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Maize uroporphyrinogen III methyltransferase: Overexpression of the functional gene fragments in *Escherichia coli* and one-step purification

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Abstract

S-Adenosyl-L-methionine: uroporphyrinogen III methyltransferase (SUMT), a key regulatory enzyme, converts uroporphyrinogen III to precorrin-2 in the porphinoids biosynthesis. In this study, the mature SUMT was signified that the maize SUMT precursor encoded by the open reading frame of maize SUMT cDNA was deleted the first 91 amino acids constituting the postulated signal peptide. Several mature SUMT fusion and deletion mutants were conducted. It actively expressed in *Escherichia coli* that the mature SUMT, or the truncated one deleting the C-terminal extra 52 amino acids based on SUMT sequence comparisons. On the contrary, it expressed as an inclusion body in *E. coli* that the mature SUMT fusion mutant, the SUMT precursor, or the mature SUMT deleting the N-terminal 36 amino acids including glycine-rich region involved directly in SAM binding. The purified His_6-tagged mature SUMT was homodimer with a molecular weight of 34 kDa, as shown by SDS–PAGE, 52 kDa using gel-filtration chromatography, and 79 kDa by dynamic light scattering assay. Red fluorescent compounds were associated with the recombinant mature SUMT which were identified as sirohydrochlorin and trimethylpyrrocorphin by spectroscopic analysis. This association slightly altered the protein secondary structure confirmed by circular dichroism assay.

Keywords: Uroporphyrinogen III methyltransferase; Maize; Purification; Red fluorescent compounds

1. Introduction

Siroheme is the prosthetic group of both sulfite and nitrite reductases in most bacteria and plants. It is biosynthesized via a branched pathway from uroporphyrinogen III, the last common tetrapyrrole primogenitor representing the branch point in porphyrins and porphinoids biosynthesis. Precorrin-2 is produced from uroporphyrinogen III catalyzed by the enzyme called *S*-adenosyl-L-methionine: uroporphyrinogen III methyltransferase (SUMT). It is either oxidized to form sirohydrochlorin and subsequently converted to siroheme, or transformed into other corresponding intermediates in the biosynthesis of several porphinoids including vitamin B_{12}, coenzyme F_{430} and heme d_1 [1].

SUMT catalyzes two succeeding methylation reactions of uroporphyrinogen III at C-2 and C-7. The recombinant SUMT has been purified to homogeneity from several bacteria [1]. The enzyme is homodimer from most bacteria except being monomer from *Bacillus*

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megaterium [2]. SUMT from *Pseudomonas denitrificans* is inhibited by substrate uroporphyrinogen III and product *S*-adenosyl-L-homocysteine (SAH)[1] [3]. According to the SUMT gene product from several bacteria, three groups are separated [4]: the sole functional enzyme CobA with SUMT activity, the bifunctional enzyme HemD/CobA with uroporphyrinogen III synthase and SUMT activity, and the tri-functional enzyme CysG synthesizing siroheme from uroporphyrinogen III. *Escherichia coli* CysG contains two functional domains. The carboxyl domain $CysG^A$ is homologues to CobA and the amino-terminal domain $CysG^B$ is responsible for the oxidation and ferrochelation of precorrin-2 [5]. The structural and functional studies on CobA from *P. denitrificans* and CysG from *Salmonella typhimurium* help to elucidate the SUMT catalyzing mechanism [6,7].

SUMT from several bacteria has an overmethylation property that synthesizes the tri- and tetra-methylated compounds. Overproduction of bacterial SUMT in *E. coli* causes the accumulation of red fluorescent compounds, sirohydrochlorin and trimethylpyrrocorphin [8,9]. This character, denoting that enzyme activity can easily be identified in vivo in a range of cell types, allows the SUMT gene applied as a method for the selection of recombinant plasmids in *E. coli*, and as a red fluorescent transcriptional reporter expressed in *E. coli*, yeast and mammalian cells [10,11].

