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Review

Catalase in peroxidase clothing: Interdependent cooperation of two cofactors in the catalytic versatility of KatG

Olive J. Njuma, Elizabeth N. Ndontsa [1], Douglas C. Goodwin *

Department of Chemistry and Biochemistry, Auburn University, Auburn, AL 36849-5312, USA

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ABSTRACT

Catalase-peroxidase (KatG) is found in eubacteria, archaea, and lower eukaryotae. The enzyme from *Mycobacterium tuberculosis* has received the greatest attention because of its role in activation of the anti-tubercular pro-drug isoniazid, and the high frequency with which drug resistance stems from mutations to the *katG* gene. Generally, the catalase activity of KatGs is striking. It rivals that of typical catalases, enzymes with which KatGs share no structural similarity. Instead, catalatic turnover is accomplished with an active site that bears a strong resemblance to a typical peroxidase (e.g., cytochrome *c* peroxidase). Yet, KatG is the only member of its superfamily with such capability. It does so using two mutually dependent cofactors: a heme and an entirely unique Met-Tyr-Trp (MYW) covalent adduct. Heme is required to generate the MYW cofactor. The MYW cofactor allows KatG to leverage heme intermediates toward a unique mechanism for H_2O_2 oxidation. This review evaluates the range of intermediates identified and their connection to the diverse catalytic processes KatG facilitates, including mechanisms of isoniazid activation.

Introduction

In 1979, Claiborne and Fridovich reported isolation of an enzyme with robust catalase activity *and* a classical peroxidase activity [1]. The copurification of the two with a single enzyme (known then as HPI) was noteworthy. Both catalases and peroxidases were known for their two-electron reduction of H_2O_2 to water. However, the two were starkly different with respect to the oxidative half reaction. On one hand, catalatic turnover operates primarily in electron pairs. So H_2O_2 is oxidized to O_2, and even the so-called peroxidatic activity of catalases involves the two-electron oxidation of small organic substrates like ethanol. In contrast, single-electron oxidations are typical of peroxidases, converting a wide range of aromatic compounds to their corresponding free radicals. In this, HPI displayed typical peroxidatic behavior, catalyzing the oxidation of *o*-dianisidine [1] and several other compounds as well [2,3].

For many reasons, catalase-peroxidases (KatGs)[2] have been the subject of increasingly intense scrutiny. One is their remarkable catalytic versatility. In addition to the activities explicitly specified in the name, peroxynitritase [4], manganese peroxidase [5], NADH oxidase [6], hydrazinolytic [6], and isonicotinoyl-NAD (IN-NAD) synthase [7,8] activities have all been observed. The latter has drawn great attention because it is the central event in the activation of the front line anti-tubercular pro-drug isoniazid or isonicotinic acid hydrazide (INH).

It is difficult to overstate the threat to human health posed by *M. tuberculosis*. According to the WHO, 8.7 million new active cases and 1.4 million deaths from tuberculosis (Tb) were reported in 2011. Adding to this alarming situation, 3.7% of new cases and 20% of previously treated cases were from multidrug resistant (MDR) strains. Globally, combined rates of treatment failure or mortality for MDR exceed 20%, and extensively drug resistant (XDR) Tb (9% of all MDR cases) has now been reported in 84 countries [9]. INH has been one of the most widely used and effective anti-tubercular agents. However, mutations to the *katG* gene have been the most common characteristic of INH resistance [10–14].

In addition, KatG is a particularly rich target to address heme protein structure and function. Though KatGs show great versatility, the catalase activity clearly dominates over the others. In terms of maximum output (i.e., k_{cat}), ~5,000 s^{-1} is typical, and KatG efficiency with respect to H_2O_2 (i.e., k_{cat}/K_M) is ~10^6 M^{-1} s^{-1} [15]. In the latter, KatGs rival the activity of typical (i.e., monofunctional) catalases [16]. By comparison, a KatG peroxidase k_{cat} is ~100-fold less, and its k_{cat}/K_M with respect to H_2O_2 is at least tenfold lower. When one turns to other KatG activities like NADH oxidase and IN-NAD synthase, rates are substantially less than 1 s^{-1} [15].

