Chapter 6 - 4, 5, 6, 8, 9, 10, 11, 16, 23

(4) The substrate was bound by the enzyme which, in turn, stabilized the enzyme from thermal denaturation

(5) a) 145 - 270 = 125 amino acids x $\frac{5.4Å}{3.6 \text{ amino acids}}$

= 187.5Å

b) The three dimensional structure of the protein places the two residues in such proximity

(6) You could measure the decrease in the absorbance of a sample containing:

NADH
Pyruvate
Lactate Dehydrogenase
Buffer

As the enzyme catalyzes the reaction, NADH will be converted to NAD^+. This would be apparent by the decrease in absorbance at 340nm. If you made a standard curve and determined the molar extinction coefficient of NADH and then use Beer's Law to quantitate how much NADA was used per unit time.

(8) $K_{cat} = 30.0\ sec^{-1}$

$K_m = 0.005\ M$

a) What is [S] when $V_0 = 0.25\ V_m$

$$V_0 = \frac{V_m[S]}{K_m + [S]}$$

$$0.25V_m = \frac{V_m[S]}{K_m + [S]}$$

$$0.25V_m(K_m + [S]) = V_m[S]$$

$$\frac{0.25V_m K_m}{V_m} + \frac{0.25V_m[S]}{V_m} = \frac{V_m[S]}{V_m}$$

$$0.25K_m + 0.25[S] = [S]$$

$$0.25K_m = 0.75[S]$$

$$[S] = \frac{(0.25)(0.005M)}{0.75}$$

$$\boxed{[S] = 1.667 \times 10^{-3} M}$$

b) $[S] = \frac{1}{2}K_m = 0.5K_m$ | $[S] = 2K_m$ | $[S] = 10K_m$

$$V_0 = \frac{V_m(0.5K_m)}{K_m + 0.5K_m}$$

$$V_0 = \frac{V_m(0.5K_m)}{1.5K_m}$$

$$\boxed{\frac{V_0}{V_m} = 0.33}$$

$$V_0 = \frac{V_m(2K_m)}{K_m + 2K_m}$$

$$\boxed{\frac{V_0}{V_m} = 0.667}$$

$$V_0 = \frac{V_m(10K_m)}{K_m + 10K_m}$$

$$\boxed{\frac{V_0}{V_m} = 0.91}$$

c) Same V_m but different K_m for Substrate, X

Enzyme A: $K_m = 2.0\,\mu M$ Enzyme B: $K_m = 0.5\,\mu M$

The graph shows the rate of formation of product Y versus time. The higher the slope in the linear portion of the curve at early times, the greater the initial activity.

The enzyme with the lower K_m for substrate X will have a higher activity @ $1\,\mu M$. The red plot represents a higher initial activity. The red plot is enzyme B.

(4) a) $[E_T] = 4\,nM$ $V_m = 1.6\,\mu M/sec$

$$K_{cat} = \frac{V_m}{[E_T]} = \frac{1.6 \times 10^3\,nM \cdot sec^{-1}}{4\,nM} = \boxed{400\,sec^{-1}}$$

b) $[E_T] = 1\,nM$, $[S] = 30\,\mu M$, $V_0 = 300\,nM/sec$

$K_{cat} = V_m / [E_T]$

$V_m = (K_{cat})([E_T])$

$V_m = (0.001\,\mu M)(400\,sec^{-1})$

$V_m = 0.4\,\mu M/sec$

$$V_0 = \frac{V_m[S]}{K_m + [S]}$$

$$K_m = \frac{V_m[S] - V_0[S]}{V_0}$$

$\boxed{K_m = 10\,\mu M}$

c) $V_m = 4.8\ \mu M \cdot sec^{-1}$ in expt. A
$K_m = 15\ \mu M$ in expt B

Calculate α and α'

1st off: Since both changed, the inhibitor is a mixed inhibitor.

