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Research review paper

Recent insights into microbial catalases: Isolation, production and purification

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ABSTRACT

Catalase, an oxidoreductase enzyme, works as a detoxification system inside living cells against reactive oxygen species formed as a by-product of different metabolic reactions. The enzyme is found in a wide range of aerobic and anaerobic organisms. Catalase has also been employed in various analytical and diagnostic methods in the form of biosensors and biomarkers in addition to its other applications in textile, paper, food and pharmaceutical industries. New applications for catalases are constantly emerging thanks to their high turnover rate, distinct evolutionary origin, relatively simple and well-defined reaction mechanisms. The following review provides comprehensive information on isolation, production and purification of catalases with different techniques from various microbial sources along with their types, structure, mechanism of action and applications.

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Introduction

Catalase (EC 1.11.1.6) is a haem-containing enzyme belonging to the oxido-reductase family. It is a significant component of the cell defense

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mechanism against oxidative stress, as it scavenges hydrogen peroxide (H_2O_2) to oxygen and water (Foyer and Noctor, 2000). All aerobic microorganisms have evolved complex inducible repair mechanisms, in the form of this enzyme, to alleviate the damaging effects of active oxygen (McCord and Fridovich, 1988). Catalase also plays a role in maintaining redox homeostasis of the cell as a part of the antioxidant response system (Dat et al., 2003; Mullineaux et al., 2006). Catalase is present in a wide range of plants, animals and microorganisms. It is typically localized in peroxisomes in eukaryotes, and cytosol in prokaryotes. Peroxisomes are essential sub-cellular organelles present in almost all eukaryotic cells, and are lacking in DNA and translational machinery. Hence, all peroxisomal matrix proteins are encoded by the nuclear genome and synthesized in the cytosol on free polyribosomes. These proteins are post-translationally imported into the pre-existing peroxisomes in a folded and even oligomerized form in eukaryotes (Williams et al., 2012; Wolf et al., 2010). The peroxisome is considered to be a protective compartment within eukaryotic cells that shields the surrounding cytoplasm from many toxic or harmful compounds which are produced and detoxicated by enzymes within this organelle (Wolf et al., 2010). A variety of indispensable metabolic reactions occurs in peroxisomes and the majority of these produce hydrogen peroxide as their by-product (Schrader and Fahimi, 2006). Therefore, catalase is generally targeted to these organelles because it is a peroxisome-localized enzyme that efficiently decomposes hydrogen peroxide. The presence of catalase at the site of hydrogen peroxide production (peroxisomes) is also thought to prevent the leakage of this reactive compound from the organelle to other cellular compartments (Gabaldon, 2010). However, some evidence of cytosolic catalases from eukaryotes such as *Saccharomyces cerevisiae* (Wieser et al., 1991), *Caenorhabditis elegans* (Taub et al., 1999), and *Neurospora crassa* (Schliebs et al., 2006) has also been reported. In prokaryotes, translational processes for synthesis of catalase occur in cytosol because they lack well-defined cellular organelles.

Types, structure and mechanism of action

Catalases, depending on their physical and biochemical properties, are divided into four types: monofunctional haem catalases (typical or classical catalase), catalase–peroxidases (atypical), non-haem catalases (pseudocatalases) and minor catalases. The ribbon representation of different types of catalases has been presented in Fig. 1. The first class, monofunctional haem catalase, is the original class of catalases seen ubiquitously in animals, plants and microorganisms. The structure of catalases varies with respect to the number and identity of domains in different species of organisms. Typically catalases exist as a dumbbell-shaped tetramer of four identical subunits. The molecular mass of monofunctional catalases is 200–340 kDa with a haem prosthetic group at the catalytic center. The haem group is responsible for catalase enzymatic activity and is located between the internal walls of the beta barrel and several helices (Frankenberg et al., 2002; Lee et al., 2007; Zamocky and Koller, 1999). The classification of haem catalases into three clades has evolved from at least two gene duplication events (Klotz et al., 1997). Clade 1 catalases have about 500 residues per subunit and are mainly of plant origin, including a subgroup of bacterial origin. Clade 2 catalases, with approximately 750 residues per subunit, are mostly of bacterial or fungal origin. These are also reported in several archaea due to horizontal gene transfer events (Diaz et al., 2012; Nicholls et al., 2001). The presence of large subunits in clade 2 catalase is due to a conspicuous C-terminal domain of nearly 150 residues (Horvath and Grishin, 2001). Clade 3 catalases with nearly 500 residues per subunit are found in archaea, bacteria, fungi and some eukaryotes. The absence of these older taxonomic groups of catalases suggests that they arose later in its evolution (Diaz et al., 2012). The crystal structure of a monomer of typical haem catalase (Fig. 2) from *Saccahromyces cerevisiae* comprises four distinct structural regions, namely, the N-terminal arm (containing 70 amino acids); the β-barrel domain (positions 72–318); a domain connection, also called wrapping domain (319–439 residues); and a α-helical domain (positions 440–503) (Zamocky and Koller, 1999). An extra domain called C-terminal region (also known as "Flavodoxin-like" domain) of about 150 residues was also observed in a small subgroup of the typical catalases studied so far. Further, it has been reported that β-barrel domain of catalase is well conserved from lower prokaryotes to higher eukaryotes, although the highest degree of divergence was observed in the C and N-terminal areas. Some variability in the domain connection and α-helical domain was also prevalent in typical catalase structures of different organisms.

