SAND2022-2129C

Structure and kinetics of a faster, non-metal dependent 5-Carboxyvanillic acid decarboxylase from *Sphingomonas paucimobilis* SYK6, LigW2

Paul Wolski,[1,2] Andy DeGiovanni,[1,3] Andria Rodrigues,[1,3] Blake A. Simmons,[1,3] Paul D. Adams,[1,3] Jose Henrique Pereira[1,3] and Kenneth L. Sale[1,2]
1. Joint Bioenergy Institute, Emeryville, CA 94608 2. Sandia National Laboratories, Livermore, CA, 94550, 3. Lawrence Berkeley National Laboratory, Berkeley, CA 94720

JBEI Joint BioEnergy Institute

Sandia National Laboratories

Abstract

Lignin modifying enzymes (LMEs) such as laccases and peroxidases are produced by soil fungi and bacteria. They catalyze the breakdown of lignin polymers into many smaller chemical species. Many of these enzymes require radical chemistry, which can produce unpredictable products and potentially even repolymerize those products. However, a class of enzymes grouped into the “Lig” family does not require radical chemistry to break either carbon-carbon or carbon-oxygen linkages in phenolic dimers and monomers. Also, unlike laccases and peroxidases, the “Lig” family of enzymes are generally intracellular and part of a metabolic pathway that work in a sequence, gradually catabolizing lignin derived dimers and monomers to directly usable molecules such as pyruvate. Here we describe our work with LigW2, a 5-carboxyvanillate decarboxylase from the soil bacterium *Sphingomonas paucimobilis* SYK6. In contrast to its functional homologue, LigW, which requires Mn to catalyze 5-carboxyvanillate decarboxylation, our results show LigW2 does not require a metal cofactor in the active site. To further investigate this result, we solved several structures of LigW2 with and without substrate and used the structure to study substrate binding and catalytic mechanisms. Our results from chelation and amino acid knockout studies point to how LigW2 functions without a metal. We also show that this enzyme is evolutionarily conserved with *Cutaneotrichosporon moniliiforme* salicylate decarboxylase, which does not require a metal cofactor, and that the salicylate decarboxylase and LigW2 might have coevolved from an ancestral enzyme to which LigW is more closely related than is LigW2.

Materials and Methods

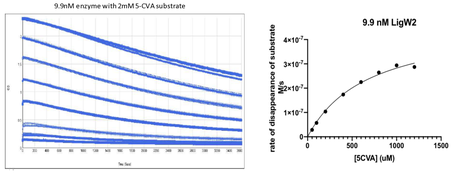
Wild type LigW2 as well as 9 active site mutants were ordered from Genscript and expressed in E.coli. The mutants were chosen based on a similar study done on LigW by others*. The proteins were expressed with a His tag and purified by IMAC, immobilized metal affinity chromatography, to >95% purity. The enzymes were then used for activity assays based on the observation of the consumption of substrate, 5-carboxy vanillic acid, by measuring the absorbance at 310nM over time. The reaction mix was further analyzed by HPLC for the appearance of a peak at an elution time specific to the product, vanillate. Kinetic parameters for the WT and mutants were analyzed and compared to understand how the mutations affected the enzyme in light of the proposed mechanism.

In addition, LigW from *Sphingomonas paucimobilis* was also expressed, purified and studied for its manganese dependence along with WT LigW2. Both enzymes were also subject to size exclusion chromatography.

Then, ICP-MS (inductively coupled plasma mass spectroscopy) was performed to confirm the presence of Mn or any extraneous metals in LigW2.

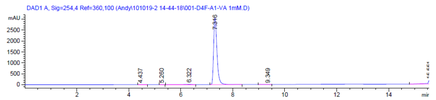
Results

Below is kinetic data as well as a typical analysis for wild type LigW2 ran at 9.9nM enzyme concentration and 50 – 1200uM substrate concentration in triplicate over the course of 60 minutes.

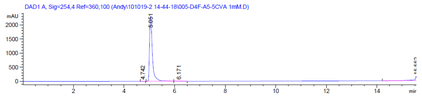


Below are traces from the reverse phase HPLC assay showing pure product, pure substrate, and a reaction with a mutant that produced some product.

