COMP 150 CSB – Computational Systems Biology

Enzymes: Basic Concepts & Kinetics

Based on
Chapter 8, Biochemistry, by Berg, Tymoczko, Stryer
(from a set of slides on Chapter 8 from 2007)

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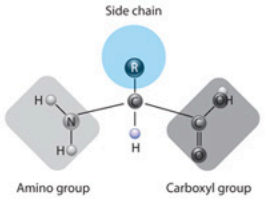
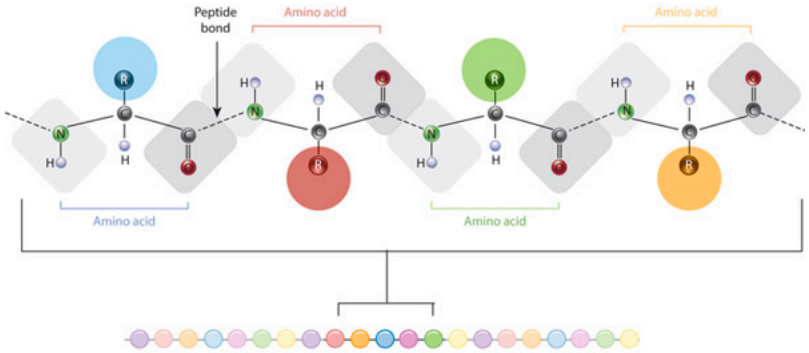
What is a protein

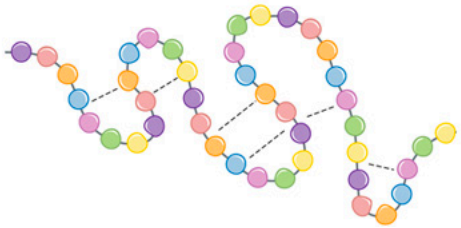
- Building block of proteins are amino acids



- Amino acids are linked using peptide bonds



- Amino acid sequences and their interaction determine shape of folded protein



https://www.nature.com/scitable/topicpage/protein-structure-14122136

What is an enzyme?

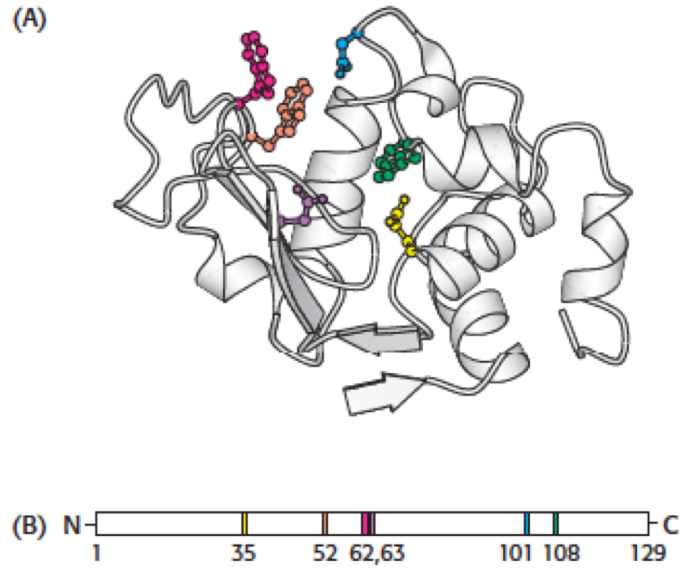


Figure 8.6 Active sites may include distant residues. (A) Ribbon diagram of the enzyme lysozyme with several components of the active site shown in color. (B) A schematic representation of the primary structure of lysozyme shows that the active site is composed of residues that come from different parts of the polypeptide chain. [Drawn from 6LYZ.pdb.]

