Cao et al. SpringerPlus (2016) 5:818
DOI 10.1186/s40064-016-2532-7

SpringerPlus

RESEARCH Open Access

Enzymatic characterization of two acetyl-CoA synthetase genes from *Populus trichocarpa*

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Abstract

The acetyl-CoA synthetase (ACS) family is a subfamily of adenylate-forming enzymes, which has a close evolutionary relationship with the 4-coumarate:CoA ligase (4CL) family. In this study, two ACS genes were cloned from *Populus trichocarpa* and were named *PtrACS1* and *PtrACS2*. Bioinformatics characterization of *PtrACS1* and *PtrACS2* showed that they contained the key ACS residues and a putative peroxisome targeting sequence 1 (PTS1) at the end of the C-terminal sequence. Real-time PCR results showed that *PtrACS*1 and *PtrACS*2 were expressed in the phloem, xylem, leaves, and roots of one-year-old *P. trichocarpa*, but were expressed primarily in the leaves. The ACS enzyme activity was higher in leaves than other tissues in *P. trichocarpa*. Two overexpressed recombinant proteins showed no catalytic activity toward the substrates of 4CL, but did have notable catalytic activity toward sodium acetate and substrates of ACS. The relative activities of PtrACS1 and PtrACS2 were 194.16 ± 11.23 and 422.25 ± 21.69 μM min^{-1} mg^{-1}, respectively. The K_m and V_{max} of *PtrACS*1 were 0.25 mM and 698.85 μM min^{-1} mg^{-1}, while those for *PtrACS*2 were 0.72 mM and 245.96 μM min^{-1} mg^{-1}, respectively. Our results revealed that both proteins belong to the ACS family, and provide a theoretical foundation for the identification and functional analysis of members of the adenylate-forming enzyme superfamily.

Keywords: Acetyl-CoA synthetase (ACS), Prokaryotic expression, Enzyme activity, *Populus trichocarpa*

Background

The adenylate-forming enzyme superfamily is characterized by the presence of a highly conserved putative AMP-binding domain (PROSITE PS00455) and a shared ATP-dependent, two-step reaction mechanism as follows (Schneider et al. 2005): Acid + ATP → Acyl-AMP + PPi (REACTION 1) and Acyl-AMP + CoA → acyl-CoA + AMP (REACTION 2), which contributes to the biosynthesis or degradation of diverse compounds such as fatty acids, amino acids, and a variety of secondary metabolites. In fact, diverse proteins such as fatty acyl-CoA synthetases, acetyl-CoA synthetases (ACS), 4-coumarate:CoA ligases (4CL), chlorobenzoate:CoA ligase, non-ribosomal polypeptide synthetases, and firefly luciferases are classified in one superfamily of adenylate-forming enzymes (Stuible and Kombrink 2001).

4CL is an important enzyme in lignin biosynthesis. It plays a key role in general phenylpropanoid metabolism, which is the link between lignin precursors and branched products. 4CL catalyzes the activation of various cinnamic acid derivatives (coumarate, caffeate, and ferulate) to form their corresponding CoA esters, and these activated phenolic acids serve as precursors for the biosynthesis of lignin (Stuible and Kombrink 2001; Weisshaar and Jenkins 1998). Previous studies showed that 4CL enzymes are encoded by multigene families in all vascular plants (Hamberger et al. 2007; Lindermayr et al. 2002; Kumar and Ellis 2003), and that isoenzymes of 4CL had differential enzymatic activity toward different

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