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Electron Transfer in Catalases and Catalase-Peroxidases

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Definition

Catalases (EC 1.11.1.6) are enzymes that catalyze the disproportionation of hydrogen peroxide into water and molecular oxygen by means of a heme iron or a dimanganese active site. They are crucial metalloproteins regulating the cellular concentration of hydrogen peroxide, which has a concentration-dependent dual role in cell signaling and oxidative stress.

Catalase-peroxidases, also named KatGs from the encoding *katG* gene, are heme-containing oxidoreductases capable of both the disproportionation of hydrogen peroxide and the oxidation of substrates via high-valent heme intermediates. KatGs also generate superoxide via an oxidase reaction and activate the antitubercular prodrug isoniazid (INH) through its conversion to isonicotinyl-NAD.

Basic Characteristics

Catalases are protective enzymes that efficiently decompose H_2O_2 into H_2O and O_2. They fall in three distinct phylogenetic groups: heme monofunctional catalases, heme catalase-peroxidases, and manganese catalases. The key catalytic reactions are expected to be driven primarily by the heme active site. Yet, there is increasing evidence that nature has developed strategies to expand such reactivity by using long-range electron transfer pathways (LRET) and protein-based radicals as alternative catalytic sites. Evidence for LRET related to catalysis exists for the two heme-containing groups. Both monofunctional catalases and catalase-peroxidases react with hydrogen peroxide directly at the heme iron active site, where it serves as oxidant ($2e^-$ oxidation, R1) of the resting ferric state to generate the oxoferryl porphyrin radical intermediate ($Fe^{IV}{=}O\ Por^{\bullet+}$), known as Compound I (Cpd I) (Nicholls et al. 2001). A second molecule of H_2O_2 reduces Cpd I back to the resting state ($2e^-$ reduction, R2). Alternatively, electron-donating substrates (S) can lead to two subsequent $1e^-$ reductions in a peroxidase-like cycle (R3), with substrates binding remotely from the heme and inducing LRET events.

$$Fe^{III}Por + H_2O_2 \rightarrow Fe^{IV}{=}O\ Por^{\bullet+} + H_2O \quad \text{(R1)}$$

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G. C. K. Roberts, A. Watts (eds.), *Encyclopedia of Biophysics*,
https://doi.org/10.1007/978-3-642-35943-9_51-1

