assignment 2

(due before class on 23 Oct 2020)

1. Derive an expression for the rate of an Enzyme catalysed reaction using the modified Michaelis-Menten mechanism:

$$\mathrm{E+S} \underset{k_a'}{\overset{k_a}{\rightleftharpoons}} \mathrm{ES} \underset{k_b'}{\overset{k_b}{\rightleftharpoons}} \mathrm{P+E}$$

Show that at the initial stages, when $[S] >> [P]$, the expression reduces to the usual Michaelis-Menten rate expression.

Ans. SSA $\Longrightarrow$ $\frac{d[\mathrm{ES}]}{dt} = k_\mathrm{a}[\mathrm{E}][\mathrm{S}] - k_\mathrm{a}'[\mathrm{ES}] - k_\mathrm{b}[\mathrm{ES}] + k_\mathrm{b}'[P][E] = 0$

or, $[\mathrm{ES}] = \frac{\mathrm{k_a}}{\mathrm{k}_a'+\mathrm{k_b}}[\mathrm{E}][\mathrm{S}] + \frac{\mathrm{k_b'}}{\mathrm{k}_a'+\mathrm{k_b}}[P][E] = K_M^{-1}[\mathrm{E}][\mathrm{S}] + \frac{\mathrm{k_b'}}{\mathrm{k}_a'+\mathrm{k_b}}[P][E]$

Using $[\mathrm{E}] = [\mathrm{E}]_0 - [\mathrm{ES}]$; $\quad K_M[\mathrm{ES}] = ([\mathrm{E}]_0 - [\mathrm{ES}])[\mathrm{S}] + \frac{\mathrm{k_b'}}{\mathrm{k}_a'+\mathrm{k_b}}[P]([\mathrm{E}]_0 - [\mathrm{ES}])$

or, $\left(K_M + [\mathrm{S}] + \frac{\mathrm{k_b'}}{\mathrm{k}_a'+\mathrm{k_b}}[P]\right)[\mathrm{ES}] = [\mathrm{E}]_0[\mathrm{S}] + \frac{\mathrm{k_b'}}{\mathrm{k}_a'+\mathrm{k_b}}[P][\mathrm{E}]_0$

or, $[\mathrm{ES}] = \frac{\left(1+\frac{\mathrm{k_b'}}{\mathrm{k}_a'+\mathrm{k_b}}\frac{[P]}{[S]}\right)[\mathrm{E}]_0}{1+\frac{K_M}{[S]_0}+\frac{\mathrm{k_b'}}{\mathrm{k}_a'+\mathrm{k_b}}[P]} \approx \frac{\left(1+\frac{\mathrm{k_b'}}{\mathrm{k}_a'+\mathrm{k_b}}\frac{[P]}{[S]_0}\right)[\mathrm{E}]_0}{1+\frac{K_M}{[S]_0}+\frac{\mathrm{k_b'}}{\mathrm{k}_a'+\mathrm{k_b}}[P]}$

$v = k_b[\mathrm{ES}] = \frac{k_b\left(1+\frac{\mathrm{k_b'}}{\mathrm{k}_a'+\mathrm{k_b}}\frac{[P]}{[S]_0}\right)[\mathrm{E}]_0}{1+\frac{K_M}{[S]_0}+\frac{\mathrm{k_b'}}{\mathrm{k}_a'+\mathrm{k_b}}[P]}$

clearly, when $[S] >> [P]$, the expression reduces to the usual Michaelis-Menten rate expression,

$v = \frac{k_b[\mathrm{E}]_0}{1+\frac{K_M}{[S]_0}}$

2. Hydrogen iodide undergoes decomposition into H_2 + I_2, when irradiated with radiation having a wavelength of 207 nm. When 1 J of energy is absorbed, 440 μg of HI is decomposed. How many molecules of HI are decomposed by one photon of radiation of this wavelength? Suggest a mechanism that is consistent with this result.

Ans. Energy in 1 Einstein of 207 nm radiation $\equiv \frac{N_A hc}{\lambda} = \frac{6.023\times10^{23}\times6.627\times10^{-34}\times2.889\times10^{8}}{207\times10^{-9}} =$ 577.9kJ/mol

$\therefore$ no. of moles of HI are decomposed by one Einstein$= 577.9 \times 10^3 \times \frac{440\times10^{-6}}{127.91} \approx 2$

