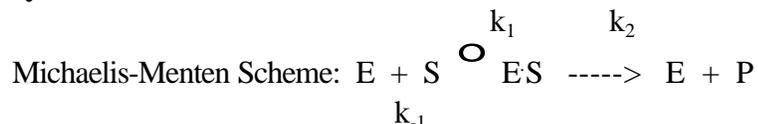


REVIEW QUESTIONS FOR ENZYME KINETICS: ANSWERS

1. **What are the two basic observations made in the laboratory to study enzyme kinetics?**

The velocity is directly proportional to enzyme concentration and hyperbolic with respect to the substrate concentration.

2. **What is the Michaelis-Menten kinetic scheme and how does this explain generally the observed kinetics?**



This scheme generally explains the observed kinetics since it shows the rate being proportional to the amount of ES whose quantities are proportional to the amount of E and S. Very importantly, it implies that at high S levels, all of the E would be present as ES, so the maximum amount of ES occurs and the rate is maximum.

3. **Why is the rate of an enzyme-catalyzed reaction proportional to the amount of E.S complex?**

The rate of an enzyme-catalyzed reaction is proportional to the amount of ES since the formation of product occurs after the formation of such a complex. No product is formed by the simple collision of E with S. E and S must bind together before product is formed.

4. **What is meant by saturation of the enzyme?**

Saturation of the enzyme means that all of the E is bound to S and no free E exists. The enzyme has bound to as much substrate as possible. This situation occurs at high levels of S.

5. **What is meant by saturation kinetics?**

Saturation kinetics refers to the situation of an enzyme reaction reaching a maximal velocity at high levels of S. All of the E present is present as ES, so the maximum amount of ES is formed. Since the rate is proportional to the amount of ES, the rate is at a maximum value. The enzyme is said to be saturated with S (see 4.).

6. **How does the formation of an E.S complex explain the reaching of a maximal velocity in the V_o vs S_o graph?**

The formation of an ES complex in an enzyme-catalyzed reaction means that all of the E is bound as ES at high levels of S. The maximum amount of ES is formed under these conditions. Since the rate is proportional to the amount of ES, the rate is at a maximum value under these conditions.

REVIEW QUESTIONS FOR ENZYME KINETICS: ANSWERS, continued

7. Explain mathematically how a value for K_m can be obtained from the V_o vs S_o graph when $V_o = 1/2 V_{max}$.

When $V_o = V_{max}/2$, then $V_{max}/2 = \frac{V_{max}S_o}{K_m + S_o}$,

$$K_m + S_o$$

cancelling V_{max} ,

$$1/2 = \frac{S_o}{K_m + S_o} \quad \text{or} \quad K_m + S_o = 2S_o$$

$$\text{or} \quad K_m = S_o \text{ at } V_o = (\text{value of}) V_{max}/2$$

8. What is the Michaelis-Menten equation and its Lineweaver-Burk form?

M-M equation $V_o = \frac{V_{max} S_o}{K_m + S_o}$ L-B eqn: $1/V_o = K_m/V_{max}(1/S_o) + 1/V_{max}$

9. How does the Michaelis-Menten equation explain why the rate of an enzyme-catalyzed reaction is proportional to the amount of enzyme?

In the M-M equation above $V_{max} = k_2 E_o$. In the experiment of V_o vs E_o , S_o is held constant so all other terms, S_o , K_m , are constant. So $V_o = \text{constant } E_o$ or V_o is proportional to E_o .

10. How does the Michaelis-Menten equation explain why the rate of an enzyme-catalyzed reaction reaches a maximum value at high substrate?

At high S_o , $K_m \ll \ll \ll S_o$ (numerically), so the term $K_m + S_o$ in the M-M equation becomes equal to S_o . $V_o = (V_{max} S_o)/S_o$, and S_o cancels. Therefore at high S_o then, $V_o = V_{max}$.

11. What type of enzyme inhibition does the following graph indicate? What can you say about the chemical similarities or differences between the substrate and the inhibitor?

The type of inhibition is noncompetitive. This implies that the substrate and the inhibitor are not structurally and chemically alike.

REVIEW QUESTIONS FOR ENZYME SPECIFICITY AND CATALYSIS: ANSWERS

1. What is the chemical basis of enzyme specificity?

The chemical basis of enzyme specificity is the complimentary relationship between the enzyme active site and the substrate that binds in that site. This complimentary relationship involves a structural fit of the S into the E active site (a "lock and key" kind of fit) and a chemical complementarity. For example if the E has H-bond donating groups, the S will have H-bond accepting groups and vice versa, or that there will be an opposite charge relationship between groups on the E and S. In addition if there are nonpolar groups on the S, the E will have a nonpolar region into which these groups will fit.

2. Describe generally what an enzyme-substrate complex "looks" like.

An enzyme-substrate complex is a combination of the enzyme and the substrate in which the two are bound together very closely so that atoms on each are essentially in physical contact with each other. The physical contact regions involve H-bonding, ionic bonds, hydrophobic interactions, and occasionally, covalent bonds.

3. What is the chemical basis of enzyme catalysis?

The chemical basis of enzyme catalysis involves the enzyme stabilizing the transition state of the reaction by helping to orient the substrate(s) in the active site and by bringing together functional groups on the enzyme and the substrate such that the enzyme functional groups can participate in the chemical catalysis events. These events include covalent bond formation between the E and S, acid or base catalysis by functional groups on the enzyme donating or accepting H⁺'s to and from the S, and by the introduction of strain (bond angle and bond length distortions) into a substrate making it more easily converted to product.

4. What kinds of functional group catalysis by the enzyme is occurring in the following diagram in both the forward and reverse directions? Indicate clearly what each functional group is doing.

Acid-base catalysis is occurring to assist the addition of water across a double bond in the forward direction. In the reverse direction, acid-base catalysis is occurring to assist in the removal of H₂O.

REVIEW QUESTIONS FOR ENZYME KINETICS: ANSWERS, continued

5. What kinds of functional groups would you expect to be involved in the binding of pyridoxal-5'-phosphate to transaminase enzymes requiring this cofactor (derived from Vitamin B6)? Diagram appropriate functional groups around this cofactor.

Possible functional groups for pyridoxal-5'-phosphate binding to transaminases:

Diagram prepared 10-20-02

