Kinetic Study of the Enzyme Lactase

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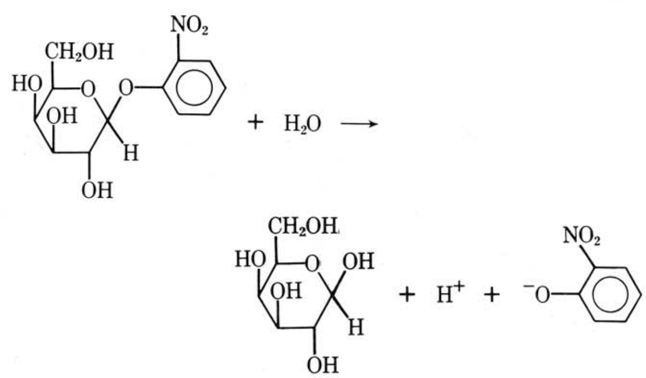
Lactase is a specific intestinal β-galactosidase that is responsible for catalyzing the hydrolysis of lactose to D-glucose and D-galactose. For most of the milk-drinking population of the world, the amount of lactose entering the small intestines is greater than the hydrolyzing ability of lactase.[1] The undigested lactose may be eventually excreted in the urine. Alternatively, it goes on to the large intestine where the polar nature of lactose draws water out of the tissues and into the intestines. Microbial degradation of lactose by bacteria in the colon eventually produces organic acids and carbon dioxide. Thus, the individual suffers from fermentative diarrhea. Despite commericals to the contrary, milk is not for everybody! Adults with high activity of lactase are tolerant to lactose and are generally of northern European background. Adults with lower activity are intolerant to it.[2]

A great deal of effort has been expended to alleviate the problem of lactose intolerance because of the importance of milk as a foodstuff. One strategy is to pass lactose through a column containing immobilized enzyme so that D-glucose and D-galactose emerge in the eluate. Another strategy is to add *Lactobacillus acidophilus* to milk which produces lactate due to homolactic fermentation at body temperature. A third strategy is to add commerical enzyme to milk and allow an incubation to take place for 24 hours in the refrigerator.

In this experiment a synthetic substrate, O-nitrophenyl-β-D-galactopyranoside (ONPG), is used in place of lactose.



The hydrolysis of this substrate catalyzed by a commerical enzyme produces *o*-nitrophenolate, which can be measured by its yellow color.

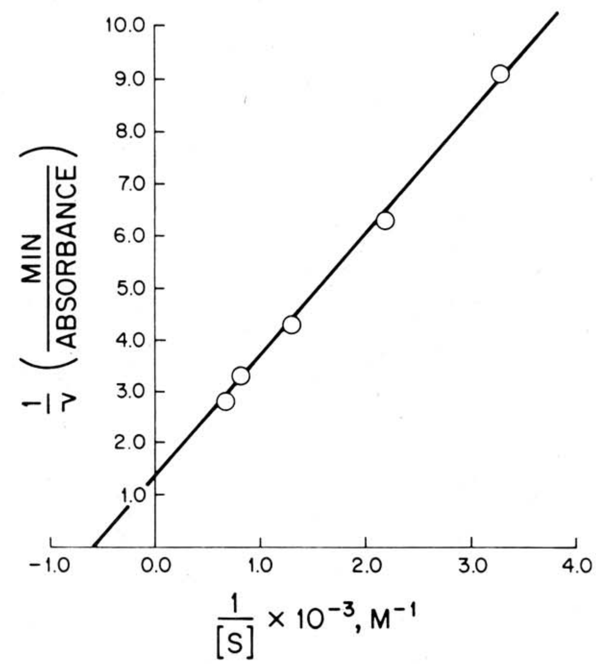
Experimental

Solutions

Substrate: ONPG may be purchased at an approximate cost of $25 for 5 g from Sigma Chemical Company, P. O. Box 14508, St. Louis, MO 63178. Prepare a 1.5×10^{-2} *M* aqueous solution and the following dilutions of the stock: 8.0 mL of stock diluted to a total 10.0 mL, 5.0 mL of stock diluted to a total 10.0 mL, 3.0 mL of stock diluted to a total 10.0 mL, and 2.0 mL of stock diluted to a total of 10.0 mL. Store the substrate solutions at 3–5°C.

Enzyme: Lact-Aid® is a yeast-derived enzyme that may be purchased from local health food stores or from Lact Aid Inc., P. O. Box 111, Pleasantville, NJ 08232 at an approximate cost of $3 for 3 mL. Prepare a stock enzyme solution by adding 3 drops from a Pasteur disposable pipet to 25.0 mL distilled water and store the enzyme solution at 3–5°C.[3]

Buffer: 0.1 *M* potassium phosphate at pH 8.0.



Lineweaver-Burk plot for lactase-catalyzed hydrolysis of ONPG at pH 8.0.

Special Equipment

Spectronic 20 Visible Spectrophotometer
pH Meter

Enzyme Assay

A cuvette is needed for the sample and one for the blank. Add 0.5 mL stock ONPG to both blank and sample cuvettes followed by 4.0 mL buffer to each. In the cuvette labelled blank add 0.5 mL distilled water and mix by inversion. Use this to calibrate the single beam instrument at zero absorbance (100% transmittance) at 420 nm. Then add 0.5 mL lactase solution to the sample cuvette, mix by inversion, and place in the spectrophotometer cell holder. The addition of enzyme defines the beginning of the rate assay. Record absorbance values at 30-s intervals for 10 min. In using a Spectronic 20 it is easier and more precise to record % transmittance and convert mathematically to absorbance. Alternatively, a baseline may be recorded on a double-beam spectrophotometer by using a blank in both sample and reference beams. Repeat the procedure for each dilution of the substrate and the stock making sure that a new mixture is made for both sample and blank.

Results

For each assay, plot absorbance versus time and determine the initial slope, which is equal to the initial rate, *v*.

[1] Bayless, T.M.; Rosensweig, N. S. *J. Amer. Med. Assoc.* **1966,** *197*, 968.

[2] Kretchmer, N., *Sci. Amer.* **1972,** *227*, 70.

[3] Lact-Aid® is highly viscous. Using three drops from a Pasteur disposable pipet is not exactly reproducible but is preferable to weighing. The K_M value is independent of enzyme concentration.

According to the Michaelis-Menten mechanism

$$v = \frac{V_{max}[S]}{K_M + [S]}$$

where V_{max} is the maximum velocity, [S] is the initial substrate concentration, and K_M is the Michaelis-Menten constant. The results are plotted in reciprocal form

$$\frac{1}{v} = \frac{K_M}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

which is known as a Lineweaver-Burk plot. A graph of $1/v$ versus $1/[S]$ should yield a straight line where the intercept on the $1/v$ axis is $1/V_{max}$ and the intercept on the $1/[S]$ axis is $-1/K_M$.

Results that conform to the Michaelis-Menten rate law are shown in the figure. V_{max} is calculated to be 0.73 absorbance/min and K_M is 1.7×10^{-3} *M* which is similar to $K_M = 2.7 \times 10^{-3}$ *M* at pH 6.6 for the enzyme from *Kluyveromyces fragilis* (formerly *Saccharomyces fragilis*).[4] The Michaelis-Menten constant is an index of how easily the enzyme can be saturated by the substrate being studied under the pH and temperature conditions of the experiment.

[4] Mahoney, R. R.; Whitaker, J. R. *J. Food Biochem.* **1977,** *1*, 327.