* Corresponding author. Fax: +1 334 844 6959.
E-mail address: goodwdc@auburn.edu (D.C. Goodwin).

[1] Current address: Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037, USA.

[2] *Abbreviations used:* KatG, catalase-peroxidase; INH, isoniazid; IN-NAD, isonicotinoyl-NAD; Tb, tuberculosis; MDR, multidrug resistant; XDR, extensively drug resistant; CcP, cytochrome c peroxidase; APx, ascorbate peroxidase; MtKatG, *Mycobacterium tuberculosis* KatG; BpKatG, *Burkholderia pseudomallei* KatG, SyKatG, *Synechocystis* KatG; EcKatG, *E. coli* KatG; MYW, Met-Tyr-Trp cofactor; rR, resonance Raman; HS, high-spin; LS, low-spin; QS, quantum mechanically mixed spin; 5c, pentacoordinate; 6c, hexacoordinate; PAA, peracetic acid; $por^{\bullet+}$, porphyrin cation radical; SOD, superoxide dismutase; X/XO, xanthine/xanthine oxidase.

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Yet, even before the first structure was known [17], sequence data was clearly showing that KatG had no relationship with the large- or small-subunit heme catalases, its activity profile notwithstanding. Rather, KatG fit squarely within the non-animal peroxidases [18–22], now known as the peroxidase-catalase superfamily [23]. Comparisons of their active sites leave no doubt that KatG belongs in class I of the superfamily along with cytochrome *c* peroxidase (CcP) and ascorbate peroxidase (APx) (Fig. 1 inset). KatG also strongly resembles peroxidases from classes II and III [17,24–29]. However, conspicuous by its absence is catalase activity. Except for KatG, no member of the superfamily produces catalatic turnover much in excess of 10 s^{-1}.

How is it that KatG, an enzyme with a peroxidase active site, has catalase activity whereas its closest relatives are nearly completely incapable of the same? The answer is directly connected to the cooperative and reciprocal relationship between two cofactors: one is among the most widely utilized cofactors in biology, and the other is entirely unique to KatG. Together they impart a catalytic versatility that is not observed in typical catalases, nor in the other members of KatG's own superfamily. This review is focused on the nature of these cofactors as they appear in KatG, the intermediates they support, and the novel catalytic mechanisms that result. In addition, participation of these cofactors in the activation of the antitubercular agent INH is examined.

The KatG resting state

Heme

KatG uses heme *b* with a His imidazole as the protein-derived fifth ligand. The heme plane bisects the active site, and by convention, the proximal half is that which contains this His ligand. As with every other member of its superfamily, the proximal His (^{Px}His) ligand of KatG is modulated by a strong H-bond with a strictly conserved Asp (^{Px}Asp) (Fig. 1). The analogous Asp in CcP imparts a strong anionic character to the ^{Px}His, and a low reduction potential for the heme iron (−182 mV) is the result [30]. Consequently, reduction potentials for multiple KatGs have been measured between −186 and −226 mV [31–33]. This is at variance with earlier studies on *M. tuberculosis* KatG (MtKatG) ($Fe^{III}/Fe^{II} \sim -60$ mV) [34]. Nevertheless, the potential of the heme iron is sufficiently negative that all KatG enzymes isolated to date have been found in the Fe^{III} state.

Generally, the structure of KatG ensures the predominance of high-spin (HS) heme iron with low-spin (LS) states observed either in very low abundance or not at all [35–38]. This depends to some extent on the gene-duplicated C-terminal domain. KatG lacking this domain displays exclusively hexacoordinate (6c) LS heme where the sixth strong-field ligand appears to be the distal His (^{Ds}His) [39,40]. The mechanism by which the C-terminal domain exerts this influence over the N-terminal domain active site (>30 Å away) is not completely understood, but it may involve a series of H-bonded interactions at the intersubunit interface [41].

