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Stabilization of bovine lactoperoxidase in the presence of ectoine

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ABSTRACT

Lactoperoxidase (LPO) is a heme peroxidase with various applications in industry and medicine. In this study, the effects of ectoine, as a compatible solute, on the structure, thermal stability, thermodynamic parameters, activity, and stability of LPO have been investigated. The results showed that the catalytic activity of LPO was improved by increasing ectoine concentration. The UV–visible absorption spectroscopy and FTIR spectra studies indicated that ectoine could bind to the LPO spontaneously. Moreover, ectoine increased the enzyme Tm and Gibbs free energy. The fluorescence measurements showed that LPO fluorescence was quenched in the presence of ectoine. The quenching mechanism was probably a static quenching by forming a ground state complex. The thermodynamic parameters indicated that hydrogen bonding and Vander Waals forces played a key role in the LPO-ectoine interaction process. The findings suggest that ectoine could be used as a lactoperoxidase stabilizing agent for industrial or medical purposes.

1. Introduction

The study of proteins stability is an interesting topic in the field of biotechnology, pharmaceutical, food industries, and the protein research. Protein stability studies are necessary to understand folding, structure and activity of protein as well as protein–ligand interactions. The stability of proteins change due to the fluctuations of environmental organic compounds, which it might effect on the folding and function of proteins (Stepankova et al., 2013).

Lactoperoxidase (EC 1.11.1.7) is a heme-containing glycoprotein with a single polypeptide chain that has a molecular weight of 78,000. This oxidoreductase enzyme together with thiocyanate ion (SCN) and hydrogen peroxide (H_2O_2) produce hypothiocyanite ion (OSCN) which has a broad-spectrum antimicrobial activity against pathogenic microorganisms (Hayashi et al., 2012; Bafort et al., 2014). In recent years, a particular attention has been paid to stabilization of LPO due to its various applications in food industry (as natural bio preservative), cosmetics, tumor therapy, and biotechnological processes (textile, and paper) (Boscolo, Leal, Ghibaudi, & Gomes, 2007; Göller and Galinski, 1999; Seifu, Buys, & Donkin, 2005). Several strategies have been used to increase LPO stability such as tragacanth-chitosan nano biopolymer, polyaniline polymer, and sugars (Altinkaynak et al., 2016; Jafary et al., 2012; Nayeri, Fattahi, Iranpoor-mobarakeh, & Nori, 2015; Samsam Shariat, Jafari, Tavakoli, & Bahri Najafi, 2015). Use of stabilizing additives is a usual practice in the enzyme technology. Moreover, the shelf life of the enzyme products relies on such additives (Andersson, Breccia, & Hatti-Kaul, 2000).

Extremolytes, which also called organic osmolytes, constitute a diverse class of low molecular weight compounds i.e. sugars, polyols, and amino acid derivatives. Organic osmolytes play an essential role in maintaining intracellular homeostasis in organisms ranging from bacteria to humans (Ablinger, Hellweger, Leitgeb, & Zimmer, 2012; Arakawa and Timasheff, 1985). Many of these compounds are widely used as stabilizing excipients of proteins due to their known stabilizing impacts on the conformational stability of proteins (Avanti, Saluja, van Streun, Frijlink, & Hinrichs, 2014). There are several theories that explain the possible protective role of compatible solutes in proteins stability, including water replacement hypothesis, transfer free energy, excluded volume, contact interaction, and preferential exclusion (Lentzen & Schwarz, 2006). The preferential exclusion model, an increasing (preferential) hydration of proteins, is the most explanation for compatible solutes that effects on the proteins conformational stability (Galinski, 1993). Thus, preferential hydration of the proteins causes less thermodynamically unfolding and in consequence increases proteins native conformational stability in the presence of extremolytes (Ratnaparkhi and Varadarajan, 2001; Street, Bolen, & Rose, 2006). Ectoine ((S)-2-methyl-4-carboxyl-1,4,5,6-tetrahydropyrimidine) is an extremolyte that is produced by aerobic chemoheterotrophic and halophilic/halotolerant bacteria. Ectoine (Ect) protects extremophilic microorganisms from extreme environmental stresses, such as high (or low) temperature, excessive pressure, and severe salt concentrations. Moreover, Ect has been used as a substance in the cosmetic market and in skin care products (Cánovas et al., 1999; Bownik & Stępniewska, 2016). It has been reported that Ect stabilizes a variety of proteins,