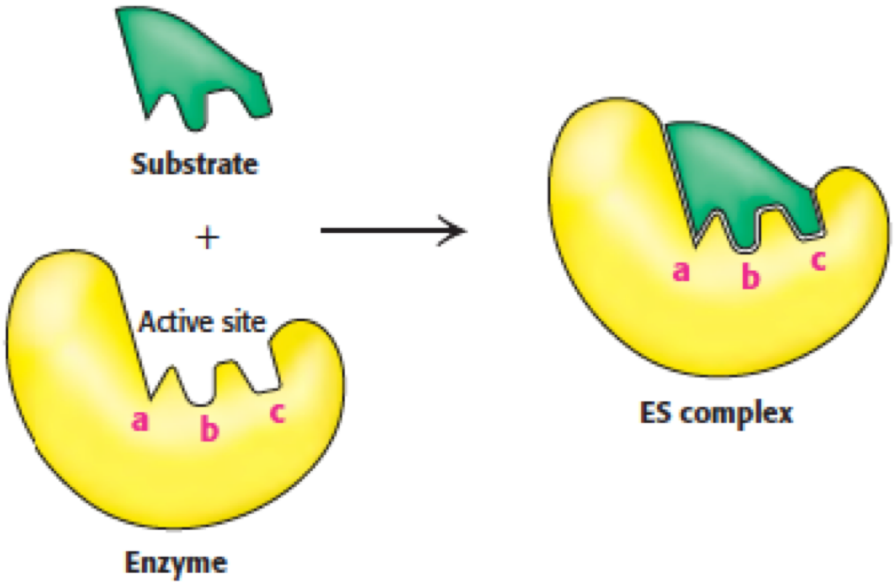


Figure 8.8 Lock-and-key model of enzyme–substrate binding. In this model, the active site of the unbound enzyme is complementary in shape to the substrate.

- Nearly all known enzymes are proteins (long chain of 100+ amino acids)
- Some amino acids (residues) form one or more active sites
- Speeds the rate of a biochemical reaction by binding with substrate

Enzyme structure

- Active site is a region along the chain that
  - a 3-D cleft or crevice
  - contains amino acid residues that participate directly in making/braking of bonds
  - occupies a small percentage of the total surface area of an enzyme
    - Enzymes are big molecules
    - Amino acids not participating in active site provide a scaffold
  - Specificity of binding depends on defined arrangements in active site
    - Substrate must have matching shape to fit into the site
    - But enzymes are flexible and shapes an be modified by binding of a substrate
  - binds substrates, cofactors, and coenzymes

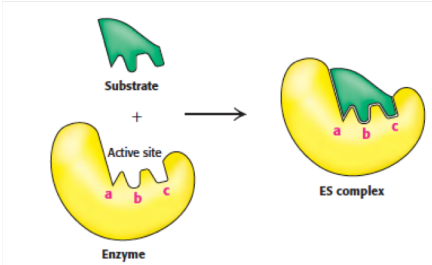


Figure 8.8 Lock-and-key model of enzyme–substrate binding. In this model, the active site of the unbound enzyme is complementary in shape to the substrate.

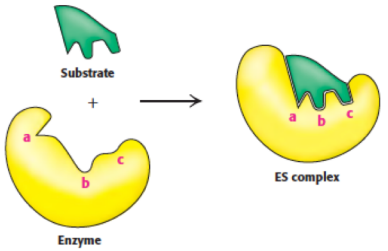


Figure 8.9 Induced-fit model of enzyme–substrate binding. In this model, the enzyme changes shape on substrate binding. The active site forms a shape complementary to the substrate only after the substrate has been bound.

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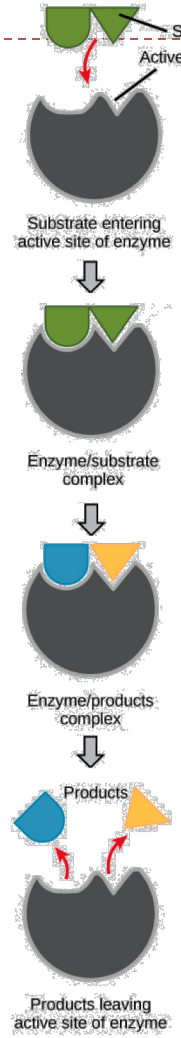
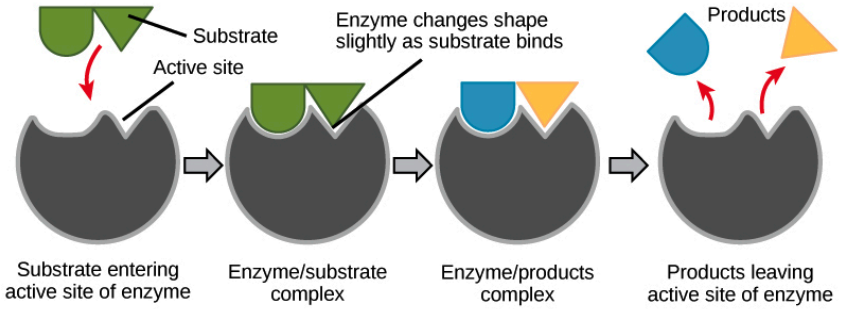
Enzyme/Substrate complex



