1. A student needed to know the activity versus pH profile for an enzyme that he was going to use in a later application. He collected the appropriate data and determined $K_M$ and $V_{\text{max}}$ at pH 6.8. They were about 0.1mM and 800mM/min, respectively. He then measured $V_o$ at $[S]_0 = 0.002$ mM and at $[S]_0 = 10$ mM, and obtained the data given in the table below.

<table>
<thead>
<tr>
<th>pH</th>
<th>$V_o$ at $[S]_0 = 0.002$ mM (mM/min)</th>
<th>$V_o$ at $[S]_0 = 10$ mM (mM/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>precipitated</td>
<td>precipitated</td>
</tr>
<tr>
<td>3</td>
<td>1.7</td>
<td>728</td>
</tr>
<tr>
<td>4</td>
<td>2.4</td>
<td>732</td>
</tr>
<tr>
<td>5</td>
<td>7.0</td>
<td>756</td>
</tr>
<tr>
<td>6</td>
<td>13.7</td>
<td>783</td>
</tr>
<tr>
<td>7</td>
<td>15.2</td>
<td>776</td>
</tr>
<tr>
<td>8</td>
<td>13.5</td>
<td>661</td>
</tr>
<tr>
<td>9</td>
<td>6.1</td>
<td>268</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
<td>43</td>
</tr>
</tbody>
</table>

a. Graph the data so that you can interpret how the velocity changes with pH.

Raw data (not necessary)
b. Interpret the data in terms of ionizations of the various forms of the enzyme that alter binding or catalysis.

\[
\begin{align*}
\text{EH}_2 & \quad + \quad S & \quad \xrightarrow{X} & \quad \text{EH}_2S & \quad \xrightarrow{} & \quad \text{EH}_2 & \quad + & \quad P \\
\quad \quad \downarrow & & \quad \quad \downarrow & & \quad \quad \downarrow & & \quad \quad \downarrow \\
\text{EH}^- & \quad + \quad S & \quad \xleftrightarrow{} & \quad \text{EHS}^- & \quad \xrightarrow{} & \quad \text{EH}^- & \quad + & \quad P \\
\quad \quad \downarrow & & \quad \quad \downarrow & & \quad \quad \downarrow & & \quad \quad \downarrow \\
\text{E}^{2-} & \quad + \quad S & \quad \xleftrightarrow{} & \quad \text{ES}^{2-} & \quad \xrightarrow{X} & \quad \text{E}^{2-} & \quad + & \quad P
\end{align*}
\]

pKa = 5.2

pKa = 8.8

c. What are the pKa’s involved?

\(-5, \sim 9\) (5.2, 8.8)

d. What other situations other than effects on \(K_M\) and \(V_{\text{max}}\) could give rise to similar observations?

- You’d want to check for denaturation of the enzyme
- Ionizations of substrate, buffer, etc., that could confuse the interpretation of the results
e. Indicate how you could rule out each of the possibilities you listed in part d (or, find that it, rather than an ionization in the active site, controlled the behavior).

Denaturation: Pre-incubate the enzyme at various test pH’s over the range, but assay it at the pH optimum.

Look up pKa’s of other components, to be sure they are not the one you observe in the V vs. pH plot.

e. The ΔH_{ionization} was found to be 0.5 kcal/mole for the acidic ionization and 12 kcal/mole for the basic ionization observed for the enzyme. Suggest what groups on the enzyme are the most likely to be responsible for the pH behavior.

