

T. D. n° 5 (enzyme inhibitors)

Exercise n° 1 :

Calculate the inhibition constant for a competitive inhibitor, knowing that :

$$K_m = 6,7 \cdot 10^{-4} \text{ M}$$

$$V_{\max} = 330 \text{ } \mu\text{M}/\text{min}$$

$$V_i = 1,5 \text{ } \mu\text{M}/\text{min} \text{ for : } [I] = 1 \cdot 10^{-5} \text{ M and } [S] = 2 \cdot 10^{-5} \text{ M}$$

Exercise n° 2 :

What concentration of competitive inhibitor should be used to achieve 75% inhibition with a substrate concentration equal to $1,5 \cdot 10^{-3} \text{ M}$?

$$\text{We give : } K_m = 2,9 \cdot 10^{-4} \text{ M}$$

$$K_i = 2 \cdot 10^{-5} \text{ M}$$

Exercise n° 3 :

S-adenosylhomocysteinase is inhibited by adenine and its derivatives. This inhibitory effect on the hydrolysis rate of S-adenosylhomocysteine (SAH) was studied by working with different concentrations of substrate and adenine. The following table shows the rates, expressed in micromoles of substrate hydrolyzed per minute per milligram of protein :

[SAH] (μM)	[adenine] (μM)				
	0	1	3	10	20
1	0.30	0.25	0.19	0.10	0.061
3	0.41	0.38	0.32	0.22	0.15
10	0.47	0.46	0.43	0.36	0.29

Using **Dixon's** representation, determine the type of adenine inhibition and its inhibition constant.

Exercise n° 4 :

Glucose oxidase from *Aspergillus niger*, which catalyzes the oxidation of D-glucose by molecular oxygen to produce D-glucono- δ -lactone and H_2O_2 , is inhibited by a glucose derivative, D-glucal. The inhibitory effect on each of the reaction substrates was studied. The measured rate values were divided by the enzyme concentration. They are given in the table in $10^{-3} \cdot \text{S}^{-1}$.

1. We first worked with different concentrations of D-glucose in the presence of oxygen (0.27 mM) :

[D-glucal] (mM)	[D-glucose] (mM)				
	5	10	20	40	80
0	0.066	0.110	0.165	0.220	0.260
79	0.044	0.078	0.126	0.183	0.235
113	0.040	0.070	0.115	0.170	0.225

2. We then varied the oxygen concentration by working in the presence of D-glucose (20 mM) :

[D-glucal] (mM)	[oxygène] (μM)					
	75	100	135	150	200	250
0	0.068	0.080	0.094	0.100	0.112	0.122
110	0.050	0.056	0.062	0.0645	0.070	0.073

Determine the type of inhibition of D-glucal relative to D-glucose and relative to oxygen, and the corresponding inhibition constants.

Exercise n° 5 :

An enzyme that hydrolyzes the phosphodiester bond between cyclic AMP and cyclic CMP was purified from pig liver. To determine whether these two activities were localized at the same active site, the Michaelis constant of cAMP was compared to its inhibition constant with respect to cCMP hydrolysis. The experiments were conducted in the presence of 0.2 μg of enzyme, and the rates are expressed in picomoles of substrate hydrolyzed per minute.

1. Determine the Michaelis constant.

[AMPc] (μM)	13.5	20	27	35	55	75
Rate	112	142	166	187	220	240

2. Determine the inhibition constant.

[AMPc] (μM)	[CMPc] (μM)			
	67	100	130	300
0	280	370	433	640
50	104	148	184	340

3. Compare the Michaelis constant of cAMP with its inhibition constant. What can be deduced ?