

a. Is the inhibitor competitive or noncompetitive?

b. Find K_I .

3.6. During a test of kinetics of an enzyme-catalyzed reaction, the following data were recorded:

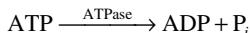
E_0 (g/l)	T (°C)	I (mmol/ml)	S (mmol/ml)	V (mmol/ml-min)
1.6	30	0	0.1	2.63
1.6	30	0	0.033	1.92
1.6	30	0	0.02	1.47
1.6	30	0	0.01	0.96
1.6	30	0	0.005	0.56
1.6	49.6	0	0.1	5.13
1.6	49.6	0	0.033	3.70
1.6	49.6	0	0.01	1.89
1.6	49.6	0	0.0067	1.43
1.6	49.6	0	0.005	1.11
0.92	30	0	0.1	1.64
0.92	30	0	0.02	0.90
0.92	30	0	0.01	0.58
0.92	30	0.6	0.1	1.33
0.92	30	0.6	0.033	0.80
0.92	30	0.6	0.02	0.57

a. Determine the Michaelis–Menten constant for the reaction with no inhibitor present at 30°C and at 49.6°C.

b. Determine the maximum velocity of the uninhibited reaction at 30°C and an enzyme concentration of 1.6 g/l.

c. Determine the K_I for the inhibitor at 30°C and decide what type of inhibitor is being used.

3.7. An enzyme ATPase has a molecular weight of 5×10^4 daltons, a K_M value of $10^{-4} M$, and a k_2 value of $k_2 = 10^4$ molecules ATP/min molecule enzyme at 37°C. The reaction catalyzed is the following:



which can also be represented as



where S is ATP. The enzyme at this temperature is unstable. The enzyme inactivation kinetics are first order:

$$E = E_0 e^{-k_d t}$$

where E_0 is the initial enzyme concentration and $k_d = 0.1 \text{ min}^{-1}$. In an experiment with a partially pure enzyme preparation, 10 µg of total crude protein (containing enzyme) is added to a 1 ml reaction mixture containing 0.02 M ATP and incubated at 37°C. After 12 hours the reaction ends (i.e., $t \rightarrow \infty$) and the inorganic phosphate (P_i) concentration is found to be 0.002 M, which was initially zero. What fraction of the crude protein preparation was the enzyme? Hint: Since $[S] \gg K_m$, the reaction rate can be represented by

$$\frac{d(\text{P})}{dt} = k_2 [\text{E}]$$