The Cloning and Expression of β-Hydroxyisobutyryl Coenzyme A Hydrolase from *Arabidopsis thaliana*

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Abstract

An essential metabolic process in the plant *Arabidopsis thaliana* is the degradation of the amino acid valine. One enzyme necessary for this process isβ-hydroxyisobutyryl coenzyme A hydrolase (CHY). *A. thaliana* expresses 8 of these hydrolases (CHY1-8), localized to different parts of the cell. Preliminary data has shown that mutants of *A. thaliana* that lack CHY4 do not germinate, but individuals that lack CHY5 (the most similar *A. thaliana* hydrolase) do not suffer any adverse effect. In order to gain a greater understanding of the roles of these enzymes in the degradation of valine, the activity of CHY5 will be compared to CHY4 and a known hydrolase from humans (HHYD). To do so, the chy5 gene was cloned in *E. coli,* which served as an expression system for purifying the CHY5 enzyme. Once purified, the activity of CHY5 can be analyzed with respect to CHY4 and HHYD. Based on previous work from this lab, we hypothesize that CHY5 will have lower activity compared to CHY4 and HHYD. The cloning and purification of CHY5 will be presented here.

Introduction

The amino acids leucine, isoleucine, and valine are 3 essential amino acids and make up the group known as branched-chain amino acids. These play important roles in the membrane spanning stretches of most proteins and make up 35% of the necessary amino acids in the mammalian diet (1). They are broken down in the mitochondria of humans and their digestion yields the molecule propionyl-CoA which may be further digested to yield products used in the TCA cycle and in lipid synthesis (2). The ubiquity of the branched-chain amino acids in mammalian proteins and use in energy production makes understanding how organisms digest and utilize these amino acids of paramount importance for furthering knowledge on protein metabolism and energy storage.

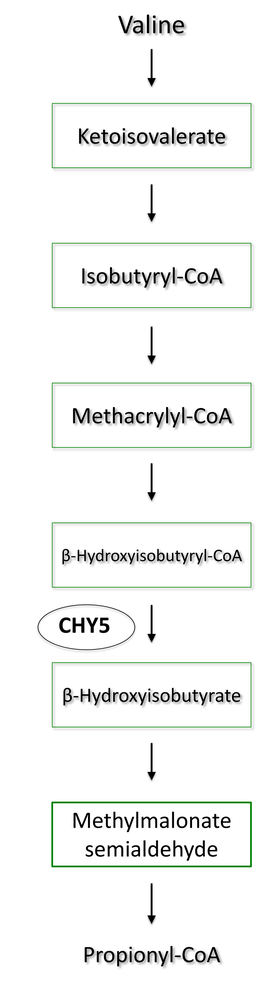

Fig 1. Catabolic pathway of valine in mitochondria of *A. thaliana*.

The research in Prof. Rouhier's lab focuses on the enzymes in the second half of valine degradation, specifically the enzymes active in the mitochondria. Of particular interest is an enzyme involved in the degradation of valine; β-hydroxyisobutyryl-CoA hydrolase. This enzyme catalyzes the breakdown of β-hydroxyisobutyryl-CoA to the intermediate β-hydroxyisobutyrate (fig 1). There is only a single gene which codes for β-hydroxyisobutyryl-CoA hydrolase in humans (2). In the plant species *Arabidopsis thaliana* there are eight of these enzymes, dubbed CHY1-8. CHY4 appears to be active in the mitochondria of *A. thaliana* (unpublished). Mutant *A. thaliana* seedlings which do not have the CHY4 gene have a lethal phenotype. Similarly, infants born without this gene suffer from severe birth defects and very high mortality rates. CHY5 shares high sequence homology with CHY4 and is also believed to be localized to the mitochondria. (unpublished).

To gain insight into the difference between these two enzymes, the chy5 gene sequence was inserted into the protein expression vector pET-28a and this construct cloned into *E. coli*. Once this was achieved this protein along with CHY4 and the human hydrolase (HHYD) could be expressed and their activities with different starting materials, assessed.

