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Characterization of partially purified catalase from camel (*Camelus dromedarius*) liver

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The liver of camel has high level of catalase (32,225 units/g tissue) as commercially used bovine liver catalase. For the establishment of the enzyme, the rate of catalase activity was linearly increased with increase of the catalase concentration and incubation time. The procedure of partial purification of catalase from camel liver included preparation of crude extract, ammonium sulphate precipitation and chromatography on diethylaminoethyl cellulose (DEAE)-Sepharose. One peak catalase activity was obtained from the chromatography. The enzyme had optimum pH of 7.0. Camel liver catalase had broad optimum temperature between 25 and 40°C and was stable up to 25°C. The Km and Vmax values were found to be 22.7 mM H_2O_2/ml and 7.9 units/ml, respectively. All metal cations partially inhibited camel liver catalase with the exception of Hg^{2+} which had strong inhibitory effect, whereas 95% of its activity was lost at 1.0 mM. In conclusion, camel liver catalase can be used as an alternative commercial bovine liver catalase.

Key words: Camel, liver, catalase, purification, characterization.

INTRODUCTION

Camels are very versatile animals. They are well adapted to life in the desert because of their unique metabolic pathways which enable them to survive without food and water for a few days (Haghkhah and Madjlesi, 1999). Their capacity to thrive well and reproduce in semi-arid and arid areas, where other livestock hardly survive, makes them the most important domestic animal in these areas (Woldemeskel et al., 2001). Around the world, camels are kept for breeding, various agricultural operations, milk and meat production and transportation (Azwai et al., 1996). In the Middle East, camels are also reared as racing animals. Breeding and management of racing camels is a very lucrative business (Tinson et al., 2000).

Mammalian catalase belongs to the family of Fe-protoporphyrin IX containing proteins that include a variety of cytochromes, globins and peroxidases, and is one of the best characterized antioxidant enzymes (Deisseroth and Dounce, 1970). Bovine liver catalase consists of four identical subunits, and each of the four active sites contains a ferriporphyrin IX as a prosthetic group. As a homotetrameric heme-containing enzyme, catalase is known for its ability to convert hydrogen peroxide into water and oxygen. Now, there is no doubt that the contribution of catalases to protect the organisms from damages caused by reactive oxygen species is essential. Hydrogen peroxide, a by-product of various oxidases and superoxide dismutases (SODs), is not only toxic by itself, but in a Fenton-type reaction that can decompose to form the even more reactive hydroxyl radical (Zamocky and Koller, 1999). This radical is probably the most deleterious of the activated intermediates of oxygen, reacting with DNA, proteins and lipids in its proximity (Lindau-Shepard and Shaffer, 1993). This protective function is not only directly achieved by rapid cleavage of hydrogen peroxide and small organic peroxides, but also by other cellular enzymes, mainly SODs (Amstad et al., 1991; Michiels et al., 1994). Catalases are thought to protect SODs against inactivation by higher levels of hydrogen peroxide (Fridovich, 1995).

Catalase is used in the food industry for removing hydrogen peroxide from milk prior to cheese production (Schroeder et al., 1969). Another use is in food wrappers, where it prevents food from oxidizing (Murthy et al., 1981). It is used in the textile industry, for removing

hydrogen peroxide from fabrics to make sure the material is peroxide-free (Yoshimoto et al., 2007). The catalase is also utilized in an enzymatic oxidation reaction to depress the deactivation of the relevant enzyme and/or side reaction due to excessive production of H_2O_2 (Upadhya and Bhat, 2000; Yoshimoto et al., 2005). A minor use is in contact lens hygiene (a few lens); it is used as cleaning products that disinfect the lens using a hydrogen peroxide solution; a solution containing catalase is then used to decompose the hydrogen peroxide before the lens is used again (Cook et al., 1996). Recently, catalase has been used in the aesthetics industry. Several mask treatments combine the enzyme with hydrogen peroxide on the face with the intent of increasing cellular oxygenation in the upper layers of the epidermis. The catalase test is also one of the main tests used by microbiologists to identify species of bacteria (Brioukhanov et al., 2006).

