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Biophysical Chemistry 125 (2007) 254–259

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Two-state irreversible thermal denaturation of *Euphorbia characias* latex amine oxidase

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Received 11 May 2006; received in revised form 7 August 2006; accepted 17 August 2006
Available online 23 August 2006

Abstract

Thermal denaturation of *Euphorbia* latex amine oxidase (ELAO) has been studied by enzymatic activity, circular dichroism and differential scanning calorimetry. Thermal denaturation of ELAO is shown to be an irreversible process. Checking the validity of two-state it really describes satisfactorily the thermal denaturation of ELAO. Based on this model we obtain the activation energy, parameter T^* (the absolute temperature at which the rate constant of denaturation is equal to 1 min^{-1}), and total enthalpy of ELAO denaturation. HPLC experiments show that the thermal denatured enzyme conserves its dimeric state. The $N_2 \xrightarrow{k} D_2$ model for thermal denaturation of ELAO is proposed: where N_2 and D_2 are the native and denatured dimer, respectively.

Keywords: Copper containing amine oxidase; Differential scanning calorimetry; Kinetic models; Irreversible denaturation

1. Introduction

Cu/TPQ amine oxidases (AOs; E.C. 1.4.3.6) are a group of heterogeneous dimeric enzymes, each subunit containing one Cu (II) and one 2,4,5-trihydroxyphenyalanine quinone (TPQ) as cofactors. TPQ is formed from a posttranslational self-processing of a tyrosine residue [1] in the amino acid sequence. These enzymes catalyze the oxidative deamination of primary amines to the corresponding aldehydes, hydrogen peroxide, and ammonia.

AOs are widely distributed in nature, occurring in plants, microorganisms, and mammals [2]. Although the functional role of Cu/TPQ AOs has not been clearly determined, it has been shown that plasma level of amine oxidases varies in diabetes [3], heart failure [4], patients suffering from serious burns and solid tumors, pregnancy and age [5]. In microorganisms these enzymes have nutritional role using primary amines as a sole source of nitrogen or carbon. In plants AOs can play a role in regulating intercellular polyamine levels, morphogenesis [6], and mobilization of seed reserves [7]. The level of plant AOs changes upon auxin treatment [8], light stress [9], germination [10], anoxic and thermal stress [11], salt stress [12], and mechanical injury [13].

In spite of intensive physiological and pharmaceutical studies on Cu/TPQ AOs, there are very few literature surveys on the thermodynamics of this group of enzymes. Moosavi-Nejad et al. [14] reported that thermal denaturation of lentil seedling amine oxidase (LSAO) showed two main reversible peaks, the first broad while the second one relatively sharp. They also deconvoluted the second peak to three subpeaks and supposed that subpeaks belonged to three hypothetical structure domains for each subunit of LSAO.

Giartosio et al. [15] analyzed thermal denaturation of bovine serum oxidase (BSAO) by differential scanning calorimetry (DSC). These authors showed that the DSC profile of BSAO had three distinct peaks. The thermogram of BSAO was deconvoluted

Abbreviations: BSAO, bovine serum amine oxidase; DSC, Differential scanning calorimetry; ELAO, *Euphorbia latex* amine oxidase; PSAO, pea seedlings amine oxidase; TPQ, 2,4,5, trihydroxyphenylalanine quinone.

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doi:10.1016/j.bpc.2006.08.006