Enzyme kinetics

Summary

In this chapter, we will explain how the rate of enzymatic reactions can be measured and how it can be described both graphically and mathematically. We will focus on the Michaelis–Menten equation and on why it is sometimes advantageous to linearize the curve. We will demonstrate how enzyme activity is measured, which units are used, and why in clinical practice it is not only the amount of enzyme that is evaluated, but its actual catalytic capacity. Finally, the chapter will link theory with practice and highlight the importance of enzyme kinetics for clinical biochemistry and medicine.

Table of contents

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1 Introduction

Every cell in our body is like a small chemical factory, where billions of reactions take place every second. But it is not enough to know whether a reaction can occur (that is the role of thermodynamics); it is also important to determine how fast it occurs. This is precisely the focus of enzyme kinetics.

The rate of reactions is crucial—it determines whether the cell obtains energy in time, whether the necessary compounds are produced, or how quickly waste products are broken down. Understanding enzyme kinetics allows us to explain why enzymes function the way they do, how their activity changes under different conditions, and how it can be deliberately influenced—for example, by drugs.

2 Enzymes: How fast do enzymes work?

To measure the rate of enzyme action, we add substrate and enzyme into the reaction mixture and monitor how quickly the substrate disappears or how quickly the product appears. In practice, the initial rate is measured, i.e., the change in substrate or product concentration per unit of time immediately after the start of the reaction.

Unsurprisingly, when enzyme and substrate are present in approximately balanced amounts, no enzyme "waits" for substrate and no substrate "waits" for enzyme — the reaction proceeds efficiently, without delays due to lack of one component. Conversely, if the substrate concentration is too low, most enzymes remain unoccupied, and the overall rate is limited by substrate availability. If the substrate is in great excess, all active sites are saturated, and adding more substrates does not significantly increase the rate — the enzyme becomes the limiting factor.

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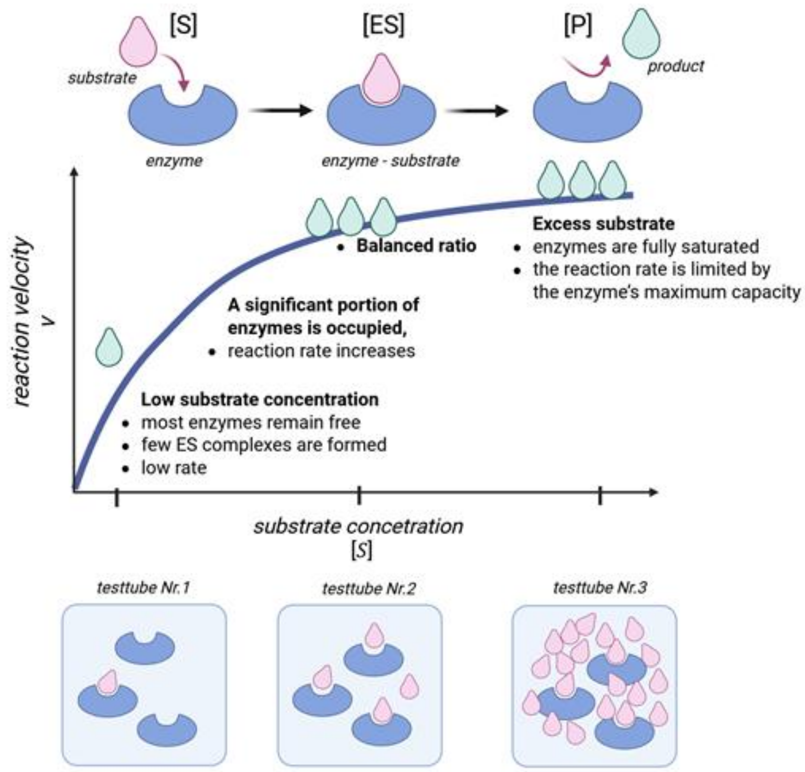


3 Reaction Kinetics — What it studies and why it matters

Unlike thermodynamics, which tells us whether a reaction is energetically feasible, kinetics explains how and how fast the conversion takes place. In biochemistry, understanding enzyme kinetics is crucial, because it allows us to describe, predict, and regulate biological processes. This knowledge has direct applications in diagnostics (measuring enzyme activity in blood), pharmacology (design of inhibitors, drug dosing), and in drug development.

