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EFFECT OF pH ON INHIBITION OF JACK BEAN UREASE

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Abstract:
The effect of pH on the inhibition of Jack Bean urease catalyzed hydrolysis of urea by chlorobenzoic acids has been carried out on the basis of kinetics of urea hydrolysis. The concentration of urea was varied from 1.00 x 10^{-2} M to 2.65 x 10^{-2} M and that of inhibitors ware maintained 2.5 x 10^{-3} M. The Michaelis constant, K_m, apperent Michaelis constant, K_{mapp} and dissociation constant of enzyme-inhibition complex, K_i were determined at pH 6.80 and temperature 37.0^{0}C. The inhibition was studied at pH 6.80 to 6.20. The percentage inhibition of each inhibitor were calculated, percentage inhibition increases with decrease in pH.
Keywords : pH, inhibition of urease, competitive inhibition, urea

Introduction:

Benzoic acids are used as a preservative in many food materials. Hence the possibility of these compounds as inhibitors of Jack bean urease has been investigated. Most of the enzymes have their optimum pH between 5 to 9. As the pH changed, the enzyme catalyzed reaction rate also changes. The types and magnitude of pH effect differs from enzyme to enzyme. Laidler and Hoare[1] have studied the inhibition of urea hydrolysis by its product[2] at pH 6.10. Wall and Laidler[3] reported pH 8.0 as maximum pH for urease catalyzed hydrolysis of urea. The pH dependency of urease inhibition was obsereved by Bannett and Janet[4]. The effect of acetohydroxamic acid on rumen urease was studied by Makkar et.al[5] and they observed that inhibition was maximum at pH 8 to 10. Barth and Michel[6] have studied the hydrolysis of urea by urease in the pH range 4-9 and showed the dependence of reaction rate on pH. Zaborska[7] investigated the effect of buffer on kinetic behaviour of urease in the pH range 5.8-8.1, according to him phosphate buffer shows competitive type of inhibition. Furthermore, another study performed in buffer-free solution K_m was found to be practically independent of pH[8] still different results were obtained for immobilized [9-11] or gel- entrapped[12] urease.
The aim of this study was to investigate the effect of Chlorobenzoic acids on the urease catalyzed hydrolysis of urea over the pH range 6.80-6.20 and determine the kinetic quantities.

Material and Methods:

All chemicals used in this study were of analytical grade. Water, double distilled in glass, was used for preparing all the solutions. Sodium dihydrogen phosphate-disodium hydrogen phosphate buffer system was used to maintain the different pH of the reaction system. The temperature of the reaction system was maintained at 37.0^{0}C.

The enzyme urease was obtained from plant source i.e. from Jack bean seeds by the method of Sumner [13]. The urea solution of 2.65 x 10^{-2} M containing 4.00 x 10^{-8} M Jack bean urease was prepared in buffer system and maintained in thermostat. The urease catalyzed hydrolysis of urea were carried out in presence of 2.50 x 10^{-3} M each of cholorobenzoic acid as a inhibitor. The study was carried out at pH 6.80, 6.40 and 6.20.

The process of hydrolysis was followed by analysis of aliquotes of reaction system for the ammonia generated. The analysis of reaction product was carried out spectophotometrically by means of ammonia-indophenol complex[14] at 580 nm. The initial rate,vwere evaluated at pH 6.80, 6.40 and 6.20. From result 1/v was plotted versus 1/[urea] and Michaelis constant, K_m and maximum velocity, v_{max} were determined. From initial rate measurement, the percentage inhibition were calculated.

Result and Discussion:

The Michaelis constant, K_m for Jack bean urease catalyzed hydrolysis of urea was found to be 6.58 x 10^{-3} M at pH 6.80 and temperature 37.0^{0}C. The rate of hydrolysis of urea decreases in the presence of cholorobenzoic acids (Table. 1). Its shows that chlorobenzoic acids inhibit the hydrolysis of urea.

The K_i values for the Chlorobenzoic acids were the dissociation constant of enzyme-inhibitor complexes. They were inversely related to the binding strength of Chlorobenzoic acids towards the enzyme Jack bean urease. From the observed K_i values, the binding strength of chlorobenzoic acids with Jack bean urease was as given below. Benzoic acid > p-chlorobenzoic acid> 2,4-dichlorobenzoic acid > o-chlorobenzoic acid.

The percentage inhibition at each pH value were calculated by

percentage inhibition = $(1 - v_I/v_0) \times 100$

Where,

v_0 = initial rate in the absence of inhibitor

v_i = initial rate in the presence of inhibitor

From the results, the increase in the percentage while changing from pH 6.80 to 6.20 for each inhibitor was also calculated (Table. 3).

It was observed from these results that by decreasing the pH even by half unit, the increase in percentage inhibition was significant. The observed trend for increase in percentage inhibition was Benzoic acid > p-chlorobenzoic acid> 2,4-dichlorobenzoic acid > o-chlorobenzoic acid.

Table. 1: Initial rate of hydrolysis of urea by Jack bean urease in presence of chlorobenzoic acids.

Temperature 37.0^{0}C pH = 6.80

[Urea] / 10^{-2} M	Without Inhibitors	Inhibitors			
		Benzoic acid	o-chloro-benzoic acid	p-chloro-benzoic acid	2,4-dichloro-benzoic acid
1.00	2.91	1.49	2.17	1.60	1.94
1.50	3.38	1.92	2.83	2.00	2.38
2.00	3.67	2.34	3.00	2.46	2.71
2.65	3.90	2.64	3.79	2.73	2.95

Table.2 : K_m, $K_{mapp}$and K_i for Jack bean urease in presence of chlorobenzoic acids.

Temperature 37.0^{0}C		pH 6.80
Inhibitors	K_{mapp} / 10^{-3} M	K_i / 10^{-3} M
-	K_m = 6.58	-
Benzoic acid	22.6	1.02
o-chlorobenzoic acid	10.8	3.90
p-chlorobenzoic acid	20.8	1.16
2,4-dichlorobenzoic acid	15.8	1.78

Table.3 : Effect of pH on inhibition of Jack bean urease in presence of chlorobenzoic acids

Benzoic acids	Initial rate v / 10^{-6} M s^{-1}			% Inhibition			Increase in % Inhibition
	pH						
	6.80	6.40	6.20	6.80	6.40	6.20	
-	3.90	3.51	2.99	-	-	-	-
Benzoic acid	2.64	2.25	1.65	32.3	35.9	44.8	12.5
o-chlorobenzoic acid	3.79	3.23	2.67	2.8	7.9	10.7	7.9
p-chlorobenzoic acid	2.73	2.30	1.75	30.0	34.5	41.5	11.5
2,4-dichlorobenzoic acid	2.95	2.50	2.00	24.4	28.9	33.0	8.6

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