The V_m changes by a factor of $1/\alpha'$
[see Table 6-9]

$$V_m' = \frac{V_m}{\alpha'}$$

$$\alpha' = \frac{V_m}{V_m'} = \frac{4.8\ \mu M\ sec^{-1}}{1.6\ \mu M\ sec^{-1}} = 3$$

$$K_m' = \frac{\alpha K_m}{\alpha'} \quad \text{(see table 6-9)}$$

$$\alpha = \frac{K_m' \alpha'}{K_m} = \frac{(10\ \mu M)(3)}{(15\ \mu M)} = 2$$

$\alpha' = 3$, $\alpha = 2$

(10) a) $K_m = 4\mu M$ $[S] = 6mM$

$K_{cat} = 20\ min^{-1}$ $V_0 = 480nM/min$

$$K_{cat} = \frac{V_m}{[E_T]}$$

$$V_0 = \frac{V_m[S]}{K_m + [S]}$$

$$V_m = \frac{V_0(K_m + [S])}{[S]}$$

$$= \frac{(0.48\mu M/min)(4\mu M + 6000\mu M)}{6000\mu M}$$

$$= \frac{1.92\mu M^2/min + 2880\mu M^2/min}{6000\mu M}$$

$$= \frac{2881.92\mu M^2/min}{6000\mu M}$$

$$= 0.480\mu M/min$$

$$K_{cat} = \frac{V_m}{[E_T]}$$

$$[E_T] = \frac{V_m}{K_{cat}} = \frac{0.480\mu M/min}{20\ min^{-1}} = 0.024\mu M$$

$$\boxed{[E_T] = 0.024\mu M \text{ or } 24nM}$$

b) $[E_T] = 0.5\,\mu M$ $[S] = ?$

$V_0 = 5\,\mu M \cdot min^{-1}$

$$K_{cat} = \frac{V_m}{[E_T]}$$

$V_m = K_{cat}[E_T] = (20 \cdot min^{-1})(0.5\,\mu M)$

$V_m = 10\,\mu M/min$

$$V_0 = \frac{V_m[S]}{K_m + [S]}$$

$V_0(K_m + [S]) = V_m[S]$

$$\frac{(5\,\mu M \cdot min^{-1})(4\,\mu M) + 5\,\mu M \cdot min^{-1}[S]}{10\,\mu M \cdot min^{-1}} = [S]$$

$$\frac{(20\,\mu M^2 \cdot min^{-1})}{10\,\mu M \cdot min^{-1}} + 0.5[S] = [S]$$

$0.5[S] = 2\,\mu M$

$[S] = 4\,\mu M$

c) $\alpha = 10$

$V_0 = 240\ nm\ min^{-1}$ $[E_T] = 0.6\,\mu M$

$0.240\,\mu M \cdot min^{-1}$

Since the $[E_T]$ is the same as in (a), the V_m must be the same as well. $V_m = 0.48\,\mu M/min$

$V_0 = 0.240\,\mu M/min$ or $0.5\,V_m$

According to Table 6-9, For a Competitive Inhibition

$K_m' = \alpha K_m$ V_m is unchanged

Since the measured V_0 is $\frac{1}{2} V_m$, the [A] must be the measured K_m value, K_m'

$K_m' = \alpha K_m$

$K_m' = 10\,(4\,\mu M) = \boxed{40\,\mu M = [S]}$

(16)

$$\alpha = 1 + \frac{[I]}{K_I}$$

$$K_I = \frac{[E][I]}{[EI]}$$

$$[EI] = \frac{[E][I]}{K_I}$$

$$[E_T] = [E] + [ES] + [EI]$$

$$[E_T] = [E] + [ES] + \left(\frac{[E][I]}{K_I}\right)$$

$$[E_T] = [ES] + [E]\left(1 + \frac{[I]}{K_I}\right)$$

$$[E_T] = [ES] + [E]\alpha$$

Remember that:

$V_0 = K_2 [ES]$ and that $[ES] = \frac{[E_T][S]}{K_m + [S]}$ from the steady state assumption

$$[E_T] = \frac{[ES] K_m + [ES][S]}{[S]} , \frac{[ES] K_m (1 + [S])}{[S]}$$

$$[E_T] = [ES] + [E] = \frac{[ES](K_m + [S])}{[S]}$$

$$\frac{[E]}{[ES]} + 1 = \frac{K_m + [S]}{[S]}$$

$$\frac{[E]}{[ES]} = \frac{K_m}{[S]} + 1 \quad (-1)$$

$$[E] = \frac{K_m [ES]}{[S]}$$

Plug $[E]$ into $[E_T] = [ES] + [E]\alpha$

$$[E_T] = \frac{[ES][S]}{[S]} + \frac{K_m [ES]\alpha}{[S]}$$

multiply $[ES]$ by $\frac{[S]}{[S]}$

$$[E_T] = [ES]\left(\frac{[S] + K_m\alpha}{[S]}\right)$$

$$[ES] = [E_T]\left(\frac{[S]}{[S]+K_m\alpha}\right)$$

$$[ES] = \frac{[E_T][S]}{K_m\alpha + [S]}$$

Plug this into $V_0 = k_2[ES]$

$$V_0 = \frac{k_2[E_T][S]}{K_m\alpha + [S]}$$ and $V_m = k_2[E_T]$

$$V_0 = \frac{V_m[S]}{K_m\alpha + [S]}$$

Chapter 6: 19, 21, 22

(19) The curves look quite different, but only the Vm is really changing.