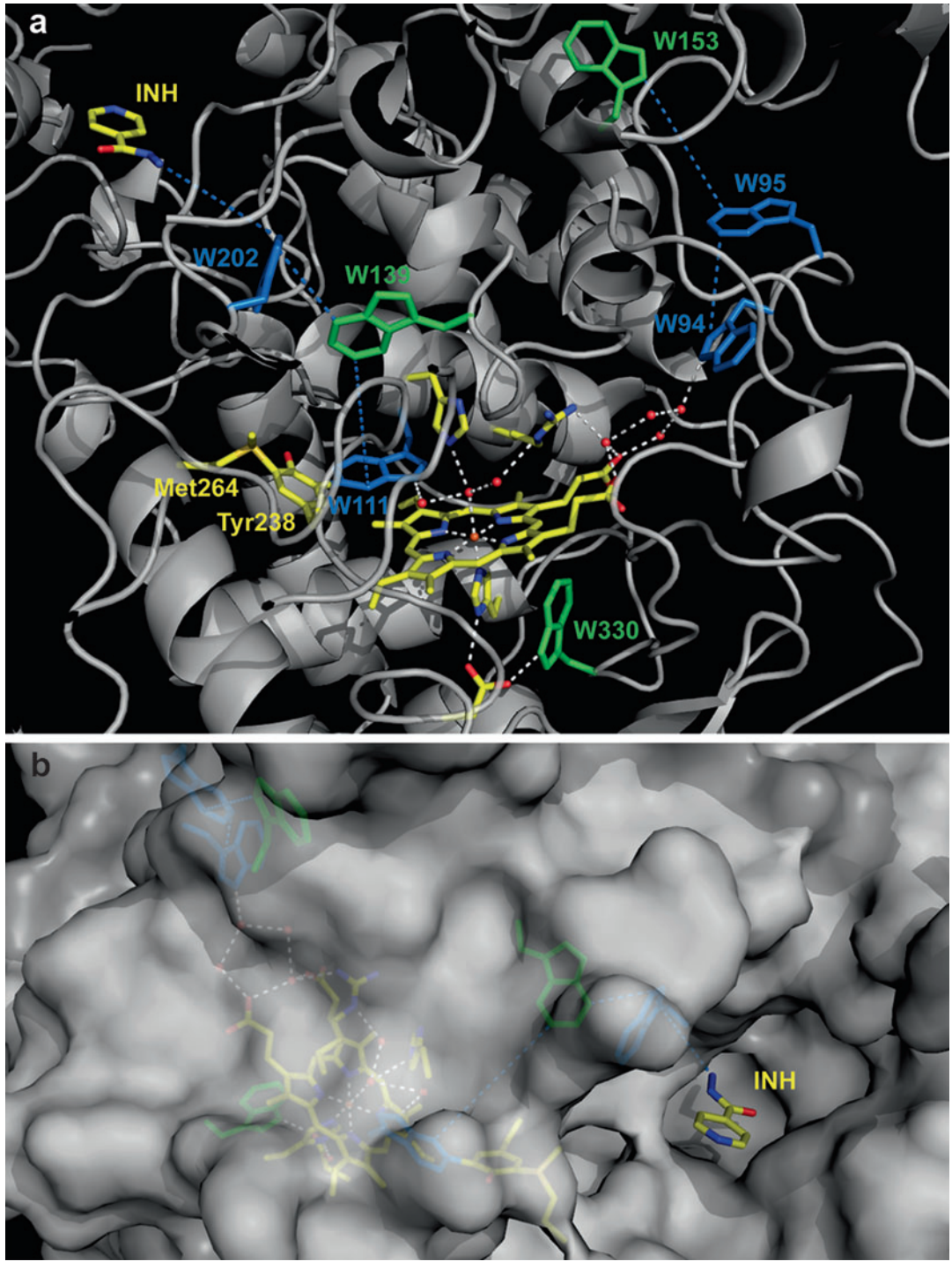

Fig. 1 (continued)

$$Fe^{IV}{=}O\ Por^{\bullet+} + H_2O_2 \rightarrow Fe^{III}Por + H_2O + O_2 \quad (R2)$$

$$Fe^{IV}{=}O\ Por^{+\bullet} + S\text{-}H \rightarrow Fe^{IV}{=}O\ Por + S_{ox} + H^+ + S\text{-}H \rightarrow Fe^{III}Por + 2S_{ox} + H_2O \quad (R3)$$

This review describes the reactions catalyzed by these heme enzymes and addresses the question of why internal LRET is needed in catalases.

Monofunctional Catalases

Two different lines of evidence suggest a role for LRET in monofunctional catalases. One involves the movement of electrons from nicotinamide adenine dinucleotide phosphate (NADPH) to the heme cavity. This cofactor, first discovered in bovine liver catalase, has been identified in catalases from bacteria to humans (Kirkman and Gaetani 2006). In conditions of low H_2O_2 concentrations, the slow $2e^-$ reduction of Cpd I back to resting state of catalases allows the formation and accumulation of the $Fe^{IV}{=}O$ Por intermediate, known as compound II (Cpd II) that is unreactive with H_2O_2. Yet, when NADPH is bound to catalases (15 Å away from the heme), an enhancement of the H_2O_2 disproportionation is observed. Electron transfer (ET) from NADPH to the heme, involving a through-bond ET process, is inferred to convert Cpd II back to resting ferric state (Olson and Bruice 1995; Sicking et al. 2008). While experimental verification of such a LRET process is missing, the evidence for electron movement makes this process reminiscent of a peroxidase-like reaction.

The second line of evidence for LRET in catalases comes from the identification of an unprecedented $Fe^{IV}{=}O\ Tyr^{\bullet}$ intermediate by multifrequency Electron Paramagnetic Resonance (EPR) spectroscopy in bovine liver catalase (Ivancich et al. 1999). In a peroxidase-like reaction, intramolecular ET from a Tyr residue to the heme converts the Cpd I intermediate of catalase into an oxoferryl heme and the $Tyr^{\bullet}$ species (R4).

$$Fe^{IV}{=}O\ Por^{\bullet+}\ Tyr \rightarrow Fe^{IV}{=}O\ Por\ Tyr^{\bullet} + H^+ \quad (R4)$$

The formation of the $Fe^{IV}{=}O\ Tyr^{\bullet}$ intermediate in a subgroup of heme catalases showing lower catalase-specific activity (Colin 2008) implies an inherent peroxidase-like reactivity, for which either the full pathway has been lost in evolution or for which the natural substrate(s) is(are) unknown. Support for this conclusion is found in the catalase from *Bacillus pumilus* (BPC), which exhibits relevant peroxidase reactivity and binds a number of substituted phenols. Co-crystallization of BPC and substrates shows that pyrogallol and catechol can access to the heme and bind on the heme distal side, while larger phenols bind at remote sites (Loewen et al. 2015). Oxidation of larger phenols must involve LRET, although the pathways are yet experimentally unidentified.

