Elucidating the Role of 4-Coumarate: CoA Ligase in Plant Cell Wall Biosynthesis

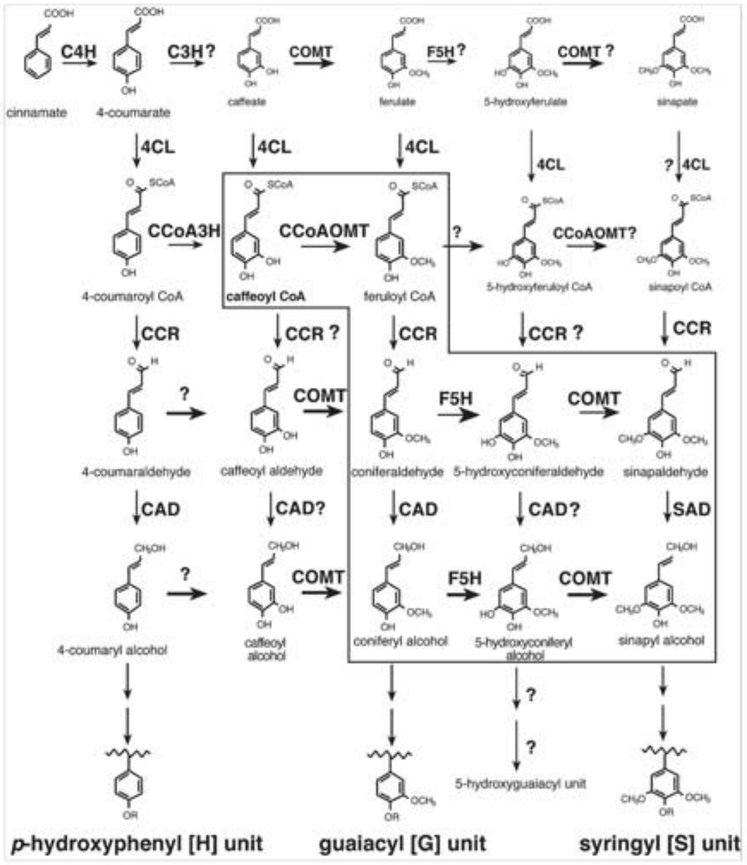
Wesley P. Morioka and Christopher M. Bianchetti

Department of Chemistry University of Wisconsin–Oshkosh, WI

Abstract

Fossil fuels are the largest contributor to global warming and our dependence on this non-renewable source of energy is unsustainable. Renewable energy sources such as bioethanol derived from plant biomass have the capability to reduce and even replace fossil fuels. Currently, bioethanol is produced from food crops, such as corn and sugar cane, and are unsustainable in the long run. Bioethanol derived from plants biomass would be sustainable. However, the carbohydrates needed to produce bioethanol are encased by ligninit and are difficult to extract. Lignin is a complex polymer found in the tissues of plants that provides the plant with shape, rigidity, and chemical transport. 4-Coumarate: CoA ligase from tobacco (Nt4CL2) plays a pivotal role in the biosynthesis of lignin by activating the building blocks of lignin that are then incorporated into a growing lignin chain. Here we present the crystal structure and enzymatic properties of Nt4CL2. Mutational analysis was also performed to investigate the key components in catalysis. An understanding how plants produce and synthesize lignin allows us to potentially create genetically modified plants with less or easier to break down lignin. A genetically modified plant reduces the time and energy intensive process of extracting the sugars from within the plant used in producing bioethanol.

Lignin Biosynthesis



Adapted from: Eckardt N.A., 2002 "Probing the mysteries of lignin biosynthesis: the crystal structure of caffeic acid/5-hydroxyferulic acid 3/5-O-methyltransferase provides new insights" Plant Cell 14: 1185–1189

Figure 1: Lignin is a heterogeneous polymer composed of the monomers p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S). The biosynthesis of lignin requires several enzymes. Nt4CL2 catalyzes a central step in the biosynthetic pathway and activates the building blocks of lignin. These activated building blocks are used as precursors for the biosynthesis of lignin and other important natural products formed via a variety of pathways.