Active sites and substrate specificity

Induced Fit



https://www.khanacademy.org/science/biology/energy-and-enzymes/introduction-to-enzymes/a/enzymes-and-the-active-site

Co-factors & co-enzymes

- Co-factor is a non protein molecule which carries out chemical reaction that cannot be performed by the standard 20 amino acids
- Typically metals or small organic molecules called co-enzymes
- coenzymes can be either tightly bound (prosthetic group) or loosely bound to the enzyme

Table 8.2 Enzyme cofactors

Cofactor	Enzyme
Coenzyme	
Thiamine pyrophosphate	Pyruvate dehydrogenase
Flavin adenine nucleotide	Monoamine oxidase
Nicotinamide adenine dinucleotide	Lactate dehydrogenase
Pyridoxal phosphate	Glycogen phosphorylase
Coenzyme A (CoA)	Acetyl CoA carboxylase
Biotin	Pyruvate carboxylase
5′-Deoxyadenosyl cobalamin	Methylmalonyl mutase
Tetrahydrofolate	Thymidylate synthase
Metal	
Zn^{2+}	Carbonic anhydrase
Zn^{2+}	Carboxypeptidase
Mg^{2+}	*Eco*RV
Mg^{2+}	Hexokinase
Ni^{2+}	Urease
Mo	Nitrate reductase
Se	Glutathione peroxidase
Mn	Superoxide dismutase
K^{+}	Propionyl CoA carboxylase

Enzymes (E) catalyze reactions with substrate S, and product P

- Substrate (S)
  - The reactant in biochemical reaction is termed as S.
- When a substrate binds to an enzyme (E) it forms an enzyme-substrate complex (ES)
- Product (P)
  - The product is formed as an outcome of the reaction
- Reactions can be reversible
  - Which is the reactant and product side?

Enzyme Nomenclature

- Enzyme is often named according to the name of the substrate it catalyzes
  - Add a –ase
  - Examples
    - Substrate: lactose, enzyme: lactase
    - Substrate: maltose, enzyme: maltase

How are enzymes related to systems biology?

- We model cells :
  - How do enzyme properties contribute to cellular behavior?
  - Link reaction properties to pathways to cellular behavior

- Let's learn about:
  - Thermodynamics provide information about the spontaneity of the reaction
  - Enzyme kinetics allow us to analyze rate of reactions

Free-energy change of a reaction (ΔG)

- Free energy (G) or Gibbs free energy is a thermodynamic property that is a measure of useful energy, or the energy that is capable of doing work

- Why do we care:
  1. The free-energy difference (ΔG, free-energy change of a reaction) between products and reactants determines if a reaction will occur spontaneously
  2. The energy required to initiate the conversion of reactants into products ($\Delta G‡$, free energy of activation) determines the reaction rate

1. Standard Free-energy change of a reaction

- Consider the reaction $A + B \rightleftharpoons C + D$

- ΔG for this reaction is calculated as: $$\Delta G = \Delta G^\circ + RT \ln \frac{[C][D]}{[A][B]}$$

where:

ΔG^o standard free-energy change (all reactants are at 1.0 M; if at ph 7, then use $\Delta G^{\circ\prime}$)

R gas constant

T is absolute temp

[X] molar concentration of reactant X

To calculate ΔG°, assume equilibrium (no net change in reactant/product; reaction has stopped): $$\Delta G^{\circ\prime} = -RT \ln \frac{[C][D]}{[A][B]}$$

KEY IDEA: Whether the ΔG for a reaction is larger, smaller, or the same as ΔG^o depends on the concentrations of the reactants and products