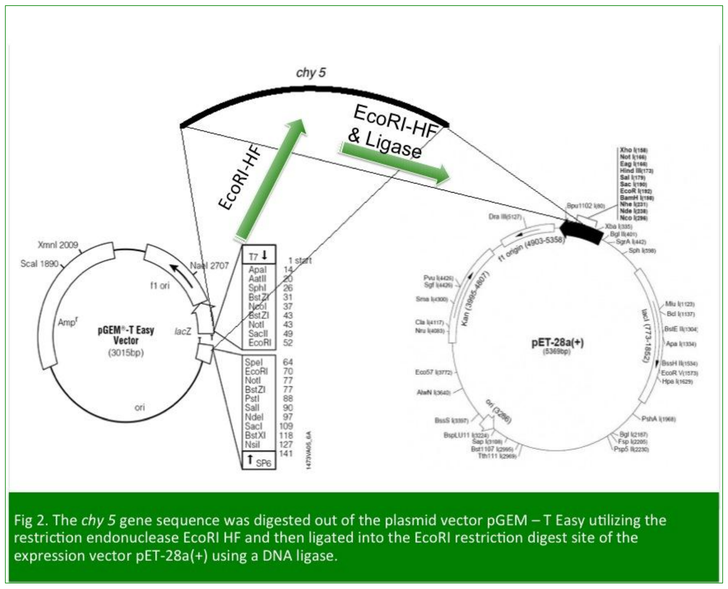

Fig 2. The *chy 5* gene sequence was digested out of the plasmid vector pGEM – T Easy utilizing the restriction endonuclease EcoRI HF and then ligated into the EcoRI restriction digest site of the expression vector pET-28a(+) using a DNA ligase.

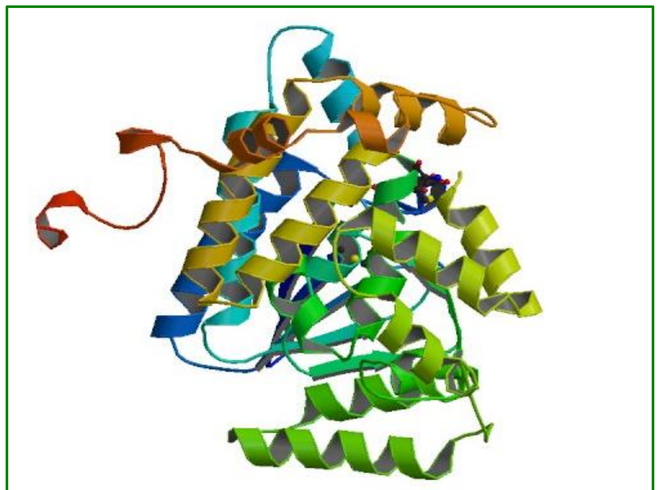

Figure 6. Crystal structure of HHYD as determined by X-ray crystallography (PDB 3BPT).

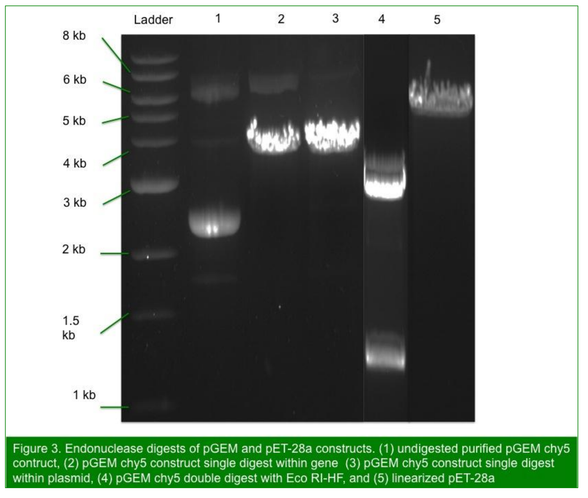

Figure 3. Endonuclease digests of pGEM and pET-28a constructs. (1) undigested purified pGEM chy5 contruct, (2) pGEM chy5 construct single digest within gene (3) pGEM chy5 construct single digest within plasmid, (4) pGEM chy5 double digest with Eco RI-HF, and (5) linearized pET-28a