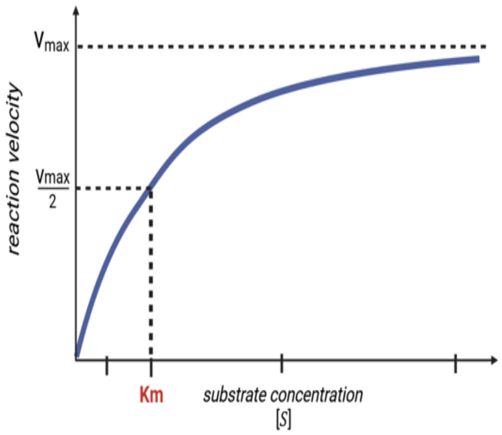
Reaction kinetics deals with the speed of chemical reactions, i.e., how quickly substrate is converted to product, and the factors influencing this rate (substrate concentration, presence of activators or inhibitors).

3.1 The Michaelis–Menten Curve

The reaction rate (v) expresses how much substrate is converted into product per unit of time (e.g., $\mu mol \cdot min^{-1}$). In practice, it is often measured spectrophotometrically as the change in absorbance over time (ΔA/min); the rate can then be calculated using the Lambert–Beer law (provided the molar absorption coefficient and cuvette path length are known).

The rate depends on the substrate concentration [S] and on conditions such as pH, temperature, ionic strength, and the presence of inhibitors/activators. The typical dependence of v on [S] has the shape of a hyperbola and is described by the Michaelis–Menten equation:

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



Key Parameters

Vmax

- The "ceiling" of the rate (the horizontal asymptote of the curve); it is reached when all active sites are saturated.
- It can only be increased by adding more active enzyme (not by adding more substrate).

Km

- The [S] value at which the rate v = ½ Vmax.
- In practice, it provides information about **affinity**: a lower Km ⇒ higher affinity (½ Vmax is reached at a lower [S]).

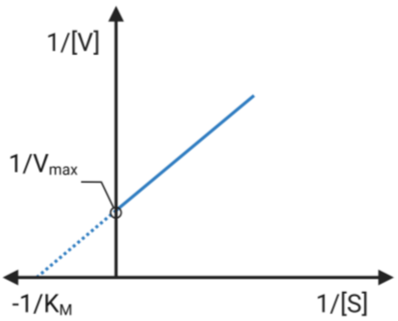
How to read the curve:

- **Low [S] (left side):** the rate increases almost linearly with [S]; the limiting factor is substrate.
- **Around Km (middle):** a small change in [S] strongly affects v; this is the area of highest sensitivity.
- **High [S] (right side):** enzyme saturation; adding more substrate hardly increases the rate—the limiting factor is the enzyme.

3.2 Why Linearize the Michaelis–Menten Curve?

The original v vs. [S] dependence is hyperbolic, which makes it difficult to determine Vmax and Km precisely. By transforming the curve into a straight line, the parameters can be easily read off from intercepts and slopes.

The Lineweaver–Burk transformation converts the hyperbola into a straight line:



Equation:

$$y = mx + b$$

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

How to construct the linearized form?

- Compute the reciprocal values of **[S]** and **v**.
- Plot 1/[S] on the horizontal axis and 1/v on the vertical axis.
- The points should now lie approximately on a straight line.
- Fit a straight line (linear regression) and obtain its equation and correlation coefficient.
- Read off the **y-intercept** (1/Vmax) and the **x-intercept** (−1/Km) from the graph (or from the regression equation).
- Convert back (take reciprocals) to get Vmax and Km.
- **Km** provides information about enzyme–substrate affinity (i.e., how much substrate is needed to reach half of Vmax.

4 Measuring Enzyme Activity — Units

Why do we measure activity rather than enzyme amount? Measuring enzyme concentration (e.g., with antibodies in ELISA) also includes molecules that are inactive (denatured, inhibited, etc.). What matters clinically is the functional fraction, i.e., how much substrate is actually converted per time unit. Therefore, activity (catalytic ability) is measured instead of total quantity.

