University of Canterbury

Mid Year Examination and Test Period 2009

Prescription Number(s):	ENCH 323
Paper Title:	Special Topic

Time Allowed:	1.5 HOURS
Number of pages:	SIX

Answer **THREE** questions out of SIX.

All questions are of equal value.

TURN OVER

Question 1

The reciprocal of the Michaelis-Menten equation is:

$$\frac{1}{v} = \left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{[S]}\right) + \frac{1}{V_{max}}$$

(a) Explain how the above form of the equation, in conjunction with data from an experiment in which an enzyme is incubated with its substrate, S, and [S] is measured at timed intervals, can be used to determine K_m and V_{max}. (*8 marks*)

(b) Outline the importance of K_m and V_{max}. (*4 marks*)

(c) What is k_{cat} and why is it useful? (*4 marks*)

(d) Show how the catalytic efficiency of an enzyme can be calculated from k_{cat} and K_m and outline its importance. (*4 marks*)

Question 2

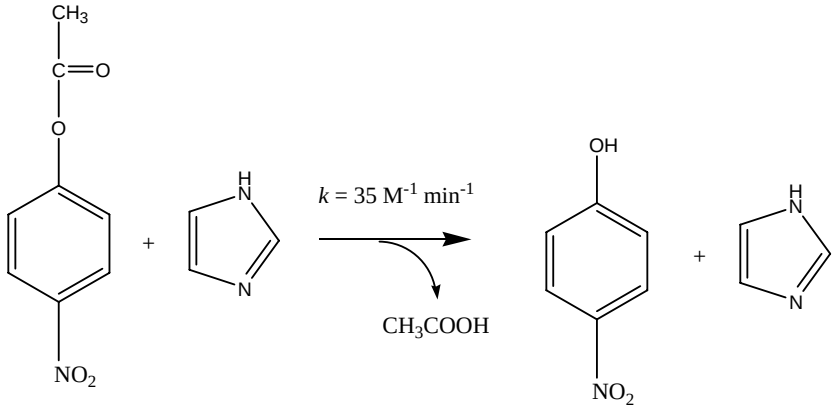
(a) Discuss the differences in inhibitor binding to an enzyme in competitive, non-competitive and uncompetitive inhibition. (*8 marks*)

(b) Using the Lineweaver-Burke plot show how it is possible to distinguish between the three types of enzyme inhibition experimentally. (*8 marks*)

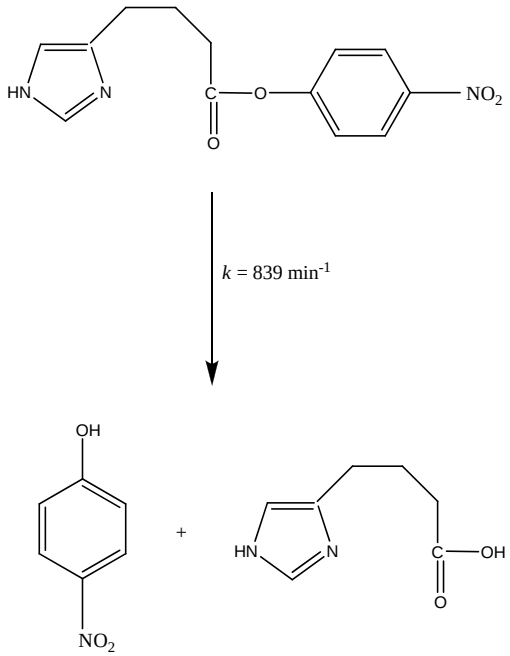
(c) Outline an important commercial use of an enzyme inhibitor. (*4 marks*)

Question 3

p-Nitrophenyl acetate is used by enzymologists to study the mechanisms of proteases. It is hydrolysed to *p*-nitrophenol in the presence of imidazole:



The hydrolysis of an imidazole analogue of *p*-nitrophenyl acetate, shown below, also forms *p*-nitrophenol:



(a) Why is *p*-nitrophenyl acetate a useful substance to study enzyme mechanisms of proteolysis? (*3 marks*)

(b) Explain the significance of the difference between the two *k* values for the hydrolysis reactions of the two *p*-nitrophenyl esters. (*8 marks*)

(c) Discuss how studying *in vitro* hydrolysis reactions of this kind has helped our understanding of protease mechanisms. (*9 marks*)

Question 4

(a) Explain why the conformational arrangement of amino acid residues in the active site of an enzyme is crucial to the mechanism of catalysis. (*8 marks*)

