The Islamic University Journal (Series of Natural Studies and Engineering)
Vol.17, No.1, pp 37- 45 , **2009**, ISSN 1726-6807, http://www.iugaza.edu.ps/ara/research/

Thermodynamic Properties of Hydrogen Peroxide Binding to Bovine Liver Catalase

Zeyad Yasseen[a*] and Salah Jadallah[b]
Faculty of Science, Islamic University-Gaza (IUG), Palestine.
Fax: +970 8 2860700.
[a]E-mail: zyasseen@iugaza.edu.ps.
[b]E-mail: sjadalla@iugaza.edu.ps.

Abstract: Catalase [CAT: EC 1.11.16] was purified from bovine liver using cellulose affinity chromatography and some enzyme characteristics were investigated. The interaction of H_2O_2 with catalase was investigated by fluorescence spectroscopy, and the change in intrinsic fluorescence intensity at 435 nm was used to estimate the association constant of the interaction. The binding process resulted in a change in the intrinsic fluorescence. The emission spectra were analyzed according to a model described by Chipman et al., to obtain the association constant (k_a) values in the temperature range of 25-50 ºC. The results showed that the association constants were temperature dependent. The value of K_a at 25°C was 2.4×10^5 M^{-1}.
Moreover, the thermodynamic parameters for the interaction H_2O_2/BLC were obtained from Van't Hoff plot. The results indicated that the interaction was enthalpically driven accompanied by negative entropic contribution in the studied temperature range. The association constant values were determined from Van't Hoff plots.
Key words: Bovine liver catalase, fluorescence emission, association constants, thermodynamic parameters, Chipman model.

دراسة الخواص الثيرموديناميكية لإرتباط إنزيم كتاليز الكبد البقري مع فوق أكسيد الهيدروجين.

ملخص: في هذه الدراسة تم تحضير إنزيم الكتاليز من عينة من كبد البقر بإستخدام تقنية الكروماتوجرافي ذو الألفة السليلوزية. وقد تمت متابعة إرتباط فوق أكسيد الهيدروجين بالإنزيم بقياس التغير في شدة التوهج الإشعاعي (الفلورسنس) على الطول الموجي 435 nm . وتم حساب قيم ثوابت الإرتباط على درجات حرارة مختلفة في المدى °C 25-50 بالإستعانة بطريقة Chipman. وبينت النتائج أن ثوابت الإرتباط كانت معتمدة على درجات الحرارة فكانت قيمة ثابت الإرتباط على 25 °C مساويةً 2.4×10^5 M^{-1}.

بالإضافة إلى أنه قد تم في هذا البحث حساب العامل الثيرموديناميكية للإرتباط الناشئ كما تم الإستعانة بمخططات فانت هوف لهذا الغرض.

وفي النهاية بينت النتائج أن الإرتباط الناشئ كان مرغوباً فيه من ناحية الإنثالبي ولكنه غير مرغوب فيه من ناحية الإنتروبي.

Introduction

Catalase [CAT: EC 1.11.16] is widely distributed in animals and plants. Catalase plays a vital role in the protection of tissues from the toxic effects of hydrogen peroxide and partially reduced oxygen species [1- 4]. Catalase catalyses the decomposition of hydrogen peroxide to molecular oxygen and water without production of free radicals [4]. Catalase is an antioxidant enzyme of high turnover rates [5]. It has been isolated from different sources such as human erythrocytes [6], yeast [7], mouse live [8] by different methods such as a ion-exchange chromatography and gel filtration [7],immobilized metal ion affinity chromatography involving chelation with zinc ions [8], Immunoaffinity Chromatography [9], and by metal ion chelating membrane medium [10].

Moreover, the techniques of fluorescence, quenching and enhancement have been used for studying the protein-ligand interaction [11]

The aim of the present work was to characterize the purified Bovine liver catalase and to study its binding properties with H_2O_2 (by the use of fluorescence measurements) at different temperatures in the range of 25-50 ºC.

Materials and methods

All chemicals were of analytical grade and were used without further purification. All solutions were prepared using distilled water. Hydrogen peroxide was purchased from Merck.

