Enzymes Problem Set 1

A) What concentration of the substrate would be required to obtain an initial rate of 0.5\(V_{\text{max}}\), 0.9 \(V_{\text{max}}\), 0.95 \(V_{\text{max}}\)?

B) The following initial rate data were obtained for an enzyme catalyzed reaction:

<table>
<thead>
<tr>
<th>Substrate Concentration ((\mu\text{mol/L}))</th>
<th>Measured Initial Rate ((\mu\text{mol/L\text{min}}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.08</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
</tr>
<tr>
<td>10</td>
<td>0.33</td>
</tr>
<tr>
<td>100</td>
<td>0.48</td>
</tr>
<tr>
<td>1000</td>
<td>0.50</td>
</tr>
</tbody>
</table>

i) What is the value of \(K_M\) and \(V_{\text{max}}\)?

ii) Calculate the concentration of substrate remaining after 60 minutes if the initial substrate concentration is 20 \(\mu\text{mol/L}\).

C) The following initial rate data were obtained by adding 2.5 \(\mu\text{g}\) of a purified enzyme having molecular weight of 42,300 Daltons into 100 mL of water with the indicated concentrations of substrate.

<table>
<thead>
<tr>
<th>(S_0) (mmol/L)</th>
<th>(v) ((\mu\text{mol/L\text{s}}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>4.50</td>
</tr>
<tr>
<td>2.00</td>
<td>7.90</td>
</tr>
<tr>
<td>4.00</td>
<td>11.10</td>
</tr>
<tr>
<td>8.00</td>
<td>15.20</td>
</tr>
</tbody>
</table>

Calculate the value of \(k_{\text{CAT}}\), \(K_M\) and the pseudo first order rate constant.
D) The following initial rate data were obtained by adding 0.35 mg of a purified dimeric enzyme having (total) molecular weight of 82,800 Daltons into 100 mL of water with the indicated concentrations of substrate.

<table>
<thead>
<tr>
<th>Substrate Conc (mM)</th>
<th>v (mM/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.55</td>
</tr>
<tr>
<td>2.0</td>
<td>0.94</td>
</tr>
<tr>
<td>3.0</td>
<td>1.32</td>
</tr>
<tr>
<td>5.0</td>
<td>1.87</td>
</tr>
<tr>
<td>10.0</td>
<td>2.59</td>
</tr>
</tbody>
</table>

i. Calculate the value of $k_{\text{CAT}}$, $K_M$ and the pseudo first order rate constant.

ii. For a solution having an enzyme concentration of 3.5 mg/L, calculate the the final substrate concentration after 5 minutes of reaction when the initial substrate concentration is 15 mM.

iii. For a solution having an enzyme concentration of 1.0 mg/L, calculate the the final substrate concentration after 5 minutes of reaction when the initial substrate concentration is 15 mM.

E) Tyrosine phenol lyase catalyzes the reversible conversion of tyrosine to phenol, pyruvate and ammonium. The enzyme uses pyridoxal 5-phosphate (PLP) as a cofactor. The enzyme catalyzes similar reactions as well, for example the reversible conversion of L-dopa (3,4-dihydroxy L-phenylalanine) to catechol, pyruvate and ammonium. An assay for the activity of this enzyme involves incubating excess phenol, pyruvate and ammonium with the enzyme sample for a measured length of time. After the termination of the reaction, the L-tyrosine present in the sample is quantified by liquid chromatography.

The following solutions are mixed:

- 500 µL 1.3 M NH₄Cl/0.05M Na₄EDTA pH adjusted to 8.5
- 100 µL 80 mM Na₂SO₃ (a reducing agent to stabilize the reaction)
- 100 µL 800 mM Pyruvate
- 100 µL 1.0 mM PLP
- 100 µL 500 mM Phenol

At t=0, you add 100 µL of an enzyme solution known to have a protein concentration of 8.5 µg/mL. After 7.0 minutes, you add 100 µL trichloroacetic acid which terminates the reaction. Using the HPLC, you find that the concentration of L-tyrosine in this final solution is 2.3 mM.

i) What is the activity of the original enzyme solution (in IU/mL)?

ii) What is the specific activity of the original enzyme solution (in IU/mg protein)?
F) DHA kinase catalyzes the conversion of dihydroxyacetone (DHA) to dihydroxyacetone phosphate (DHAP) using ATP as a co-substrate. The enzyme assay relies on a coupled reaction in which the reaction product, DHAP, is subsequently converted into glycerol 3-phosphate by the presence of the enzyme glycerol 3-phosphate dehydrogenase, which uses NADH as a co-substrate. Glycerol is added to the assay to prevent glycerol kinase interference. α,α-dipyridyl is added to the assay to prevent glycerol dehydrogenase interference. NADH absorbs light at a wavelength of 340 nm. Thus, the rate of DHA kinase activity is directly related to the rate at which NADH disappears from the solution.

The following solutions are mixed:

200 μL 200 mM triethanolamine-HCl buffer
400 μL 25 mM α,α-dipyridyl
50 μL 5 U glycerol 3-phosphate dehydrogenase from rabbit muscle
50 μL 2.0 M glycerol
50 μL 2.0 mM NADH
50 μL 20 mM MgCl₂
50 μL 20 mM dihydroxyacetone, DHA
50 μL 20 mM ATP

At t=0, you add 100 μL of an enzyme solution known to have a protein concentration of 4.3 μg/mL, and measure the rate of decrease in absorbance of the solution to be 0.52 AU/min. The molar extinction coefficient of NADH is 6.22 AU·L/mmol for the light path length used. "AU" means absorbance unit.

i) Write the reaction of the enzyme and the couple reaction, including the cofactors. Highlight the actual species measured. Why is excess glycerol 3-phosphate dehydrogenase needed?

ii) What is the activity of the original enzyme solution (in IU/mL)?

iii) What is the specific activity of the original enzyme solution (in IU/mg protein)?

G) Iodoacetamide (MW = 185) is a common irreversible inhibitor of enzymes because it binds to reactive cysteine residues in a 1:1 molar ratio.

The enzyme glyceraldehyde 3-phosphate dehydrogenase has a MW of 150,000, and is known to have one reactive cysteine residue at the active site. A 5 mL solution of this enzyme at a concentration of 1.2 mg/mL is completely inactivated by 0.03 mg iodoacetamide. How many subunits does this enzyme contain?
Invertase mediates the conversion of sucrose into glucose and fructose. You place 0.66 mg of invertase in a beaker of 500 mL buffer initially containing 20 mM sucrose and no glucose and fructose. The value of $K_M$ is 2.5 mM and the value of $k_{cat}$ is $1550 \text{ s}^{-1}$. The invertase you are using has a molecular weight of 65000.

i) What is the initial reaction rate (mmol sucrose/Lmin)?

ii) What will be the concentration of sucrose (mM) after 5 min?

iii) What will be the reaction rate (mmol sucrose/Lmin) after 5 min?
By what percentage has the reaction rate decreased after 5 min?

iv) What will be the concentration of sucrose (mM) after 10 min?
What will be the concentration of glucose after 10 min?

v) What will be the reaction rate (mmol sucrose/Lmin) after 10 min?
By what percentage has the reaction rate decreased after 10 min?

vi) If in the 500 mL solution you used 1.32 mg of the enzyme (instead of 0.66 mg) what will be the concentration of sucrose after 10 min?