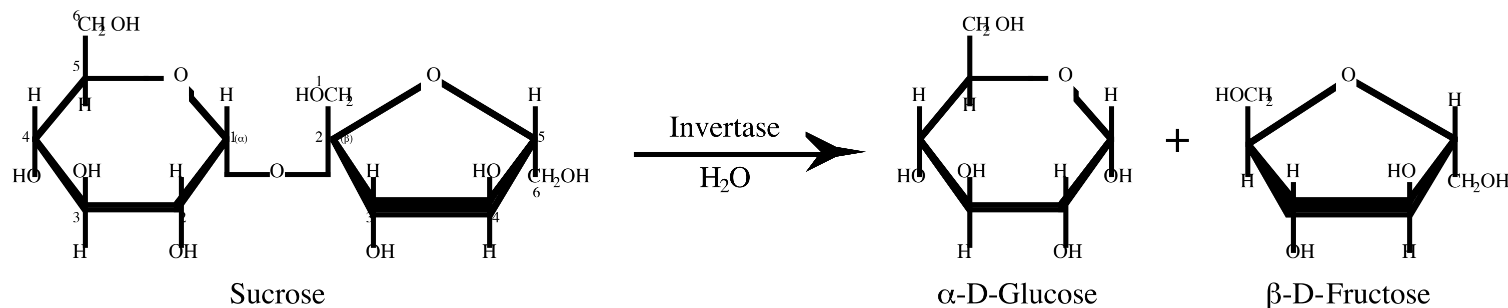
Introduction

Organisms that can metabolize sucrose have an enzyme to assist in this process. Many organisms, both eukaryotic and prokaryotic, have an enzyme called *invertase* that catalyzes the decomposition of *sucrose* into its two component monosaccharides, α-D-*glucose* and β-D-*fructose*:



It is easily seen that sucrose is composed of two different types of monosaccharide units, an α-glucose and a β-fructose (β-fructofuranose). If invertase is specific to α-glucosides, then invertase can be characterized as an α-glucosidase, and if it is specific to β-fructofuranosides, then it can be characterized as a β-fructofuranosidase. Invertase is tested for hydrolysis with the α-glucosides *maltose* and *cellobiose*, and with the β-fructofuranosides *raffinose* and *melezitose*.

The method to use to detect hydrolysis is the reduction of 3,5-dinitrosalicylate (3,5-DNS) by reducing sugars. For the sucrose hydrolysis reaction, this is straightforward since sucrose is not a reducing sugar but both glucose and fructose are reducing sugars. Product sugars catalyzed by the enzyme react with the 3,5-DNS to form a colored complex whose absorbance at 540 nm is measurable by spectrophotometry.

This method works well when the substrate is not a reducing sugar and the products are reducing sugars, as in the sucrose reaction. This is not a problem for testing hydrolysis of raffinose and melezitose, both of which are not reducing sugars. Maltose and cellobiose *are* reducing sugars, so this method needs to be modified to test these sugars. Decomposition of one molecule of either maltose or cellobiose yields two molecules of product, both of which are reducing sugars. Therefore, if hydrolysis occurs, there is a net gain of 1 mole of reducing sugars per 1 mole of analyte sugar. If hydrolysis was successful, this should indicate that a completed reaction tube treated with 3,5-DNS should have greater absorption than a tube that has not been reacted with enzyme. Care must still be taken not to use too much analyte, since the unreacted tube will still produce a considerable amount of coloring that could overwhelm the spectrophotometer's absorption range.

To verify that the modified method works, it is first used to detect hydrolysis of *lactose* by *lactase* (β-galactosidase). Lactose is a reducing sugar, which upon

hydrolysis, produces two product reducing monosaccharides, so this reaction can be used to prove that the method works before attempting to use it with invertase and maltose or cellobiose.

Two logical candidates to test for inhibition of invertase would be glucose and fructose, since many biochemical pathways use feedback inhibition to deactivate the pathway when the products of the pathway are already in ample supply. For this experiment, fructose is tested as a possible inhibitor.

Another possible inhibitor of invertase would be sucralose, a sucrose analog, which is marketed commercially as an artificial sweetener under the trade name Splenda®. Sucralose is very similar in structure to sucrose, with the only differences being that some hydroxyl groups have been replaced with chlorine atoms. Before being tested as an inhibitor, it must be tested first as a possible substrate for invertase.