit with the solvent content of 64–27% and the Matthews coefficient of 3.42–1.71 $Å^3\,Da^{-1}$ that is within the range of the values expected for most protein crystals (Matthews, 1968).

Diffraction data sets from each crystal form were collected at room temperature on a Rigaku R-AXIS IIc imaging-plate detector system using Cu $K\alpha$ X-rays from a Rigaku RU-300 rotating-anode generator operating at 40 kV and 100 mA. Oscillation images obtained from the detector were processed and reduced by a R-AXIS data-processing software package. Since the $P2_1$ crystals diffracted better, they were chosen for the structure determination. A native data set from a single $P2_1$ crystal was measured to 2.0 Å resolution with 93% completeness and $R_{sym} = 6.98\%$. The self-rotation search including the data between 15.0 and 4.0 Å was performed by using program *X-PLOR* (Brünger *et al.*, 1987). From the search, a non-crystallographic twofold axis was found at $\psi = 10.0$, $\varphi = 90.0$, $\kappa = 180.0^\circ$ with a peak height of 4.8σ. The rest of the peaks, except for a peak from the crystallographic twofold axis, had steadily decreasing heights below 3.4σ. A search for heavy-atom derivatives of the $P2_1$ crystals is in progress for the purpose of determining the three-dimensional structure by the multiple isomorphous replacement method.

This work was supported by grants from the Ministry of Science and Technology, Korea.

References

Brünger, A. T., Kuriyan, J. & Karplus, M. (1987). *Science*, **235**, 458–460.
Chae, H. Z., Kim, I. H., Kim, K. & Rhee, S. G. (1993). *J. Biol. Chem.* **268**, 16815–16821.
Chae, H. Z., Robison, K., Poole, L. B., Church, G., Storz, G. & Rhee, S. G. (1994). *Proc. Natl Acad. Sci. USA*, **91**, 7017–7021.
Chae, H. Z., Uhm, T. B. & Rhee, S. G. (1994). *Proc. Natl Acad. Sci. USA*, **91**, 7022–7026.
Fridovich, I. & Freeman, B. (1986). *Annu. Rev. Physiol.* **48**, 693–702.
Hecht, D. & Zick, Y. (1992). *Biochem. Biophys. Res. Commun.* **188**, 773–779.
Ignotz, R. A. & Massague, J. (1986). *J. Biol. Chem.* **261**, 4337–4345.
Kang, S. W. & Rhee, S. G. (1998). *J. Biol. Chem.* In the press.
Kim, K., Kim, I. H., Lee, K. Y., Rhee, S. G. & Stadtman, E. R. (1988). *J. Biol. Chem.* **263**, 4704–4711.
Lowenstein, C. J. & Snyder, S. H. (1992). *Cell*, **70**, 705–707.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Netto, L. E. S., Chae, H. Z., Kang, S. W., Rhee, S. G. & Stadtman, E. R. (1996). *J. Biol. Chem.* **271**, 15315–15321.
Ohba, M., Shibanuma, M., Kuroki, T. & Nose, K. (1994). *J. Cell. Biol.* **126**, 1076–1088.
Rhee, S. G., Kim, K. H., Chae, H. Z., Yim, M. B., Uchida, K., Netto, L. E. & Stadtman, E. R. (1994). *Ann. NY Acad. Sci.* **738**, 86–92.
Sies, H. (1993). *Eur. J. Biochem.* **215**, 213–219.
Sundaresan, M., Yu, Z.-X., Ferrans, V. J., Irani, K. & Finkel, T. (1995). *Science*, **270**, 296–299.