

1. The enzyme glucose oxidase converts glucose to glucolactone and generates one equivalent of hydrogen peroxide. The cofactor flavin adenine dinucleotide (FAD) is required for function. The nucleoside adenine monophosphate is a non-competitive inhibitor that binds to the FAD binding site with an association constant of $K_a = 10^6 \text{ M}^{-1}$. Glucose oxidase is observed to have a maximal velocity of 1.5 mmol/min for the oxidation of glucose. At a glucose concentration of 10^{-5} M the rate is 0.5 mmol/min.
- A. Determine K_M for glucose oxidase.
- B. Determine effect on the maximal rate if the concentration of adenine is 10^{-3} M .

$$K_M \text{ (50 points)} = \underline{2 \times 10^{-5} \text{ M}}$$

$$V_{\max,I} \text{ (50 points)} = \underline{1.5 \mu\text{mol/min}}$$

The Michaelis constant is:

$$\begin{aligned} V &= \frac{[S]V_{\max}}{K_M + [S]} \\ K_M &= \left(\frac{V_{\max}}{V} - 1 \right) [S] \\ &= (3 - 1)10^{-5} \text{ M} \\ &= 2 \times 10^{-5} \text{ M} \end{aligned}$$

In the presence of an inhibitor we have:

$$\begin{aligned} K_a &= \frac{[EI]}{[E][I]} \text{ is the inverse of } K_I \text{ so } K_I = 10^{-6} \\ V_I &= \frac{[S]V_{\max}}{\alpha(K_M + [S])} \text{ so } V_{\max,I} = \frac{V_{\max}}{\alpha} \\ \alpha &= 1 + \frac{[I]}{K_I} = 1 + \frac{10^{-3}}{10^{-6}} = 1001 \\ V_{\max,I} &= \frac{V_{\max}}{\alpha} = \frac{1.5 \text{ mmol / min}}{1001} \approx 1.5 \text{ } \mu\text{mol / min} \end{aligned}$$

2. The rate constant for the folding of the villin headpiece is $k = 3 \times 10^5 \text{ s}^{-1}$ at 290 K and $9 \times 10^5 \text{ s}^{-1}$ at 310 K. Determine that activation energy for the folding transition.

Solution: Use the Arrhenius equation.

$$\ln k_2 - \ln k_1 = -\frac{E_a}{R} \left(\frac{1}{T_2} - \frac{1}{T_1} \right)$$

$$\ln \left(\frac{k_2}{k_1} \right) = -\frac{E_a}{R} \left(\frac{1}{T_2} - \frac{1}{T_1} \right)$$

$$E_a = \frac{R \ln \left(\frac{k_2}{k_1} \right)}{\left(\frac{1}{T_1} - \frac{1}{T_2} \right)} = \frac{RT_1 T_2 \ln \left(\frac{k_2}{k_1} \right)}{(T_2 - T_1)}$$

Plugging in numbers we obtain:

$$E_a = \frac{(8.31 \text{ J/mol-K})(290 \text{ K})(310 \text{ K}) \ln \left(\frac{9 \times 10^5}{3 \times 10^5} \right)}{20 \text{ K}} = 41 \text{ kJ/mol}$$

E_a (10 points) = 41 kJ/mol.