4-3-2022

A chalcone synthase-like bacterial protein catalyzes heterocyclic c-ring cleavage of naringenin to alter bioactivity against nuclear receptors in colonic epithelial cells

Ebru Ece Gulsan

Farrhin Nowshad

Stephen Safe

Meredith Leigh Davis

Jimmy Walter Crott

See next page for additional authors

Authors

Ebru Ece Gulsan, Farrhin Nowshad, Stephen Safe, Meredith Leigh Davis, Jimmy Walter Crott, Arul Jayaraman, and Kyongbum Lee

A CHALCONE SYNTHASE-LIKE BACTERIAL PROTEIN CATALYZES HETEROCYCLIC C-RING CLEAVAGE OF NARINGENIN TO ALTER BIOACTIVITY AGAINST NUCLEAR RECEPTORS IN COLONIC EPITHELIAL CELLS

Ebru Ece Gulsan, Tufts University, USA
ebru_ece.gulsan@tufts.edu
Farrhin Nowshad, Texas A&M University, USA
Stephen Safe, Texas A&M University, USA
Meredith Leigh Davis, Tufts University, USA
Jimmy Walter Crott, Tufts University, USA
Arul Jayaraman, Texas A&M University, USA
Kyongbum Lee, Tufts University, USA

Key Words: Flavonoids, gut microbiome, ring cleavage, naringenin, enzyme promiscuity

Flavonoids are the largest class of naturally occurring polyphenolic phytochemicals in terms of number of structurally distinct molecules, with over 6,000 unique structures. They are particularly abundant in plant-based foods that are considered health-promoting. Upon ingestion, flavonoids are only partially absorbed, and gut microbiota plays a significant role in their metabolism. The health benefits of flavonoids are generally associated with their antioxidant and anti-inflammatory activities, although the potency varies widely even among flavonoids of the same subclass.

The enzymatic pathways of flavonoid metabolism in gut bacteria are largely unresolved. Flavonoids are not natural substrates of gut bacterial enzymes. Consequently, reactions of flavonoid metabolism have been attributed to more general classes of enzymes. However, the specific enzymes that belong to these classes and the bacterial species carrying the enzymes remain to be elucidated. To systematically characterize promiscuous enzyme activity on flavonoids, we developed a prediction tool that is based on chemical reaction similarity. The algorithm can take a single enzyme or a list of enzymes from an organism or a batch culture to match microbial enzymes with their non-native flavonoid substrates and orphan reactions. We successfully predicted the promiscuous activity of known flavonoid-metabolizing bacterial enzymes.

In vitro studies have shown that flavonoids can engage specific cellular receptors, but it remains to be established if and which of the pathways regulated by these receptors are responsible for the flavonoids' beneficial effects *in vivo*. Interestingly, these receptors can also bind phenolic acids, which are known metabolic products of flavonoids. In contrast to many flavonoid compounds, these metabolites are well absorbed in the intestine and are typically found at higher plasma concentrations. This raises the intriguing question whether flavonoid derived metabolites are quantitatively important ligands for the Aryl Hydrocarbon Receptor (AhR), nuclear receptor 4A (NR4A), and other flavonoid relevant receptors.

In this study, we investigate the microbial metabolism of a model flavonoid, naringenin, into its C-ring cleavage product, 3,4-hydroxyphenylpropanoic acid (34HPPA) through naringenin chalcone as the intermediate. Using metabolic modeling, we predicted that this metabolism in gut bacteria proceeds through a chalcone synthase-like bacterial polyketide synthase and confirmed this prediction using *in vitro* culture experiments and LCMS/MS techniques. We also showed that the formation of 34HPPA in a mixed bacterial community depends on microbiota composition due to the rarity of the chalcone synthase-like enzyme. Our gene expression assays for induction of CYP1A1, CYP1B1 and UGT1A1 in human epithelial cells showed that naringenin chalcone is an AhR ligand, while naringenin is inactive. 34HPPA alone did not affect AhR-responsive gene expression, but it significantly enhanced CYP1A1 and CYP1B1 expressions when combined with the AhR agonist TCDD (10 nM), indicating a synergistic effect. However, our binding studies, as orphan receptor gene expressions cannot be linked to their functions directly, on naringenin and 34HPPA with NR4A1/2 receptors showed that only naringenin has strong binding affinity. Having found that naringenin and its ring cleavage metabolites exhibit different AhR agonist activities and NR4A binding affinities, we next investigated whether they also show different immunomodulatory effects. We observed a significant decrease in IL-1ß induced IL-8 expression upon naringenin treatment in Caco-2 cells, whereas 34HPPA had no effect. Our findings underscore the importance of elucidating the pathways and products of gut bacterial flavonoid metabolism. Prospectively, the prediction-validation methodology developed in this work could be used to systematically characterize the metabolism of flavonoid metabolites and identify the responsible enzymes and species, directly linking the formation of bioactive flavonoids to abundance of specific gut bacteria.