Journal of Inorganic Biochemistry 104 (2010) 648–656

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

Disruption of the H-bond network in the main access channel of catalase–peroxidase modulates enthalpy and entropy of Fe(III) reduction

Jutta Vlasits [a], Marzia Bellei [b], Christa Jakopitsch [a], Francesca De Rienzo [b,c], Paul G. Furtmüller [a], Marcel Zamocky [a], Marco Sola [b,c], Gianantonio Battistuzzi [b], Christian Obinger [a,*]

[a] Department of Chemistry, Division of Biochemistry, BOKU, University of Natural Resources and Applied Life Sciences, Muthgasse 18, A-1190 Vienna, Austria
[b] Department of Chemistry, University of Modena and Reggio Emilia, via Campi 183, 41125 Modena, Italy
[c] Centro S3, CNR-Istituto di Nanoscienze, Via Campi 213A, 41125 Modena, Italy

ARTICLE INFO

Article history:
Received 11 January 2010
Received in revised form 15 February 2010
Accepted 23 February 2010
Available online 3 March 2010

Keywords:
Catalase–peroxidase
Catalase activity
Reduction potential
Redox thermodynamics
Enthalpy
Entropy

ABSTRACT

Catalase–peroxidases are the only heme peroxidases with substantial hydrogen peroxide dismutation activity. In order to understand the role of the redox chemistry in their bifunctional activity, *catalatically*-active and inactive mutant proteins have been probed in spectroelectrochemical experiments. In detail, wild-type KatG from *Synechocystis* has been compared with variants with (i) disrupted KatG-typical adduct (Trp122-Tyr249-Met275), (ii) mutation of the catalytic distal His123-Arg119 pair, and (iii) altered accessibility to the heme cavity (Asp152, Ser335) and modified charge at the substrate channel entrance (Glu253). A valuable insight into the mechanism of reduction potential ($E^{\circ\prime}$) modulation in KatG has been obtained from the parameterization of the corresponding enthalpic and entropic components, determined from the analysis of the temperature dependence of $E^{\circ\prime}$. Moreover, model structures of ferric and ferrous *Synechocystis* KatG have been computed and used as reference to analyze and discuss the experimental data. The results, discussed by reference to published resonance Raman data on the strength of the proximal iron-imidazole bond and catalytic properties, demonstrate that $E^{\circ\prime}$ of the Fe(III)/Fe(II) couple is not strongly correlated with the bifunctional activity. Besides the importance of an intact Trp-Tyr-Met adduct, it is the architecture of the long and constricted main channel that distinguishes KatGs from monofunctional peroxidases. An ordered matrix of oriented water dipoles is important for H_2O_2 oxidation. Its disruption results in modification of enthalpic and entropic contributions to $E^{\circ\prime}$ that reflect reduction-induced changes in polarity, electrostatics, continuity and accessibility of solvent to the metal center as well as alterations in solvent reorganization.

1. Introduction

Catalase–peroxidases (KatGs, EC 1.11.1.7) have raised considerable interest, since, despite their striking sequence homologies with heme peroxidases like ascorbate peroxidase (APX) or cytochrome *c* peroxidase (CcP) [1], they exhibit substantial catalase activity similar to monofunctional catalases. Thus, they are ideal model oxidoreductases to study structural modifications that enable a peroxidase to efficiently dismutate hydrogen peroxide. The available crystal structures of KatGs from *Haloarcula marismortui* (pdb code 1ITK), *Burkholderia pseudomallei* (1MWV), *Mycobacterium tuberculosis* (1SJ2), and *Synechococcus* PCC 7942 (1UB2) [2–5] revealed that the principal organization of their active site is very similar to those of CcP [6] and APX [7,8], including the proximal (His290-Trp341-Asp402; *Synechocystis* PCC 6803 numbering) and the distal (Arg119-Trp122-His123) triads (Fig. 1). Unique to KatG and essential for *catalatic* activity (that is completely absent in both APX and CcP) is a highly conserved peculiar distal site adduct that includes Trp122-Tyr249-Met275 [2–5,9] and plays a role as transient radical site in the *catalatic* turnover [10,11]. In addition, the access channel to the deeply buried heme *b* of KatGs is longer and more restricted than in typical (monofunctional) peroxidases. KatG-specific large loop insertions build up this channel and also connect the distal and proximal domains. Large loop 1 (LL1) is of particular interest, since it contains a number of highly conserved residues that are essential for efficient H_2O_2 oxidation. These include Tyr249 (part of the covalent adduct), Ile248 and Glu253, the latter creating an acidic entrance to the channel. Isoleucine 248 is hydrogen-bonded to KatG-specific Asp152 that, together with Ser335, forms the narrowest part of the channel and controls access to the distal heme cavity (Fig. 1). Resonance Raman data and structural analysis demonstrated the existence of a very rigid and ordered structure built up by the interaction of these residues with distal and (via LL1) proximal amino acids, with the heme itself, and with the solute matrix in the channel. Disruption of these interactions typically affected the catalase but not the peroxidase activity of KatG [12,13].

* Corresponding author. Department of Chemistry, Division of Biochemistry, BOKU, Muthgasse 18, A-1190 Vienna, Austria. Tel.: +43 1 47654 6073; fax: +43 1 47654 6059.
E-mail address: christian.obinger@boku.ac.at (C. Obinger).

