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Functional and conformational transitions of mevalonate diphosphate decarboxylase from *Bacopa monniera*

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ABSTRACT

Functional and conformational transitions of mevalonate diphosphate decarboxylase (MDD), a key enzyme of mevalonate pathway in isoprenoid biosynthesis, from *Bacopa monniera* (BmMDD), cloned and overexpressed in *Escherichia coli* were studied under thermal, chemical and pH-mediated denaturation conditions using fluorescence and Circular dichroism spectroscopy. Native BmMDD is a helix dominant structure with 45% helix and 11% sheets and possesses seven tryptophan residues with two residues exposed on surface, three residues partially exposed and two situated in the interior of the protein. Thermal denaturation of BmMDD causes rapid structural transitions at and above 40 °C and transient exposure of hydrophobic residues at 50 °C, leading to aggregation of the protein. An acid induced molten globule like structure was observed at pH 4, exhibiting altered but compact secondary structure, distorted tertiary structure and exposed hydrophobic residues. The molten globule displayed different response at higher temperature and similar response to chemical denaturation as compared to the native protein. The surface tryptophans have predominantly positively charged amino acids around them, as indicated by higher K_{SV} for KI as compared to that for CsCl. The native enzyme displayed two different lifetimes, $\tau 1$ (1.203 ± 0.036 ns) and $\tau 2$ (3.473 ± 0.12 ns) indicating two populations of tryptophan.

1. Introduction

Bacopa monniera is an important medicinal herb used in traditional Ayurveda. Bacopa extract is mainly used for the treatment of anxiety and improvement of intelligence and memory. Bacopa extract also possesses analgesic, sedative, anti-inflammatory, antipyretic, free radical scavenging and anti-lipid peroxidative activities [1,2]. Triterpenoid saponins, a type of terpenoid are the main active chemical constituent of this plant [3] which are responsible for the pharmacological properties of the plant extract [4]. Plant isoprenoids have essential roles in membrane fluidity, respiration, photosynthesis, and regulation of growth and development. Many isoprenoids are of economic interest for rubber production as well as for drugs, nutraceuticals, flavors, fragrances, pigments, agrochemicals, and disinfectants [5]. Terpenoids are synthesized from C5 carbon compound precursors known as isopentenyl diphosphate (IPP) [6] and its allylic isomer dimethylallyl diphosphate.

In plant kingdom IPP is derived from two distinct pathways: the mevalonate (MVA) pathway which is active in the cytosol, or from the recently unveiled plastidial 2-C methyl-D-erythritol-4-phosphate (MEP) pathway. The MVA pathway is present in the majority of organisms, including archaebacteria [7], some gram-positive bacteria (e.g., enterococci, staphylococci, and streptococci) [8], yeasts [9], and animals [10], whereas most gram-negative bacteria (e.g., *Bacillus subtilis* and *Escherichia coli*) [11], cyanobacteria [12], and green algae use only the MEP pathway [13]. The MVA pathway produces IPP through six enzymatic reactions sequentially [14]. The last of these reactions is catalyzed by the enzyme mevalonate diphosphate decarboxylase (MDD) (EC 4.1.1.33). MDD is a key enzyme in the MVA pathway [15], it catalyzes the divalent ion dependant irreversible decarboxylation of the six-carbon mevalonate diphosphate (MVA-PP) to the five-carbon IPP along with the concurrent hydrolysis of ATP to ADP and formation of CO_2 and inorganic phosphate [16]. MDD is a member of the distinct galactokinase (G), homoserine kinase (H), mevalonate kinase (M) and phosphomevalonate kinase (P) (GHMP) super family of ATP-dependent enzymes [17].

Abbreviations: BmMDD, *Bacopa monniera* mevalonate diphosphate decarboxylase; CD, circular dichroism; GdnHCl, Guanidine hydrochloride; MEP, 2-C methyl-D-erythritol-4-phosphate; MVA, mevalonate; βME, β-mercaptoethanol; IPP, isopentenyl diphosphate (IPP).

