Structural Study of Hydroxycinnamoyl-CoA:shikimate Hydroxycinnamoyl Transferase

Jose H. Pereira[1,2,*] (jhpereira@lbl.gov), Aymerick Eudes[1,3], Sasha Yogiswara[1,3,4], George Wang[1,3], Veronica Teixeira Benites[1,3,5], Edward E. K. Baidoo[1,3], Taek Soon Lee[1,3], Jay D. Keasling[1,3,4], Dominique Loqué[1,3] and **Paul D. Adams[1,2]**

[1]Joint BioEnergy Institute, EmeryStation East, 5885 Hollis St, 4th Floor, Emeryville, CA 94608, USA. [2]Molecular Biophysics and Integrated Bioimaging Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720, USA. [3]Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, Berkeley, CA 94720, USA. [4]Department of Bioengineering & Department of Chemical & Biomolecular Engineering, University of California, Berkeley, CA 94720, USA. [5]Graduate Program, San Francisco State University, San Francisco, CA 94132, USA.

Project Goals: Understand structurally the substrate promiscuity of Hydroxycinnamoyl-CoA:shikimate Hydroxycinnamoyl Transferase (HCT) from a *Panicum virgatum* (PvHCT) toward a series of benzene and benzoate derivatives. The high-resolution crystal structures of PvHCT-*p*-coumaroyl-CoA-shikimate and PvHCT-*p*-coumaroyl-CoA-protocatechuate confirmed the binding of a non-canonical acceptor in a similar manner as shikimate in the active site of the enzyme.

Lignin poses a crucial challenge in the processing of plant biomass for agro-industrial applications. For bioengineering purposes, there is a pressing interest in identifying and characterizing the enzymes responsible for the biosynthesis of lignin. Hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase (HCT) is a key metabolic entry point for the synthesis of the most important lignin monomers: coniferyl and sinapyl alcohols. In this study, we investigated structurally the substrate promiscuity of HCT from *Panicum virgatum*. The crystal structure of PvHCT consists of two domains, with the *p*-coumaryl-CoA and shikimate binding sites located between them. Domain I consists of N-terminal residues 1 to 199 and C-terminal residues 387 to 409. Domain II consists exclusively of C-terminal residues 200 to 386 and 410 to 446. The structure of PvHCT2 in complex with *p*-coumaroyl-CoA and shikimate reveals that the two molecules reacted during the soaking of the compounds into the crystal. Therefore, the product state was observed in the electron density map, given the ternary complex of PvHCT, free coenzyme A and *p*-coumaroyl shikimate. In contrast, the crystal structure of PvHCT-*p*-coumaroyl-CoA-protocatechuate is in a substrate state, i.e. the ternary complex consists of PvHCT, *p*-coumaroyl-CoA and protocatechuate.

The *p*-coumaroyl-shikimate contacts the PvHCT via the phenolic group and carbonyl group of the *p*-coumaroyl portion. The shikimate portion contacts PvHCT through both carboxyl and hydroxyl groups. The carbonyl group of the *p*-coumaroyl moiety directly contacts Trp384 and the phenolic moiety interacts via water-mediated hydrogen bonds with Ser38 and Tyr40. The carboxyl group of the shikimate moiety makes a salt bridge interaction with Arg369. While the C5 hydroxyl group contacts the catalytic residue His163, the C3 hydroxyl group contacts Thr382. PvHCT-*p*-coumaroyl-CoA-protocatechuate structure shows that the protocatechuate binds in a very similar manner as shikimate, with the carboxyl group making a tight salt bridge with Arg369, and the C3 (equivalent to C5 in shikimate moiety) hydroxyl group interacting with the nitrogen NE2 of His163. However, we see that the C5 hydroxyl (equivalent to C3 in shikimate moiety) interaction with Thr382 is lost, since this hydroxyl group is absent in protocatechuate[1].

References

1. Eudes, A., Pereira, J.H., Yogiswara, S. Wang, G., Benites, V.T., Baidoo, E.E.K., Lee, T.S., Adams, P.D., Keasling, J.D. and Loqué, D.Exploiting The Substrate Promiscuity of Hydroxycinnamoyl-CoA:shikimate Hydroxycinnamoyl Transferase to Reduce Lignin. *Plant & Cell Physiology.* IN PRESS

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