Enzyme Problem Set 2

A) The following initial rate data were obtained by adding 1.0 μg of an enzyme having molecular weight of 57,400 Daltons into 100 mL of water with the indicated concentrations of substrate alone, or substrate with one of two inhibitors, A or B. (ref. Blanch and Clark, Biochemical Engineering, p.91).

S_0	Initial Reaction Rates (v_0) (nmol/min)		
(mmol/L)	No inhibitor	A (at 5 mM)	B (at 25 mM)
0.20	8.34	3.15	5.32
0.33	12.48	5.06	6.26
0.50	16.67	7.12	7.07
1.00	25.00	13.30	8.56
2.50	36.20	26.20	9.45
4.00	40.00	28.90	9.60
5.00	42.60	31.80	9.75

i) Calculate the value of K_M and V_{MAX} for this enzyme. Note: the initial reaction rates have units of "nmol/min" (without a volume), and note that the quantity of enzyme is indicated (not the concentration).

ii) What is the value of k_{cat} or the *turnover number* (having units of s^{-1})? It is the maximum number of substrate molecules that can be converted to product per unit time per active site on the enzyme.

iii) Find the type of inhibitor and inhibition constant K_I for inhibitor A.

iv) Find the type of inhibitor and inhibition constant K_I for inhibitor B.

B) The following initial rate data were obtained using a 3.5 mg/L enzyme solution. The enzyme is a dimer having a total molecular weight of 114,500 Daltons. The rate data were obtained using the substrate alone and an inhibitor, A, at a concentration of 10 mM.

S_0	Initial Reaction Rates (v_0) (mmol/Lmin)	
(mmol/L)	No inhibitor	A (at 10 mM)
1.0	6.6	2.6
2.0	11.2	5.0
4.0	17.1	8.9
8.0	25.1	14.5
15.0	30.4	20.7

i) Calculate the value of K_M and V_{MAX} for this enzyme.
ii) What is the value of k_{cat} or the *turnover number* (having units of s^{-1})?
iii) Find the type of inhibitor and inhibition constant K_I for inhibitor A.