An enigmatic catalase of Blastocrithidia

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ABSTRACT

Here we report that trypanosomatid flagellates of the genus Blastocrithidia possess catalase. This enzyme is not phylogenetically related to the previously characterized catalases in other monoxenous trypanosomatids, suggesting that their genes have been acquired independently. Surprisingly, Blastocrithidia catalase is less enzymatically active, compared to its counterpart from Leptonomas pyrrhocoris, posing an intriguing biological question why this gene has been retained in the evolution of trypanosomatids.

Catalase (EC 1.11.1.6) is a ubiquitous enzyme, usually involved in oxidative stress protection. It contains a heme cofactor in its active site and converts hydrogen peroxide (H$_2$O$_2$) to water and oxygen [1]. The hydrogen peroxide is typically produced in the mitochondria by a superoxide dismutase using extremely harmful anion superoxide as a substrate [2]. H$_2$O$_2$ is a small molecule that can penetrate the cell membrane, often playing a role of a secondary messenger in many biochemical reactions [3]. However, in the presence of iron, H$_2$O$_2$ can be converted to a highly reactive and toxic hydroxyl radical by the Fenton reaction.

Most species use catalase to control intracellular level of hydrogen peroxide. Nevertheless, several prominent examples of organisms, lacking this enzyme, have been recently reported. It is presumed that these species rely on other biochemical pathways to detoxify intracellular H$_2$O$_2$ (reviewed in [4]).

Kinetoplastid flagellates of the family Trypanosomatidae [5] is one of such peculiar examples. These parasites infect either exclusively invertebrates (monoxenous species) or invertebrates and vertebrates or plants (dixenous species) [6]. Most of the analyzed trypanosomatids do not possess catalase-encoding gene in their genomes. The only known exception to this rule so far was a group of monoxenous relatives of Leishmania (representatives of the insect-infesting genera Crithidia, Leptomonas, and Novyomona) [7]. Conspicuously, dixenous Leishmania have secondarily lost catalase. It has been proposed that hydrogen peroxide plays a role in promastigote-to-amastigote differentiation of these parasites [8]. Thus, presence of a catalase appears to be incompatible with dixenous life cycle of Leishmania [4].

One of the trypanosomatid groups insufficiently investigated in this regard is the genus Blastocrithidia. These parasites recently came into prominence because of their confounding genetic code with all three stop codons reassigned to encode amino acids. One of these codons (UAU) also serves the genuine translation terminator [9].

Genomic analysis of two species belonging to this genus (Blastocrithidia sp. p57 and B. triatomae) reveals that both possess a catalase gene (GenBank accession numbers MK934828 and MK934829, respectively). A phylogenetically-related flagellate with standard genetic code of the so-called ‘jaculum’ group [7] (hereafter called Trypanosomatidae sp. Fi-14) also encodes this enzyme in its genome (GenBank accession number MK934827). The sequences of trypanosomatid catalases display high level of conservation (Fig. 1). All amino acids involved in the heme binding (marked by black squares in Fig. 1) are invariant in all trypanosomatid species analyzed, while sequence motifs of the NADPH binding site (green boxes in Fig. 1) and tetramer interface (blue boxes in Fig. 1) differentiate between Leishmaniana (Cerithidia and Leptonomas spp.) and members of the Blastocrithidia / ‘jaculum’ clade. The catalase sequences of Blastocrithidia spp. (but not Trypanosomatidae sp. Fi-14) contain amino acid-coding in-frame stop codons UAG (Glu), UAA (Glu), and UGA (Trp) (Fig. 1). NCBI-CDD analysis
suggests that trypanosomatid catalases belong to the catalase clade 3. Clade 3 catalases are found in all three kingdoms of life. Their tetrameric protein structure is also conserved across these different groups of bacteria. While catalase might be one of the most efficient enzymes used for removal of hydrogen peroxide, trypanosomatid catalases are primarily used for detoxification of the toxic molecule. The enzymes' low activity in comparison to that of Leishmaniinae (magenta in Fig. 2A) suggests that these genes are translated with a lowered efficiency [9] and substantially reduced levels of both heme a and b, support this hypothesis.