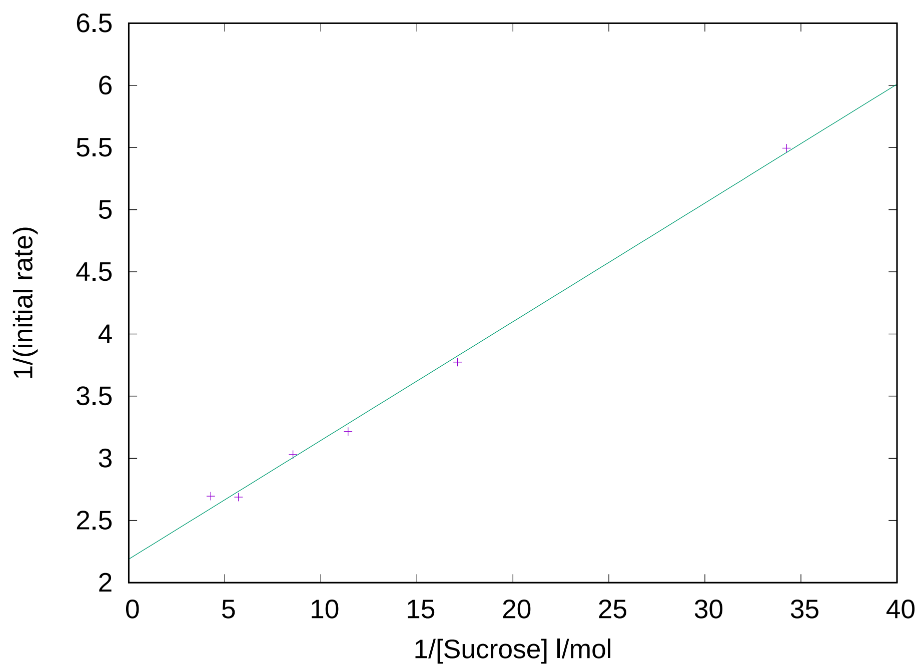
For mechanism, look at the lecture slides uploaded on Moodle.

3. The hydrolysis of sucrose by the enzyme invertase was followed by measuring the initial rate of change in polarimeter (optical rotation) readings, α, at various initial concentrations of sucrose. the reaction is inhibited reversibly by the addition of urea :

[Sucrose] (mol l^{-1})	0.0292	0.0584	0.0876	0.117	0.175	0.234
initial rate $\frac{d\alpha}{dt} = v_0$	0.182	0.265	0.311	0.330	0.372	0.371
initial rate (2M urea), v_0'	0.083	0.119	0.154	0.167	0.192	0.188

(a) Make a simple plot of the data in the absence of urea and determine the Michaelis constant for this reaction.

(b) Carry out a suitable analysis of the data in the presence of urea and determine whether urea is a competitive or a non-competitive inhibitor.

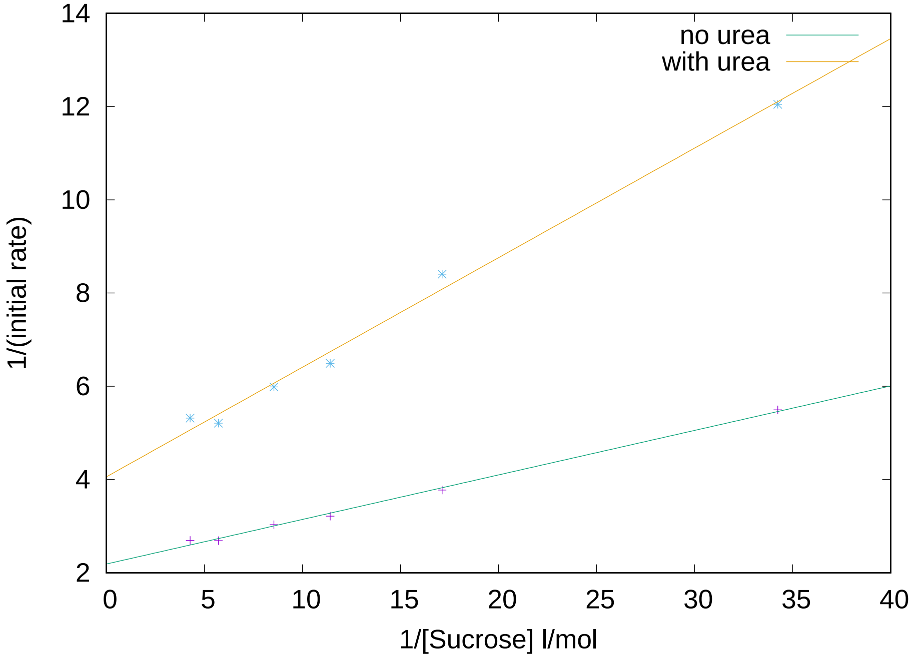


Ans. (a)

intercept$= 2.188$ and slope$= 0.0955$

$\therefore K_M = \frac{0.0955}{2.188} = 0.0436$ mol l^{-1}

(b)



The slope (greater than without area) and intercept both are different for the case with urea. This means that both non-competitive (mixed) inhibition is taking place : inhibitor binds to site other than active site, and its presence reduces ability of substrate to bind to active site.

4. The longest wavelength absorption band of chlorophyll a peaks *in vivo* at $\lambda = 680$ nm.

(a) For photons with $\lambda = 680$ nm, calculate the energy in J photon^{-1} and in J Einstein^{-1}. (1 Einstein=energy in 1 Avogadro number of photons).

(b) CO_2 fixation in photosynthesis can be represented as

$CO_2 + H_2O \rightarrow (CH_2O) + O_2$ with $\Delta_r H = 116$ kcal mol^{-1}

What is the minimum number of Einsteins of radiation that need to be absorbed to provide the energy needed to fix 1 mol of CO_2.

(c) Experimentally, the number of photons required to fix 1 mol of $CO_2$is 8 or 9.

What is the photochemical quantum yield?

Ans. (a) $E = \frac{hc}{\lambda} = 29.208 \times 10^{-23}\text{kJ/photon} = 175.921\text{kJ Einstein}^{-1}$

(b) no. of Einsteins $= \frac{116 \times 4.184}{175.921} = 2.766$

(c) correction : 'mol' should be read as 'molecule'

photochemical quantum yield$= \frac{2.766}{8.5} = 0.325$