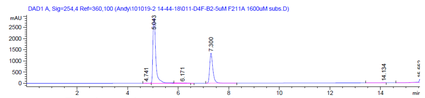
Product: vanillate



Substrate: 5-Carboxyvanillic acid

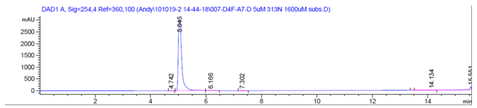


Mutant F211A



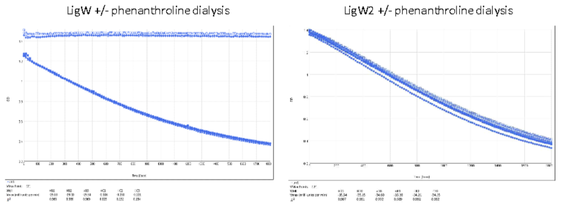
Both substrate and product seen.

Mutant D313N

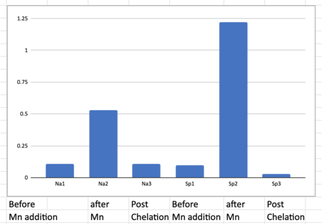


Only substrate seen.

Below are a time course of LigW2 and LigW from *S. paucimobilis SYK-6* after treatment with 10mM Phenanthroline to chelate any metals present. The horizontal line is the inactive LigW, whereas the downward sloping line is LigW2 with almost native activity.



Fraction of Protein occupied with Mn2+
Determined by ICP-MS



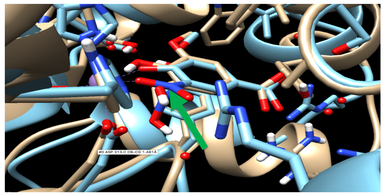
Na: LigW from *Novosphingobium*
Sp. LigW2 from *Sphingomonas*

Discussion

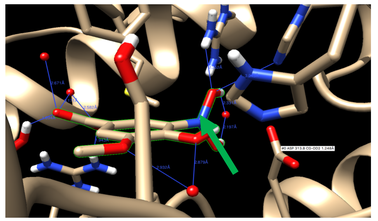
LigW2 is the family of decarboxylases, as is LigW. Our data show that LigW2 is about 10X faster in terms of Kcat than for LigW. The main differences are the lack of a metal in LigW2 and slight differences in positioning and hydrogen bonding distances .
Salicylate decarboxylase from *Trichosporon moniliiforme* has been shown to not require a metal.** We are currently comparing the sequence and structure of the phenolic decarboxylases that require a metal and ones that do not.

Salicylic acid has been shown to decarboxylate with the ring stabilizing the positive charge intermediate, and this could be how 5CVA decarboxylates in LigW2

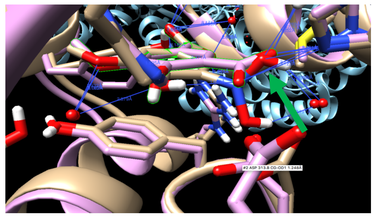
:
LigW (blue)
complexed
with 5NVA and Mn2+
Over LigW2 (gold)
With 5NVA



LigW2 complexed
with 5NVA,
Note the orientation
Of the the substrate
leaving group
compared in LigW



LigW2 complexed
with 5CVA in pink. Note the orientation
Of the the substrate leaving group



Acknowledgments

This work conducted by the Joint BioEnergy Institute was supported by the Office of Science, Office of Biological and Environmental Research, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. Thank you to the University of Georgia for assistance with ICP-MS studies. Sandia National Laboratories is a multimission laboratory managed and operated by National Technology & Engineering Solutions of Sandia, LLC, a wholly owned subsidiary of Honeywell International Inc., for the U.S. Department of Energy’s National Nuclear Security Administration under contract DE-NA0003525.

U.S. DEPARTMENT OF ENERGY | Office of Science

*A Vladimirova et. al, JACS. 2016, 138, 826-836
** K. Kirimura et. al, Biochemical and Biophysical Research Communications 394 (2010) 279–284