The Km is essentially unchanged

This is not a hallmark of a competitive inhibitor (which actually does the opposite).

Acetazolamide is a Mixed Inhibitor

(21) At pH 5.2 given: Asp^{52} $pK_A = 4.5$
Glu^{35} $pK_A = 5.9$

The Glutamate is less than 50% deprotonated
The Aspartic acid is nearly fully deprotonated.

As the pH increases, the glutamate residue which acts as a general acid in the mechanism (and must be protonated) becomes deprotonated and the activity of lysozyme drops

As the pH decreases, the aspartic acid starts to become protonated. This amino acid acts as the nucleophile and must be deprotonated for the enzyme to be active.

(11) a) When [S] increases from 0.2 to 0.4 μM,
V_0 increases 2 fold as well
When [S] = $10 \mu M$, V_0 = $50 \mu M$/sec
However the V_0 increase when [S] increases
from $100 \mu M$ to $200 \mu M$ is minimal
suggesting that the reaction is at V_m
and the enzyme molecules are saturated
at this concentration of [S]

b) When $\alpha = 2$, K_m is $\frac{1}{2}$
when $\alpha' = 3$, V_m increases 3 fold

c) When $\alpha = 2$, the x-intercept moves
(K_m increases). When $\alpha = 2$ and $\alpha' = 3$
The K_m appears to decrease.

(13) a) Arginine has a resonance stabilized positive charge on the guanidinium group whereas glutamine has an amide

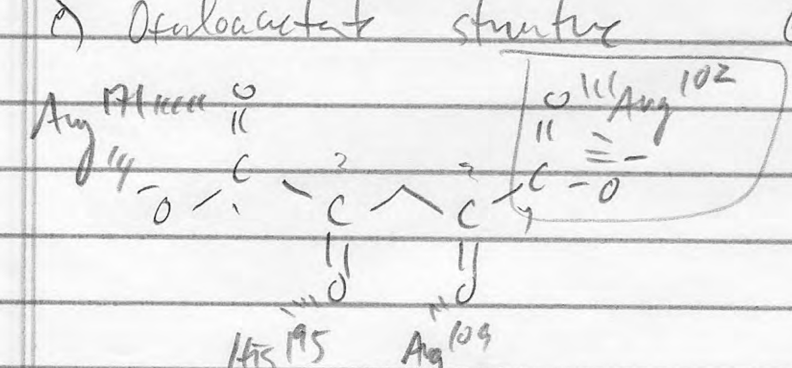
Arginine can stabiliz the C2 carboxyl oxygen when pyruvate is bound

b) Lysine can provide a single hydrogen bond whereas Arginine can provide 2

c) The resonance stabilized negative charge of pyruvate interacts with the resonance stabilized positive charge across arginine.

d) The polar glutamine residue does not interact with the aromatic ring of NADH as well as isoleucine does