Reaction Mechanism

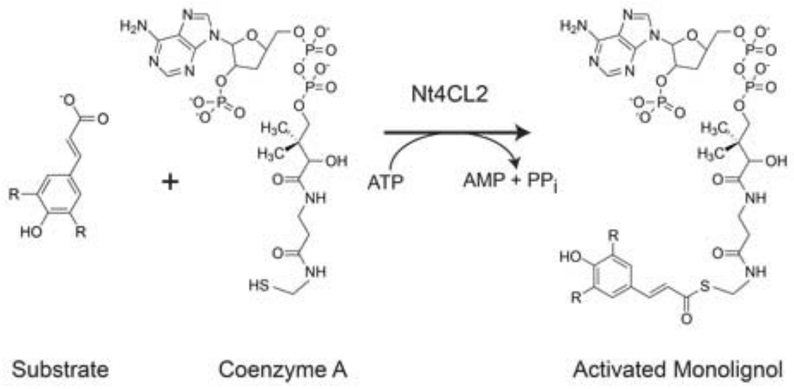


Figure 2: Generalized reaction mechanism of Nt4CL2. The reaction proceeds via a two-steps mechanism. In the first step, an adenylate intermediate is formed coupled with the release of pyrophosphate. In the second step, a thioester is formed between coenzyme A (CoA) and substrate.

Nt4CL2 Substrates

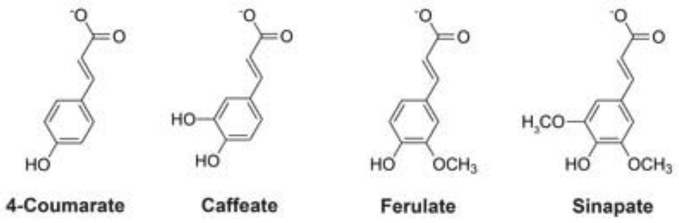


Figure 3: Substrates used to determined the kinetic parameters of Nt4CL2. Each reaction contained 30 nM Nt4CL2, 2.5 mM ATP, 0.3 mM CoA, 2.5 mM $MgCl_2$, 100 mM Tris pH 7.5 and varying concentrations of 4-coumarate, caffeate, ferulate or sinapate. The concentrations of substrate varied from 0.5 µM to 20 µM for 4-coumarate, caffeate and ferulate and from 0.3 mM to 5.0 mM for sinapate.

Nt4CL2 Assay

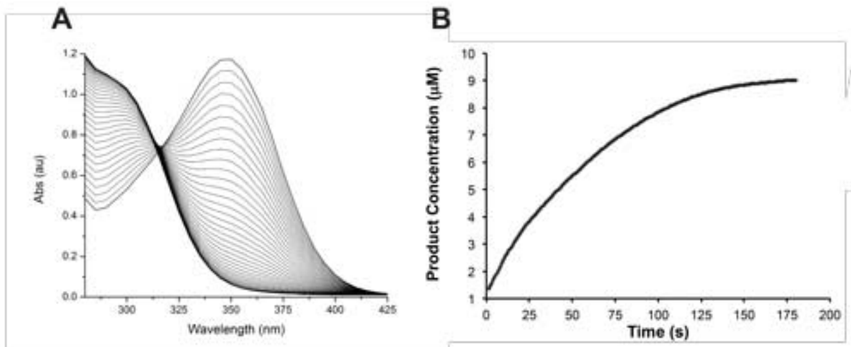


Figure 4: A. Example absorbance spectrum for the formation of Caffeoyl-CoA. Each thioester product generated had a unique absorbance spectrum.
B. Example graph of product formation versus time. The kinetic properties of Nt4CL2 were determined by plotting the rate of product formation versus substrate concentration and are shown in Table 1.