2. Enzymes accelerate reactions by facilitating the formation of the transition state

- Consider the intermediate state between the end points of a reaction:

$$S \longrightarrow X^{\ddagger} \longrightarrow P$$

  - The double dagger denotes the transition state

- The difference in free energy between the transition state and the substrate is called the Gibbs free energy of activation or simply the activation energy, symbolized by $\Delta G^{\ddagger}$

- Enzymes lower the activation energy, and equilibrium between S and P is achieved sooner (amount of product is the same)

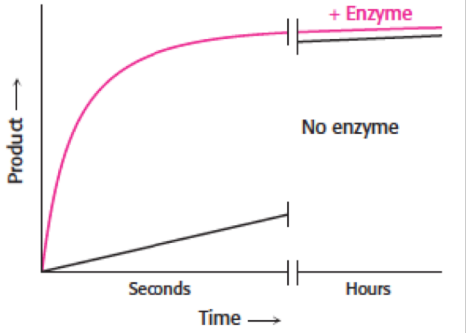


Figure 8.2 Enzymes accelerate the reaction rate. The same equilibrium point is reached but much more quickly in the presence of an enzyme.

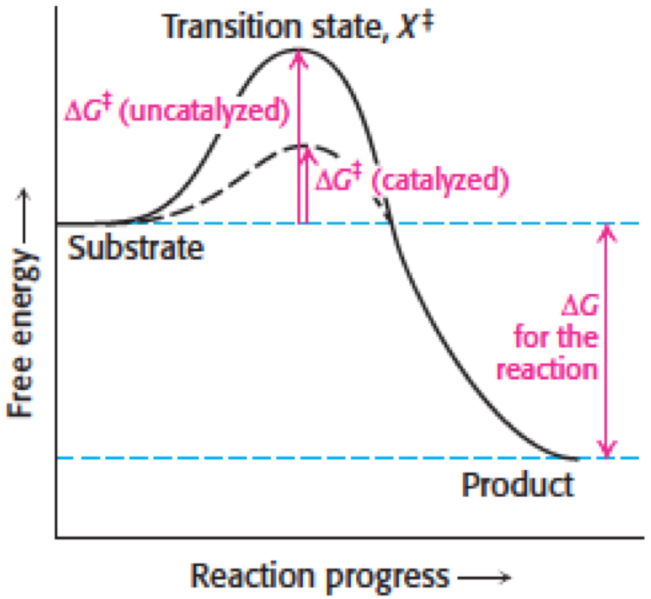


Figure 8.3 Enzymes decrease the activation energy. Enzymes accelerate reactions by decreasing $\Delta G^{\ddagger}$, the free energy of activation.

Key Ideas to remember

- A reaction can take place spontaneously only if ΔG is negative. Such reactions are said to be exergonic.

- A reaction cannot take place spontaneously if ΔG is positive. An input of free energy is required to drive such a reaction. These reactions are termed endergonic.

- If ΔG is zero, system is at equilibrium

- The ΔG of a reaction is independent of the path (or molecular mechanism) of the transformation.

- The reaction rate is a function of the the free energy of activation ($\Delta G^{\ddagger}$), which is largely unrelated to the ΔG of the reaction.

Kinetic Analysis of Enzymes

Why study enzyme kinetics:

- Allow studying complex dynamic enzyme behavior
- Systematic evaluation of enzyme function

Our questions:

- How does the activity of an enzyme vary with substrate concentrations and why?
- What are kinetic parameters, k_{cat}, V_m, and K_m, and what do they tell us about the enzymatic reaction?

Concentrations and rates

- The (molar) concentration of B, or molarity, is given by:

[B] = moles of B/ liters = molarity = mol/L (or mol/m^3)

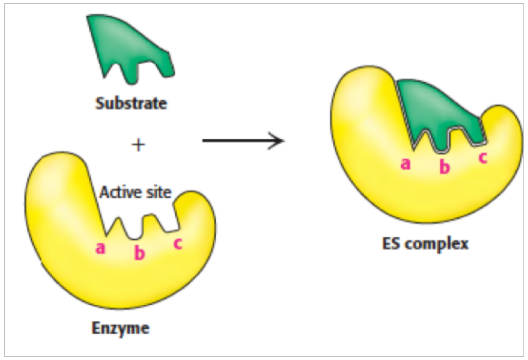
- The rate of product formation P from a substrate S can be expressed as:

$$\frac{d[P]}{dt} = k\,[S]$$

Reaction rate usually has the units of mol / (L . S)

