The effect of some osmolytes on the activity and stability of mushroom tyrosinase

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The thermodynamical stability and remained activity of mushroom tyrosinase (MT) from *Agaricus bisporus* in 10 mM phosphate buffer, pH 6.8, stored at two temperatures of 4 and 40°C were investigated in the presence of three different amino acids (His, Phe and Asp) and also trehalose as osmolytes, for comparing with the results obtained in the absence of any additive. Kinetics of inactivation obeye the first order law. Inactivation rate constant (k_{inact}) value is the best parameter describing effect of osmolytes on kinetic stability of the enzyme. Trehalose and His have the smallest value of k_{inact} (0.7×10^{-4} s^{-1}) in comparison with their absence (2.5×10^{-4} s^{-1}). Moreover, to obtain effect of these four osmolytes on thermodynamical stability of the enzyme, protein denaturation by dodecyl trimethylammonium bromide (DTAB) and thermal scanning was investigated. Sigmoidal denaturation curves were analysed according to the two states model of Pace theory to find the Gibbs free energy change of denaturation process in aqueous solution at room temperature, as a very good thermodynamic criterion indicating stability of the protein. Although His, Phe and Asp induced constriction of MT tertiary structure, its secondary structure had not any change and the result was a chemical and thermal stabilization of MT. The enzyme shows a proper coincidence of thermodyanamic and structural changes with the presence of trehalose. Thus, among the four osmolytes, trehalose is an exceptional protein stabilizer.

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1. Introduction

Tyrosinase (EC 1.14.18.1) is a copper-containing mono-oxygenase responsible for the biosynthesis of melanins and other polyphenolic compounds (Lerch 1981). It catalyses both the orthohydroxylation of monophenols and the oxidation of o-diphenols to o-quinones. Tyrosinase is widely distributed in mammals, plants and micro-organisms (Robb 1984). Various strategies to increase the stability of enzyme include chemical modification (Ryan *et al* 1994) using osmolytes (Taneja and Ahmad 1994) and special organic solvents (e.g. polyethylene glycol) (Ozaki *et al* 1998). Osmolytes can be polyols, sugars, polysaccharides, neutral polymers, amino acids and their derivatives, and large dipolar molecules like trimethylamine N-oxide (Yancey *et al* 1982; Fan-Guo *et al* 2001; Murphy 2001). It is light that physico-chemical properties of proteins affected from bulk properties of the solvent environment. Osmolytes as solvent additives favorably affect protein stability and solubility.While they can increase stability of proteins while protecting them from thermal denaturation, the enzymatic activity is not reduced (Arakawa and Timasheff 1983).

Trehalose [α-D-glucopyranosyl (1-1)- α-D-glucopyranoside] is a nonreducing disaccharide in which the two glucose

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Abbreviations used: Asp, Aspartic acid; CA, caffeic acid; DTAB, dodecyl trimethylammonium bromide; His, histidine; MRW, mean amino acid residue weight; MT, mushroom tyrosinase; PBS, phosphate buffer solution; Phe, phenylalanine; T_m, protein melting point; Tre, trehalose.