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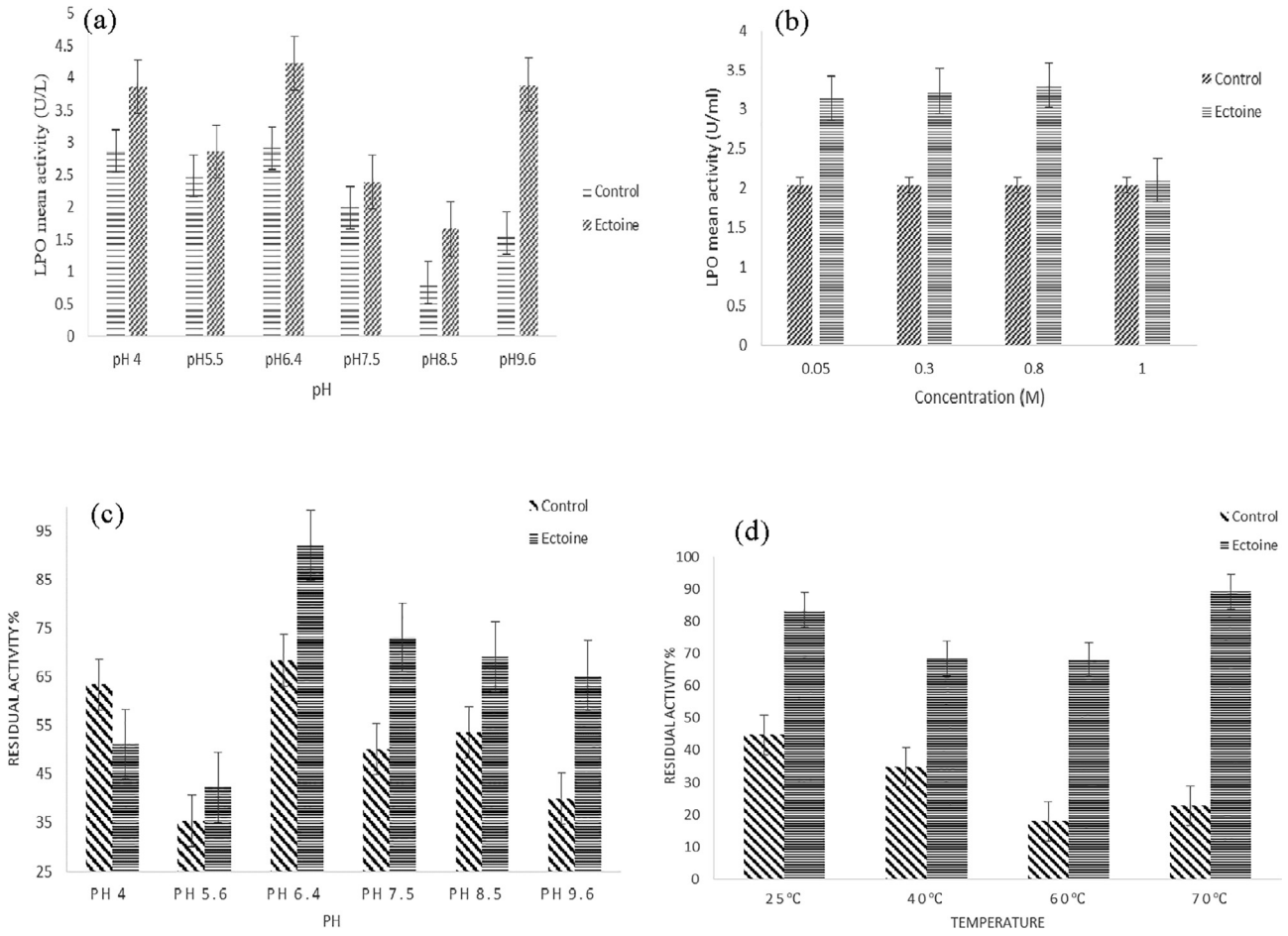

