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

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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 β = 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 β = 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