Although, there is a wide use of bovine liver catalase in different applications, very little information on liver catalase from camel has been reported (Mousa et al., 2006). This study focused only on partial purification and characterization of catalase from camel liver.

MATERIALS AND METHODS

Camel liver (male) was obtained from Jeddah slaughter house and was immediately stored at -20°C until required for analysis.

Purification of catalase from camel liver

Preparation of crude extract

The catalase crude extract was prepared by homogenizing 5 g of camel liver in 20 ml of 20 mM Tris-HCl buffer buffer, pH 7.0 using a homogenizer. The homogenate was centrifuged at 10,000g for 15 min and the supernatant was designated as crude extract.

Ammonium sulphate precipitation

Solid ammonium sulphate (80% saturation) was added to the crude extract at 4°C with continuous stirring. The precipitate formed was collected by centrifugation at 10,000g for 20 min at 4°C and dissolved in minimum volume of 20 mM Tris-HCl buffer pH 7.0 and dialyzed overnight against the same buffer.

DEAE–Sepharose chromatography

The dialyzate was loaded on a DEAE-Sepharose column (10 x 1.6 cm i.d.) pre-equilibrated with 20 mM Tris-HCl buffer, pH 7.0 at 4°C. The column was thoroughly washed with the equilibration buffer until the UV absorbance of the column eluate returned to the base line. The bound proteins were eluted with a stepwise gradient of 0.0 to 0.2 M NaCl in the same buffer at a flow rate of 60 ml/h. Active fractions were pooled at 0.1 M NaCl.

Catalase assay

Catalase activity was determined according to Bergmeyer (1974).

The activity of catalase was measured spectrophotometrically at 240 nm using specific absorption coefficient at 0.0392 cm µmol^{-1} H_2O_2. The reaction mixture contained 0.5 ml of substrate solution made up of 30 mM H_2O_2 in a 50 mM Tris-HCl buffer, pH 7.0 and enzyme solution. One unit of catalase activity is defined as the amount of enzyme which decomposes 1 µmol of H_2O_2 ($\varepsilon_{240} = 0.0392$ cm µmol^{-1}) per min and the specific activity is expressed as µmol/mg protein under the test conditions.

Protein determination

Protein is quantified by the method of Bradford (1976) with bovine serum albumin as standard.

Characterization of catalase

The optimum pH for the catalase activity was determined by assaying the activity at different pH values, using the following buffers: 50 mM sodium acetate buffer (pH 3.6 to 6.5) and 50 mM Tris-HCl buffer (pH 7.0 to 8.0). The optimum temperature for catalase activity was determined by assaying the enzyme at temperatures from 10 to 60°C at pH 7.0. Heat stability was measured by incubating the enzyme at 10 to 60°C for 15 min in 50 mM Tris-HCl buffer, pH 7.0. After heat treatment, the enzyme solution was cooled and the residual activity assayed under standard assay conditions. Kinetic parameters of the enzyme for hydrogen peroxide as substrate were determined at pH 7.0. The values of Michael's constant (Km) and the maximum velocity (Vmax) were determined from Lineweaver Burk plot. The effects of various metal ions on enzyme activity were determined by pre-incubating the enzyme with 0.5 and 1.0 mM metal ions for 15 min and then assaying the enzyme activity. The activity assayed in the absence of metal ions was taken as 100%.

RESULTS AND DISCUSSION

For the establishment of the camel liver catalase, different concentrations of enzyme were added to the assay reaction mixtures and assayed for catalase activity. The rate of H_2O_2 degradation was found to be linearly increased with increase of the catalase concentration up to 6.6 units (Figure 1). The effect of incubation time on the activity of catalase was also carried out using a fixed protein concentration of crude catalase. The rate of H_2O_2 degradation was linearly increased with increasing incubation time up to 6 min (Figure 2).

Catalase is encoded by a single gene, which is highly conserved among species (Reimer et al., 1994; Quan et al., 1986; Nakashima et al., 1989). Mammals, including humans and mice, express catalase in all tissues, and a high concentration of catalase can be found in the liver, kidneys and erythrocytes (Deisseroth and Dounce 1970; Schisler and Singh, 1987). A study of catalase activity in mice showed that high values of catalase activity were found in the liver (66 and 100 units/g tissue), lung (2390 units/g tissue) and erythrocytes (6340 units/ml blood) (Nishikawa et al., 2002). In this study, camel liver showed a high level of catalase (32,225 units/g tissue).

