

Science Education Collection

Enzyme Assays and Kinetics

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Abstract

Enzyme kinetics describes the catalytic effects of enzymes, which are biomolecules that facilitate chemical reactions necessary for living organisms. Enzymes act on molecules, referred to as substrates, to form products. Enzyme kinetic parameters are determined via assays that directly or indirectly measure changes in substrate or product concentration over time.

This video will cover the basic principles of enzyme kinetics (including rate equations) and kinetic models. The concepts governing enzyme assays are also discussed, followed by a typical colorimetric assay. The applications section discusses an enzyme assay via Förster resonance energy transfer (FRET) analysis, characterizing extracellular enzyme activity in the environment, and investigating DNA repair kinetics using molecular probes.

Enzymes are biochemical catalysts that are essential for life. Enzyme assays are used to study the kinetic properties of enzymatic reactions, elucidating the catalytic effects of enzymes. This video will cover enzyme kinetics and assays, go over a general procedure, and show some applications.

Enzymes are proteins or protein-like molecules that act on a reactant molecule, referred to as the substrate. Enzymes reduce the activation energy of biochemical reactions. This allows reactions to occur at faster rates with lower energy requirements.

Enzymatic reactions can be broken up into three elementary components. The first is the formation of the enzyme-substrate complex, formed by the binding of the substrate to the enzyme active site. The complex can decompose into its original constituents. This is the second elementary reaction. Alternatively, the complex can form the product and recover the enzyme, the third elementary reaction.

The kinetics of an elementary reaction is given by the elementary rate law equation. Rate law equations give the rate in terms of the concentration of the reactants and a rate constant. Each of the elementary reactions has an individual rate law equation, with its own rate constant. These equations can be distilled down to a kinetic model known as the Michaelis-Menten equation. This gives the reaction rate in terms of the substrate concentration; which can be experimentally determined. Some general trends for enzyme reactions can be identified using the Michaelis-Menten equation. At high substrate concentration, a saturation point is reached, called V_{max} . Here, the rate is limited by the total enzyme concentration, and the number of substrate molecules an enzyme converts into product per given time, also known as k_{cat} . In Michaelis-Menten kinetics k_{cat} is one of the two constants that govern reaction rate. The other constant, K_M , is known as the affinity constant. K_M is also equivalent to the concentration where the reaction rate is equivalent to one-half V_{max} . An enzyme with a higher affinity will have a lower K_M and reach V_{max} faster, while an enzyme with lower affinity will have a higher K_M and take longer to reach V_{max} . Knowing k_{cat} and K_M allows for enzymes to be compared. To do this we use a ratio called enzyme efficiency. Higher k_{cat} and lower K_M result in higher efficiencies, while lower k_{cat} and higher K_M results in lower.

The factors used to elucidate enzyme kinetics must be determined experimentally. These assays are typically performed by mixing an enzyme and substrate solution in a controlled environment. Observations are made by measuring the changes in concentration of the substrate, product, or byproducts with respect to time.

The change in concentration over time is used to determine the reaction rate. In order to determine the kinetics, rate data must be obtained at multiple concentrations. If a plot of the inverse initial rate vs. inverse initial concentration, known as the Lineweaver-Burk plot, is linear, then the reaction follows Michaelis-Menten kinetics. The slope and intercept of the line allow for the determination of the kinetic parameters K_M and V_{max} , which can then be used to calculate k_{cat} and the enzyme efficiency.

Now that the principles of enzyme kinetics have been discussed, let's look at how a typical enzyme assay is performed.

In this procedure a colorimetric assay is demonstrated. The first step is to generate a standard curve, which will correlate absorbance with protein concentration. Solutions of known concentration are prepared along with a control sample. A developer solution that reacts with the target protein is added to produce a colored compound. Absorbance is measured and plotted against concentration to generate the standard curve.

To perform the assay, a known concentration of substrate is prepared along with the appropriate amount of enzyme. The enzyme and substrate are mixed and allowed to incubate for a set time interval. pH and temperature are controlled with buffer solutions and heating blocks. A quenching agent is added to stop the reaction. Developer solution is then added to the reactions and mixed. The solutions are then placed in cuvettes and absorbance is measured. The amount of substrate consumed is determined by comparing the measured absorbance to the standard curve. Using the collected data, initial reaction rates are determined by plotting concentration over time. Finally, with the rate data and concentration, the Michaelis-Menten plot is made. This allows for the determination of kinetic properties for the enzyme such as turnover number and enzyme efficiency.

Now that we've reviewed an assay procedure, let's look at other ways assays are performed and their applications.

In this procedure FRET analysis is used to study the kinetics of a protease hydrolyzing a peptide bond of a protein. These emissions can be measured, allowing for a continuous and quantitative analysis of substrate consumption and production, aiding in the determination of the reaction kinetics.

Enzyme assays can be used in environmental science to determine the levels of extracellular enzyme activity in the environment. Waters, soils, and sediments can be collected from the environment and processed in the laboratory. Extracellular enzymatic activity of these materials can then be characterized using enzyme assays. This is a useful tool for understanding how the environment processes organic material.

A cell's DNA repair mechanism can be evaluated by studying the kinetics of enzymes found in the nucleus. The rate at which an enzyme removes DNA lesions, or damages, can be measured using fluorescent molecular beacons, which only fluoresce when bound to unique DNA sequences. The level of DNA repair can be measured in real time by detecting the fluorescently labeled cleavage products.

You've just watched JoVE's video on enzyme kinetics and assays. This video explained enzyme kinetics, covered assay concepts, went over a general procedure, and described some applications.

Thanks for watching!