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Catalase from larvae of the camel tick *Hyalomma dromedarii*

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ABSTRACT

Catalase plays a major role in protecting cells against toxic reactive oxygen species. Here, Catalase was purified from larvae of the camel tick *Hyalomma dromedarii* and designated TLCAT. It was purified by ammonium sulfate precipitation and chromatography on DEAE-cellulose, Sephacryl S-300 and CM-cellulose columns. Gel filtration and SDS-PAGE of the purified TLCAT indicated that the protein has a native molecular weight of 120 kDa and is most likely a homodimer with a subunit of approximately 60 kDa. The Km value of TLCAT is 12 mM H_2O_2 and displayed its optimum activity at pH 7.2. $CaCl_2$, $MgCl_2$, $MnCl_2$ and $NiCl_2$ increased the activity of TLCAT, while $FeCl_2$, $CoCl_2$, $CuCl_2$ and $ZnCl_2$ inhibited the activity of TLCAT. Sodium azide inhibited TLCAT competitively with a Ki value of 0.28 mM. The presence of TLCAT in cells may play a role in protecting *H. dromedarii* ticks against oxidative damage. This finding will contribute to our understanding of the physiology of these ectoparasites and the development of untraditional methods to control them.

1. Introduction

All aerobic organisms during the course of metabolism form reactive oxygen species (ROS) as by products. Superoxide ($^{\bullet}O_2^-$), nitric oxide ($^{\bullet}NO$), hydroxyl ion radicals ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) are the common ROS [9]. ROS are maintained under certain levels by a battery of enzymatic and non-enzymatic molecules with antioxidant capacity. The enzymatic defense against oxidative stress primarily comprises of superoxide dismutase, catalase and glutathione peroxidases [26]. Catalase (CAT, H_2O_2: H_2O_2 oxidoreductase; EC 1.11.1.6) plays a key role in protecting cells against toxic ROS [17]. It is an antioxidant and hydroperoxidase enzyme that protects the cellular environment from harmful effects of H_2O_2 by facilitating its degradation to oxygen and water [5]. Aerobic organisms benefit substantially from the high energy yields obtained via controlled conversion of molecular oxygen to water, yet reactive intermediates are burden that cause cellular damage. Catalase is one of the antioxidant enzymes, which deals with removal of the oxidative damage in cells. It has a double function; it catalyzes the decomposition of H_2O_2 into oxygen and water (catalase activity) and also oxidizes electron donors such as ethanol, methanol, or phenols (peroxidative activity) [27,28]. Catalases are classified into four groups: monofunctional heme (typical) catalases, catalase-peroxidases, manganese catalases and catalase-phenol oxidases (CATPO). CATPOs are bifunctional enzymes being capable of H_2O_2 decomposition (catalase activity) and phenolic oxidation in the absence of H_2O_2 (phenol oxidase activity) [18]. Catalase is a common enzyme found in nearly all living organisms. It was the first antioxidant enzyme to be characterized. Catalase is a tetramer of four polypeptide chains, each over 500 amino acids long. It contains four porphyrin heme (iron) groups that allow the enzyme to react with the hydrogen peroxide [22]. Each of these four protein subunits also contains a molecule of NADPH [16]. Catalase has one of the highest turnover rates of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen [2].

Ticks are members of the arthropod class Arachnida, subclass Acari, and order Parasitiformes Ticks live on all continents of the world. Many of the 899 or so species of ticks are associated with disease in humans, livestock and wild life [4]. Studies about ecology, behavior and physiology of ticks afford a better understanding of these organisms and therefore become important tools to develop new control methods. Due to the rapid increase in pesticide-resistant tick populations [6], the study of tick physiology has gained increasing importance regarding the mechanisms involved in detoxification of toxins [11,19]. These mechanisms, in general, not only act against specific toxic molecules, but also help in the maintenance of physiologic homeostasis, e.g. in avoiding oxidative damage generated by ROS [7]. Catalase was purified from

Abbreviations: BSA, bovine serum albumin; CAT, catalase; PAGE, polyacrylamide gel electrophoresis; ROS, reactive oxygen species; SOD, superoxide dismutase; TLCAT, tick larvae catalase

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developing embryo and cuticle of the camel tick *Hyalomma dromedarii* [14,8] and three SOD isoenzymes were purified from its larvae [13]. This study aims at purification and characterization of CAT from the larvae of the camel tick *H. dromedarii* to investigate its role as antioxidant enzyme.

2. Materials and methods

2.1. Tick material

Engorged camel tick *H. dromedarii* females were collected from a Camel market near Cairo and held at 28 °C and 85% relative humidity. Eggs were collected daily from fertilized oviposition female ticks and incubated under the same condition until hatching larvae at day 27 then frozen immediately at −40 °C.

2.2. Chemicals

Phenylmethylsulfonylfluoride (PMSF), carboxymethyl-cellulose (CM-cellulose), diethylaminoethyl-cellulose (DEAE-cellulose), molecular weight marker kits for gel filtration and Sephacryl S-300 were purchased from Sigma Chemical Co. All other chemicals were of analytical grade.

