High-Throughput In Vitro Screening for Inhibitors of Cereal α-Glucosidase

The hydrolysis of starch is a key step in plant germination, which also has relevance in the malting and brewing processes for beer and spirit production. Gaps in knowledge about this metabolic process exist that cannot easily be addressed using traditional genetic techniques, due to functional redundancy in many of the enzyme activities required for alpha-glucan metabolism in cereal crop species. Chemical inhibitors provide opportunities to probe the role of carbohydrate-active enzymes and the phenotypes associated with inhibition of specific enzymes. Iminosugars are the largest group of carbohydrate-active enzyme inhibitors and represent an underused resource for the dissection of plant carbohydrate metabolism. Herein we report a method for carrying out a reverse chemical genetic screen on α-glucosidase, the enzyme that catalyzes the final step in starch degradation during plant germination, namely the hydrolysis of maltose to release glucose. This chapter outlines the use of a high-throughput screen of small molecules for inhibition of α-glucosidase using a colorimetric assay which involves the substrate p-nitrophenyl α-D-glucopyranoside. Identified inhibitors can be further utilized in phenotypic screens to probe the roles played by amylolytic enzymes. Furthermore this 96-well plate-based method can be adapted to assay exo-glycosidase activities involved in other aspects of carbohydrate metabolism.

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