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Cloning, Expression and Purification of Wheat Acetyl-CoA Carboxylases CT Domain in *E. coil*

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Abstract The entire gene of carboxyltransferase(CT) domain of acetyl-CoA carboxylase(ACCase) from Chinese Spring wheat(CSW) plastid was cloned firstly, and the 2.3 kb gene was inserted into PET28a$^+$ vector and expressed in *E. coil* in a soluble state. The $(His)_6$ fusion protein was identified by SDS-PAGE and Western blot. The recombinant protein was purified by affinity chromatography, and the calculated molecular mass(M_r) was 88000. The results of the sequence analysis indicate that the cloned gene(GeneBank accession No. EU124675) was a supplement and revision of the reported ACCase CT partial cDNA from Chinese Spring wheat plastid. The recombinant protein will be significant for us to investigate the recognizing mechanism between ACCase and herbicides, and further to screen new herbicides.

Keywords Chinese Spring wheat; Acetyl-CoA carboxylase; Carboxyltransferase(CT) domain; Cloning; Expression; Purification

1 Introduction

Acetyl-CoA carboxylase(EC 6.4.1.2) plays critical roles in fatty acid metabolism. It is a biotin-dependent enzyme that catalyzes the first committed step of *de novo* fatty acid biosynthesis[1]. Two isoforms of ACCase have been identified in plant cells. Heteromeric ACCase, locating at plastid in most plants, is composed of four subunits, biotin carboxyl carrier protein(BCCP), biotin carboxylase(BC), carboxyltransferases α and β(CT-α and CT-β). And homomeric ACCase, locating at cytosol, is composed of a single strand peptide including four function domains, BC, BCCP, CT-α and CT-β. But there is an exceptional case in Gramineae(grasses), the ACCase in plastid is homomeric[2].

Aryloxyphenoxypropionates(APPs) and cyclohexanediones(CHDs) are two classes of herbicides of gramineous grasses[3—5], which selectively inhibit the carboxyltransferase(CT) activity of ACCases and block the growth of gramineous grasses[6—8]. However, the molecular recognizing mechanism between ACCase CT and inhibitors is not known. Owing to the low yields and instability of ACCase[9—11], it is a challenge to obtain sufficient quantities of purified enzymes by extraction from grasses. But it will be a right choice to get recombinant protein by overexpressing *in vitro*. Recently Tong's group[12,13] has expressed the homomeric ACCase CT domain from the yeast in *E. coil*, whereas the expression of the CT domain from plant plastids has not been reported yet.

In this study, the plastid ACCase CT gene from Chinese Spring wheat was cloned and overexpressed in *E. coil*. The expression of CT domain gene will facilitate the studies including the kinetics assays, recognizing mechanism of ACCase and inhibitors, and screen of the new herbicides.

2 Materials and Methods

2.1 Plant Material and cDNA Cloning

Chinese Spring wheat seeds were planted at room temperature for 10—15 d. Total RNA from the wheat stems[14] was isolated with trizol reagents (Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, China) according to the manufacturer's protocol. The synthesis of the first cDNA strand was carried out in the presence of Superscript II

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RNase H⁻ Reverse Transcriptase (Invitrogen Corporation, California, USA) with oligo(dT) as primer at 42 °C for 40 min.

2.2 Cloning of ACCase CT Domain Gene

Coding gene of plastid ACCase CT domain from Chinese Spring wheat was obtained from two fragments, CT1 and CT2, which were amplified, respectively, from wheat cDNA by PCR in the presence of *Taq* DNA polymerase(Fermentas) with the gene specific primers(See Table 1)[15]. The 1.1 kb(CT1) and 1.5 kb(CT2) PCR products were isolated and inserted into PMD18 T-vector[Takara Biotechnology(Dalian) Co., Ltd.]. Then CT1 and CT2 in PMD18 were digested with Nhe I, Bpu1102 I and Bpu1102 I, EcoR I, respectively. Two digested fragments(CT1: 1 kb, CT2: 1.3 kb) were isolated and linked together by T4 DNA ligase(Fermentas). Then the linked product(2.3 kb) was inserted into PET 28a$^+$ vector and transformed in *E. coil* DH5α(DE3)[16]. The positive clones were identified by PCR, double restriction enzymes digestion and sequencing(Sangon Biological Engineering Technology & Services Co., Ltd., Shanghai, China).

Table 1 Gene specific primers*

Code	Gene target	Upstream primer (5′ to 3′)	Downstream primer (5′ to 3′)	Restriction sites	Product size/bp
CT1	4638—5732	CACTCTGCTAGCTCGTCATCTGGTCCT	GATGTAAGCAACGGGTCTGTCAGGTGG	Nhe I, —	1095
CT2	5453—6992	ACTTGGCATACGGTGCATACAGCGTA	GTCGACGAATTCATCTGATCATTTAAGGACTT	—, EcoR I	1540
RCP18-5	4638—6992	CACTCTGCTAGCTCGTCATCTGGTCCT	GTCGACGAATTCATCTGATCATTTAAGGACTT	Nhe I, EcoR I	2354

* Restriction sites are underlined in primers sequence.

2.3 Expression and Purification of Recombinant Prqtein

The recombinant plasmid(RCP18-5) was transformed into expression strain BL21(DE3) pLys[17], and the target protein was expressed in the presence of 0.2 mmol/L IPTG at 21 °C for 10 h.

Cultured cells were centrifuged at 4 °C, and the precipitation was resuspened in 50 mmol/L Tris-HCl (pH 8.0) and freezed at −40 °C, at least, 2 h. Cells were lysised by sonication in the presence of 1 mmol/L HEPES. After adding NaCl at a final concentration of 0.5 mol/L, the target protein was purified by Chelating Sepharose Fast Flow(GE Health Care, Piscataway, USA) charged with Ni^{2+} ions as described[18]. The purified product was identified with 12% SDS-PAGE, and stored in 5% glycerol at 4 °C.

2.4 Western Blot Assay

To determine whether the purified protein was the target recombinant protein, Western blot was carried out according to the typical Western blot protocol with the His-tag antibody as the primary antibody(anti mouse) and HRP-antibody as the secondary antibody[anti mouse, Tiangen Biotechnology(Beijing) Co., Ltd.].

2.5 Sequence Analysis

Some internet and biology softwares were used to analyse the sequence of cloned gene and the characteristics of the recombinant protein, such as http://www.ncbi.nlm.nih.gov/blast/bl2seq/wblast2.cgi, DNASTAR 5.0, Primer Premier 5.0 and so on.

3 Results and Discussion

3.1 Gene Specific Primers

Only partial CSW plastid ACCase CT cDNA(GeneBank accession No. Z23038) has been reported[19,20], and the sequence identity between this cDNA and that from Hard Red Winter wheat Tam107(Genebank accession No. AF029895) is 97.1%. So Tam107 plastid ACCase gene was selected as the template to design PCR primers of CSW ACCase CT. The location of CT domain in ACCase was confirmed according to the Tong's report[12]. Since the entire CT gene was failed to be cloned with one pair of primers, the two cDNA fragments(CT1 and CT2) were generated by PCR with two pairs of primers. The full-length CT gene was obtained from linage of CT1 and CT2 at the Bpu1102 I position.

3.2 Plant Tissues and RNA Isolation

Piotr Gornicki reported that higher transcription level of ACCase was found in the stems of wheat than those in other tissues[14], and therefore the stems of the wheat seedling were used preferentially to isolate total RNA. The products were monitored by denatured agarose gel electrophoresis[17]. The electrophoresis result indicates that the extracted RNA is considerably intact(Fig.1).