Enzyme Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$



E = enzyme concentration
S = Substrate concentration
ES = Enzyme-substrate complex concentration
P = product concentration
k_1 = rate constant for formation of ES from E + S
k_{-1} = rate constant for decomposition of ES to E + S
k_2 = rate constant for decomposition of ES to E + P

Assumptions:
- Only ES exists; no EP
- P→ S is negligible
- [S] >> [E]
- Rate of ES formation = Rate of ES decomposition

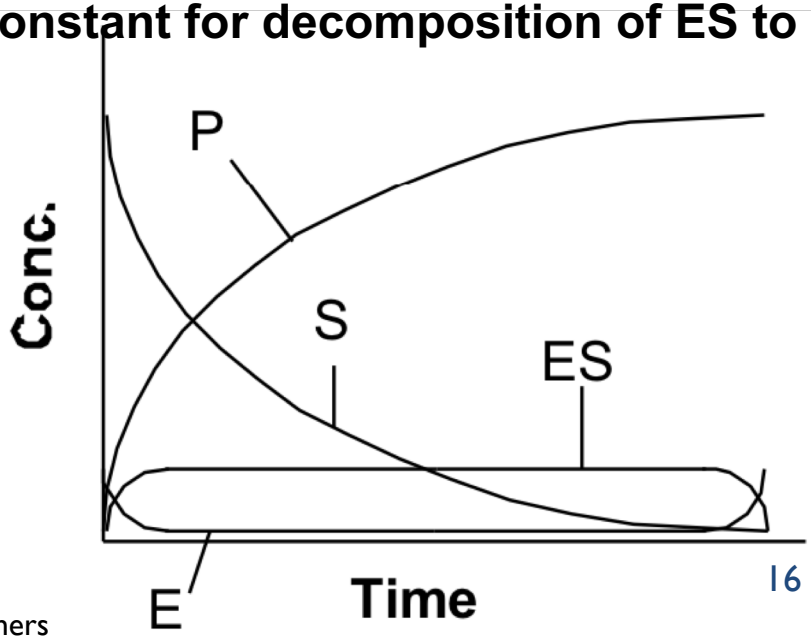


Figure from Tony Carruthers

Michaelis-Menton Derivation

1. The overall rate of product formation: $v_o = k_2 [ES]$
2. Rate of formation of [ES]: $v_f = k_1[E][S]$
3. Rate of decomposition of [ES]:

   $v_d = k_{-1}[ES] + k_2 [ES]$
4. Rate of ES formation = Rate of ES decomposition (steady state)
5. So: $k_1[E][S] = k_{-1}[ES] + k_2 [ES]$

