PROBLEM SET 2 KEYS

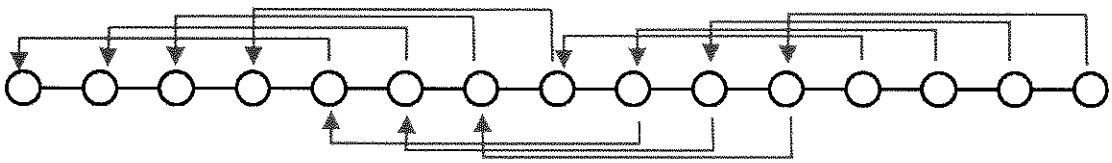
1.

(a) One turn contains 3.6 residues; therefore 15 residues form 4 full turns (15/3.6 = 4.2).

(b) The length of one turn is 5.4 Å, so a 15-residues α-helix will be 15/3.6 x 5.4 Å = 22.5 Å long

(c) We can figure this out using the following simple considerations. 1 residue can be involved in max 2 H-bonds, therefore 15 residues can make up to 2x15=30 H-bonds. In the α-helix, 4 residues at the N-terminus and 4 at the C-terminus make only 1 bond pre residue. This makes the total number of H-bonds 30-2x4=22. When calculating this number, each H-bond was counted twice: one time for the donor residue and one time for the acceptor. The real number of H-bonds is then 22/2=11.

We can also draw the H-bond connections (between res. ***i*** and ***i+4***) and count them. The acceptors (CO) are indicated by an arrow.

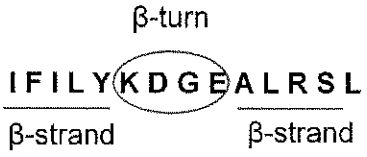


2.

Peptide (c) is most likely to form α-helix with its three charged residues (Lys, Glu, and Arg) aligned on one face of the helix. Peptide (a) has adjacent basic residues (Arg and Lys), which would destabilize α-helix. Peptide (b) contains Gly and Pro, both of which are helix-breaking (table 6.1).

3.

(a)

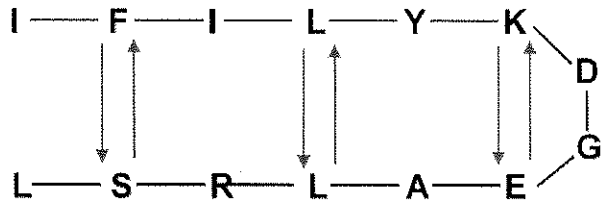


Since F is H-bonded to S, this leads to the H-bonding pattern shown below. This identifies the four residues in the middle as belonging to the β-turn: the CO of the first residue in the turn (K) is H-bonded to NH of the fourth residue (E).

(b)

Here is a simplified scheme of H-binding in the β-hairpin. Arrows are used to indicate the direction from donor (NH group) to acceptor (CO group). More detailed as well as

less detailed drawing were considered OK, if they correctly addressed the question. Altogether there are 6 H-bonds in this hairpin: two per each residue that has its NH and CO groups oriented toward the interacting strand. Due to the nature of the β-conformation, the NH and CO groups of alternate residues (here L1, I3, Y5, A10, R12, and I14) are oriented outside the area between the strands and, therefore, cannot participate in the H-bonding between these strands.



4.

(a) Use the equation $k_{cat} = V_{max} / [E_t]$. k_{cat} = 1600 nM•s^{-1} / 4 nM = 400 s^{-1}.

(b) Use the equation $V_{max} = k_{cat} [E_t]$. When $[E_t]$ = 1 nM, V_{max} = 400 nM•s^{-1}.
v / V_{max} = 300 nM•s^{-1} / 400 nM•s^{-1} = 3/4
Rearrange the Michaelis-Menten equation, substitute for v / V_{max}, and solve for K_m.
$v / V_{max} = [S]/(Km + [S])$
$3/4 = [S]/(Km + [S])$
$Km = [S]/3$
In this experiment, the concentration of the substrate, A, was 30 μM, so Km = 10 μM.

(c) Catalytic efficiency = k_{cat} / K_m = 400 s^{-1}/ 10 μM = 4 x 10^7 M^{-1}• s^{-1} . A perfect enzyme is an enzyme that catalyzes so efficiently, that almost every time enzyme (E) meets its substrate (S), the reaction occurs. k_{cat} / K_m of such enzymes is in the diffusion-limited range (10^8 M^{-1}• s^{-1} – 10^9 M^{-1}• s^{-1}). Therefore, we can say the enzyme we studied is a highly efficient and almost perfect enzyme.

5.

