p-AMINO BENZOIC ACID and p-HYDROXY BENZOIC ACID as JACKBEAN UREASE INHIBITORS

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***Abstract*- Inhibition of jackbean urease catalysed hydrolysis of urea by p-amino benzoic acid and p-hydroxy benzoic acid has been studied on the basis of kineic measurement of urea hydrolysis. The concentration of urea was varied in the range of 1.00 10-2 to2.65 10-2 and that of inhibitor was maintained at 2.5 10-3. The Michaelis constant,Km, apperent Michealis constant,Kmapp and dissociation constant of enzyme-inhibitor complex,Ki were determined at pH6.80 and temperature 37.0 0c. Both the inhibitor shows competitive type of inhibition.**

***Key words*- Inhibition,Jackbean urease, Urea hydrolysis, Competitive inhibition,p-amino benzoic acid, p-hydroxy benzoic acid.**

I. INTRODUCTION

Enzyme inhibition is an important area of medicinal research since studies in this field has already led to the discovery of variety of drugs useful in a number of diseases. Specific inhibitor interact with enzyme and block their activity towards their corresponding substrates. The importance of enzymes inhibitors as drug is enormous since thee molecules have been used for treating a number of physiological conditions[1-15]. Enzyme inhibitors mascurading as drug, antibiotics and preservatives. Benzoic acids are often used as acidic preservatives. Hence investigation of p-amino benzoic acid and p-hydroxy benzoic acid on urease catalysed hydrolysis of urea is of great importance.

Various workers have studied the inhibition of urease. Hydroxamic acid and acetohydroxamic acid as urease inhibitors was reported by Makkar et-al [17] and Acker [18]. Sahrawat [19] evaluated the effect of chelating compounds on the retardation of urea hydrolysis in the soil and noticed that citric, tartaric and oxalic acids had very little effect on the urea hydrolysis. The effect of p-hydroxy mercury benzoate on blue green algae urease was studied by Singh Surendra [20] and he reported that it was the potent inhibitor. Gould et-al [21] have studied the inhibition of urease activity by heterocyclic sulphur compounds and observed that these are the potent inhibitors of Jackbean urease. Mulvaney et-al [22] have studied the effect of p-benzoquinone and hydroquinone on the hydrolysis of urea. Zhang et-al [23] have reported the effect of hydroquinone as urease inhibitor on the fertilizer efficiency of urea , while Goos et-al [24] have shown the effect of ammonium thiosulphate on the urease catalysed hydrolysis of urea. D`Arrigo et-al [25] showed that fentine acetate competitively inhibited the urea hydrolysis by Jackbean urease and non-competitively by flurenol.

The study of inhibition of Jackbean urease catalysed hydrolysis of urea by p-amino benzoic acid and p- hydroxyl benzoic acid was still lacking behind. Therefore the present work has been undertaken to study the effect of these ompounds on the Jackbean urease catalysed hydrolysis of urea by spectrophotometric technique.

II. MATERIALS AND METHODS

All chemicals used in the present study were of analytical greade. Water doubly distilled in glass was used for preparing the various solutions. Phosphate buffer system was used to maintain pH of the reaction system at 6.80. The temperature of the reaction system was maintain at 37.0 oc.

The enzyme urease was obtained from plant source i.e. from Jackbean seed. The Jackbean seeds were purchased from local market and enzyme urease was extracted by the method of sumner [26].

The urea solution of 2.65 10-2 M containing 4.00 10-8 M urease aws prepared in the buffer solution of 6.80 pH and maintained at temperature 37.0 oc. The progess of the urea hydrolysis was followed by analysis of aliquots of the reaction system for the amount of ammonia generated. The analysis was carried out spectrophptometrically by means of ammonia-indophenol complex [27] at 580 nm. The hydrolysis was followed for about 40 minutes. The amount of urea hydrolysed was determined at various time interval and average rate of urea hydrolysis at every instant of time was

evaluated. From the average rate, the initial rate of reaction, v was determined.

From the results, reciprocal of initial rate, 1/V was plotted versus reciprocal of urea concentration, 1/[Urea]. From the plot Michaelis constant,Km and maximum velocity,Vmax were determined.

Similar experiment were carried out with 2.50 10-3 M p-amino benzoic acid and p-hydroxy benzoic acid concentration in the reaction system, keeping all other reaction condition unaltered.

III. RESULTS AND DISCUSSION

The Michaelis constant, Km and maximum velocity, Vmax for the Jackbean seed urease catalysed hydrolysis of urea was evaluated from Lineweaver-Burk [28] plot of 1/V versus 1/ [Urea]. The Michaelis constant, Km was found to be 6.58 10-3 M and maximum velocity, Vmax was 4.95 10-6 MS-1 at pH 6.80 and temperature 37.0 oc (Table.1 and Fig. 1). The rate of hydrolysis of urea by jackbean urease decreases in the presence of p-amino benzoic acid and p-hydroxy benzoic acid (Table.2). It shows that both compound inhibited the urease catalysed hydrolysis of urea and thereby acts as an inhibitor.