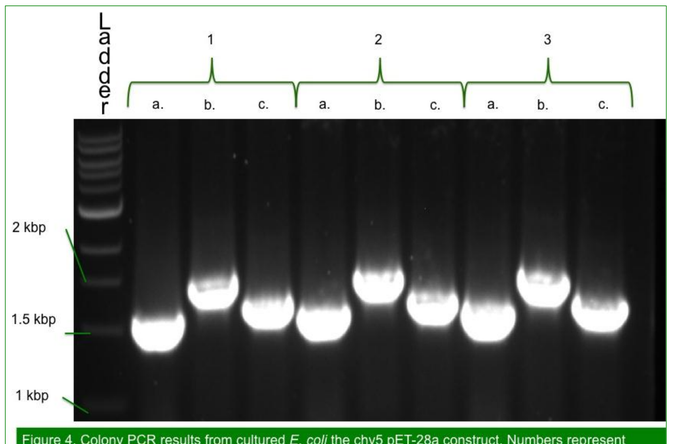

Figure 4. Colony PCR results from cultured *E. coli* the chy5 pET-28a construct. Numbers represent different colonies. Letters corespond to PCR with primers specific to the chy5 gene (a), the T7 promoter and terminator of pET-28a (b), and the T7 promoter and end of the chy 5 gene sequence (c).

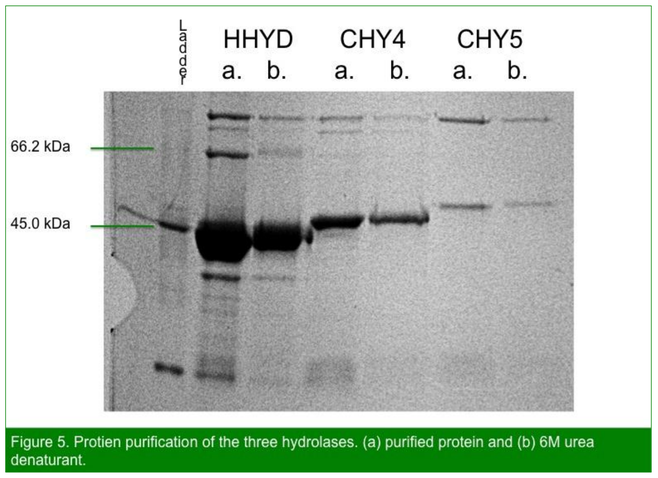

Figure 5. Protien purification of the three hydrolases. (a) purified protein and (b) 6M urea denaturant.

Materials & Methods

The *Arabidopsis thaliana* chy5 gene sequence was expressed in the promega pGEM T-Easy vector in the bacteria *Escherichia coli*. This construct was then purified from *E. coli* and the gene sequence excised out of pGEM using the restriction endonuclease EcoRI-HF. The expression vector pET-28a was then linearized with the same restriction endonuclease and the chy5 gene sequence, ligated into pET-28a to make the expression construct. This construct was then cloned into *E. coli*. The presence of the chy5 gene was confirmed by sequencing and colony PCR. The CHY4, HHYD (previously cloned into pET-28a), and CHY5 proteins were all expressed and purified utilizing affinity column chromatography.

Results & Discussion

The chy5 gene sequence was successfully cloned out of the pGEM plasmid, into the pET-28a plasmid, and uptaken by *E. coli* as confirmed by colony PCR (figure 4). The chy5 sequence in pET-28a was confirmed by DNA sequencing (data not shown).

All three proteins were expressed in *E. coli* however, each copurified with many other contaminating proteins (figure 5 (a) lanes). These contaminating proteins did not wash out of the purified fraction even in the presence of the 6M urea denaturing buff (figure 5 (b) lanes). Thus, while chy5 was successfully cloned and expressed along side CHY4 and HHYD, these proteins were not pure enough to have their activities analyzed.

Future Work

Experiments will focus on expressing proteins without contaminating proteins from *E. coli*. Once proteins have been expressed as pure fractions, experiments will be performed assessing their individual activities with different β-Hydroxyisobutyryl Coenzyme A and other molecules that resemble it.

Acknowledgements

I would like to thank Dr. Kerry Rouhier for her guidance in all parts of this project as well as in creating the chy4 and hhyd pET-28a constructs. I would also like to thank the Kenyon Summer Science Program for funding this project.

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