ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

p-NITRO BENZOIC ACID AND p-CHLORO BENZOIC ACID AS JACK BEAN UREASE INHIBITOR

Yogesh Thawari

Department of Chemistry, N. H. College, Bramhapuri

**Corresponding author: thawariyogesh@gmail.com*

ABSTRACT

Enzyme inhibition is an important area of medicinal research. Enzyme inhibitor possess a wide application in agriculture, clinical science and used as drug, antibiotics and preservatives. Benzoic acid is used as preservative in many food materials. Hence inhibition of Jack bean urease catalysed hydrolysis of urea by p-nitro benzoic acid and p-chloro benzoic acid has been studied on the basis of kinetic study of hydrolysis of urea. During the experimental study the concentration of substrate was varied in the range of 1.00×10^{-2} to 2.65×10^{-2} and that of inhibitor was maintained at 2.5×10^{-3}. The results of the inhibition effect are quantitatively expressed in terms of Apparent Michaelis constants K_{mapp} and enzyme-inhibitor constant, K_i at pH 6.80 and temperature 37.0°C. Both the inhibitor shows competitive type of inhibition where both substrate and inhibitor compete with each other to react with active site of enzyme.

Keywords: Enzyme, Inhibition, Jack bean urease, Urea hydrolysis, Michaelis constant, Competitive inhibition.

1. INTRODUCTION

Enzyme acts as a catalyst for biochemical reaction and greatly enhances the rate of specific biochemical reactions. Urease is a highly competent biocatalyst for the hydrolysis of urea [1, 2]. It is absolutely definite enzyme for the hydrolysis of urea [3]. Enzyme urease catalysed the hydrolysis of urea and converts it into ammonia and carbon dioxide. This enzyme occurs in bacteria, algae and many higher plant species. While the studies on the enzyme catalysed biochemical reactions are important, the possibilities of retardation of rate of such enzyme catalysed reactions are equally significant and of great importance. The inhibition of enzyme catalysed reactions has wide applications in the field such as agriculture, medicinal, pharmacology and toxicology. Benzoic acids are used as preservative in many food materials. Hence the possibility of these compounds as inhibitor for Jack bean urease has been investigated.

Many co-workers have studied the inhibition of urease. Mishra, Faig and Soechting [4] had carried out the inhibition of urease by 1,4-napthoquinone, 2-methyl-1,4-napthoquinone, 2,3-dichlorohydroquinone, 4,6 diter-butylpyrocatachol and 4-tertiary Butyl pyrocatachol. They investigated that coating of urea with these chemicals was more effective than direct application to soil. Du et al. [5] has studied the inhibition of jack bean urease by Hg^{2+} and reported that it show non-competitive type of inhibition. Yang Hou and co-workers [6] studied the inhibition action of acetyl-hydroxamate oxovanadium complex and found that it has strong inhibitory activity. Du et al. [7] investigate the inhibitory action of boric acid and found that it shows competitive type of inhibition. Kobashi and co-workers [8] studied the specific inhibition of urease by N-acylphosphoric triamides. They investigated that benzoyl and isopentenoyl phosphoric triamides strongly inhibited the Jack bean, soyabean and watermelon seed urease. The effect of p-hydroxy mercury benzoate on blue green algae urease was studied by Singh Surendra [9] and he reported that it was the potent inhibitor. Zhang et-al [10] has reported the effect of hydroquinone as urease inhibitor and its application in the fertilizer efficiency of urea. Goos et-al [11] has shown the inhibitory effect of ammonium thiosulphate on the urease catalysed hydrolysis of urea. Gould et al [12] have carried out the study of inhibition of urease activity by heterocyclic sulphur compounds and observed that 5-mercapto-3-phenyl-1,3,4-thiadiazole-2-thione and 5-amino-1,3,4-thiadizole-2-thiol were strong inhibitors of jack bean urease. While Ackers [13] studied the effect of primary hydroxamic acid on Jack bean urease and concluded that it was an inhibitor of urease. Hydroxamic acid and acetohydroxamic acid as urease inhibitors was reported by Makkar et-al [14]. Sahrawat [15] 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 slight effect on the urea hydrolysis. Mulvaney and Bremer [16] studied the effect of p-benzoquinone and hydroquinone on hydrolysis of urea in soil. They observed that the effect of these compounds increased markedly with amount of p-benzoquinone and hydroquinone added and decreased markedly with time and with increase in temperature from 10 to 40°C.