Both the compounds were tested for competitive, non-competitive and un-competitive type of inhibition by plotting the Lineweaver-Burk plot.

TABLE I
EVALUATION OF MICHAELI'S CONSTANT FOR UREASE CATALYZED HYDROLYSIS OF UREA

Urease concentration	4.00 x 10^{-8}		
Temperature	37.0°C		
pH	6.80		
Urea concentration/ 10^{-2} M	Initial rate, v/ 10^{-6} Ms^{-1}	M/[Urea]	$10^4 Ms^{-1}/v$
2.65	3.90	37.7	25.6
2.00	3.67	50.0	27.2
1.50	3.38	66.7	29.6
1.00	2.91	100.0	34.4
Michaeli's constant, K_m = 6.58x10^{-3} M			

TABLE II
INITIAL RATE OF HYDROLYSIS OF UREA BY UREASE IN PRESENCE OF P-AMINOBENZOIC ACID AND P-HYDROXYBENZOIC ACID

Temperature	37.0°C			
pH	6.8			
Urease	4.00 x 10-8M			
Inhibitor	2.50x10-3M			
Inhibitor	Intial rate, v/$10^{-6}Ms^{-1}$			
	Urea concentration/ 10^{-2}M			
	2.65	2.00	1.50	1.00
----------	3.90	3.67	3.38	2.91
p-aminobenzoic acid	2.85	2.51	2.13	1.67
p-hydroxybenzoic acid	3.28	2.88	2.63	2.13

TABLE III
DECREASE IN INITIAL RATE OF HYDROLYSIS OF UREA IN PRESENCE OF P-AMINO BENZOIC ACID AT VARIOUS UREA CONCENTRATION

Urease concentration		4.00 x 10^{-8}M	
p-aminobenzoic acid concentration		2.50x 10^{-3}M	
Temperature		37.0°C	
pH		6.80	
Urea concentration/ 10^{-2} M	Initial rate, v/ 10^{-6} Ms^{-1}	M/[Urea]	$10^4 Ms^{-1}/v$
2.65	2.85	37.7	35.1
2.00	2.51	50.0	39.8
1.50	2.13	66.7	47.0
1.00	1.67	100.0	60.0
Apparent Michaeli's constant, K_{mapp} = 19.8x10^{-3} M			

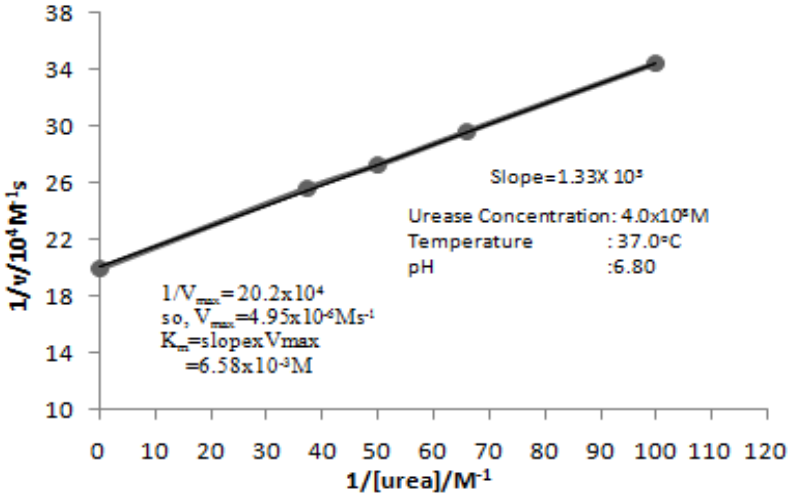

Fig. 1. Linewaver-Burk plot for urease catalyzed hydrolysis of Urea

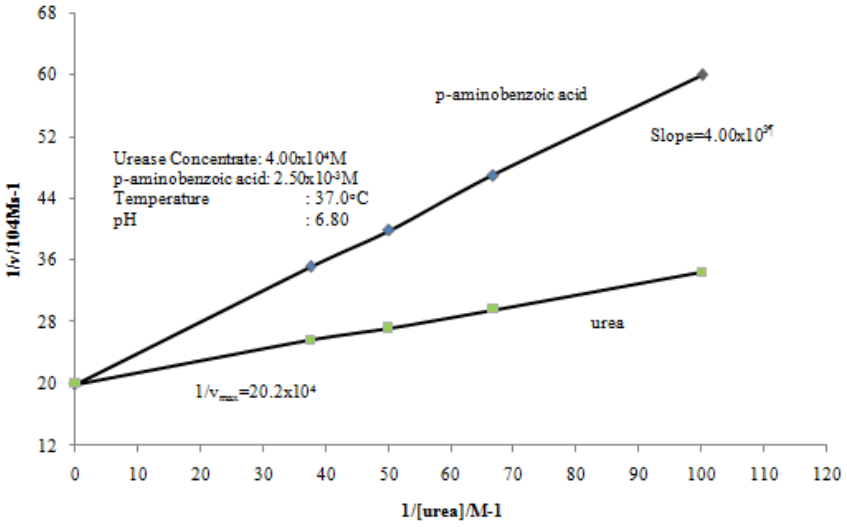

Fig 2: Lineweaver-Burk plot for urease catakyzed hydrolysis of urea inhibited by p-aminobenzoic acid