The procedure of partial purification of catalase from camel liver included crude extract preparation,
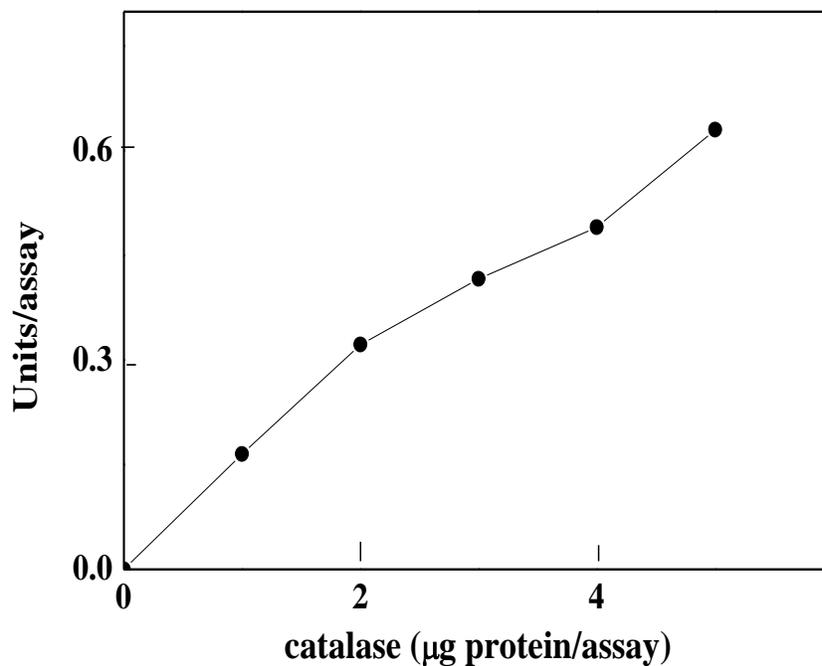
Figure 1. Effect of camel liver catalase concentration on the rate of catalase activity. Reaction mixture contained in 1.0 ml: 50 mM Tris-HCl buffer, pH 7.0, 30 mM H_2O_2 and different concentrations of enzyme. The reaction mixtures were incubated at room temperature for one minute and assayed for catalase activity under standard assay conditions.
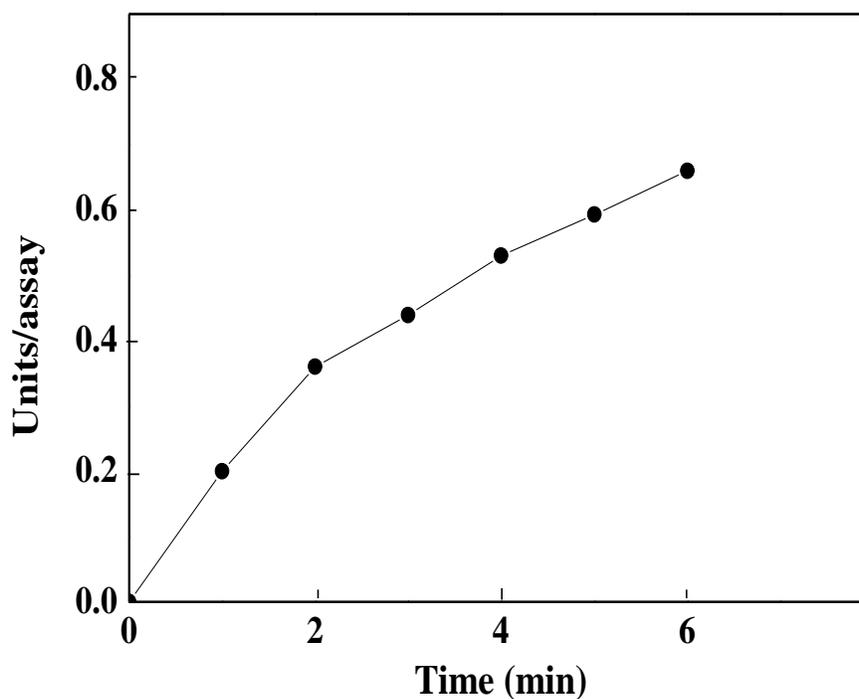

