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Thermodynamic Analysis of Lactoperoxidase activity in camel milk

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***Abstract*—Lactoperoxidase (LP) is a glycoprotein that occurs naturally in colostrum, milk, and many other human and animal secretions. The present study was undertaken to explore thermodynamic parameters of LP in camel milk in a temperature range of 67-73°C. The H_2O_2-mediated oxidation of pyrogallol at 430 nm was used to assess the peroxidase activity. Thermal denaturation of LP, measured by loss in activity, showed good agreement with a first-order reaction. The low values obtained for activation energy (349.05 kJ/mol) and change in enthalpy of activation (~346 kJ/mol) indicate that low amount of energy is required to initiate denaturation, probably due to the molecular conformation of camel LP.**

Keywords- Camel milk; Lactoperoxidase; Thermal stability

I. INTRODUCTION

Lactoperoxidase (LP) is an oxidoreductase enzyme secreted into milk, saliva, and tears [1]. LP catalyzes the peroxidation of thiocyanate, generating hypothiocyanite and other products that impair the function of bacterial metabolic enzymes [2, 3]. The biological significance of LP is its involvement in the natural host defense system against invading microorganisms [4]. Also degradation of various carcinogens and protection of animal cells against peroxidative effects for LP system reported by Tenovuo [5]. LP system can be activated in milk after heat treatment, thus contributing to extend the shelf-life of pasteurized milk in locations with inefficient cold storage conditions [6]. The purpose of this study was to determine thermodynamic parameters of LP in camel milk in a temperature range of 67 to 73 ◦C.

II. MATERIALS AND METHODS

A. Milk Sampling

Fresh raw camel (*Camelus bactrianus*) milk were supplied from Khokhor and Tabriz (East-Azerbaijan province, Iran) and divided into small portions (50 ml) and stored at -20 °C until analysis.

B. Enzymatic activity assay

Milk LP activity was measured by following the H2O2-dependent oxidation of pyrogallol at 430 nm, using an extinction coefficient of 2470 M^{-1} cm^{-1}. 3 mL of TS buffer (0.1 M Citrate-phosphate-borate buffer, pH 6.5), 0.15 mL pyrogallol (200 mM) and milk sample (0.05 mL) were added together in cuvette. The reaction was initiated by the addition of 0.03 mL hydrogen peroxide solution (61 mM) and immediately the measurement of absorbance started at 430 nm as a function of time for 2 min at 15 sec intervals using an UNICO UV-2100 PC (USA) spectrophotometer. Measurements were carried out against the reagent blank containing pyrogallol and enzyme solution only. Reaction velocity was computed from linear slopes of absorbance-time curve. One unit of activity is defined as the amount of enzyme that catalyzes the oxidation of 1 µmol of pyrogallol per min at room temperature (~22-25°C).

C. Heat incubation study

Thermal stability of milk LP was studied by incubating aliquots of milk at various temperatures (67, 69, 71 and 73 °C) up to 60 min in a thermostatic water bath and measuring their activity at room temperature after brief cooling in ice. The incubation was carried out in sealed vials to prevent change of volume of the sample and, hence, the enzyme concentration due to evaporation. Assays at the different temperatures were done at least in 3 separate experiments and the mean values of data were used to obtain the different kinetic and thermodynamic parameters.

D. Kinetic data analysis

Inactivation kinetics of milk LP toward thermal processes was subjected to reaction kinetic analysis. This process behaves in an analogous way to a general rate reaction of order n according to this equation:

$$-dA/dt = k.A^n \quad (1)$$

where -dA/dt represents the loss of LP activity rate, k the inactivation rate constant (min^{-1}), A the LP activity at each time of treatment, and n the order of reaction. The experimental points are plotted according to the equation $\ln A/A_0 = k.t$ derived from Eq. 1, where A_0 is the initial response value (e.g initial enzyme activity at isothermal condition at time t_0), A is the response value after heating treatment and t is the exposure time (min). Linear regressions were performed using the SigmaPlot for windows version 10.0 (Systat software, Germany). The rate constant in a denaturation process and the temperature of treatment are related according to the Arrhenius equation:

$$\ln k = \ln A - Ea/RT \quad (2)$$

where k is the rate constant, A is the Arrhenius constant, Ea the apparent activation energy, R the universal gas constant, and T the absolute temperature. The slope of the line obtained permits to calculate the activation energy.

