Conclusions

- **Invertase hydrolyzes β-fructofuranosides**

Invertase hydrolyzed both of the candidate β-fructofuranosides, raffinose and melezitose. When raffinose, melezitose, and sucrose are compared using a *Lineweaver-Burk* plot, as *Figure 2* shows, sucrose has the smallest K_m, and therefore invertase binding has greatest affinity for sucrose. Raffinose has the next lowest K_m, followed by melezitose, indicating that invertase had the most difficulty binding melezitose. Melezitose is a substituted fructofuranoside, while sucrose and raffinose are unsubstituted fructofuranosides. Invertase would seem to prefer an unhindered fructose component when binding the substrate, although this is not required since invertase was able to hydrolyze some melezitose.

Invertase should have been optimized over the course of evolution to hydrolyze sucrose most efficiently, but as *Figure 2* shows, it can actually hydrolyze raffinose (V_{max} = 0.383 mMol/min) more rapidly than sucrose (V_{max} = 0.209 mMol/min). Additional experimentation is required to verify this conclusion; for example, the experiment was carried out at room temperature, and not the enzyme's optimal temperature of 55 °C. Melezitose is hydrolyzed at the slowest rate (V_{max} = 0.105 mMol/min, as might be

expected from the extra steric hindrance caused by the substituted group on the fructose component.

- **Hydrolysis of a Reducing Disaccharide Can Be Detected**

When a disaccharide is a reducing sugar, detection of hydrolysis is possible if both monosaccharide products are also reducing sugars. The lactose reacted with lactase had a significantly higher absorbance (0.676 A) than a sample with lactose alone (0.535 A).

- **Invertase does not hydrolyze α-glucosides**

Maltose and cellobiose were not hydrolyzed by invertase. Maltose reacted with invertase did not have a significantly higher absorbance (0.495 A) than a maltose sample alone (0.514 A). The same was true for cellobiose (0.462 A, 0.496 A). Some anomalous behavior in these results is that for both maltose and cellobiose, there was actually less absorbance with added enzyme. Unfortunately, only one tube was made for the samples without enzyme (but four samples were made for those with added enzyme so an average of $n = 4$ samples could be computed). It could be just experimental error, but the experiment could be repeated with more tubes to be sure.

- **Invertase is a β-fructofuranosidase only**

  Invertase could hydrolyze the two candidate β-fructofuranosides (and is therefore a β-fructofuranosidase), but not the two α-glucosides. Therefore, when hydrolyzing sucrose, invertase is specific for the fructose group, and not the glucose group.

- **Sucralose is not hydrolyzed by invertase**

  A set of tests revealed that sucralose is not hydrolyzed by invertase, despite its close structural resemblance to sucrose. However, unlike sucrose, sucralose was found to be able to reduce 3,5-dinitrosalicylate, as reducing sugars are able to do.

- **Fructose is an inhibitor, but more investigation is needed**

  The *Lineweaver-Burk* plot in *Figure 3* shows that fructose's curve is parallel to that of the control without added inhibitor, and that this would seem to indicate uncompetitive inhibition of invertase. However, the linear regression line is a poor fit ($R^2 = 0.814$), so it is inconclusive as to which type of inhibitor that fructose is.

- **Sucralose is an uncompetitive inhibitor of invertase**

  Figure 3 also shows that sucralose is acting as an uncompetitive inhibitor, which means that sucralose is binding directly to the enzyme-substrate complex but not to the free enzyme. This is surprising, since the structure is so close to that of sucrose, and it doesn't seem logical that sucralose would be binding a complex of invertase and sucrose, rather than being bound directly by invertase at the active site. Competitive inhibition had been expected.