The second catalases group, catalase–peroxidase, is a less prevalent class (Chelikani et al., 2004). This catalase is found primarily in aerobic bacteria and has a molecular mass in the range of 120–340 kDa (Nagy et al., 1997; Obinger et al., 1997). Catalase–peroxidases are not found in plants and animals (Zamocky et al., 2008) but they do resemble plant and fungal peroxidases (Chelikani et al., 2004). These bifunctional catalases are haem enzymes characterized by their peroxidative activity in addition to the catalytic activity (Kapetanaki et al., 2007; Welinder, 1991). A stable tetramer form of this enzyme has been observed in HPII of *Escherichia coli*, although, catalase–peroxidase generally occurs in dimer forms usually having about 750 residues in each subunit. In dimer forms, the two subunits of catalase–peroxidase are organized into separate N-terminal and C-terminal globular domains and these are arranged along the axis of longest subunit (Diaz et al., 2012). Wilming and Johnsson (2001) have observed that the haem group is present only in the N-terminal domain and this domain includes an extension of about 60 residues, where at least 30 residues are disordered in all structures analyzed. The C-terminal domain of this enzyme occludes the site corresponding to the haem pocket with the segment of the protein. A distinctive feature in most catalase–peroxidase structures is the presence of a covalent adduct in which tyrosine is attached at its ortho position to methionine on one side and a tryptophan is linked on the other side (Donald et al., 2003).

The third group comprises non-haem catalases, a minor bacterial protein family with a di-manganese active site rather than a haem. These are also called manganese catalases or pseudo-catalases. These enzymes exhibit molecular weight in the range of 170–210 kDa. The structure of the manganese catalase of *Lactobacillus plantarum*, in a homohexameric form contains approximately 30 kDa subunits. A bridged binuclear manganese center is located in each subunit within a conserved packed 4-helix bundle domain (Barynin et al., 2001). The highly conserved essential ligands (glutamate, histidine) form typical signatures for the manganese catalase sequence. The classification of these non-haem catalases into five distinct clades has occurred due to lateral gene transfer between various bacterial taxa. Clade 1 is spread among archaebacteria and at least one firmicutes, while clade 2 includes actinomycetes and firmicutes. Clade 3 is distributed among proteobacteria and firmicutes. Clade 4 comprises mainly the *Bacteroides* genus as well as many cyanobacteria, whereas clade 5 includes proteobacteria. It has been observed that clades 1 and 2 share a common ancestor, while, clade 5 is the most distantly related to the other clades (Zamocky et al., 2008).

The fourth minor class of catalases includes several haem-containing proteins and these exhibits a very low level of catalytic activity, possibly due to the presence of haem. These include bifunctional enzymes such as chloroperoxidases, bromoperoxidase (Nicholls et al., 2001), and catalase–phenol oxidases (Vetrano et al., 2005). Chloroperoxidase occurs as a 42 kDa monomer having one haem b group bound in an eight-helical-segment array. The gross structure and active site of this enzyme are different from other catalases and peroxidases. The major difference is the presence of cysteine in the proximal side, as a fifth ligand to the haem iron, rather than histidine or tryptophan. The other significant difference is the presence of glutamic acid, as a single catalytic residue on the distal side of the haem, rather than histidine in combination with asparagine or arginine as found in other catalases. Catalase–phenol oxidase, the other minor bifunctional catalase, is a tetrameric haem protein with molecular mass of 320 kDa. This enzyme has unique