Finally, we measured whether the lower catalase activity makes Blastocrithidia cells more sensitive to hydrogen peroxide in comparison to their Leishmaniinae counterparts. The ability of parasites to survive in different concentrations of H2O2 was determined by the base assay employing utilization of resazurin (Alamar Blue). Blastocrithidia sp. p57 demonstrated lower sensitivity (p < 0.0001) to the hydrogen peroxide than L. pyrrhocoris sp. p57 with IC50 of 0.35 and 0.15 mM, respectively (Fig. 3B). We concluded that Blastocrithidia spp. mainly rely on other (catalase-independent) mechanisms of oxidative stress protection. While catalase might be one of the most efficient enzymes used for removal of hydrogen peroxide, trypanosomatid catalases are primarily used for detoxification of the toxic molecule. The enzymes' low activity in comparison to that of Leishmaniinae (magenta in Fig. 2A) suggests that these genes are translated with a lowered efficiency [9] and substantially reduced levels of both heme a and b, support this hypothesis.

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two related evolutionary questions worth considering in this regard: i) why catalase has been acquired only in some trypanosomatid groups?, and ii) why has it been retained in Blastocrithidia / "jaculum" lineage, while it does not appear to be important for oxidative stress protection in these flagellates? The acquisition of such a robust biochemical system to combat harmful molecules [14] should be advantageous for the majority of these parasites. However, many trypanosomatid lineages do not have it, yet they survive in the same conditions using alternative detoxification pathways. Some of them can even lose catalase secondarily, as exemplified by Leishmania spp., which have disposed of this enzyme in evolution when it has become incompatible with their dixenous life cycle [4]. The key to answer this question may be the peculiarity of catalase as compared to other enzymes used for H2O2 decomposition: it is the only one producing O2. And while Leishmaniinae are very diverse in terms of host adaptation,

Blastocrithidia appear to be more uniform. We speculate that Blastocrithidia spp. have retained catalase to sense the gradient of hydrogen peroxide by the mean of the produced oxygen. H2O2 is produced by the cells of the insect intestinal wall to control microflora [15] and sensing it may be important for proper localization of trypanosomatids. In contrast to L. pyrrhocoris, which resides in the midgut lumen and never attaches to enterocytes [16], Blastocrithidia spp. tend to reach and anchor on the epithelium of the midgut wall and/or Malpighian tubules [17–19]. In these conditions, sensing peroxide molecules might help in orientation of the flagellates. Other potential explanations for the observed phenomenon can be proposed, and more work is needed to understand the biological role of catalase trypanosomatids.

Fig. 2. Catalase phylogeny reconstructed in IQ-TREE based on amino acid alignment prepared in MAFFT and trimmed in trimAl. The dataset was obtained using a BlastP search with the catalase sequence of Blastocrithidia sp. p57 as a query against NCBI nr database. It was then purged from duplicates (multiple sequences for one species), closely related sequences for prokaryotic species of one genus, and sequences of unidentified organisms. Numbers at branches are ultrafast bootstrap supports; the scale bar corresponds to the number of the substitutions per site. A – a subtree containing catalases of trypanosomatids, demonstrating their evolutionary remoteness and independent origin of this enzyme in Blastocrithidia / "jaculum" and Leishmaniinae clades; B – a subtree with the catalases of Leishmaniinae; C – a subtree with the catalases of Blastocrithidia spp. and Trypanosomatidae sp. Fi-14.

Fig. 3. Oxygen production upon addition of H2O2, levels of heme a and b (A) and cell survival after exposure to hydrogen peroxide (B). Numbers in A were measured as in [11]. For cell survival analysis, 10^6 parasites in 100 μl medium were exposed to a serial dilution of H2O2, starting from 51.6 mM. The plates were incubated for 1 h at room temperature, and then 10 μl of 5% resazurin solution was added to each well. After 47 h incubation at 23 °C, fluorescence (excitation wavelength 540 nm, emission wavelength 590 nm) was measured. Data from three independent biological replicates are presented in both panels.
Author contributions

VY conceived the study; VY and JL supervised the study; NK, EH and AYK designed experiments; CB, NK, KZ, EH, and RS performed experiments; CB, AYK, NK, EH, and RS analyzed data; CB and VY wrote the manuscript; JL, AYK, NK, and EH made manuscript revisions.

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