Fig. 1. LPO mean activity, (a) in the absence and presence of 0.8 M Ect at different pH (4, 5.5, 6.4, 7.5, 8.5 and 9.6) at 25 °C, (b) in the absence and presence of different Ect concentrations (0.05, 0.3, 0.8 and 1 M) at pH 6.4 at 25 °C, (c) LPO residual activity in the presence of 0.8 M ectoine at different pH (4, 5.5, 6.4, 7.5, 8.5 and 9.6) at 25 °C; (d)) in the presence of 0.8 M ectoine at different temperatures (25, 40, 60 and 70) at pH 6.4. Data are mean ± SD. statistically significant differences (SPSS, two-way ANOVA) are indicated $p < 0.05$.

nucleic acids, membranes and the whole cell from thermal stress, proteolysis, freezing, freeze–thawing, drying, oxidative damage, and change in pH or salt concentration (Graf, Anzali, Buenger, Pfluecker, & Driller, 2008; Lippert and Galinski, 1992). Ectoine has no toxic side effect (Kanapathipillai et al., 2010), and has this ability to be used as a therapeutic agent or stabilizer in biopharmaceutical formulations. Ect stabilizes proteins, especially globular proteins like antibodies and provides an integrated solution for the optimization of protein productions (Abdel-Aziz et al., 2013; Bownik & Stępniewska, 2016). These wide range applications of Ect is leading to an increasing interest in the usage of Ect as a potential agent for stabilization of proteins.

The aim of the present study is to determine the Ect effects on catalytic activity of LPO in different temperatures and pH. The changes in the secondary structure, thermodynamic parameters and activity of this enzyme are monitored by using spectroscopic methods such as fluorescence spectroscopy, UV–visible absorption spectroscopy and FTIR.

2. Material and methods

2.1. Materials

LPO from bovine milk, Ect, ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) and H_2O_2 were obtained from Sigma Chemical Company (St. Louis MO). All other reagents used in this study were analytical grade. Double distilled water was used for solutions preparation.

2.2. Preparation of solutions

Tow buffer, 100 mM potassium phosphate buffer and 100 mM citric acid buffer, were used during the experiment of different pH range (4, 5.5, 6.4, 7.5, 8.5, 9.6). The pH was measured with a glass electrode and a Metrohm 827 pH lab pH meter. Protein solutions were prepared by dissolving LPO into the buffer (1 mg/ml). The stock solutions of Ect (3 M) was prepared by dissolving appropriate amount of Ect powder in 0.1 M potassium phosphate buffer pH 6.4.

2.3. Enzymatic activity assay

Measurements were performed by Shimadzu UV2600 Spectrophotometer according to the Barrett method (Barrett, Grandison, & Lewis, 1999). This method is based on oxidation of ABTS by H_2O_2, which generates $ABTS^{\cdot +}$, a product that is absorbent at 412 nm. One unit of enzyme will oxidize 1.0 µmole of ABTS per minute at pH 6.4 at 25 °C. The reaction mixture contained 0.11 ml of 100 mM potassium phosphate buffer (pH 6.4), 1 ml ABTS, 1.1 ml H_2O_2, and 0.1 ml of the enzyme solution. The absorbance was continuously monitored at 412 nm for 2 min. Each measurement was repeated three times and the results were presented as mean ± SD for each experiment.

2.4. The optimum Ect concentration

The different concentrations of Ect (0.05, 0.3, 0.8, 1 M) (Boscolo