(b) Outline the mechanism of catalysis of a named enzyme example. (*8 marks*)

(c) Why is the stability of $EX^{\ddagger}$ (transition state complex) important in catalysis? (*4 marks*)

Question 5

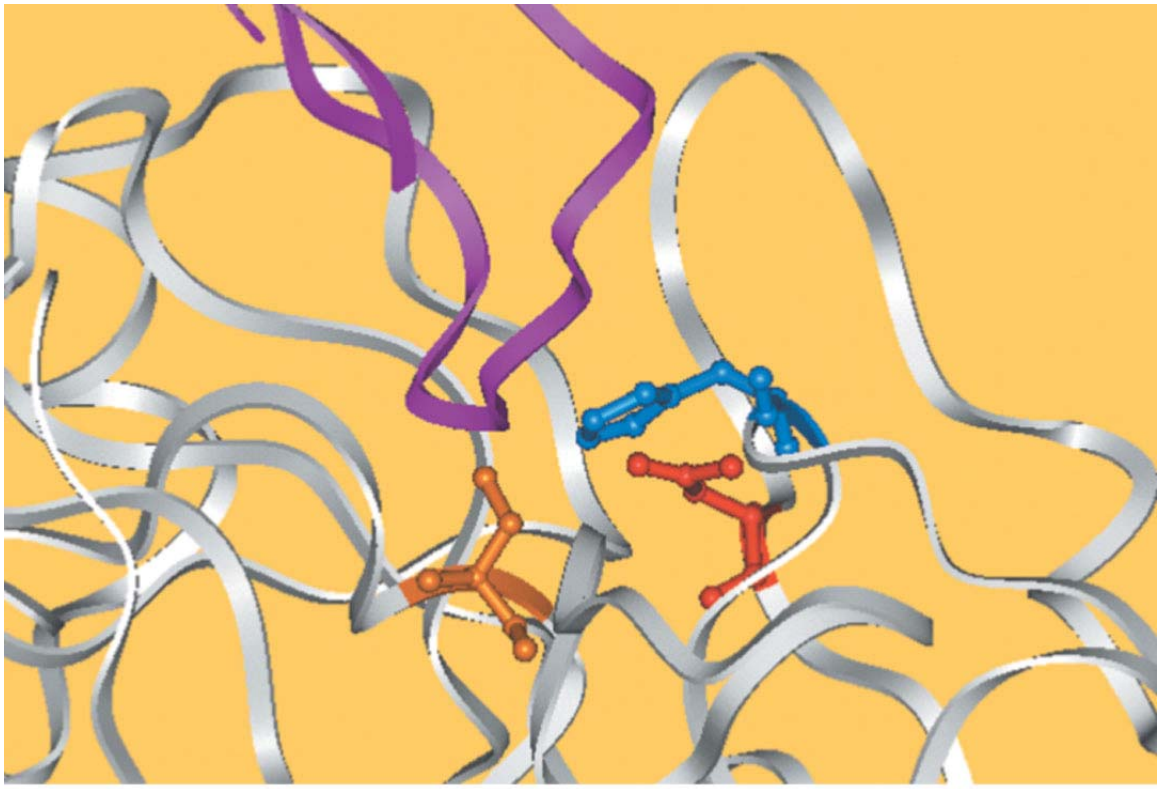
(a) Explain co-operativity in relation to substrate binding to an enzyme. (*5 marks*)

(b) If rate, *v*, is plotted against [S] for an allosteric enzyme what shape is the graph? Explain the importance of the above relationship between [S] and *v* in enzyme regulation. (*8 marks*)

(c) Using an enzyme example show how allosteric effectors enhance the efficiency of cellular metabolism. (*7 marks*)

Question 6

The diagram below shows the conformational structure of the active site of chymotrypsin. Three important amino acid residues are shown: Blue = His; Red = Asp; and Orange = Ser. A section of the protein substrate is shown in purple.



(a) Explain the roles of the three identified amino acids in the mechanism of chymotrypsin catalysis. (*7 marks*)

(b) Discuss the importance of chymotrypsinogen in the regulation of chymotrypsin activity. (*7 marks*)

(c) Explain why cysteine residues are crucial for chymotrypsin activity. (*4 marks*)

(d) Why is diisopropylfluorophosphate a potent inhibitor of serine proteases? (*2 marks*)

END OF PAPER