4.1 Catalytic Activity of an Enzyme

Catalytic activity is a physicochemical quantity that expresses the rate of an enzymatic reaction—that is, how many moles of substrate are converted into product per unit of time. It represents the "power of the enzyme," independent of the volume of the solution.

- **SI unit: katal (kat)**
  1 kat = 1 mol/s
  This unit is far too large for practical use and is therefore rarely applied in routine laboratory diagnostics.
- **Practical unit: U (international unit, IU)**
  1 U = 1 µmol/min
  This unit is suitable for routine measurements of enzyme activity in serum, plasma, or other biological fluids.

4.2 Catalytic Concentration of an Enzyme

Catalytic concentration expresses how much enzymatic activity corresponds to a unit volume of solution (usually 1 liter). It is denoted, for example, in kat/L or U/L. Unlike absolute catalytic activity (which indicates how much substrate an enzyme converts per unit time), catalytic concentration also takes into account the volume of the solution. Therefore, it is very useful in clinical biochemistry—it allows for comparison of results between different biological fluids (e.g., serum, plasma, urine).

Conditions for Measurement

To ensure that catalytic concentration values are reliable and reproducible, measurements must be carried out under standardized conditions:

- **Substrate and cofactor** (if required) must be present in excess so that the reaction is not limited by their shortage.
- **Temperature** is standardized at **37 °C** in clinical measurements, corresponding to physiological conditions in the human body.
- **Incubation time and measurement** duration must be precisely defined and consistent across all assays.
- **The pH of the environment** is stabilized by a buffer, ensuring that the enzyme operates under optimal conditions.

Units

- **katal (kat)** – the SI unit of catalytic activity, defined as 1 mol of substrate converted per second.
- **U (international unit, IU)** – a practical laboratory unit, defined as 1 μmol of substrate converted per minute.

When expressing catalytic concentration, these units are related to the volume of solution (e.g., U/L, kat/L).

Conversion of Units

$$1\ U = 16.67\ nkat$$

For example, if enzyme activity in serum is **50 U/L**, this corresponds to **833 nkat/L**.

Summary

Enzyme kinetics describes how fast enzymes work and which factors influence this rate. The reaction rate is usually measured by monitoring the change in substrate or product concentration over time, most often at the very beginning of the reaction (the initial velocity). The typical mathematical model is the **Michaelis–Menten equation**, which shows the dependence of reaction velocity on substrate concentration. Two key parameters are **Vmax**, the maximum attainable rate, and **Km**, the substrate concentration required to reach half of Vmax, which reflects the enzyme's affinity for the substrate.

Since the hyperbolic Michaelis–Menten curve is not always easy to evaluate, **linearization methods** (e.g., the Lineweaver–Burk transformation) are used to more precisely determine kinetic parameters. In clinical practice, instead of the amount of enzyme, its **activity** is measured, i.e., the ability to convert substrate per unit time. Absolute activity is expressed in **kat** (1 mol/s), but in laboratories, the more practical unit **U** (1 μmol/min) is commonly used. When activity is expressed relative to volume, we refer to **catalytic concentration**, usually given in **U/L**, which is a key diagnostic parameter.

Understanding enzyme kinetics is crucial not only for biochemistry but also for medicine—it enables proper interpretation of laboratory results, understanding of how drugs act on enzymes, and optimization of their dosing.

Control Questions

1. What does "initial velocity" of an enzymatic reaction mean, and why is it measured?
2. What is the shape of the dependence of reaction velocity on substrate concentration?
3. What do the parameters **Vmax** and **Km** express in the Michaelis–Menten equation?
4. How does the course of the reaction differ at low, medium, and high substrate concentrations?
5. Why is linearization of the Michaelis–Menten curve used, and how does the Lineweaver–Burk plot work?
6. Why is enzyme **activity** evaluated in clinical practice instead of the total amount of enzyme?
7. What are the practical units of enzyme activity, and how do you convert between **kat** and **U**?