(a) $V = \frac{V_{max}[S]}{Km + [S]}$ invert & multiply with V_{max}

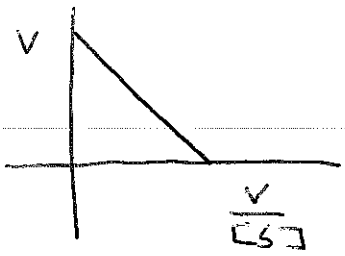
$$\frac{V_{max}}{V} = \frac{V_{max}(Km + [S])}{V_{max}[S]} = \frac{Km + [S]}{[S]}$$

$$V_{max} = \frac{V(Km + [S])}{[S]}$$

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

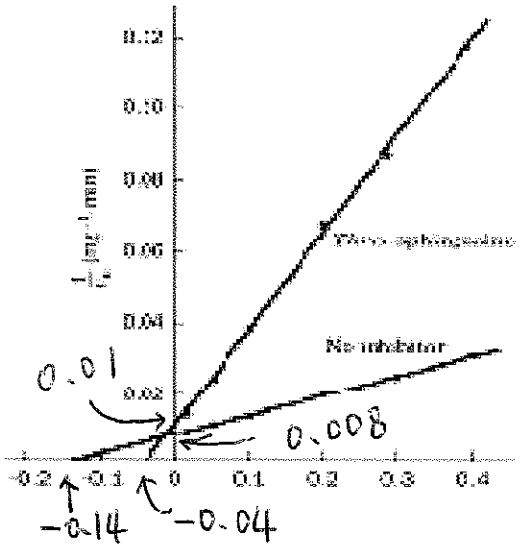
$$V = -Km\frac{v}{[S]} + V_{max}$$

(b) Slope = -Km, x-intercept = V_{max}/K_m, y-intercept = V_{max}



6.

(a)



K_m is determined from the x-intercept (= $-1/K_m$). In the absence of inhibitor, K_m = 1/0.14 μM^{-1} = 7 μM. In the presence of inhibitor, K_m = 1 / 0.04 μM^{-1} = 25 μM. V_{max} is determined from the y-intercept (= 1/ V_{max}). In the absence of inhibitor, V_{max} = 1/0.008 mg^{-1}•min = 125 mg•min^{-1}. In the presence of inhibitor, Vmax = 1/0.01 mg^{-1}•min = 100 mg•min^{-1}

(b)

The lines in the double-reciprocal plots intersect very close to the 1/v axis. Hence, inhibitor A is most likely a competitive inhibitor. Competitive inhibition is likely also because of the structural similarity between the inhibitor and the substrate, which allows them to compete for binding to the enzyme active site.

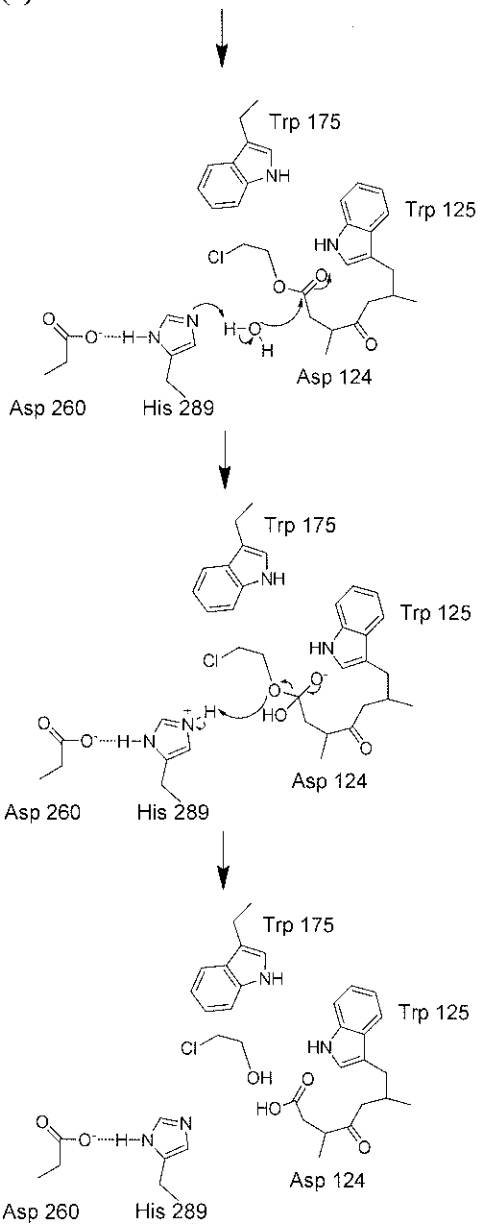
7.

Inhibitor A is an uncompetitive inhibitor. The ratio of Vi (velocity with inhibitor) to Vo (velocity without inhibitor) decreases as the [S] increases (Vi/Vo at low [S] = 0.84 and Vi/Vo at high [S] = 0.50). This means as more substrate is added the reaction is more inhibited. This is because the inhibitor binds the ES complex. As [S] increases there is more ES for the inhibitor to bind to, and therefore the reaction is more inhibited. Competitive inhibitors are the opposite, as more [S] is added the enzyme is less inhibited. The competitive inhibitor competes with the substrate for the substrate binding site on the enzyme. Noncompetitive

inhibitors give the same ratio of Vi/Vo because the inhibitor binds to both forms and changing the [S] will not change the amount of inhibition.

8.

(a)



(b)

(1).By formation of a hydrogen bond to His 289, it immobilizes and orients His 289 for its role in catalytic triad. (2). Asp 260 stabilizes the positive charge that develops on His 289 during hydrolysis of the chloroethyl-enzyme ester intermediate.

(c)

His acts as a general base, accepting a proton from the attacking water molecule.

(d)
Because there is no other residue that can work as a general base, water molecule cannot attack carbonyl group of the chloroethyl-enzyme ester intermediate. Therefore, the expected product is the chloroethyl-enzyme ester intermediate.