Figure 2. Effect of incubation time on catalase activity. Reaction mixture contained in 1.0 ml: 50 mM Tris-HCl buffer, pH 7.0, 30 mM H_2O_2, and fixed amount of camel liver catalase crude extract. The reaction mixtures were incubated for different time intervals and assayed for catalase activity under standard assay conditions.

Table 1. Purification scheme of catalase from camel liver.

Sample	Total units*	Total protein (mg)	Specific activity (units/mg protein)	Fold purification	Recovery (%)
Crude extract	161,125	220	732	1.0	100
Ammonium sulphate precipitation	99,750	85	1173	1.6	62
DEAE- Sepharose	21,200	12	4416	6.0	32

*One unit of catalase activity is defined as the amount of enzyme which decomposes 1 µmol of H_2O_2 (ε_{240} = 0.0392 cm µmol^{-1}) per min under standard assay conditions.
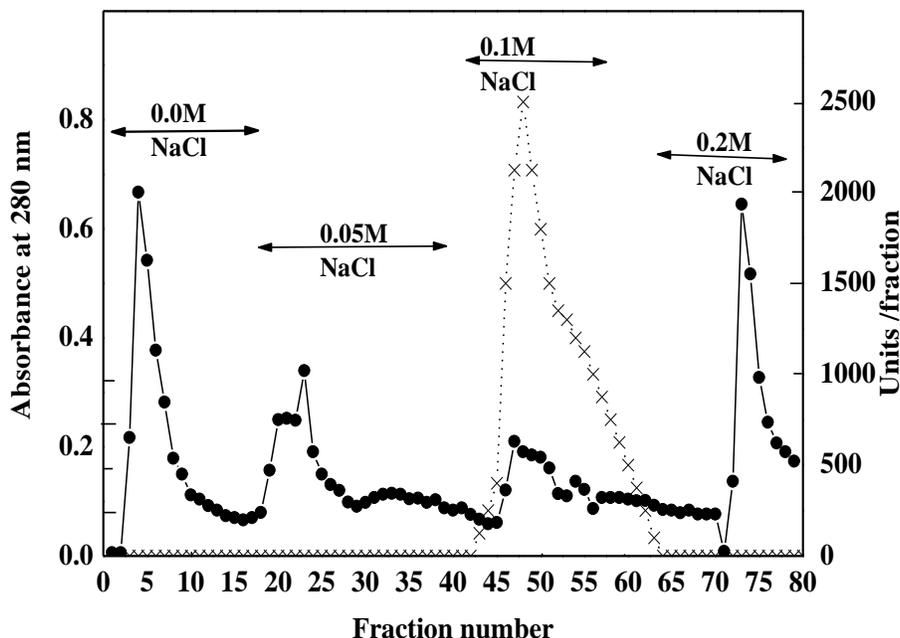

Figure 3. A typical elution profile for the chromatography of catalase from camel liver crude extract on DEAE-Spharose column (10 x 1.6 cm i.d.) equilibrated with 20 mM Tris-HCl buffer, pH 7.0 at a flow rate of 60 ml/h and 3 ml fractions. Absorbance at 280 nm (•—•), catalase activity (x---x).

ammonium sulphate precipitation and chromatography on DEAE-Sepharose (Table 1). Catalase was separated into one activity peak and eluted from the column at 0.1 M NaCl (Figure 3). The specific activity was found to be 4416 units/mg protein with a fold purification of 6.0. The specific activities of catalases from 16 different organisms ranged from 20,700 to 273,800 units/mg protein (Switala and Loewen, 2002).

