Clinical and thermodynamical properties of catalase enzyme in sera of Iraqi diabetic patients

Hussein Najm Abed¹, Mahmoud Hussein Hadwan²*
¹Chemistry Dept., College of Science, Al-Qadisiyah University, (IRAQ)
²Chemistry Dept., College of Science, University of Babylon, (IRAQ)
E-mails: mahmoudhadwan@gmail.com

ABSTRACT
Catalase is an antioxidant enzyme existing in most of the animal cells; it defends them from oxidative damage by catalyzing the rapid decay of hydrogen peroxide in two kinds of reactions. The aim of the present study was to assess the catalase activity in normal subjects and patients who suffer from diabetes mellitus. Serum catalase activity was assessed by spectrophotometric method in 120 diabetic patients (type II) and 60 healthy subjects. Catalase activity increased in diabetic patients compared to control group. The increase in the serum catalase enzyme is belonged to the oxidative damage of human tissues by increment oxygen free radicals in the body. To correlate our results with the catalase thermodynamically behavior, the thermodynamic constants of activation ΔH, Ea and ΔS, were determined using Arrhenius plot and found to be 11.96 KJ/Mol, 22.44 KJ/male and 2.39 KJ/Mol/K, respectively.

INTRODUCTION
Diabetes mellitus is a collection of metabolic disorders illustrated by hyperglycaemia that is produced from defects in insulin secretion, insulin action or both[1]. It is classified into two types. Type 1 diabetes, which is known as juvenile onset diabetes, and type 2 diabetes that known as non-insulin dependent diabetes. Type 2 diabetes induces by two primary metabolic defects: insulin resistance and progressive pancreatic β-cell dysfunction[2]. β-Cell dysfunction applied on insulin resistance results in hyperglycaemia and consequently to type 2 diabetes[3]. Treatment is aimed to decrease blood glucose levels to normal or near normal values[4,5]. Exercise and diet are the first management of selection for patients with type 2 diabetes mellitus. When they do not complete sufficient blood glucose control, they start with oral antidiabetes treatment is then advocated[6]. First-line monotherapy usually initiates with metformin or sulfonylurea (an insulin Secretagogue), which inhibits hepatic gluconeogenesis[7]. When monotherapy failure, these agents are often given in combination[8]. Through diabetes, constant hyperglycemia causes raised production of free radicals, particularly reactive oxygen species (ROS), for all
tissues from glucose auto-oxidation and protein glycosylation\cite{9,10,11}. Oxidative stress results from an imbalance between radical-scavenging systems and radical-generating, i.e., decreased activity of antioxidant defenses or free radical production or both. The oxidative stress may be implicated in the pathogenesis of diabetes, not only by oxygen free-radical production, but also because of auto-oxidation of glucose, non-enzymatic protein glycosylation\cite{12}, impaired glutathione metabolism\cite{13}. There are defense mechanisms from free radicals as the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), whose activities contribute to remove superoxide, hydrogen peroxide and hydroxyl radicals\cite{14}. The catalase reaction is essentially a dismutation reaction resembling SOD; one H$_2$O$_2$ is reduced to H$_2$O and the other is oxidized to O$_2$\cite{15}. Red blood cells (RBCs) are the major source of antioxidants\cite{16,17} and the Catalase is a significant antioxidant formed by RBC; hence, catalase levels can indicate ROS levels\cite{18}. The present study evaluates the clinical and thermodynamic properties of catalase enzyme activity in sera of Iraqi diabetic patients.

**MATERIALS AND METHODS**

**Patients**

A clinical study was performed in Al-Najaf Center for Diabetes and Endocrinology (Al-Najaf City, Iraq) during 2015. The inclusion criteria were clinical T2DM with duration of diabetes of at least one year. Controls were selected from healthy adult volunteers with established levels of FBS<126 mg/dl. Patient characteristics and laboratory values are shown in TABLE 1. A total of 120 subjects with T2DM and 60 healthy persons were enrolled. The patients were under treatment with glibenclamide or metformin.

**Thermodynamic studies**

The relation between the rate of an enzymatic reaction and activation energy is obtained by the empirical formula of the Arrhenius equation (equation 1)

$$E_a = R \cdot \ln \left( \frac{V_2}{V_1} \cdot \left( \frac{1}{T_1} \cdot \frac{1}{T_2} \right) \right)$$  \hspace{1cm} (1)$$

Where $V_1$ and $V_2$ are the enzyme activities at the temperatures $T_1$ and $T_2$; $E_a$ is the energy of activation (kJ mol$^{-1}$) which can be determined from the slope of the Arrhenius plot of ln($V$) against $1/T$.

The activation enthalpy ($\Delta H$) can be calculated by equation 2.

$$\Delta H = E_a - RT.$$  \hspace{1cm} (2)$$

Finally the entropy ($\Delta S$) was calculated by equation 3 (Eyring-Polanyi), which correlates $\Delta H$, $E_a$, and Arrhenius equation (equation 1);

$$\ln \left( \frac{V_{\text{max}}}{T} \right) = \ln \left( \frac{K_B}{h} \right) + \frac{\Delta S}{R} - \frac{\Delta H}{R} \cdot \frac{1}{T}.$$  \hspace{1cm} (3)$$

Where $T$, $K_B$, $h$, and $R$ are absolute temperature, Boltzmann constant, Planck constant and gas constant respectively.

