

Effect of pH on enzymatic activity

Exercise 1

The hydrolysis of acetyl-phenylalanine-glycine p-nitroacetanilide (a synthetic substrate) by papain was studied under conditions where ($S \ll K_m$), allowing for the determination of the k_{cat}/K_m ratio. Experiments were conducted at different pH levels to determine the pK values of the groups that modify the enzyme's activity.

pH	$k_{cat}/K_m \text{ M}^{-1} \cdot \text{s}^{-1}$
3,7	300
3,9	430
4,2	660
4,6	1020
5,1	1330
5,5	1400
6,0	1450
7,2	1300
7,7	1100
8,0	900
8,2	770
8,4	590
8,8	350
9	220
9,3	170

Determine the pK values of the groups that modify the enzyme's activity when their protonation state changes.

Solution : $pK_1 = 4,2$
 $pK_2 = 8,2$

Exercise 2

The effect of pH on the behavior of an enzyme can be described by the model discussed in class. V_m (expressed in $10^{-4} \text{ M} \cdot \text{min}^{-1}$) and K_m (expressed in mM) were measured at different pH levels, with an enzyme concentration of $1 \mu\text{M}$. The results are presented in the following table:

pH	Vm	Km
3	0,057	1
4	0,537	1,07
5	3,21	1,45
6	6,4	1,89
7	7,05	1,99
8	6,55	2
9	3,6	2
10	0,655	2
11	0,07	2

- 1) Determine pK_1 , pK_2 , pK'_1 and pK'_2
- 2) Calculate k_{cat} (en s^{-1}).

Solution : 1) $pK_a = 9$; $pK_b = 4,8$
 $pK'_a = 9$; $pK'_b = 5,1$
le pH optimal est 7
2) $k_s = 12 s^{-1}$