Kevin Walker

Functional Analysis of Enzymes on Biosynthetic Pathways of Plant-derived Bioactive Compounds

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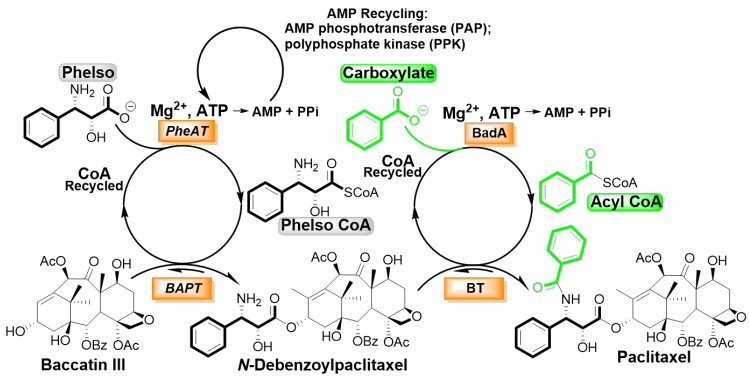
We use interdisciplinary methods to evaluate enzyme catalysts from various sources, such as bacteria, plants, and yeast, with non-natural substrates. Our vision is to transform natural compounds or synthetically-derived chemicals to novel products. Transfer of the genes encoding these enzymes into a chassis organism can potentially make various bioactive molecules *in vivo* or *in vitro*.

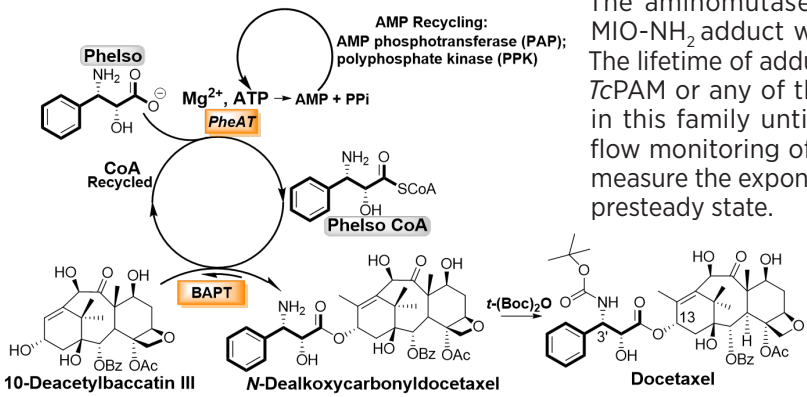


Taxane analogues (docetaxel, paclitaxel, cabazitaxel, paclitaxel C, and tesetaxel) are used ***1)*** for breast, ovarian, and prostate cancers, ***2)*** to stem complications from stent implants in heart surgery, and ***3)*** to work potentially as neuroprotectants against stroke.

Biocatalysis of Docetaxel – Current methods to make docetaxel still use an 11 to 12-step semisynthesis, which involves protecting group chemistry that compromises yields and reduces atom economy.

We use regioselective biocatalysts (*Taxus* Acyltransferases (AT) and Bacterial CoA Ligases) to bypass protecting group chemistry to make docetaxel.

Streamlined 3-Step Biocatalysis of Docetaxel—An alternative to make docetaxel:

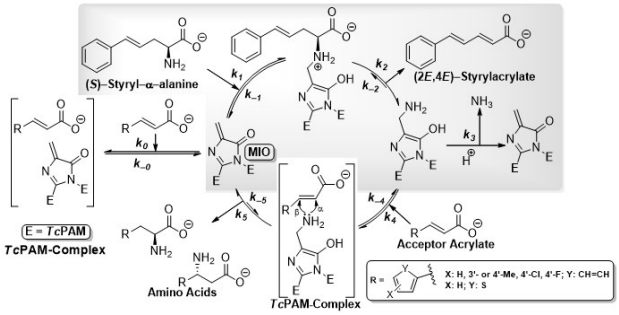


Coupling acyltransferases with CoA ligases (above) provides a Green source of docetaxel and its drug analogues.

Four-Enzyme Cascade Reaction to Paclitaxel – PheAT and BadA (CoA Ligases), BAPT and BT (Acyltransferase) are used in an alternative route to make paclitaxel; we use regioselective biocatalysts (*Taxus* Acyltransferases (AT) of the BAHD Superfamily and Bacterial CoA Ligases) that streamline the production pipeline to the target pharmaceutical paclitaxel (Taxol). No taxane side products accumulate and cofactors (ATP and Coenzyme A) can be recycled. This method provides a Green source of a taxane drug precursor.

Importance of β-Amino Acids as Bioactive Products

Paclitaxel (Taxol) Pathway Aminomutase – A Taxus phenylalanine aminomutase (*Tc*PAM) converts (2*S*)-α-phenylalanine ((2*S*)-α-Phe) to (3*R*)-β-Phe and lies on the paclitaxel (Taxol™) biosynthetic pathway in *Taxus* plants.



To understand how to use *Tc*PAM chemistry to biocatalyze β-amino acids, it is necessary to understand the subtleties of its mechanism.

The aminomutase forms a transient MIO-NH_2 adduct with a finite lifetime. The lifetime of adduct was unknown for *Tc*PAM or any of the several enzymes in this family until we used stopped-flow monitoring of product release to measure the exponential burst phase at presteady state.

A new graduate student can embark on studies involving organic chemistry synthesis of novel surrogate substrates. Other areas of training include molecular cloning techniques, expression of various enzymes in *E. coli*, and assay development. Included are basic biochemical applications and molecular engineering approaches related to enzyme kinetics, enzyme purification and characterization, and various analytical techniques (such as NMR, GC/ MS, LC-MS(/MS), and X-ray crystallography).

Selected Publications

Understanding Which Residues of the Active Site and Loop Structure of a Tyrosine Aminomutase Define its Mutase and Lyase Activities, G. Attanayake; T. Walter; K. D. Walker, *Biochemistry (ACS)*, **2018**, *DOI: 10.1021/acs.biochem.8b00269*.

Biocatalysis of a Paclitaxel Analogue: Conversion of Baccatin III to N-Debenzoyl-N-(2-furoyl)paclitaxel and Characterization of an Amino Phenylpropanoyl CoA Transferase, C.K. Thornburg; T. Walter; K.D. Walker, *Biochemistry (ACS)* **2017**, *56 (44)*, 5920–5930.

Paclitaxel Biosynthesis: Adenylation and Thiolation Domains of an NRPS TycA PheAT Module Produce Various Arylisoserine CoA Thioesters, RMuchiri; K.D.Walker, *Biochemistry (ACS)* **2017**, *56 (10)*, 1415–1425.

Identification and characterization of the missing phosphatase on the riboflavin biosynthesis pathway in Arabidopsis thaliana, N. Sa; R. Rawat; C.K. Thornburg; K.D. Walker; S. Roje, *Plant J.* **2016**, *88 (5)*, 705–716. **Featured Article**.

Mutation of aryl binding-pocket residues results in an unexpected activity switch in an Oryza sativa tyrosine aminomutase, T. Walter; D. Wijewardena; K.D. Walker, *Biochemistry* **2016**, *55 (25)*, 3497–3503.