Figure 4 shows that the optimum pH for camel liver catalase was detected at 7.0. Optimum pH of the crystalline bovine milk catalase was 8.0 (Ito and Akuzawa, 1983). Several catalases had broad optima pH. The activities of normal and acatalasemic dog liver catalases showed different pH profiles in a pH range from 3 to 11. The activity of catalase purified from normal dog liver was almost unchanged in a wide pH range and was still detectable even at 11. In contrast, the activity of the purified catalase from acatalasemic dog liver was stable

only in a narrow pH range of 6 to 9 (Nakamura et al., 2000). Although, the optimum pH for catalatic activity of *Hyalomma dromedarii* developing embryo catalase was found to be between 6.5 and 8.0, it exhibited a relatively sharp maximum at pH 8.5 for 3,4-dihydroxyphenylalanine (DOPA) oxidation (Kamel and Hamed, 1982). The broad optimum pH (5.0 to 9.0) was also reported for active fraction from human white adipose tissue (van Eyk et al., 1992). For silkworm recombinant catalase, the pH optimum was about 8.0 (Yamamoto et al., 2005).

The optimum temperature for camel liver catalase activity was broad between 25 and 40°C (Figure 5).

Optimum temperature of the crystalline bovine milk catalase was reported to be 20°C (Ito and Akuzawa, 1983). Camel liver catalase activity was stable up to 25°C and the enzyme retained 65 and 60% of its activity after 15 min of incubation at 40 and 50°C (Figure 6). At 37°C, the initial activity of normal dog liver catalase was
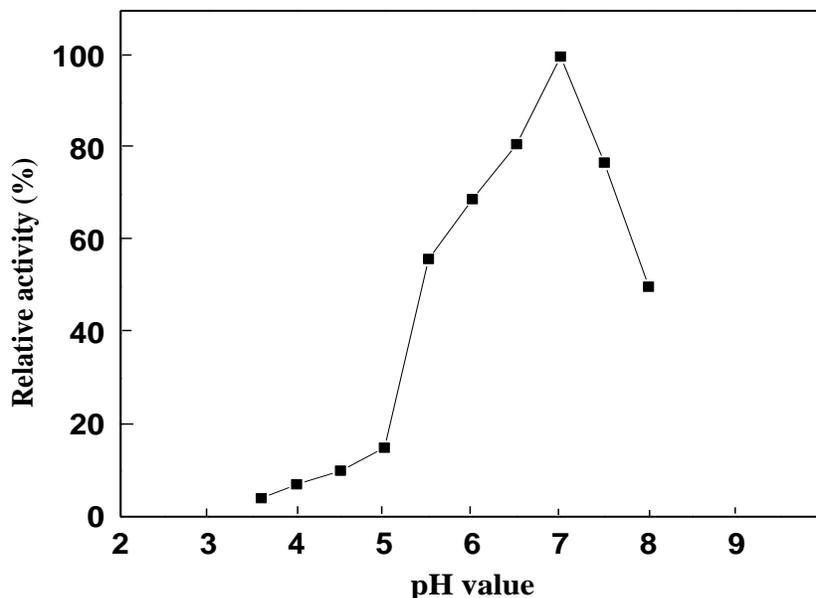
Figure 4. pH Optimum of catalase from camel liver DEAE-Sepharose fraction. The reaction mixture contained in 1 ml: 30 mM H_2O_2, suitable amount of enzyme and 50 mM sodium acetate buffer (pH 3.6-6.5), 50 mM Tris-HCl buffer (pH 7.0 to 8.0). Each point represents the average of two experiments.
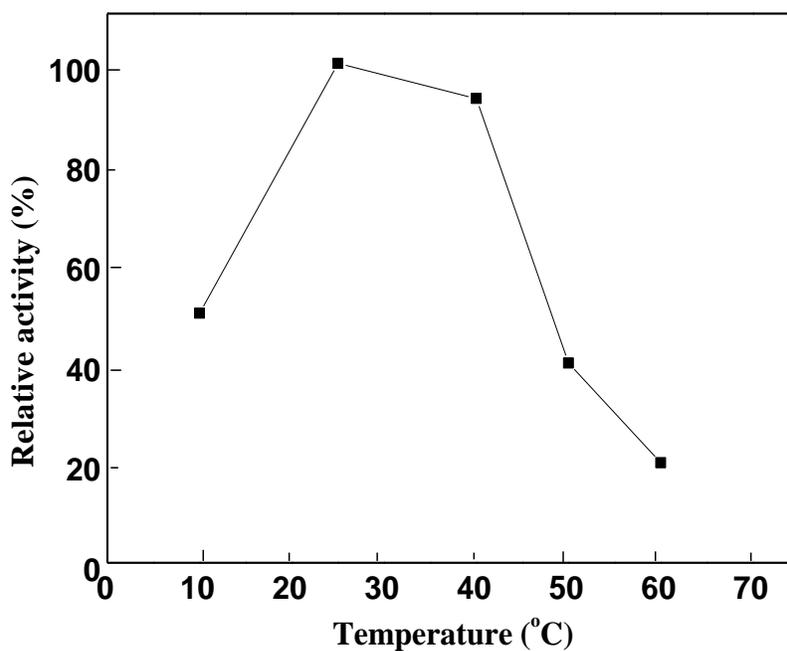