Kinetic Properties of Nt4CL2

Table 1: Nt4CL2 Kinetic Parameters

Substrate	V_{max} (µmol/s)	K_m (µmol)	K_{cat} (s^{-1})	K_{cat}/K_m ($\mu mol^{-1}s^{-1}$)
4-Coumarate	0.128	2.23	4.25	1.91
Caffeate	0.056	1.96	1.85	0.95
Ferulate	0.074	1.33	2.48	1.86
Sinapate	ND	ND	ND	ND

Data for the kinetic properties of Nt4CL2. ND, turnover detected, kinetic parameters not determined due to low solubility of the compound.

X-ray Crystal Structure

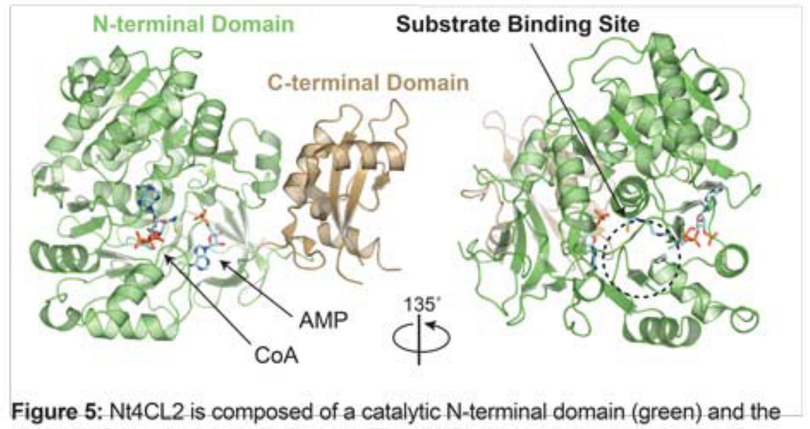


Figure 5: Nt4CL2 is composed of a catalytic N-terminal domain (green) and the dynamic C-terminal domain (brown). The Nt4CL2 structure was not solved in complex with CoA and ATP, however, structural alignments with 5BSR allowed for the identification of these binding sites. The Nt4CL2 C-terminal domain is in the open conformation. The right panel is a 135° rotation relative to the left panel. The substrate-binding site is outlined with a dashed circle.

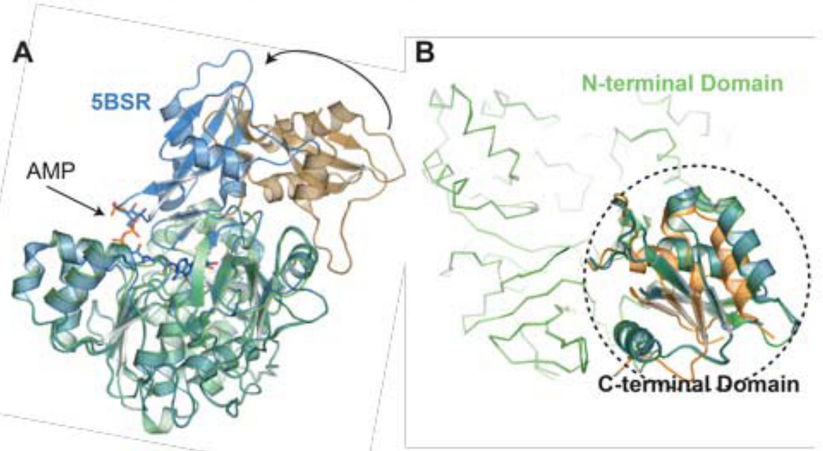


Figure 6: The C-terminal domain of Nt4CL2 is dynamic. **A.** A structural alignment of 5BSR (blue) and Nt4CL2 (same as in Figure 5) shows the C-terminal domain in dramatically different conformations. **B.** Alignment of the B (green), C (orange) and D (blue) monomers show the different conformations of the C-terminal domain observed in the Nt4CL2 crystal structure. The C-terminal domain of the A monomer was not observed due to large domain movements.

Acknowledgments: This project was supported by the Office of Student Research and Creative Activity. Use of the LS-CAT beamline at the APS was supported by Grant 085P1000817.