Michaelis-Menton Derivation

6. In solving for [ES], use the enzyme balance to eliminate [E]. $E_T = [E] + [ES]$

7. $k_1 (E_T - [ES])[S] = k_{-1}[ES] + k_2 [ES]$

$k_1 E_T[S] - k_1[ES][S] = k_{-1}[ES] + k_2 [ES]$

8. Rearrange and combine [ES] terms:

$k_1 E_T[S] = (k_{-1} + k_2 + k_1 [S])[ES]$

9. Solve for $[ES] = \dfrac{k_1 E_T[S]}{(k_{-1} + k_2 + k_1 [S])}$

Michaelis-Menton Derivation

10. Divide through by k_1: $[ES] = \dfrac{E_T[S]}{(k_{-1} + k_2)/k_1 + [S]}$

11. Defined Michaelis constant: $K_M = (k_{-1} + k_2) / k_1$

12. Substitute K_M into the equation in step 10.

13. Then substitute [ES] into $v_o = k_2 [ES]$ from step1 and replace $k_2 E_T$ with V_{max} to give:

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

Michaelis-Menton Plot

Relates reaction velocity and substrate concentration

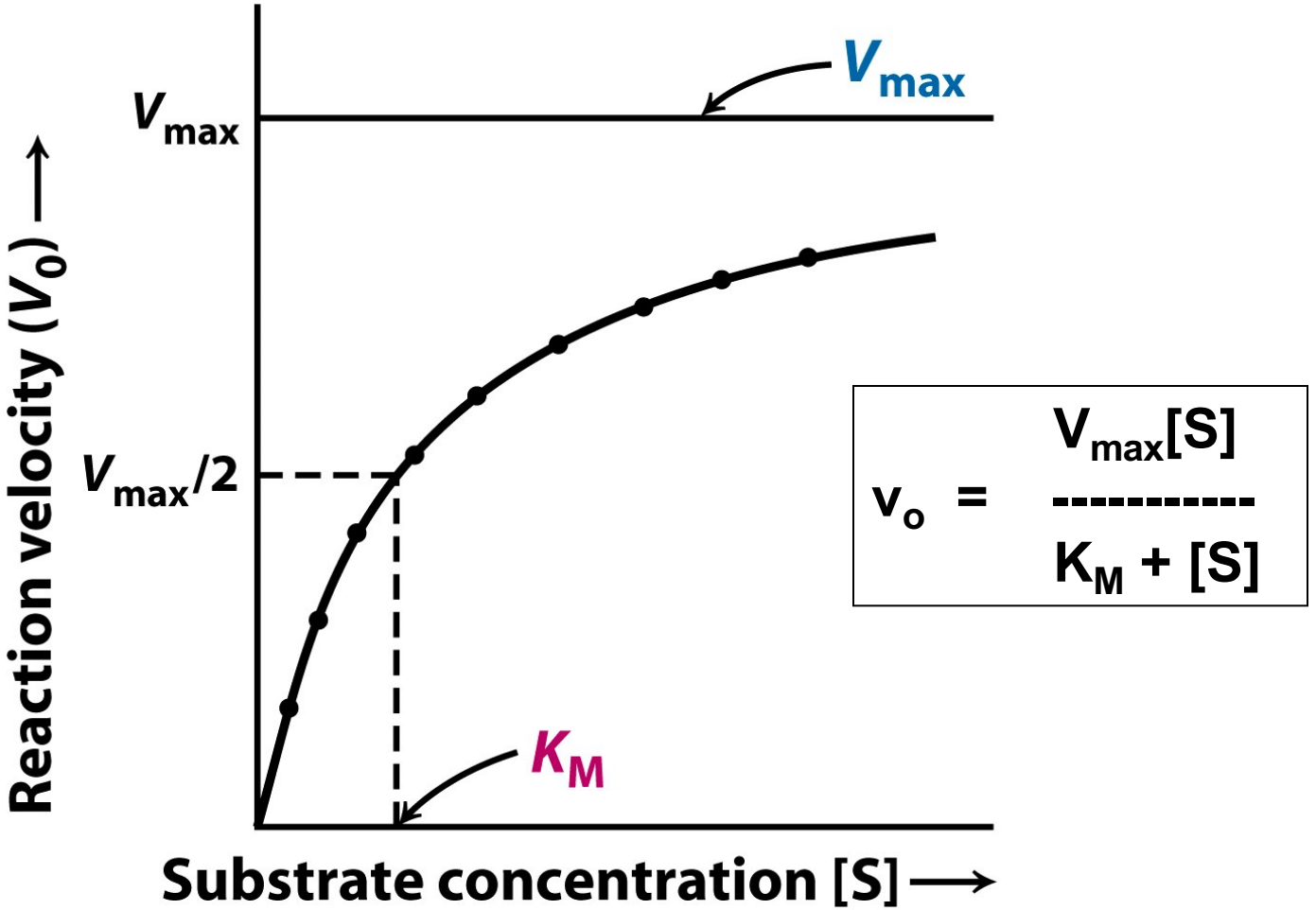


Figure 8-12
Biochemistry, Sixth Edition

The Michaelis-Menton Equation

Define Michaelis constant:

$K_M = (k_{-1} + k_2) / k_1$

It quantifies the instability of the ES complex.

