Structural Changes and Aggregation Process of Cu/Containing Amine Oxidase in the Presence of 2,2,2'-Trifluoroethanol

M. Amani[1,2], R.Yousefi[1], A.A. Moosavi-Movahedi[1,*], F. Pintus[3], A. Mura[3], G. Floris[3], B.I. Kurganov[4] and A.A. Saboury[1]

[1]*Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran;* [2]*Medical Faculty, Ardabil University of Medical Sciences (ArUMS), Ardabil, Iran;* [3]*Department of Applied Science in Biosystems, University of Cagliari, Cagliari, Italy;* [4]*Bakh Institute of Biochemistry, Russian Academy of Sciences, Leninskii prospect 33, Moscow, Russia*

Abstract: Conformational and structural changes of lentil seedlings amine oxidase (LSAO) were studied in the presence of trifluoroethanol (TFE) by spectroscopic and analytical techniques. At TFE concentrations up to 5%, the induction of a structural transition from β-sheet to α-helix and up to 10% TFE a structural transition from α-helix to β-sheet as well as inactivation of the enzyme are observed. At TFE concentrations between 10-35%, LSAO proves to be prone to aggregation and beyond 35% TFE leads to a non–native protein structure with a high α-helix content. The obtained results revealed that the aggregation of LSAO is strongly linked to the nature of secondary structures.

Keywords: Amine oxidase, TFE, Secondary structure, Circular dichroism, Fluorescence, Aggregation.

INTRODUCTION

Cu/TPQ-containing amine oxidases (AOs) are widespread family of quino-proteins found in every kingdom of life. These enzymes are involved in the catabolism of biogenic amines showing an important role in various processes e.g. tissue differentiation, growth of tumors, transformation of cultured cells, wound healing and programmed cell death [1]. One important structural feature in all AOs is the presence of both a copper ion and the tyrosine–derived cofactor topa quinone (TPQ) in close proximity [2].

AOs catalyze the oxidation of amines as follows:

$$RCH_2NH_2 + O_2 + H_2O \rightarrow RCHO + H_2O_2 + NH_3$$

In plants, amine oxidases are involved in process of yellowing, senescence, wound response, cell wall biosynthesis, growth factor and alkaloid biosynthesis [3], well studied examples are the enzymes from the pulses pea (*Pisum sativum*) [4], lentil (*Lens esculenta*) [5] and *Euphorbia* latex [6]. Pea seedling amine oxidase (PSAO) is the only plant enzyme so far crystallized [4].

Each subunit of the enzyme comprises three domains termed D2, D3 and D4. The largest domain of each subunit (D4) has a β-sandwich structure, comprising two twisted anti-parallel β-sheets. Each of two smaller domains of the protein (D2 and D3) consists of two α-helices followed by a slightly curved anti-parallel four stranded β–sheets with a hairpin turn between strands 3 and 4. The active site (Cu-TPQ) of each subunit, located towards one edge of the β-sandwich domain D4, is not directly accessible from the solvent and the substrate access to it appears to require a substantial rearrangement of the polypeptide chain [4]. Lentil seedling amine oxidase (LSAO) is very similar to PSAO in amino acid sequences and in the reactivity towards substrates and inhibitors [7]. So we can hypothesize that LSAO can be very similar to PSAO in crystalline structure.

Organic cosolvent TFE, which mimics a partial hydrophobic environment found *in vivo* in or next to the membrane, is commonly used as a structurally inducing agent [8]. This cosolvent is known to destabilize hydrophobic interactions within a polypeptide chain and to stabilize the local H-bonds between residues close in the amino acid sequence [9]. Recently several researcher groups have analyzed the folding kinetics of individual proteins in the presence of TFE. However, results obtained in different proteins led to a variety of interpretations on the effects of TFE on the folding process. TFE destabilizes the native structure of proteins and promotes amyloid type aggregates [10]. The aggregation process is really believed to be involved in several disorders such as Alzheimer's, cystic fibrosis, and Prion diseases [11]. In plants, it was shown that the water stress is one of the factors which cause protein aggregation [12]. Because of the higher reactivity of amine oxidase products (H_2O_2 and aldehyde), they are believed to be more poisonous than its substrates. For example aldehydes, was reported to introduce protein aggregation [13]. So it is reasonable to conclude that a portion of protein aggregation during water stress can arise from amine oxidase activity. Aggregation occurs from denatured or partially denatured conformations. Denaturants such as trifluoroethanol (TFE) cause the equilibrium between N and D to be shifted toward the latter, thus favoring aggregation and subsequent fibril formation [14].

The mechanism(s) by which proteins can aggregate must be well characterized in order to develop strategies preventing the protein aggregation process. In presented paper we studied the aggregation of the LSAO in the presence of different concentrations of TFE as effective agent for partial unfolding of proteins.

*Address correspondence to this author at the Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran; Tel: (+9821) 66403957; Fax: (+9821) 66404680; E-mail: moosavi@ibb.ut.ac.ir

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