e) Oxaloacetate structure   Gln^{102} → Arg Mutation



The Arginine would form an interaction withe the C-4 carboxylate

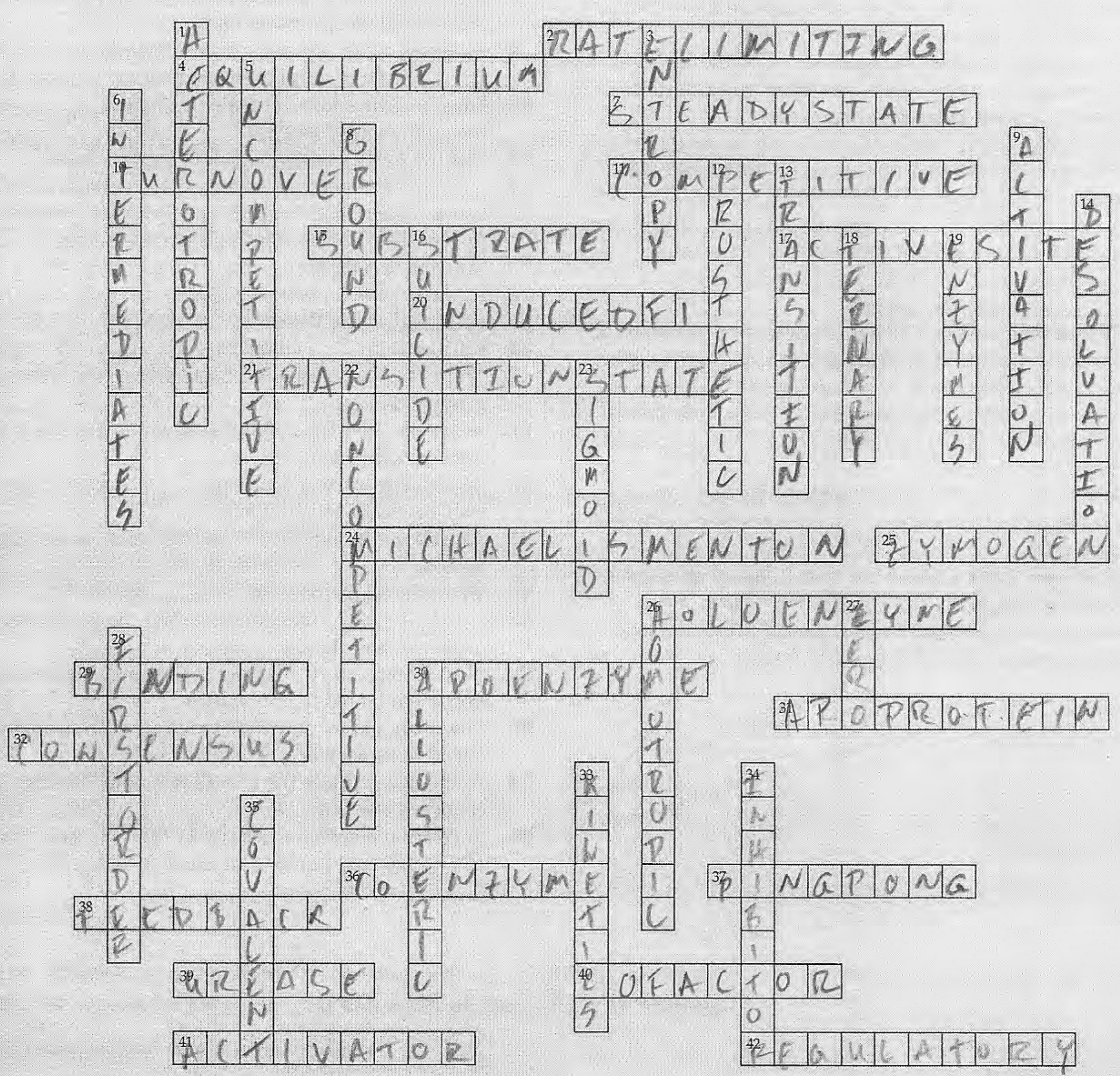
f) The mutant uses oxaloacetate because of the attraction between the C4 carboxylate and the guanidinium group's positive charge on Arginine.

g) Induced fit allows for some "wiggle" in the active site of an enzyme.

SELF-TEST

Enzymes and the study of enzyme reaction rates (enzyme kinetics) are among the most difficult areas of biochemistry for students to assimilate. Consequently, more problems have been included in this chapter's Self-Test. If you work through these problems carefully, your understanding of this material will be greatly enhanced. However, these problems will be beneficial only if you work through them to completion without looking at the answers.

Do You Know the Terms?



ACROSS

2. The slowest reaction in a sequence is the _______-_______ step.
4. State of a system in which no further net change is occurring.
7. The assumption that the rate of formation of ES is exactly equal to the rate of breakdown of ES is called the _______ _______ assumption. (2 words)
10. k_{cat} is known as the _______ number. At saturating substrate concentrations, $k_{cat} = V_{max}/[E_t]$.
11. Type of inhibitor that alters the K_m of an enzyme without altering V_{max}.
15. Molecule that binds to the active site of an enzyme.
17. Relatively small portion of an enzyme that is involved in substrate binding. (2 words)