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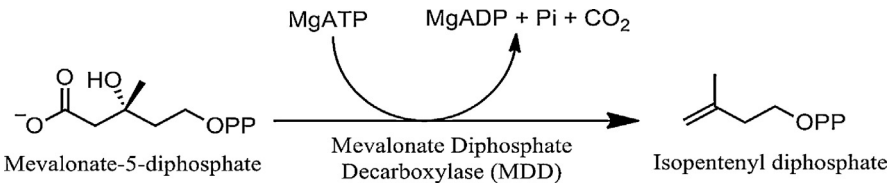

MDD has been purified and studied from various sources, like human, rat and yeast [18–20]. Crystal structure for MDD has been reported from sources like human, rat and *Staphylococcus epidermidis*, however no structure has been reported from plant source. Preliminary down regulation studies of MDD in yeast and rat indicate that MDD is important for normal growth and viability, therefore indicating that MDD is key enzyme of MVA pathway in isoprenoid biosynthesis [14,21], however further studies need to be done to understand the role of MDD.

Furthermore it has also been reported that MDD can also catalyze the conversion of 3-hydroxy-3-methylbutyrate to isobutene [22]. Isobutene is a product of petroleum refining process and is used for the production of renewable fuels and chemicals [23–25]. MDD can therefore be used for the production of renewable isobutene.

For many globular proteins unfolding/refolding has been well described in terms of two-state (native ↔ denature) model. However, many studies have showed that the protein folding involves a discrete pathway with distinct intermediate states between native and denatured states. These intermediate states have been observed under different conditions which do not appear to be native or completely unfolded states [26]. In order to understand the protein folding and its stability, it is essential to characterize the unfolded and partially folded state of protein [27,28]. In this regard, different agents such as pH, temperature, chaotropic agents and denaturants, either individually or in combination can be used as they can really transform proteins in a more or less completely unfolded state. By recording changes in intrinsic tryptophan fluorescence and in the secondary and tertiary structural features of protein in response to tailored changes in surroundings, one can establish presence of interesting structural intermediates relevant to structure-function relationship of the protein.

Although MDD has been characterized in terms of its activity and structure as its crystal structure has been reported [18,29], no studies regarding the functional and conformational transitions of MDD have been reported so far. Tryptophan environment can be probed by monitoring tryptophan fluorescence and by quenching studies using small chemical molecules [30,31]. The recombinant BmMDD from *B. monniera* has already been purified and characterized by us [32] and the enzyme is an interesting candidate for performing conformational characterization. In the present report, we have addressed these aspects to reveal the structural dynamics of the protein in solution by subjecting BmMDD to acid, alkali, thermal and chemical induced denaturation. Biophysical techniques were used to monitor the structural transitions in BmMDD to understand its conformational stability. Here, we also report the existence and characterization of an acid induced molten globule like intermediate at pH 4.0.

2. Materials and methods

2.1. Materials

Guanidine hydrochloride (GdnHCl) and 1-anilino-8-napthalenesulfonate (ANS) were procured from Sigma Aldrich Ltd. USA. All the other reagents were of analytical grade. For spectroscopic measurements solutions were prepared in sterile Milli *Q* water.

2.2. Isolation and cloning of BmMDD

Detailed protocols for isolation and cloning of BmMDD are described in Supplementary methods S1.

2.3. Heterologous expression and purification of recombinant BmMDD

Detailed protocols for heterologous expression and purification of recombinant BmMDD are described in Supplementary methods S2.

2.4. Enzyme assay

The activity for BmMDD was measured using a coupled spectrophotometric assay as described earlier [32]. The assay was carried out in reaction mixture containing 100 mM Tris–HCl buffer (pH 8.0), 100 mM KCl, 40 mM NH_4Cl, 12 mM $MgCl_2$, 10 mM ATP, 1 mM phosphoenolpyruvate, 0.2 mM NADH, 81 U pyruvate kinase and 75 U lactate dehydrogenase, 0.21 μM purified BmMDD and 0.3 mM mevalonate 5-diphosphate. Decarboxylation of mevalonate 5-diphosphate to IPP by purified BmMDD was monitored by measuring the decrease in NADH absorbance at 340 nm (A_{340}) as shown in the following representation.