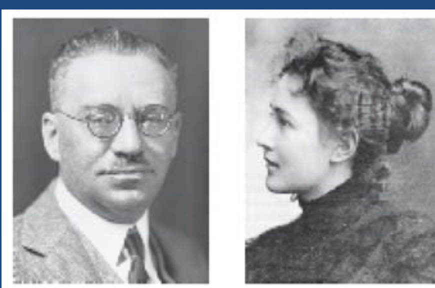
ENZYMOLOGY

Presented by Dr. A.H.Mahdavi

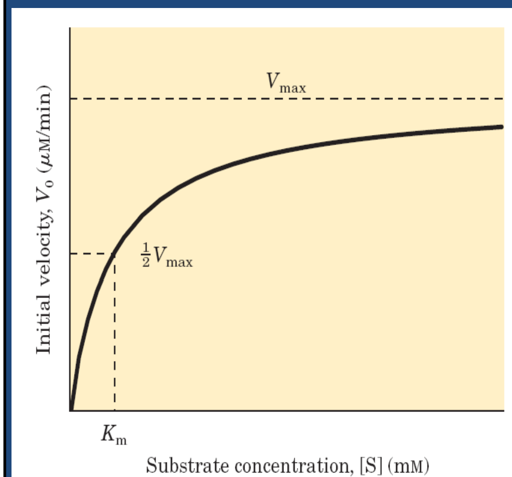
Enzyme Kinetics

- the *rate* of the reaction
- *how it changes* in response to changes in experimental parameters



Leonor Michaelis, 1875–1949   Maud Menten, 1879–1960

Michaelis-Menten Equation



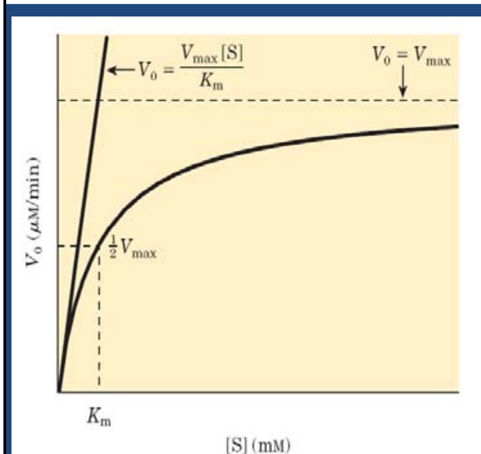
$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES$$

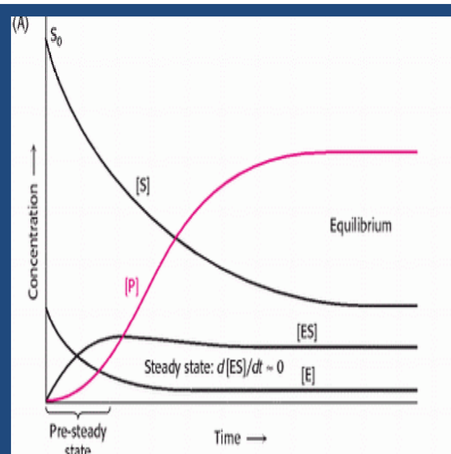
$$ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

$$V_0 = \frac{V_{max}\,[S]}{K_m + [S]}$$

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

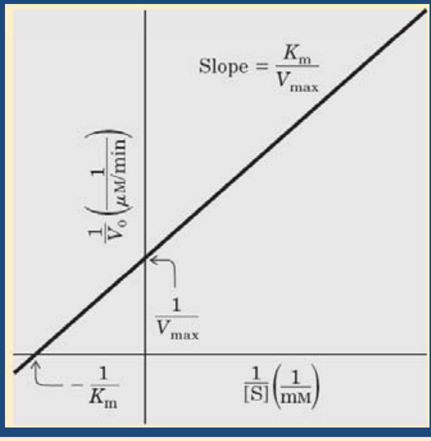
$$V_0 = k_2[ES]$$





Lineweaver-Burk equation

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$



A double-reciprocal or Lineweaver-Burk plot.

- ***kcat,*** *to describe the limiting* rate of any enzyme-catalyzed reaction at saturation. **"turnover number"**
- *kcat/Km* **"specificity constant"**

TABLE 6-6 K_m for Some Enzymes and Substrates

Enzyme	*Substrate*	K_m (mM)
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

TABLE 6-7 Turnover Numbers, k_{cat}, of Some Enzymes

Enzyme	*Substrate*	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

TABLE 6–8 Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)

Enzyme	*Substrate*	k_{cat} (s^{-1})	K_m (M)	k_{cat}/K_m ($M^{-1}s^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1×10^0	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^2	2.5×10^{-5}	3.6×10^7
β-Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-5}	1×10^8

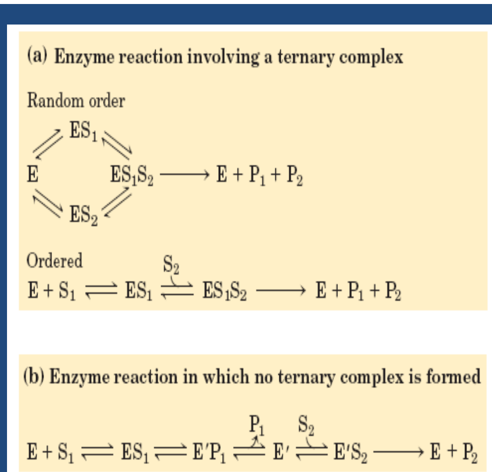


FIGURE 6–13 Common mechanisms for enzyme-catalyzed bisubstrate reactions. (a) The enzyme and both substrates come together to form a ternary complex. In ordered binding, substrate 1 must bind before substrate 2 can bind productively. In random binding, the substrates can bind in either order. **(b)** An enzyme-substrate complex forms, a product leaves the complex, the altered enzyme forms a second complex with another substrate molecule, and the second product leaves, regenerating the enzyme. Substrate 1 may transfer a functional group to the enzyme (to form the covalently modified E′), which is subsequently transferred to substrate 2. This is called a Ping-Pong or double-displacement mechanism.