Teaching Enzyme Kinetics Using a Commercial Diagnostic Assay for Glucose in Plasma

Pamela J. Bryer

Biology Department, Bowdoin College
Brunswick, Maine 04011
pbryer@polar.bowdoin.edu

Introductory biology students often have difficulty with the concepts involved in enzyme kinetics. One would hope that the laboratory would be a source of clarification for these problems but, depending on the enzyme system being utilized, that may not be the case. While having students extract the enzyme that will be used in the assay has many merits, the data obtained from this method are often skewed or fluctuate from one run to the next which can be both confusing and frustrating to the introductory level student. I have found that performing a colorimetric assay using a commercial preparation of an enzyme mixture used for the diagnostic determination of glucose in blood plasma eliminates the disparity between runs and is virtually foolproof. This assay also has the added advantages of requiring very little preparation time, it can easily illustrate a number of principles relating to enzymes, and the experiment length can be tailored to meet one's needs.

The enzyme mixture (Sigma Diagnostics Glucose Trinder #315, Sigma Chemical Co., St. Louis, MO 63103) once reconstituted with deionized water is stable for up to 3 months if refrigerated. The basic assay I have worked out using this enzyme is the following: 0.5 ml glucose (0.2 mg/ml) + 1.5 ml dH_20 + 1.0 ml enzyme. Once mixed (time zero), spectrophotometric readings at 510 nm are taken at 15, 30, 45, 60 120, and 180 seconds. This gives a nice linear graph.

I have the students perform this assay several times, changing parameters as they go. They test the specificity of the enzyme for its substrate by performing the assay not only with glucose, but also with the isomers of glucose, mannose and galactose (it is readily evident from the data that this enzyme complex is specific for glucose and the relative position of an -OH on the ring does indeed make a difference). The students also test the effect temperature has on the enzymatic reaction (room temperature, 4°C, 37°C, and 65°C are adequate to demonstrate the changes), and I also introduce the students to the concept of Michaelis-Menton kinetics by having them perform an experiment where the enzyme concentration is kept constant and the glucose concentration is varied (I have them use 0.1, 0.3, 0.5, 0.7, and 1.0 ml of 0.2 mg/ml glucose). They may then determine for the enzyme the maximal velocity for the enzymatic reaction (Vmax) and the Michaelis constant (Km, a measure of the strength of the enzyme-substrate complex).

This assay, by virtue of its simplicity, also lends itself quite nicely to independent projects. Students can be given the general assay procedure and be asked to hypothesize the effects of temperature, pH, or substrate concentration on the enzyme's effectiveness. They can then test their hypotheses quickly and build upon them. The quick, positive results they obtain from experiments using this assay tend to elicit a positive attitude toward future experiments, which is indeed an added benefit!