Figure 5. Temperature optimum of catalase from camel liver DEAE-Sepharose fraction. The enzyme activity was measured at various temperatures using the standard assay method as previously described. Each point represents the average of two experiments.

unaffected during 30 min of incubation. Under the same temperature condition, the acatalasemic dog liver catalase showed a decrease in the activity by about 10%.

When it was incubated at 45°C, the activity of the normal catalase decreased slowly, reaching almost 70% of the initial activity after 30 min. In contrast, the activity of the
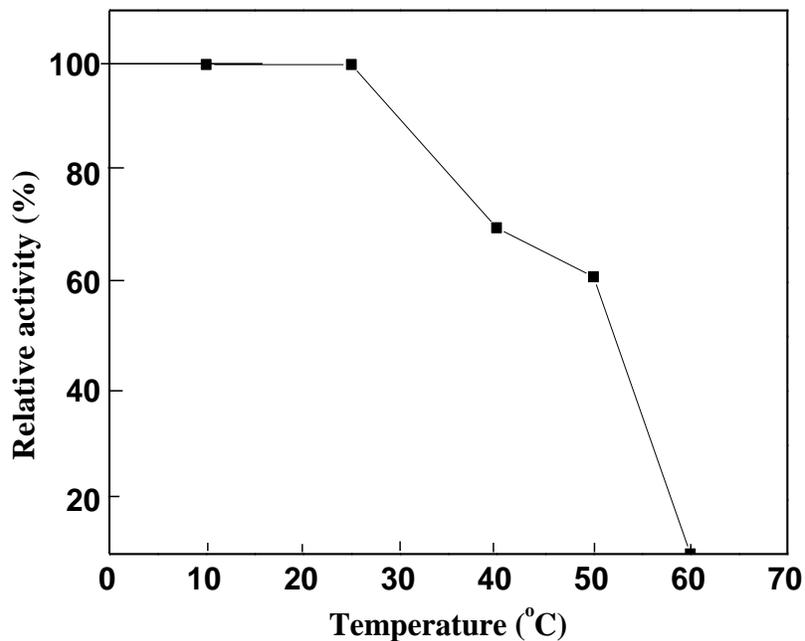
Figure 6. Effect of temperature on the thermal stability of catalase from camel liver DEAE-Sepharose fraction. The reaction mixture (1.0 ml) contained: 50 mM Tris-HCl buffer, pH 7.0. The reaction mixture was preincubated at various temperatures for 15 min prior to substrate addition, followed by cooling in an ice bath. The enzyme activity was measured using the standard assay method as previously described. Activity at zero time was taken as 100% activity. Each point represents the average of two experiments.
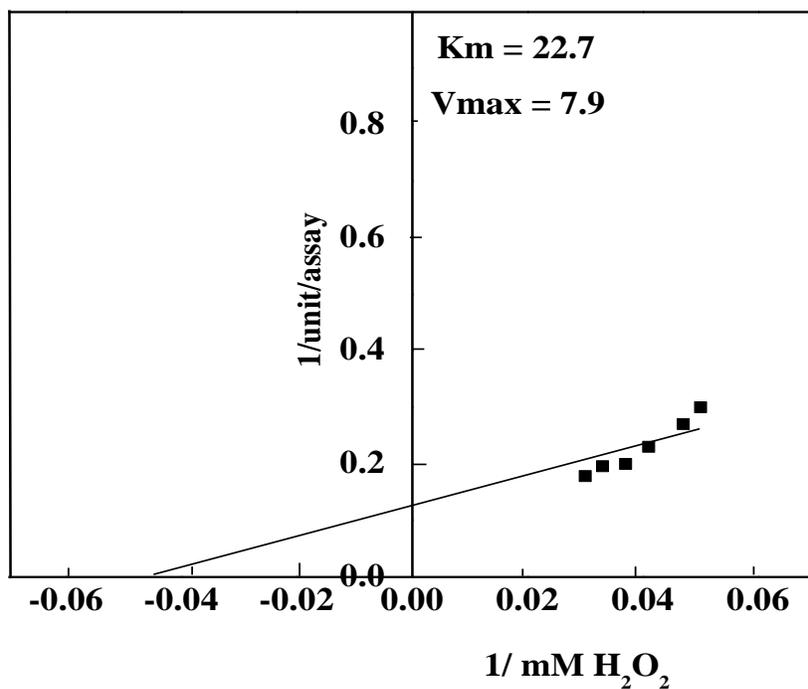