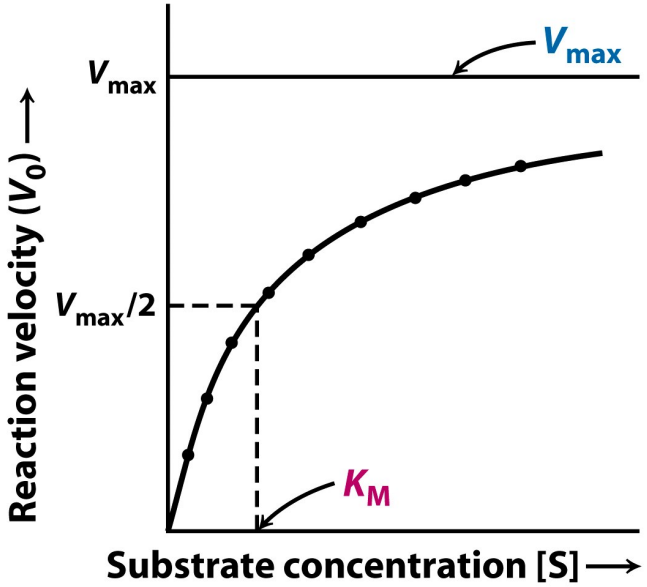


Figure 8-12
Biochemistry, Sixth Edition

The overall velocity of an enzyme-catalyzed reaction is given by rate of conversion of ES to E + P.

$$v_o = k_2[ES] = k_{cat}[ES]$$

Enzyme Kinetics - Units

- Write the rate of change of S:

  $d[S]/dt = - k_1[E][S] + k_{-1}[ES]$

- What are units of k_1 and k_{-1} ?
- k_1 has units of concentration^{-1}time^{-1}
- k_{-1} has units of time^{-1}

- Similarly, k_2 has units of time^{-1}

- $K_M = (k_{-1} + k_2) / k_1$ will have units of concentration

K_{cat} or k_{cat}

In an enzyme catalyzed reaction, the overall rate of product formation is $v = k_2 [ES]$.

If all of the enzyme molecules are complexed with substrate (excess [S]) then the maximum velocity occurs and $V_{max} = k_{cat} E_T$ where k_{cat} is the overall reaction rate constant.

This can also be written as $k_{cat} = V_{max} / E_T$.

k_{cat} is called the turnover number (TON).

Table 8.5 Turnover numbers of some enzymes

Enzyme	Turnover number (per second) = k_{cat} (s^{-1})
Carbonic anhydrase	600,000
3-Ketosteroid isomerase	280,000
Acetylcholinesterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Tryptophan synthetase	2
Lysozyme	0.5

K_M

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

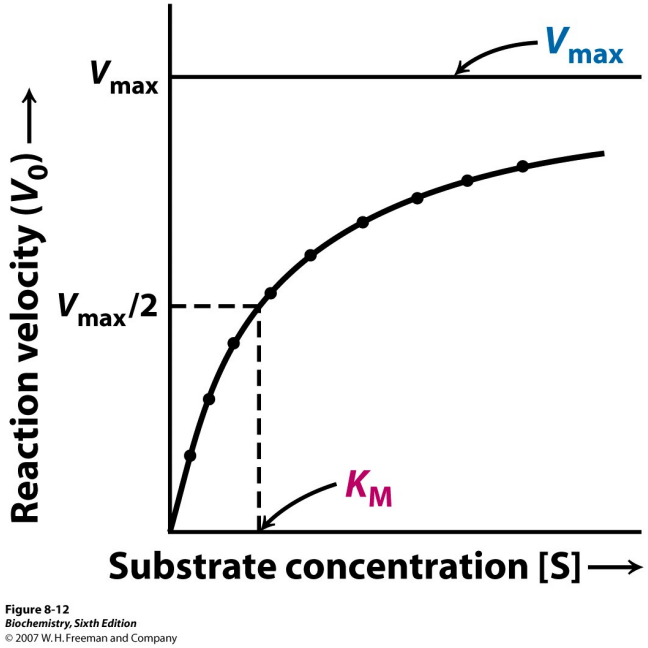


Figure 8-12
Biochemistry, Sixth Edition
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- **K_M has two meanings:**

1. K_M = [S] when v_o = 1/2 V_{max}

K_M provides a measure of the substrate concentration required for significant catalysis to take place

2. K_M is related to the rate constants of the individual steps:

$K_M = (k_{-1} + k_2) / k_1$

$K_M \cong k_{-1} / k_1 = K_s$ (the enzyme-substrate dissociation constant) when k_2 is small (<< either k_1 or k_{-1}).