Study of substituted benzoic acid as a inhibitor for enzyme urease is still an area of interest. Therefore, the current work has been carried out to study the effect of this substituted benzoic acid on the Jack bean urease catalysed hydrolysis of urea.

2. MATERIAL AND METHODS

All the chemicals used like urea, sodium phosphate, sodium hypochlorite were of analytical grade. Fresh solutions were prepared for each set of experiments. Redistilled and ion free water was used for the solutions preparation. Phosphate buffer were used to maintain pH of the reaction mixture. Enzyme Urease was extracted from jack bean seed purchased from local market by the reported method of sumner [17]. The urea solution of $2.65x10^{-2}$ M containing $4.00x10^{-8}$ M urease was prepared in the buffer solution of 6.80 pH and maintained at temperature 37.0°C. The progress of the enzyme reaction were analysed by measuring the ammonia generated during the course of reaction. The analysis was carried out spectrophotometrically by means of ammonia-indophenol complex [18] at 580 nm. Form the absorbance value concentration of urea hydrolysed and average rate of reaction was estimated. Similar set of experiments were carried out at various urea concentrations ranging from 1.00×10^{-2} M to 2.65×10^{-2} M that of urease concentration was maintained at $4.00x10^{-8}$ M. Initial rate of the reaction was determined by plotting average rate Vs time. Maximum velocity, V_{max} and Michaelis constant, K_m were determined by plotting graph between reciprocal of initial rate, 1/V versus reciprocal of urea concentration, 1/[Urea].

Similar experiment were carried out with 2.50×10^{-3} M p-nitro benzoic acid and p-chloro benzoic acid concentration in the reaction system, keeping all other reaction conditions unaltered.

3. RESULTS AND DISCUSSION

In the present investigation the Michaelis constant (K_m) for the urease catalysed hydrolysis of urea was found to be 6.58×10^{-3}M at temperature 37.0^0C, pH 6.80 and urease concentration of 4.00×10^{-8}M (Table 1 and Fig.1). In presence of added substituted benzoic acids initial rate of the reaction decreases (Table 2).

Table 1: Evaluation of Michaelis constant (K_m) for Urea hydrolysis catalysed by enzyme.

Urease concentration		4.00×10^{-8}M	
Temperature		37.0°C	
pH		6.80	
Urea concentration $/10^{-2}$M	Initial rate, ν $/10^{-6}$Ms^{-1}	M/[Urea]	10^4 Ms^{-1}/ν
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
Michaelis constant, K_m =		**6.58×10^{-3}M**	

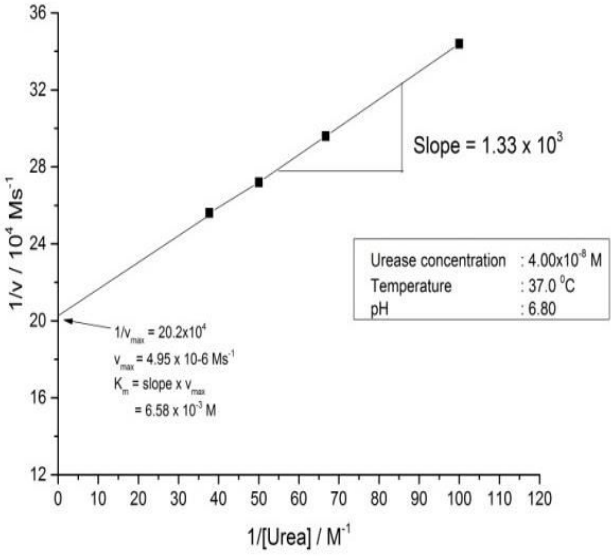

Fig. 1: Lineweaver-Burk plot for hydrolysis of urea catalysed by enzyme urease

Table 2: Initial rate of hydrolysis of urea by urease in presence of p-nitrobenzoic acid and p-chlorobenzoic acid