2.3. Assay of catalase activity

The assay of CAT activity was carried out according to the method described by Aebi [1]. The assay reaction mixture contained in 3.0 ml total volume of 0.05 M potassium phosphate buffer pH 7.0 containing 0.02 M H_2O_2 and the reaction was started by addition of enzyme solution. The decomposition of H_2O_2 was followed as a decline in absorbance at 240 nm for 1 min. One unit of CAT activity was defined as the calculated consumption of 1 μmol of H_2O_2/min at 25 °C. The extension coefficient of H_2O_2 was taken to be 43.6 M^{-1} cm^{-1}.

2.4. Staining of CAT activity on native PAGE

Activity staining of CAT was determined as described by Harris and Hopkinson [10]. After electrophoresis, the gel is incubated in 3% H_2O_2 for about 15 min. Rinse the gel with distilled water and then immerse it in a 1:1 mixture of 2% Potassium ferricyanide and 2% Ferric chloride. Gently agitate the tray containing the gel for few minutes. Yellow bands of CAT activity appear on a blue green background.

2.5. Purification of camel tick larval catalase

2.5.1. Preparation of crude extract

Two grams of camel tick larvae were homogenized in 10 ml 0.02 M K-phosphate buffer pH 7.0, using a Teflon-pestled homogenizer. Cell debris and insoluble materials were removed by centrifugation at 12,000 × *g* for 20 min and the supernatant was saved and designated as crude extract.

2.5.2. Ammonium sulfate precipitation

The crude extract was brought to 70% saturation by gradually adding solid $(NH_4)_2SO_4$ and stirred for 30 min at 4 °C. The pellet was obtained by centrifugation at 12,000 × *g* for 30 min and dissolved in 0.02 M K-phosphate buffer pH 7.0 and dialyzed extensively against the same buffer.

2.5.3. DEAE-cellulose column chromatography

The dialyzed sample was chromatographed on a DEAE-cellulose column (12 × 2.4 cm^2 i.d.) previously equilibrated with 0.02 M K-phosphate buffer pH 7.0. The adsorbed proteins were eluted with a stepwise NaCl gradient ranging from 0 to 1 M prepared in the equilibration buffer at a flow rate of 60 ml/h. 5 ml fractions were collected and the fractions containing CAT activity were pooled and lyophilized.

2.5.4. Sephacryl S-300 column chromatography

The concentrated solution containing the CAT activity was applied onto a Sephacryl S-300 column (142 cm × 1.75 cm i.d.). The column was equilibrated and developed with 0.02 M K-phosphate buffer pH 7.0 at a flow rate of 30 ml/h and 2 ml fractions were collected.

2.5.5. CM-cellulose column chromatography

The concentrated solution containing the CAT activity obtained from the Sephacryl S-300 column was chromatographed on a CM-cellulose column (4 × 1.6 cm i.d.) previously equilibrated with 0.02 M Na-acetate buffer pH 5.6. The adsorbed proteins were eluted with stepwise NaCl gradient ranging from 0 to 0.3 M prepared in the equilibration buffer at a flow rate of 30 ml/h and 2 ml fractions were collected.

2.6. Electrophoretic analysis

Native gel electrophoresis was carried out with 7% PAGE according to Smith [30]. SDS-PAGE was performed with 12% polyacrylamide gel according to Laemmli [21]. The subunit molecular weight of the purified CAT enzyme was determined by SDS-PAGE as described by Weber and Osborn [32]. The proteins were stained with 0.25% coomassie brilliant blue R-250.

2.7. Protein determination

Protein was determined by the dye binding assay method of Bradford [3] using BSA as a standard protein.

3. Results

3.1. Purification of CAT from camel tick larvae

The CAT specific activity of the larval crude extract was found to be 25.9 units/mg protein. A typical purification scheme of CAT from the camel tick *H. dromedarii* larvae is presented in Table 1. After ammonium sulfate precipitation, most of the CAT activity was precipitated so that 89.7% of the activity was recovered. The DEAE-cellulose elution profile (Fig. 1a) revealed the presence of one major peak containing CAT activity designated TLCAT and eluted with 0.0 M NaCl. The DEAE-cellulose fractions were pooled, concentrated by lyophilization and applied onto a Sephacryl S-300 column. The elution profile of TLCAT on the Sephacryl S-300

Table 1
A typical purification scheme of catalase from the camel tick *H. dromedarii* larvae.

Purification step	Total mg	Total units	Recovery (%)	Specific activity	Fold purification
Crude extract	242	6260	100.0	25.9	1.0
70% $(NH4)_2SO_4$ fraction	192	5616	89.7	29.2	1.13
DEAE-cellulose fraction	25.8	4170	66.6	161.6	6.2
Sephacryl S-300 fraction	8.6	2988	47.7	347.4	13.4
CM-cellulose fraction	1.7	2120	33.8	1247.0	48.1