The values of the activation energy (Ea) allow the determination of different thermodynamic parameters such

as variations in enthalpy (ΔH°), Gibbs free energy (ΔG°) and entropy (ΔS°) according to the following expressions:

$$\Delta H° = Ea - RT \quad (3)$$

$$\Delta G° = -RT \ln (kh/KT) \quad (4)$$

$$\Delta S° = (\Delta H° - \Delta G°)/T \quad (5)$$

where h and K are the Planck's and the Boltzmann's constants, respectively.

III. Results and Discussion

Effect of heat treatment on the enzymatic activity of LP on pyrogallol substrate at different temperatures showed that the degree of LP denaturation increases with temperature and time of treatment and linear relationship with high coefficients of correlation was observed between residual LP activity and time for each temperature. This result indicates that thermal inactivation of camel milk LP follows a first-order kinetic model.

Inactivation rate constants were used to drawn the Arrhenius plot, from which slope activation energy was calculated and found to be 349.05 kJ/mol (Fig. 1). This value is lower than the values obtained for bovine milk LP by Martin-Hernandez [7] and Marin [8] which was 737.69 and 800 kJ/mol, respectively. The lower value found for the activation energy means that a lower amount of energy is needed to initiate denaturation [9], may be due to the less compactness of camel milk LP in comparison to bovine milk LP.

Table 1 shows the thermodynamic values of variation in activation enthalpy (ΔH°), variation in activation entropy (ΔS°) and variation in Gibbs free energy (ΔG°) calculated for the different temperatures.

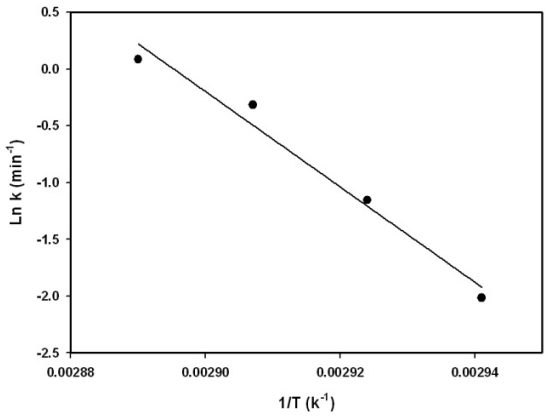

Figure 1. Effect of temperature on the rate constant (k)for the process of loss of lactoperoxidase activity on pyrogallol substrate. 1/T represents the reciprocal of the absolute temperature

TABLE 1: Thermodynamic parameters for inactivation of camel milk LP.

Temperature (°C)	?H° (kJ/mol)	?G° (kJ/mol)	?S° (kJ/mol)
67	346.22	100.91	0.721
69	346.21	99.07	0.722
71	346.19	97.27	0.723
73	346.17	96.69	0.721

The values of the change in enthalpy of activation obtained at this study for camel milk LP (~346 kJ/mol) are lower than the values obtained for bovine milk LP (~734 kJ/mol) which reported by Marin [9]. These results confirming the camel milk LP probably is less stable than bovine milk LP toward thermal processes, as suggested by lower value of activation energy. The positive values found for the variation in entropy of activation indicates that there are no significant processes of aggregation, since, if this would happen, the values of entropy would be negative [10].

CONCLUSION

The investigation of thermal inactivation of camel milk LP indicated a monophasic inactivation pattern or first-order kinetic model. The lower values of activation energy and enthalpy for camel milk LP in comparison to bovine counterpart suggest that camel milk LP is less stable than bovine milk LP toward thermal processes.

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