Tutorial #X: Searching for a Synthesis Enzyme

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Enzymes can be recruited to perform reactions in the service of organic chemistry. Can we find a mutant of β-galactosidase that will enable us to produce a glycosylated polyphenolic neutraceutical?

This document was produced using the LaTeX typesetting language with the Tufte-handout document class. Images of proteins were created using *UCSF Chimera*. Chemical diagrams were made with *ChemDoodle* and further edited with *Affinity Designer*.

Enzymes Kinetics and Synthesis

Enzymes catalyze many biological reactions. Often the reaction catalyzed is a desirable synthetic reaction. The stereospecificity and high catalytic efficiency of enzymes makes them prime candidates for use in organic synthesis. In this exercise we will choose a target reaction, find an enzyme that can catalyze the reaction and then attempt to improve the enzyme by generating mutants. We will focus on the enzyme kinetics of the enzyme candidates with an assay reaction and explore the use of computational tools to deal with large amounts of data.

A Desirable Reaction

Daidzin is a natural product found in Japanese kudzo vines and soybean leaves. It is believed to be the active ingredient in herbal medicinal extracts that have been used to treat alcoholism over hundreds of years. Toxic alcohol is removed from the body by the action of *alcohol dehydrogenase*, which catalyzes the oxidation of alcohol by NAD^+ to give acetaldehyde, an even more toxic substance.[1,2] Fortunately another enzyme, *aldehyde dehydrogenase*, quickly oxidizes the aldehyde to give acetic acid that can be ligated to coenzyme-A and used for energy or, more likely, to make fats. daidzin inhibits *aldehyde dehydrogenase* and this will allow acetaldehyde to build up and make a person very uncomfortable.

Natural products can be expensive to extract and often the extraction method degrades the material. I have found a cheap source of a related compound, daidzein, from coal tar. This simpler compound is also an inhibitor of *aldehyde dehydrogenase* but it is not soluble in water without the sugar group. I want to glycosylate the daidzein with galactose, rather than the glucose that is present in daidzin. I plan to use an enzyme to catalyze the condensation reaction.

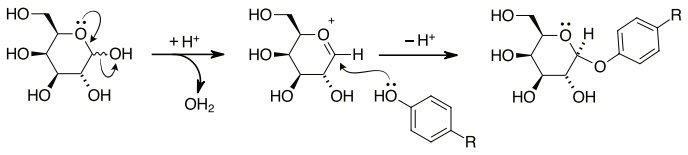
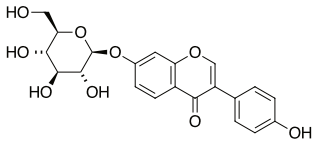
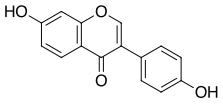


Figure 2: Condensation of a hemiacetal with a phenol in the synthesis of 7-(β-D-Galactopyranosyloxy)-4'-hydroxyisoflavone

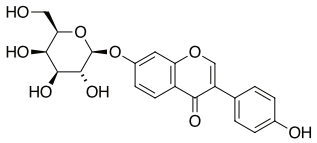
Figure 1: The natural products daidzin and daidzein compared to our synthetic target, 7-(β-D-Galactopyranosyloxy)-4'-hydroxyisoflavone



Daidzin



Daidzein



7-(β-D-Galactopyranosyloxy)-4'-hydroxyisoflavone

[1] "Daidzin: A Potent, Selective Inhibitor of Human Mitochondrial Aldehyde Dehydrogenase." W.-M. Keung, B.L. Vallee, *Proc. Natl. Acad. Sci. USA*, **1993**, *90*, 1247–1251. https://www.jstor.org/stable/2361166

[2] "Structure of Daidzin, a Naturally Occurring Anti-Alcohol-Addiction Agent in Complex with Human Mitochondrial Aldehyde Dehydrogenase." E.D. Lowe et al., *J. Med. Chem.*, **2008**, *51*, 4482–4487. https://doi.org/10.1021/jm800488j