Although HS heme dominates, KatGs have long shown a multiplicity of HS states [2,3,42–44]. By EPR, these are distinguished by their *g*-tensor anisotropy, most readily apparent in the $g \sim 6$ region of the spectrum. At a minimum, two signals corresponding to endpoints on a scale of rhombic distortion have been widely observed. At one end of the continuum, is a wide rhombic signal with $g_x \sim 6.6$ and $g_y \sim 5.1$. At the other is an axial signal with $g_\perp \sim 5.9$. Other signals with intermediate rhombic distortion are also routinely detected, including a narrow rhombic species ($g_x \sim 6.0$ and $g_y \sim 5.6$) and an intermediate species ($g_x \sim 6.3$ and $g_y \sim 5.3$) [35,45–47]. The number of states and the degree to which they are populated relate to the position and proximity of H_2O around the sixth coordination site of the heme iron. As such, the distribution of these HS coordination states is highly sensitive to pH, buffer identity, enzyme age, substrates, and amino acid substitution in or around the enzyme active site [35,37,45,46].

Likewise, resonance Raman (rR) studies consistently identify a multiplicity of HS coordination states (5c and 6c) from the core-size marker bands (ν_3 in particular) [33,36,38,48,49]. Some have noted contributions from a 6c LS species [36,48,49]. In these reports, the 5c HS state appeared to dominate. Others noted that what at first appeared to be a 6c LS state (ν_3 feature at 1503 cm^{-1}) did not behave as such upon closer examination, prompting its assignment as a quantum mechanical admixture (QS) of 5/2 and 3/2 spin states. At neutral pH there was an equal distribution between the 5c HS, 6c HS, and QS states [38]. As observed with EPR, the distribution of coordination states is greatly sensitive to pH, substitution of active site residues and other fac-

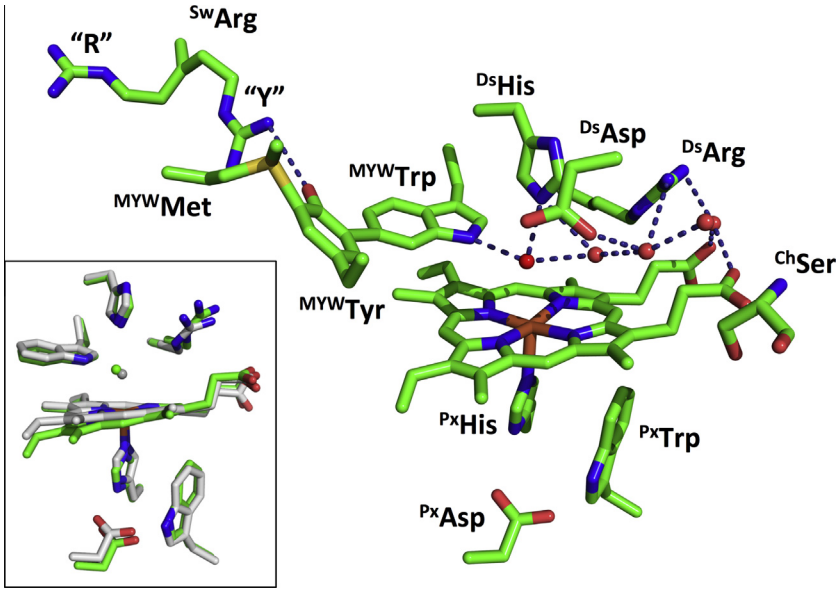

Fig. 1. The KatG active site. Residues of the proximal cavity are indicated by Px (e.g., ^{Px}His). Residues of the distal cavity are indicated by Ds (e.g., ^{Ds}His). Members of the MYW cofactor are indicated (e.g., ^{MYW}Trp) as are the "R" and "Y" conformations the arginine switch (^{Sw}Arg) and the access channel serine (^{Ch}Ser). Inset: An overlay of the conserved active site residues of cytochrome *c* peroxidase (carbons in gray) (PDB: 2CYP [24]) and KatG (carbons in green) (2CCA [107]). Images constructed using PyMOL v.1.6.0.0 [127].