Electron Transfer in Catalases and Catalase-Peroxidases, Fig. 1 **Panel A**: Structure of the N-terminal domain of *B. pseudomallei* KatG with isoniazid (INH) bound (PDB reference 3N3N). The cross-sectional view, perpendicular to the main access channel of hydrogen peroxide to the heme active site, shows the heme environment. The three radical sites (Trp330, Trp139, and Trp153) for the $Fe^{IV}{=}O\ Trp^{\bullet}$ intermediates, identified by multifrequency EPR spectroscopy, are shown in green. The tryptophan residues in blue and the structural waters in red are required for electron transfer between Trp153 or Trp139 and the heme, with the paths indicated by blue and white dashed lines. The long distance (24.6 Å) between the INH substrate and the heme precludes single-step tunneling phenomena to occur at biologically relevant rates. Hence, multistep tunneling or electron hopping is likely to occur, with distances of 8.1 Å from INH to Trp202, 4.5 Å from Trp202 to Trp139, and 9.7 Å from Trp139 to Trp111. Interestingly, the distance between INH and Trp139 is 13.3 Å and thus well within the predicted limit of 14 Å (Page et al. 1999), but Trp202 appears to be required as electron relay even in this case. **Panel B**: Surface view of BpKatG with isoniazid (INH) bound in a lateral funnel-shaped channel. The surface is rendered with partial transparency to reveal the buried heme site on the opposite side of the protein, with a distance of ca. 25 Å between the heme iron and the INH substrate

Catalase-Peroxidases

Catalase-peroxidases, commonly called KatGs, contain an unprecedented adduct of three cross-linked residues Met-Tyr-Trp (Fig. 1) in the heme microenvironment that, along with a mobile Arg, is required for the catalase reaction (R1 and R2). Radical formation on this adduct has been proposed as essential to the catalase reaction in *M. tuberculosis* KatG (Zhao et al. 2010, Njuma et al. 2017). A comprehensive mechanism for the catalase reaction in KatGs has been proposed, based on DFT calculations and involving a transient radical on the adduct, an ionizable Trp, and the mobile arginine (Loewen et al. 2014; Kruft et al. 2015).

Multifrequency EPR studies have unequivocally identified specific tryptophan radicals ($Trp^{\bullet}$) in KatGs from *Synechocystis* (Ivancich et al. 2003), *Mycobacterium tuberculosis* (Singh et al. 2007), and *Burkholderia pseudomallei* (Colin et al. 2009) formed as stable and catalytically competitive oxoferryl-$Trp^{\bullet}$ intermediates in the peroxidase reaction. *B. pseudomallei* KatG represents the more general case among bacterial KatGs, with three unique $Trp^{\bullet}$ intermediates formed on Trp330, Trp156, and Trp139 (Fig. 1). Trp153, being close to the enzyme surface, and Trp139 being halfway between the heme and the surface, both require a LRET pathway for the intramolecular ET to the heme, involving structural waters and Trp94, Trp 95, and Trp111 acting as electron relays (Fig. 1). Trp139 is close to a funnel-shaped channel, where isoniazid binds (Wiseman et al. 2010), and thus consistent with the Fe^{IV}=O $Trp^{\bullet}$ species identified earlier as the reactive intermediate for INH oxidation (Singh et al. 2007). $Tyr^{\bullet}$ radicals are also formed in KatGs with no specific role in the peroxidase reaction but possibly related to transporting away from the metal active site; the potentially damaging oxidizing equivalents formed with very high excess of oxidant (Ivancich et al. 2013; Gray and Winkler 2015). The multiplicity of LRET pathways in KatGs implies possible intramolecular LRET for other substrates, which may not access to the heme site or may require oxidation potentials unattained by the heme (Smith et al. 2009).

Catalase-peroxidases also catalyze the reduction of molecular oxygen to superoxide using electron donors such as NADH (Singh et al. 2004) and isonicotinyl hydrazide (INH) (Wagenack et al. 1999). The route for electron transfer from NADH to the heme is not known, but the pathway leading through Trp139 is used for transfer from INH (Wiseman et al. 2010). The role of superoxide formation in normal metabolism is not clear, but the process of prodrug INH activation in *M. tuberculosis* KatG has adapted superoxide to the formation of IN-NAD. In conclusion, superoxide formation and the peroxidatic process are quite different processes, but they use the same underlying electron transport pathways to bring electrons to the heme cavity from a surface-bound electron donor.

Cross-References

- ▶ Ascorbate Peroxidase
- ▶ Cytochrome c Peroxidase
- ▶ Electron Transfer Theory
- ▶ Electron Transfer Through Proteins
- ▶ EPR
- ▶ Heme
- ▶ Nicotinamide Adenine Dinucleotide
- ▶ Peroxidases

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