Figure 7. Lineweaver-Burk plot relating catalase from camel liver DEAE-Sepharose fraction reaction velocities to starch as substrate concentration. The reaction mixture contained in 0.5 ml: 50 mM sodium acetate buffer, pH 5.5, suitable amount of enzyme and concentrations of starch ranging from 1 to 6 mg.

Table 2. Effect of metal cations on catalase activity from camel liver DEAE-Sepharose fraction.

Metal cation	Relative activity (%)	
	0.5 mM	1.0 mM
Pb^{2+}	79	77
Ni^{2+}	77	71
Ca^{2+}	74	68
Zn^{2+}	67	65
Cu^{2+}	61	50
Co^{2+}	54	41
Hg^{2+}	42	5

Enzyme was preincubated for 15 min at room temperature with listed metal ions prior to substrate addition. Activity in the absence of metal cations was taken as 100% activity. Each value represents the average of two experiments.

acatalasemic dog liver catalase decreased dramatically during incubation at 45°C, reaching approximately 20% of its initial activity after 30 min (Nakamura et al., 2000). The temperature stability of catalase from human white adipose tissue was detected up to 50°C (van Eyk et al., 1992). Silkworm recombinant catalase was relatively stable during incubation at temperatures between 20 and 40°C, but catalase activity was lost at 70°C (Yamamoto et al., 2005). The crystalline bovine milk catalase had a thermostable range below 40°C (Ito and Akuzawa, 1983).

The Km and Vmax values for camel liver catalase was found to be 22.7 mM H_2O_2/ml and 7.9 units/ml, respectively (Figure 7). Similar Km values for catalases from beef liver (Km 25 mM) (Abe et al., 1979) and *H. dromedarii* developing embryo (Km 28 mM) (Kamel and Hamed, 1982) were reported. The low Km value (8 mM) for catalase was reported for human white adipose tissue (van Eyk et al., 1992).

The effect of metal cations at 0.5 and 1.0 mM concentrations on camel liver catalase activity was studied (Table 2). Pb^{2+}, Ni^{2+}, Ca^{2+} and Zn^{2+} partially inhibited the enzyme activity with 65 to 79% of its activity which was retained. A moderate inhibitory effect was obtained by Cu^{2+} and Co^{2+}, where 41 and 50% of activity were retained, respectively. Hg^{2+} strongly inhibited camel liver catalase, where 95% of its activity was lost at 1.0 mM. The decomposition of H_2O_2 by catalase was measured in a cell-free *in vitro* system in the presence of 0 to 24 mM Ni^{2+} or Mg^{2+} as well as in red blood cells (RBCs), and in post-mitochondrial fractions of liver and kidney of rats. The inhibition of the catalytic activity of catalase was directly proportional to Ni^{2+} concentration, while Mg^{2+} had no effect (Rodriguez et al., 1990).

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