**Analytical methods**

**Preparation of catalase from human erythrocytes**

Blood was drawn onto citrate phosphate-dext-
trose tube. The erythrocytes were pelleted by centrifugation at 1800xg for 2 min. The pellet was resuspended in 5 volumes of 20 mM phosphate bufferin 0.9 % NaCl containing 1 mM EDTA and pelleted as above. The washing process was repeated 3 times. Finally, a 50 ml erythrocyte pellet was lysed in 9950 ml of distilled water and the hemolysate was centrifuged at 13000 xg for 5 min. Supernatant was kept in the refrigerator and used for the measurement of catalase activity.

Determination of catalase activity

The assessment of catalase activity is based on the reaction of undecomposed hydrogen peroxide with ammonium molybdate to give a yellowish color, which has a maximum absorbance at 354 nm. 20 ml of sample was incubated with 1.5 ml of 60 mM phosphate buffer solution (pH 7.4) at 37°C. The reaction was started by the addition of 0.5 ml of 30 mM hydrogen peroxide. After incubation for three minutes, catalase activity was determined by mixing the reaction mixture with ammonium molybdate. Absorbance was measured at 354 nm. Catalase activity was calculated from the rate constant of a first-order reaction equation.

Effects of pH and temperature

Catalase activity was measured in a pH range of 3.0-11.0. Different buffer systems were used in accordance with respective pH ranges: 50 mM citrate-phosphate buffer for pH 3.0-6.0, 50 mM potassium phosphate buffer for pH 6.0-7.0, 50 mM Tris-HCl buffer for pH 7.0-9.0, and 50 mM carbonate buffer for pH 9.0-10.0. To characterize the effects of temperature, standard reaction mixtures were assayed for catalase activity at different temperatures, in a range of 15°C-80°C.

Statistical analysis

Data analysis was performed using SPSS 17 for Windows (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± SD were evaluated by one-way analysis of variance (ANOVA). The statistical significance level was considered at P < 0.05.

RESULTS

Our study has shown that the level of CAT was elevated significantly in diabetic patients than control.

Effect of temperature on enzyme activity

Figure 1 showed the optimum temperature curve for Catalase. The complete assays of enzyme were incubated at different temperatures from 25 to 45°C for 3 minutes. Results showed that catalase had an optimum temperature at 35°C and after 35°C the temperature is decreased because of denaturation of enzyme.

The enzyme activity, when studied at different pH values ranging from 3.0 to 10.0 is found to be maximum at pH 7.0 (Figure-2)

Thermodynamic parameters (Ea, ΔH, and ΔS)

The activation energy (Ea) can be determined from the slope of the empirical formula of the Arrhenius plot of natural logarithm of the catalase activity versus the reciprocal value of temperature (Figure 3a). The activation energy was found to be 22.44 kJ/mol. Both enthalpy of activation (ΔH) and

![Figure 1: The effect of temperature on enzyme activity.](image-url)
entropy of activation ($\Delta S$) were calculated using Arrhenius plot as shown in Figure 3b and were found to be 11.96 kJ/mol, 2.39 kJ/mol respectively.

**DISCUSSION**

Catalase is one of numerous antioxidant defense enzymes (Peroxidase, SOD, Glutathione peroxidase) that catalyses dismutation of hydrogen peroxide into oxygen and water. It is a tetrameric heme including enzyme present in all aerobic organisms is acting as a key role in protecting cells versus oxidative stress\[19\]. The increment of catalase activity in patients with diabetes as compared to the control group may be related to promotion ROS levels. Higher levels of ROS have been exposed to play a role in the progress of diabetic complications. An accumulation of ROS in diabetes cannot be accurately balanced by antioxidant systems. Consequently, when oxidative stress growths as a result of a pathologic state, a protection system stimulates the regulation and expression of antioxidant enzymes. The results of the present study designates the presence of some change in oxidant antioxidant balance in diabetic patients. The increase in the serum catalase is associated with the oxidative stress. The pH has an important role in the activity of the enzyme. The catalase was found to yield maximum activity at pH 7 (Figure 2). These results, perhaps clarified by the fact that acidic pH has an inhibitory effect on the enzyme leading to decreasing its activity. Also, the survival of the active sites in amino acids will be affected by the change in pH, which may modify the ionization of these amino acids. The optimum temperature, where the maximum catalase activity carries out, is equal to 35°C. The kinetic energy of molecules raises with an increase in temperature. When the temperature was additional increased, the molecules of enzyme surpass the barrier of energy. This lead to the breakage of hydrophobic and hydrogen bonds that are dependable for preserving the three dimensional structure of the enzyme\[20, 21\].

![Figure 2: the effect of pH on catalase activity](image)
![Figure 3: Arrhenius plot for the activity of catalase, for the calculation of (a) activation energy, and (b) entropy of activation and enthalpy of activation.](image)
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REFERENCES