Purification of Bovine Liver Catalase

B L C was extracted essentially as described previously by Aydemir, T., and Kuru, K. [3] and Coban, A., et al., [4] with some modifications. Up to 500 g of fresh bovine liver bought from a local slaughterhouse was taken immediately to the laboratory in crushed ice. At First Small portions of the liver were mixed with citric acid (up to obtain 10 % w/w) and homogenized in a blender, and then the homogenate was centrifuged at 4000×g for 30 min in a high speed Sorvall RC 5C Plus Centrifuge. The precipitate was dispersed in potassium phosphate buffer solution of pH 7.0 stirred for 15 min and centrifuged at 8000 ×g for 30 min. The extraction was fractionated and stirred with solid ammonium sulfate then centrifuged for 30 min at 8000×g.

Fractions containing BLC were loaded directly to a cellulose affinity column (1.5×25cm) previously equilibrated with 20 mM potassium phosphate buffer solution (pH 7.0). after the enzyme was bound to cellulose matrix, it was washed with 20 mM potassium phosphate buffer solution and then eluted with the same buffer at a flow rate of 20 ml/h. The active fractions of 3 mL were collected and the absorbance at 280 nm for each

fraction was separately determined until the absorbance of the column eluate at 278 nm is < 0.05. The purified BLC was stored at – 20 ºC in 20 mM potassium phosphate buffer of pH 7.0.

Concentration of BLC enzyme

Concentration of BLC was determined spectrophotometrically using a Perkin Elmer UV/Vis (Lambda 20) spectrometer at 25 ºC.

The value of the enzyme molar extinction coefficient was 13,600 M^{-1} cm^{-1} at 278 nm [12]. Absorbance measurements were carried out using a Beckman DU-64 spectrophotometer. A molar mass of 247.5 kDa (as an average value for BLC of higher organisms) was used in calculation [3].

Fluorescence measurements

Spectrofluorimetric titrations of H_2O_2 with BLC were performed over a range of temperatures (25 – 50 ºC) in a Perkin Elmer luminescence (series no. 70412). The spectrometer equipped with a water jacket cuvette holder to maintain the desired temperature. The excitation wavelength was 290 nm, and the emission was recorded in the wavelength range of 350 – 625 nm. The excitation and emission slits were both 10 nm. The spectra were monitored at 435 nm. After the spectrum of enzyme alone has been recorded, successive addition (20 µl) of 15 mM H_2O_2 were added to BLC of concentration 1.73 µM; both in phosphate buffer solution pH 7.0. The fluorescence intensities were measured after incubation in the cuvette for 2 min.

Data analysis

The fluorescence data were analyzed according to double-logarithmic plots as described by Chipman et al [13]. The association constants at each temperature from 25 to 50 ºC was estimated from the intercept of the plot at the desired temperature.

Results and discussion

The interaction H_2O_2/BLC was monitored by intrinsic fluorescence as a function of molar ratio of $[H_2O_2]$/[BLC] in 20 mM phosphate buffer solution of pH 7.0. Fig. 1 displays the fluorescence titrations of BLC with H_2O_2. Fluorescence emission spectra obtained after the interaction of H_2O_2 with catalase were recorded in the wavelength range of 350-625nm corresponding to exciting at 290 nm using excitation and emission slit widths of 10nm. Emission titration of H_2O_2/catalase resulted in a substantial increase in the fluorescence intensity at 435 nm and it can be clearly seen that the fluorescence intensity at this wavelength increases with successive addition of H_2O_2. However, no shift in the emitted spectra was noticed.

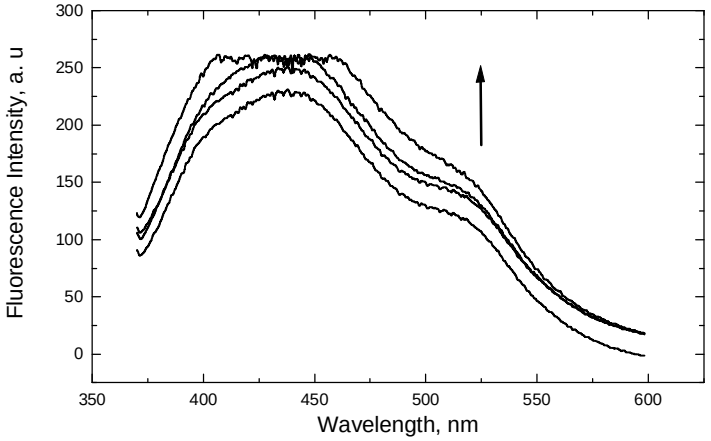

Fig. 1: Fluorescence emission spectra corresponding to titration of H_2O_2 with BLC at 25 ºC. The direction of arrow correspond to successive additions of 20 µl of 15 mM H_2O_2 aliquots.