•K_M then measures strength of ES complex: Generally, the lower the numerical value of K_M, the tighter the substrate binding.

•K_M is used as a measure of the affinity of E for S when $k_{-1} >> k_2$

TABLE 8.4 K_M values of some enzymes

Enzyme	Substrate	K_M (μM)
Chymotrypsin	Acetyl-L-tryptophanamide	5000
Lysozyme	Hexa-*N*-acetylglucosamine	6
β-Galactosidase	Lactose	4000
Threonine deaminase	Threonine	5000
Carbonic anhydrase	CO_2	8000
Penicillinase	Benzylpenicillin	50
Pyruvate carboxylase	Pyruvate	400
	HCO_3^-	1000
	ATP	60
Arginine-tRNA synthetase	Arginine	3
	tRNA	0.4
	ATP	300

Table 8-4
Biochemistry, Sixth Edition

k_{cat}/K_M

k_{cat}/K_M is taken to be a measure of the catalytic efficiency of an enzyme because it takes into account rate of catalysis (k_{cat}) and strength of E-S interaction (K_M)

Rewriting k_{cat}/K_M in terms of the kinetic constants gives:

$$\frac{k_{cat}}{K_M} = \frac{k_1 k_2}{k_{-1} + k_2}$$

So, when k_2 is small, the denominator becomes k_{-1} and k_{cat}/K_M is small. →continued

k_{cat}/K_M

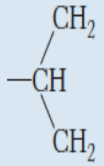
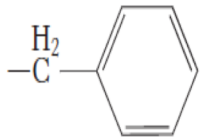
$$\frac{k_{cat}}{K_M} = \frac{k_1 k_2}{k_{-1} + k_2}$$

And where k_2 is large, the denominator becomes k_2 and k_{cat}/K_M is limited by the value of k_1 or formation of the ES complex. This formation is in turn limited by the rate of diffusion of S into the active site of E. So, the maximum value for this second-order rate constant (k_{cat}/K_M) is the rate of diffusion (~10^9 sec^{-1} M^{-1}).

If k_{cat}/K_M is at the upper limit (10^8 or 10^9), the enzyme has attained kinetic perfection – every interaction between substrate and enzyme is productive

k_{cat}/K_M

Table 8.6 Substrate preferences of chymotrypsin

Amino acid in ester	Amino acid side chain	k_{cat}/K_M ($s^{-1} M^{-1}$)
Glycine	—H	1.3×10^{-1}
Valine		2.0
Norvaline	$-CH_2CH_2CH_3$	3.6×10^{2}
Norleucine	$-CH_2CH_2CH_2CH_3$	3.0×10^{3}
Phenylalanine		1.0×10^{5}

Source: After A. Fersht, *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding* (W. H. Freeman and Company, 1999), Table 7.3.

Table 8.7 Enzymes for which k_{cat}/K_M is close to the diffusion-controlled rate of encounter

Enzyme	k_{cat}/K_M ($s^{-1} M^{-1}$)
Acetylcholinesterase	1.6×10^{8}
Carbonic anhydrase	8.3×10^{7}
Catalase	4×10^{7}
Crotonase	2.8×10^{8}
Fumarase	1.6×10^{8}
Triose phosphate isomerase	2.4×10^{8}
β-Lactamase	1×10^{8}
Superoxide dismutase	7×10^{9}

Source: After A. Fersht, *Structure and Mechanism in Protein Science: A Guide to Enzyme Catalysis and Protein Folding* (W. H. Freeman and Company, 1999), Table 4.5.

Summary

- Enzymes have active sites that allow substrate binding
- Gibbs Free Energy:
  - Spontaneity of the reaction is a function of ΔG, which is a function of reactant concentrations
- Enzymes lower the free energy of activation ($\Delta G‡$) as reaction proceeds
- Making simplifying assumptions, we derived the Michaelis-Menten equations
- We discussed K_m, K_s, K_{cat} and V_m discussed their biological implications

References

- Download PDF for biochem book

https://archive.org/details/BiochemistryStryer7th

- More detailed slides on kinetics:
  - http://elte.prompt.hu/sites/default/files/tananyagok/IntroductionToPracticalBiochemistry/ch09s02.html