0162-0134/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2010.02.006

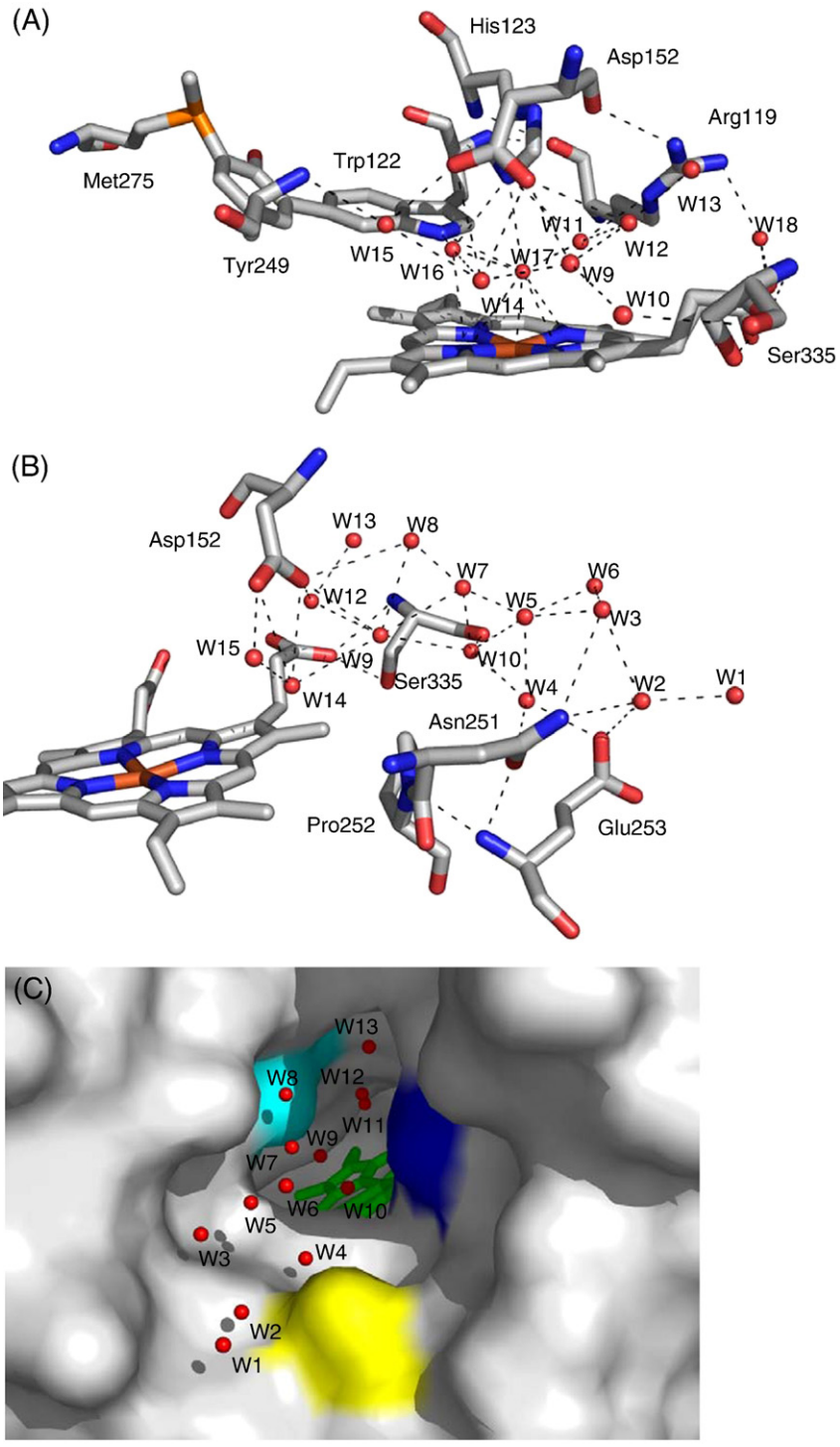

Fig. 1. (A) Detailed view of the active site of KatG from *B. pseudomallei* (*Synechocystis* numbering) with the interacting water (W) molecules starting at the constriction of the channel. Water molecules are numbered 9–18 and correspond to the following structural water molecules of *Burkholderia*. W9, position 3086; W10, position 3180; W11, position 2653; W12, position 2511; W13, position 3138; W14, position 3071; W15, position 2764; W16, position 3897; W17, position 3085; W18, position 2229. (B) Detailed view of the main channel showing the residues investigated in this study, including the amino acids asparagine and proline, and the interacting water molecules. W9, W10 and W12–W15 correspond to the positions in (A). W1, position 3558; W2, position 2472; W3, position 2860; W4, position 3149; W5, position 3620; W6, position 4107; W7, position 3898; W8, position 3770. (C) Surface structure of the main cannel of KatG. *Green*, heme; *blue*, Ser335 (*Burkholderia*: Ser324); *cyan*, Asp152 (Asp141); *yellow*, Glu253 (Glu242); *red*, water molecules; W1–W8 correspond to the positions in (B) and W9–W13 to the positions in (A). The figures were constructed using the coordinates deposited in the Protein Data Bank (accession code 1MWV).

Here, we addressed the impact of the protein matrix and of the organization of ordered and oriented water dipoles on the reduction potential of the heme iron. We have investigated the thermodynamics of the one-electron reduction of ferric heme in wild-type KatG from *Synechocystis* and in variants that lack (i) the KatG-typical adduct (Trp122Phe, Tyr249Phe), (ii) the peroxidase-typical catalytic residues (Arg119Asn, His123Gln) and (iii) that feature an altered solute matrix in the access channel (Asp152Ser, Ser335Gly and Glu253Gln). Analysis of the temperature dependence of the reduction potentials ($E^{\circ\prime}$), allowed parameterization of the corresponding enthalpic and