Acidic ionization: pKa = ~5, ΔHion = ~0, looks like a carboxyl group…Aspartic acid, Glutamic Acid, C-terminus

Basic ionization: Pka ~ 9, ΔHion = ~0, looks like a primary amine…Lysine or the N-terminus

2. Aldolase catalyzes the following reaction, written in the direction of glycolysis (as opposed to glyconeogenesis):

Fructose-1,6-bisphosphate ⇌ Glyceraldehyde-3-phosphate + Dihydroxyacetone phosphate

The thermodynamics of this reaction (in the direction written) were studied at pH 9, and the following data were obtained:

<table>
<thead>
<tr>
<th>T (K)</th>
<th>Keq</th>
</tr>
</thead>
<tbody>
<tr>
<td>278.15</td>
<td>4.3 x 10^{-4}</td>
</tr>
<tr>
<td>298.15</td>
<td>1.8 x 10^{-3}</td>
</tr>
<tr>
<td>313.15</td>
<td>6.37 x 10^{-3}</td>
</tr>
</tbody>
</table>

a. From this information, calculate ΔG°, ΔH° and ΔS° for the reaction under these conditions (use 25°C if the parameter is dependent on temperature).

Plot ln Keq vs 1/T, the slope is –ΔH/R:

- 1.98 cal/mole•K * -6650 K = 13.2 kcal/mole

Intercept is ΔS/R:

1.98 cal/mole•K * 16 = 31.7 cal/mole•K

At 25°

ΔG° = ΔH° - TΔS°

= 13,200 cal/mole – 298K•31.7 cal/mole•K

= 3.75 kcal/mole
b. Calculate the value of the equilibrium constant at 30°C. Which direction, glycolysis or glyconeogenesis, is favored?

For 303K, I got
\[ \Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \]
\[ = 13,200 \text{ cal/mole} - 303K \times 31.7 \text{ cal/mole} \times K \]
\[ = 35.95 \text{ kcal/mole} \]
\[ \ln K_{eq} = -\Delta G/RT = -3595 \text{ cal/mole}/1.98\text{cal/mole}K \times 303K = 5.99 \]
\[ K_{eq} = 2.4 \times 10^{-3}. \]
Clearly, the reverse direction (glyconeogenesis) is favored.

3. At saturating levels of substrate, an enzyme-catalyzed reaction was found to be 2.5 times faster at 35°C than at 20°C.

a. Assuming \( \ln k \) is linear with \( 1/T \) in this temperature range, calculate \( E_a \) for the reaction.

Plot \( \ln k \) vs \( 1/T \), and the slope is \( -E_A/R \)… in finding the slope, just use rise over run:

\[ \text{rise} = \Delta \ln k = \ln (k_{T_2}) - \ln (k_{T_1}) = \ln (k_{T_2}/k_{T_1}) = \ln 2.5 = 0.916 \]
\[ \text{run} = \Delta (1/T) = (1/308) - (1/293) = 1.66 \times 10^{-4}. \]

Slope = \( 5.51 \times 10^3 K. \) \( E_A = -5.51 \times 10^3 \times 1.98 \text{ cal/mole} \times E_A = 10.4 \text{ kcal/mole} \)

b. Calculate \( Q_{10} \) for the reaction.

Since the two temperatures we know are not 10° apart, we’ll have to calculate the ratio for a new pair of temperatures. The thing to remember is that the ratio of rates (actually, rate constants) at any two temperatures is \( \frac{-E_A}{e^{RT}} \), since the pre-exponential factor cancels out.

So, just choose two temperatures that are 10 degrees apart, and calculate the ratio. I got \( Q_{10} = 1.97 \) for 283 and 273. But, the fraction goes down slightly as the T goes up. So, you see that \( Q_{10} \) doesn’t agree entirely with Arrhenius.

c. Calculate \( \Delta H^\ddagger \) for the reaction.

This plot is just like the Arrhenius plot, except that you divide \( k \) by \( T \) before you take the \( \ln \).

Slope = \( 5.79 \times 10^3 \), \( \Delta H^\ddagger = 11.5 \text{ kcal/mole} \)

4. Describe three distinctly different ways that a change in temperature can alter the velocity of an enzyme-catalyzed reaction.

- Denature the protein
- Change the pKa of a group that is important for binding or catalysis
- Change the pH
- Change substrate concentration (gasses or partially soluble solids)
- Change \( K_M \)