Effect of temperature on binding H_2O_2/BLC

To study the influence of temperature on the binding H_2O_2/BLC and to obtain the thermodynamic parameters, a series of fluorescence titrations similar to those mentioned above were performed at 25,30, 40 and 50 ºC.

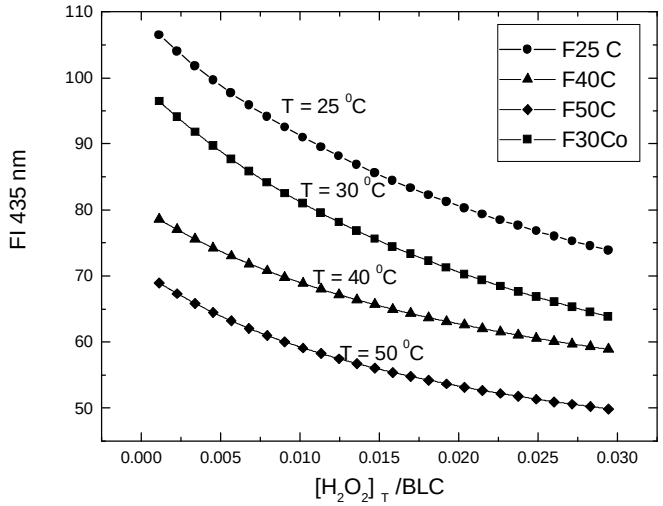

Fig.2: Representative plots of fluorescence intensities at 435 nm vs. the molar ratio $[H_2O_2]_T$ /[BLC]. Concentrations of BLC and H_2O_2 were 1.73 μM and 15 mM. respectively. The continuous lines are the best fitted curves at different temperatures. (●) 25 ºC, (■) 30 ºC , (▲) 40 ºC and (♦) 50 ºC.

Fig.2 shows the best fits representing the intrinsic fluorescence at 435 nm versus the molar ratio $[H_2O_2]_T$/BLC at selected temperatures in the range of 25 – 50 ºC. The fluorescence titration data at each temperature were analyzed using a model described by Chipman et al [13,14]. The experimental data were analyzed using equation (1) from which the association constant was estimated. Double logarithmic plots were constructed and the binding constants were obtained from the abscissa of the linear fits obtained (Fig. 3)

$$Log\frac{(F-F_\infty)}{(F_0-F_\infty)} = \log K_a + Log[H_2O_2]_f \qquad (1)$$

Where F_0, F, and F_∞ are the fluorescence intensities account for enzyme solution without H_2O_2, in presence of H_2O_2, and Fluorescence intensity at saturation by H_2O_2, respectively.

The changes in enthalpy (ΔH) and entropy (ΔS) were estimated from Van't Hoff plots. The change in free energy (ΔG) was obtained from the association constant, K_a, according to the expression $\Delta G = -RT\ln K_a$. The association constants obtained at different temperatures in the range of 25-50 ºC are listed in Table 1. It clearly seen that the values of association constant decrease with temperature, suggesting that the interaction is an exothermic process with better contacts of H_2O_2 in the active sites of the BLC enzyme via van der Waals and/or hydrogen-bonding interactions. The association constants and the thermodynamic parameters (ΔH^{VH} , ΔS and ΔG) associated with the formation of H_2O_2/BLC conjugate are summarized in Table 1

Table (1):**Association constants (K_a), and thermodynamic parameters (ΔH , ΔS, and ΔG) for with the interaction H_2O_2/BLC at pH 7.0** as a function of temperature.