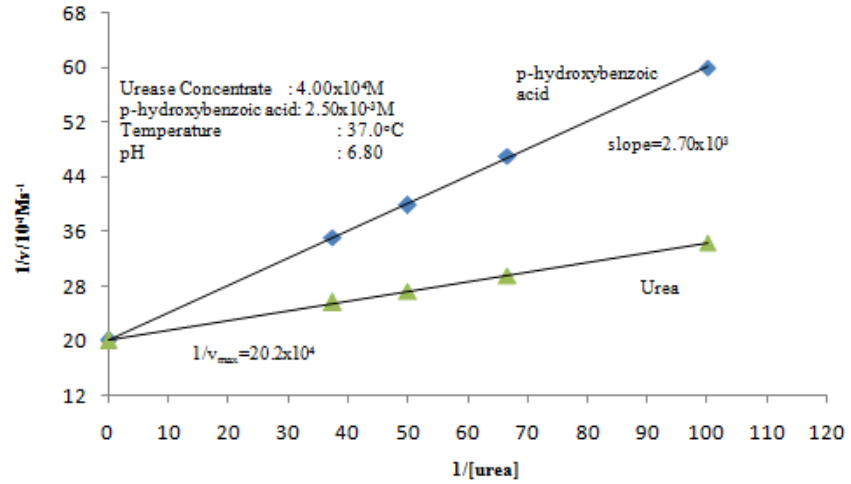

Fig. 3: Lineweaver-Burk plot for urease catalyzed hydrolysis of urea inhibied by p-hydroxyybenzoic acid

The Lineweaver-Burk plot for both the compound showed that the maximum velocity, Vmax remains constant while the Michaelis constant, Km changes to apperent Michaelis contant, Kmapp. Therefore both the compounds were obviously the cases of competitive inhibition (Table.3-4 and Fig.2-3). It confirms that p-amino benzoic acid and p-hydroxy benzoic acid are the competitive inhibitors of the Jackbean seed urease catalysed hydrolysis of urea.

TABLE IV
DECREASE IN INITIAL RATE OF HYDROLYSIS OF UREA IN PRESENCE OF P-HYDROXY BENZOIC ACID AT VARIOUS UREA CONCENTRATION

Urease concentration	4.00 x 10^{-8}M
p-hydrobenzoic acid concentration	2.50x 10^{-3}M
Temperature	37.0°C

pH	6.80		
Urea concentration/ 10^{-2} M	Initial rate, v/ 10^{-6} Ms^{-1}	M/[Urea]	$10^4Ms^{-1}/v$
2.65	3.28	37.7	30.5
2.00	2.88	50.0	34.0
1.50	2.63	66.7	38.0
1.00	2.13	100.0	47.0
Apparent Michaeli's constant, K_{mapp} = 13.4x10^{-3} M			

In the present case, the urea, p-amino benzoic acid and p-hydroxy benzoic acid competes for the same active sie of the enzyme urease and forms a complex with the urease. The complexes, urease-p-amino benzoic acid and urease-p-hydroxy benzoic acid does not react with urea to give product.

From the Michaelis constant,Km, apperent Michaelis constant,Kmapp and concentration of the inhibitor compounds, the corresponding dissociation constants, Ki of urease-p-amino benzoic acid and urease-p-hydroxy benzoic acid complexes were calculated using the equation,

$$Kmapp = Km [1 + [I] / Ki]$$

The Ki values shows the binding strength of the p-amino benzoic acid and p-hydroxy benzoic acid towards the Jackbean urease. The result shows that the binding strength of p-amino benzoic acid towards Jackbean urease was more than the binding strength of p-hydroxy benzoic acid (Table.5).

TABLE V
APPARENT MICHAELI'S CONSTANT AND ENZYME-INHIBITOR CONSTANT FOR UREASE CATALYZED HYDROLYSIS OF UREA IN PRESENCE AND ABSENCE OF P-AMINOBENZOIC ACID AND P-HYDROXYBENZOIC ACID.

Temperature	37.0°C	
pH	6.80	
Urease	4.00×10^{-8}M	
Inhibitor	2.50×10^{-3}M	
Inhibitor	$K_{mapp}/10^{-3}$M	Dissociation constant of enzyme-inhibitor complex, $K_i/10^{-3}$M
———	$K_m = 6.58$	———
p-amino benzoic acid	19.8	1.24
p-hydroxy benzoic acid	13.4	2.41

ACKNOWLEDGEMENT

All the authors thank to Director, Institute of Science, Nagpur, for the necessary facilities to carry out the present work.

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