BIOC4540 Enzymology
Problem Set #1 ANSWERS

1. The activity of the enzyme(s) that convert sugars to starch is destroyed by heat inactivation.

2. The cytoplasmic density is 1.20 gm/mL and is 20% soluble protein. Thus the concentration of soluble protein (all enzymes in this case) is: 0.20 x 1.20 gm/mL = 0.24 gm/mL protein. However, there are 1000 different enzymes ∴ the concentration of each enzyme is: 0.24 mg/mL. The molar concentration of each enzyme (MW = 100,000 g/mol) is: 0.24 mg/mL x 1 mmol/10^5mg = 2.40 x 10^{-6} M = 2.40 μM.

3. Urease enhances urea hydrolysis by 10^{14} fold at 20°C and pH 8.0. If a given quantity of urease hydrolyzes a given amount of urea in 5 min then it would take: 5 min x 10^{14} to hydrolyze this amount of urea in the absence of urease. Thus, 5 x 10^{14} min x 1h/60min x 1d/24h x 1y/365.25 d = 9.506 x 10^8 y.

4. The substrate complexes the enzyme and this E-S complex is more stable than the enzyme alone. In other words, the substrate stabilizes the structure of the enzyme.

5. (a) The number of residues spaced between Arg^{145} and Glu^{270} is 270 – 145 = 125 residues. The translation distance between residues is 5.4 Å/3.6 residues = 1.5 Å/residue. Therefore, the linear or extended distance between Arg^{145} and Glu^{270} is 125 residues x 1.5 Å/residue = 187.5 Å.

   (b) The three-dimensional folding of the enzyme brings these two residues into close proximity.

6. The reaction rate can be measured by following the decrease in the absorption of NADH (at 340 nm) as the reaction proceeds. Determine the K_m value from kinetic experiments that give data that can be plotted according to a Michaelis-Menten graph. Next, using substrate concentrations well above the K_m, measure the initial rate (rate of NADH disappearance with time as determined spectrophotometrically) at several known enzyme concentrations, and make a plot of initial rates at increasing concentrations of enzyme. The plot should be linear, with a slope that provides a measure of LDH concentration.

7. (a) 1.6 x 10^{-3} μmoles/min
   (b) (1.6 x 10^{-3} μmoles/(min x 0.1 mL x 1L/10^3 mL)) = 16 μmoles/L·min
   (c) (1.6 x 10^{-3} μmoles/min) x (1 mL/(24 mg x 0.020 mL)) = 3.33 x 10^{-3} μmoles/min·mg
   (d) 1.6 x 10^{-3} μmoles/(min x 0.020 mL) = 0.08 U/mL
   (e) from (c) the specific activity is 3.33 x 10^{-3} μmoles/min·mg which is 3.33 x 10^{-3} U/mg

8. (a) In 7(d) above the extract contained 0.08 U/mL x 50 mL = 4.00 U (total). After fractionation and dialysis the sample had: 5.9×10^{-3} µmoles/min = 5.9×10^{-3} U/0.020 mL = 0.295 U/mL x 12 mL sample volume = 3.54 U (total). The percent recovery of enzyme activity is: 3.54/4.00 x 100% = 88.5%.

   (b) In 7(e) above the specific activity = 3.33×10^{-3} U/mg. After fractionation and dialysis the specific activity = 5.9×10^{-3} µmoles/(min x 30 mg/mL x 0.020 mL) = 9.8×10^{-3} U/mg. The degree of purification is: $(9.8 \times 10^{-3}$ U/mg)/ $(3.33 \times 10^{-3}$ U/mg) = 2.95 fold.

9. v_0 = 8.5 mg maltose/min = 8.5×10^{-3} g/min x 1mole/342 g = 2.49×10^{-5} moles/min.
   [$(2.49 \times 10^{-5}$moles/min)/15×10^{-3} mg amylase] x (10^6 µmole/1mole)
   = 1660 µmole $min^{-1}mg^{-1}$ = 1660 U/mg.
   1.66×10^3 µmole $min^{-1}mg^{-1}$ x (1mole/10^6 µmole) x (1 min/60s) x (10^6mg/1kg) = 27.67 mole $s^{-1}kg^{-1}$ or 27.67 katal/kg.

10. (i) original stock = 3 mL; dilute stock by taking 12.5 µL (80 X) to 1000 µL
    (ii) remove 5.5 µL aliquots ⇒ assay for activity (5.5, 5.9, 5.75 µg PNP; mean = 5.72 µg)
    (iii) secondary stock = 0.23 mg mL^{-1} protein
    (iv) (5.72 µg PNP/125 sec) x (60 sec/1min) = (2.75 µg/min) x (1 µmole/139.11µg) = 1.98×10^{-2} U.
    (v) 1.98×10^{-2}U/(5.5×10^{-3}mL x 0.23 mg/mL) = 15.65 U/mg protein; note: same specific activity for primary and secondary stocks since only diluting with fluid (15.65 µmole PNP/(min x mg protein)) x (1 mole/10^6 µmoles) x (1 min/60sec) x (10^6 mg protein/1kg protein) = 0.261 katal/kg.