Temperature, ºC	**$K_a \times 10^{-4} M^{-1}$**	**ΔH^{VH} kJ/mol**	**ΔS J/mol.K**	**ΔG (kJ/mol)**
25 ºC	24.0	-136.7	-354.2	- 30.8 (at 25 ºC)
30 ºC	13.2			
40 ºC	2.45			
50 ºC	0.347			

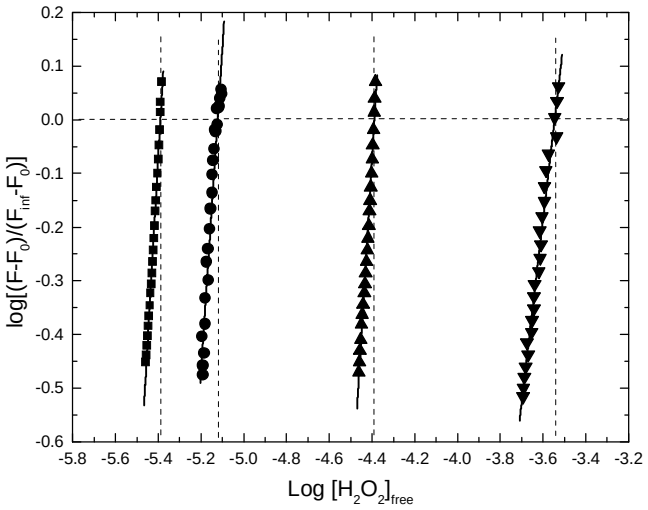

Fig.3: Double logarithmic plots for the interaction H_2O_2/BLC in phosphate buffer (pH 7.0) at four different temperatures. (■) 25 ºC , (●) 30 ºC , (▲) 40 ºC and (▼) 50 ºC.

Temperature dependence of the association constants has been estimated from Van't Hoff plots of log K_a vs the reciprocal of temperature (Fig. 4). A linear fit has been obtained from which the Van't Hoff enthalpy (ΔH^{VH}) and entropy ($\Delta S°$) contributions were estimated.

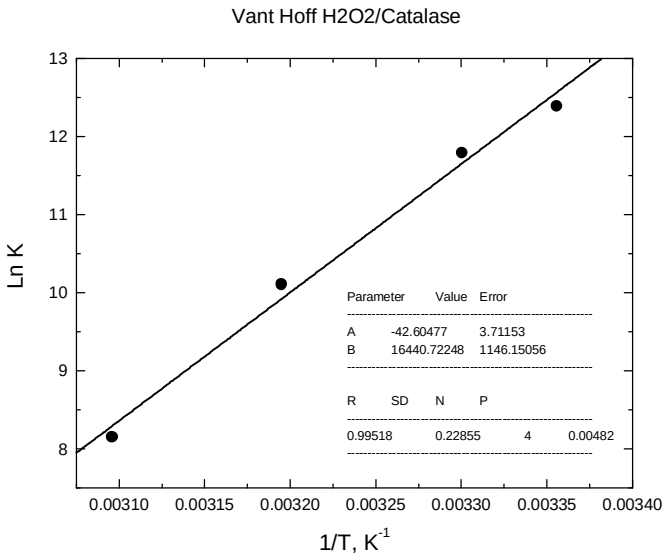

Fig.4: Van't Hoff plot corresponding to interaction of H_2O_2 with BLC enzyme in phosphate buffer solution pH 7.0 and at different temperatures in the range of 25-50 ºC

The association constants of the conjugate H_2O_2/BLC was used for calculation of the corresponding ΔG value at each temperature. Moreover, the temperature dependence of $_{Ka}$ for the conjugate was analyzed by Van't Hoff plot of LnK_a vs 1/T and linear fit was obtained. From the slope of the straight line, the value of Van't Hoff enthalpy (ΔH^{VH}) was calculated. The entropy change (ΔS) was calculated from the X-intercept of the line. The results showed that, the interaction was enthalpically driven with negative values of entropic contribution.

The negative value of ΔH^{VH} can be explained by the formation of H-bonding and Van der Waals forces during the conjugation [15,16]. The unfavorable entropic contribution may arise from the loss of vibration degrees of freedom in the catalase enzyme when bounded to hydrogen peroxide. Thus, for stable binding to occur, the enthalpic contribution must be sufficient high to offset of unfavorable entropy of the conjugate formed [17, 18].