The product from *Arabidopsis thaliana* and maize SUMT gene is formed as a higher molecular weight precursor with a plastid-targeting signal peptide [12,13]. Plant SUMT only participates in the siroheme biosynthesis and is not well studied partly due to the difficulty of enzyme activity assay in vitro. As SUMT plays the key regulating role in porphinoids biosynthesis, here, we reported the overexpression and purification of recombinant maize mature and truncated SUMT in *E. coli*. The both forms of enzyme were associated with red fluorescent compounds which were identified as sirohydrochlorin and trimethylpyrrocorphin from the mature SUMT. This association were slightly altered the mature SUMT secondary structure.

2. Materials and methods

2.1. Plasmids and bacteria strains

The pZmSUMT plasmid containing the maize SUMT cDNA fragment was provided by Sakakibara H. The pUC18 plasmid (Takara, Japan), pQE plasmids (Qiagen, Germany), and pET plasmids (Novagen, USA) were used for the construction of two forms of the mature SUMT and three other mature SUMT fusion and deletion mutants under the control lac promoter, T5 promoter and T7 promoter, respectively. *E. coli* strain JM109 was used for cloning all recombinant plasmids and expressing the pUC18-derived plasmid. *E. coli* strain S13009 and BL21(DE3) were used for expressing the pQE-derived plasmids and the pET-derived plasmid, respectively.

2.2. The construction of plasmids encoded individual two forms of mature SUMT

The different gene fragments from the pZmSUMT plasmid were excised with the different restriction endonucleases and inserted into the different plasmids (Fig. 1). The *Pst*I/*Hin*dIII fragment encoded the mature SUMT named SUMT1 which the SUMT precursor was deleted the first 91 amino acids assumed to constitute the postulated signal peptide. SUMT1 was thought the native enzyme in this study. This fragment was ligated into pQE 31 plasmid with the same digestion, given p31S1 plasmid which encoded SUMT1 with 22 amino acids including a His_6-tag and 12 amino-acid spacer at N-terminus. The p31S1 plasmid was re-digested with *Kpn*I and *Hin*dIII and the excised fragment was re-inserted into the corresponding sites of pUC18 plasmid, yielding p18S1 plasmid which encoded SUMT1 with the N-terminal extra 10 amino acids without a His_6-tag.

2.3. The construction of plasmids encoded individual the mature SUMT fusion and deletion mutants

The *Nco*I/*Hin*dIII fragment from the pZmSUMT plasmid representing the open reading frame encoding the SUMT precursor named SUMT2 indicating that the signal peptide fused with SUMT1, was inserted into the appropriately digested pET 15b plasmid, then re-excised with *Bgl*II and *Hin*dIII. The *Bgl*II/*Hin*dIII fragment was re-inserted into pET 22b plasmid with the same enzymes

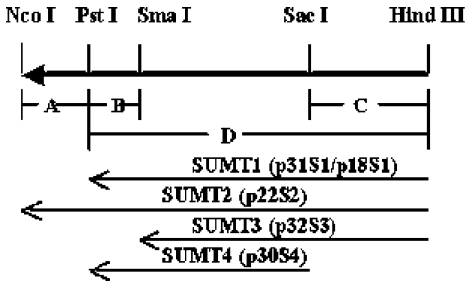

Fig. 1. The different deletion design based on the restriction endonucleases in the ZmSUMT cDNA. A, represented that gene product was the postulated signal peptide. B, designated the gene product of 36 amino acids in which the last five amino acids GAGPG were the well conserved glycine-rich region involved directly in SAM binding. C, the product of C-terminal extra amino acid region based on SUMT sequence comparisons. D, the mature SUMT. The mature SUMT and its three different fusion and deletion mutants were designed and named. The recombinant plasmids were signified in the brackets (see the plasmid construction in the text).

[1] *Abbreviations used*: SAH, *S*-adenosyl-L-homocysteine; SAM, *S*-adenosyl-L-methionine; LB, Luria–Bertani; IPTG, isopropyl-β-D-thiogalactopyranoside; Ni–NTA, nickel–nitrilotriacetic acid; FPLC, fast protein liquid chromatography; UV–Vis, ultraviolet–visible.