Temperature	37.0°C			
pH	6.80			
Urease	4.00×10^{-8} M			
Inhibitor	2.50×10^{-3} M			
Inhibitor	Initial rate v/10^{-6} Ms^{-1}			
	Urea Concentration/10^{-2} M			
Initial rate without inhibitor	3.90	3.67	3.38	2.91
With p-Nitro benzoic acid	2.79	2.45	2.00	1.56
With p-Chloro benzoic acid	2.73	2.46	2.02	1.60

It is observed that substituted benzoic acids do have an inhibitory effect on the hydrolysis of urea. What was more significant observation in Lineweaver-Burk [19] plot however was that these plots had the same intercept on the y-axis. It indicates that in both the cases the v_{max} was the same but K_m values changes to Apparent Michaelis constant, K_{mapp}. Therefore obviously the inhibition by p-nitro benzoic acids and p-chloro benzoic acid were a case of competitive type of inhibition where both substrate and inhibitor compete for same active site of enzyme (Tables 3-4 and Figs. 2-3).

Table 3: Decrease in initial rate of hydrolysis of urea in presence of p-nitro benzoic acid at various urea concentrations

Urease Concentration			4.00×10^{-8} M
p-Nitro benzoic acid concentration			2.50×10^{-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	2.79	37.7	35.8
2.00	2.45	50.0	40.8
1.50	2.02	66.7	49.5
1.00	1.56	100.0	64.0
Apparent Michaelis constant, K_{mapp} = **21.8×10^{-3}M**			

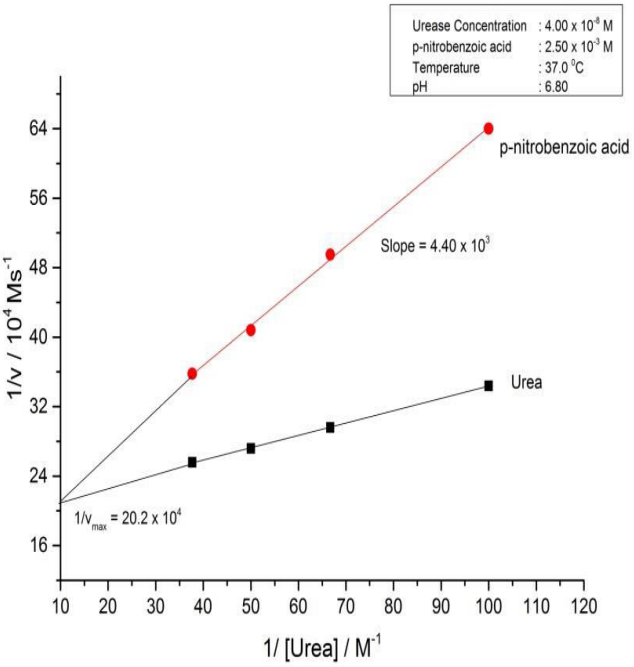

Fig. 2: Lineweaver-Burk plot for urease catalysed hydrolysis of urea inhibited by p-nitro benzoic acid

Table 4: Decrease in initial rate in presence of p-chloro benzoic acid at various urea concentrations.

Urease Concentration			4.00×10^{-8} M
p-Chloro benzoic acid concentration			2.50×10^{-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	2.73	37.7	36.6
2.00	2.46	50.0	40.6
1.50	2.00	66.7	48.0
1.00	1.60	100.0	62.5
Apparent Michaelis constant, K_{mapp} = **20.8×10^{-3}M**			

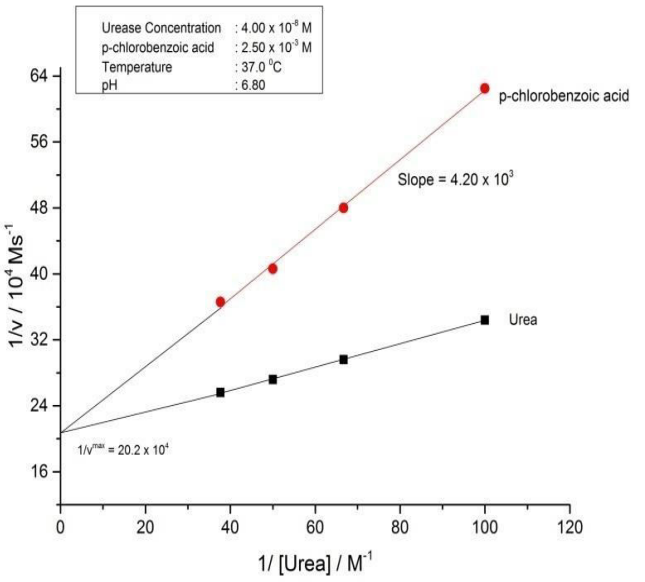

Fig. 3: Lineweaver-Burk plot for urease catalysed hydrolysis of urea inhibited by p-chlorobenzoic acid