Conclusion

The values of association constants (K_a) for the conjugate H_2O_2/BLC were temperature dependent in the studied range of temperature . The interaction of H_2O_2/BLC was enthalpically driven and associated with negative values of the entropy over the studied temperature range .

Acknowledgement:

We would like to express our special thanks to Nisreen el-Gamal and Sahr Bader for their grateful experimental help in this work

References

[1] Yörük, I. H., et al. Purification and properties of catalase from van apple (golden delicious), Pakistan Journal of Nutrition, 4, (1): (2005), pp 8-10.

[2]Yamaguchi, J., et al. Purification and characterization of heme-containing low-activity form of catalase from greening pumpkin cotyledons. Eur. J. Biochem. 159 (1986), pp 315-322.

[3] Aydemir, T., and Kuru, K., purification and partial characterization of catalase from chicken erythrocytes and the effect of various inhibitors on enzyme activity. Turk J. Chemistry. 27 (2003), pp 85-97.

[4] Coban, A., et al. Purification and characterization of catalase enzymes from chicken liver and sheep erythrocytes. Asian journal of chemistry. Vol. 19, No. 5 (2007), pp 3941-3953.

[5] Kim K. B., and Lee BM, Oxidative stress to DNA, protein, and antioxidant enzymes (superoxide dismutase and catalase) in rats treated with benzo(a)pyrene. Cancer Lett. (1997) Feb 26;113(1-2):205-12.

[6] Goth L. Human erythrocyte catalase, isolation with an improved method, characterization and comparison to bovine liver catalase. Enzyme. (1989) 41(4) pp191-9.

[7] Potapovich, M.V., et al. Isolation, Purification, and Characterization of Catalase from the Methylotrophic Yeast Pichia pastoris. J. Biochemistry (Moscow), Volume 66, Number 6, June 2001 , pp. 646-657(12)

[8] Yang, Q., et al. Rapid One-Step Isolation of Mouse Liver Catalase by Immobilized Metal Ion Affinity Chromatography, Protein Expression and Purification, Volume 12, Issue 2, March (1998) pp 277-283

[9] Ito, O., et al. Purification of Milk Catalase by Immunoaffinity Chromatography, Journal of Food Science (1990) 55 (4), pp 1172–1173

[10] Li Yang, et al. Immobilized iminodiacetic acid (IDA)-type Cu^{2+}-chelating membrane affinity chromatography for purification of bovine liver catalase. J. Biomedical chromatography 13, (1999) pp 229-234.

[11] Athar, H., et al. Use of fluorescence enhancement technique to study bilirubin-albumin interaction. International Journal of Biological Macromolecules 25 (1999), pp 353-358.

[12] Yuichiro J. et al. Thioctic Acid and Dihydrolipoic Acid are Novel Antioxidants Which Interact With Reactive Oxygen Species, J. Free Radical Research Volume 15, Issue 5 October (1991) pp 255-263

[13] Chipman, D. M., et al. The binding of oligosaccharides containing N-acetylglucasamine and N-acetylmuramic acid to lysozyme. J. Biol. Chem. 242, (1976) pp 4388-4394.

[14] Sultan, N. A., and Swamy, M. J., Thermodynamic analysis of binding of 4-methyllumbelliferyl-α-and β-D-galactopyranosides to Momordica charantia lectin. Current Science, vol. 84, No. 2, 25 January (2003) pp. 200-203.

[15] Connelly, P.R., et al. Thermodynamics of protein-peptide interactions in the ribonuclease S. system studied by titration Calorimetry. *Biochem.*, 29 (25) (1990) pp 6108-6114.

[16]Connelly, P.R., et al. Probing hydration contributions to the thermodynamics of ligand binding by proteins. *Biochem.*, 32 (1993) pp 5583-5590.

[17] Eftink, M.R., et al. Enthalpy-entropy compensation and heat capacity changes for protein-ligand interactions: general thermodynamical models and data for the binding of nucleosides to ribonuclease A. *Biochem.*, 22 (1983), pp3884-3896.

[18] Komath, S. S., et al. Thermodynamic analysis of saccharide binding to snake gourd (trichosanthes anguina) seed lectin. Fluorescence and absorption spectroscopic studies. Eur. J. Biochemistry. 286 (2001) pp 111-119.