The increase in the slope in Lineweaver-Burk plot indicates an increase in the strength of binding of the competitive inhibitor. Thus higher the K_{mapp} value means stronger binding of inhibitor to the enzyme.

From the observed values of K_{mapp} and K_m we had also calculated the K_i values of enzyme-inhibitor constant (Table 5).

The Ki values shows the binding strength of the p-nitro benzoic acid and p-chloro benzoic acid towards the Jack bean urease. Lower the K_i value higher will be the binding strength of benzoic acids towards the enzyme urease. From the results it is observed that the binding strength of p-nitrobenzoic acids towards enzyme urease is comparatively more than p-chloro benzoic acid.

Table 5: Apparent Michaelis constant (K_{mapp}) and Enzyme-inhibitor constant (K_i) for urease catalysed hydrolysis of urea in presence and absence of p-nitrobenzoic acid and p-chlorobenzoic 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 $Ki/10^{-3}$ M
Michaelis constant K_m	$K_m = 6.58$	----------
p-Nitro benzoic acid	21.8	1.08
p-Chloro benzoic acid	20.8	1.16

4. ACKNOWLEDGEMENT

Thanks to the Director, Institute of Science, Nagpur, for providing all the facilities to carry out the present work.

Conflict of interest

The author declares that there are no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

5. REFERENCES

1. Blackeley RL, Webb EC, Zerner B. et al. *Biochemistry,* 1969; **8**:1884-1890.
2. Gazzalo C, Blackeley RL, Zerner B. et al. *Can. J. Biochemistry,* 1973; **51**:1325-1330.
3. Estiu G, Merz KM. *Journal of American Chemical Society,* 2004; **126**:6932-6944.
4. Mishra MM, Flaig W, Soechting H et al. *Plant soil,* 1980; **55**:25-33.
5. Nana D, Mingming C, Zhaodi L, Liangquan S, Huajie X, Shuisheng CD et al. *Chemistry Central Journal,* 2012; **6**:154-160.
6. Yang H, Yu-Ting Y, Xiao-Shan C, Zhong-Lu Y et al. *Inorganic Chemistry Communications*, 2014; **45**:131-134.
7. Nana D, Liangquan S, Huajie X, Chongfu S, Shuisheng C et al. *Journal of Molecular Catalysis B: Enzymatic,* 2012; **82**:53– 58.
8. Kobashi K, Tekede S, Nuumata A. *J. Biochem. (Tokyo),* 1985; **98**:1881-1888.
9. Singh S., *Curr. Sci.,* 1989; **58:**443- 449.
10. Zhang Z, Lui T, Zhou L, Tuurang T. et al. *Biol Fertil Soils,* 1987; **18:**214-219.
11. Goos RJ, Fairlie TE. *Soil. Sci. Soc. Am. J.*, 1988; **15**:222-229.
12. Gould WD, Cook FD, Bular JA. et al. *Soil. Sci. Sac. Am. J.,* 1978; **42**:66-69.
13. Ackers HA. *Experientia,* 1981; **37**:229-230.
14. Makkar HPS, Sharma OP, Dawara R K, Negi SS et al. *J. Dairy Sci.,* 1981; **64**:643-651.
15. Sahrawat KL. Div. *Agric. Chem., Indian Agric. Res. Inst. New Delhi-110012, India. Plant and Soil,* 1978; **50**:521-526.
16. Mulvaney RL, Bremner JM. *Soil. Boil. Biochem.*, 1978; **10:** 287-294.
17. Sumner JB. *J. Biol. Chem.,* 1926; **69**:435-439.
18. Crother AB, Large BS. *Analyst,* 1956; **81**:64-70.
19. Lineweaver H, Burk D. *J. Am. Chem. Soc.,* 1934; **56**, 658-667.