THE COMPETITIVE INHIBITION OF THE UREASE-CATALYZED HYDROLYSIS OF UREA BY PHOSPHATE

By KENT M. HARMON* and CARL NIEMANN

(From the Gates and Crellin Laboratories of Chemistry,† California Institute of Technology, Pasadena)

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The inhibition of the urease-catalyzed hydrolysis of urea by phosphate has been noted by a number of investigators (1–3), but there appears to be no information available regarding the nature of this inhibitory action. Assuming the validity of the Michaelis-Menten equation (4), it can be shown (5, 6) that for a system containing enzyme, substrate, and inhibitor

$$\frac{1}{v} = \left(1 + \frac{i}{K_c}\right)\left(\frac{K_m}{V}\right)\left(\frac{1}{s}\right) + \left(1 + \frac{i}{K_n}\right)\left(\frac{1}{V}\right) \tag{1}$$

where

e = total enzyme concentration
s = substrate concentration
i = inhibitor "
p = concentration of enzyme-substrate complex
q = " " enzyme-inhibitor "
r = " " enzyme-substrate-inhibitor complex
v = observed rate for a given initial concentration of e, s, and i
$K_m = s(e - p - q - r)/p$
$K_c = i(e - p - q - r)/q$
$K_n = ip/r$
V = rate where $i = 0$ and $p = e$

It follows from equation (1) that when $1/v$ is plotted (usually as the ordinate) against $1/s$ a straight line will be obtained with inhibitory action influencing either the slope or the ordinate intercept or both. Thus the type of inhibition may be defined on the basis of the effect of the inhibitory action upon the slope and intercept in the above plot.

In the absence of an inhibitor ($i = 0$) equation (1) reduces to the Michaelis-Menten equation

$$\frac{1}{v} = \left(\frac{K_m}{V}\right)\left(\frac{1}{s}\right) + \left(\frac{1}{V}\right) \tag{2}$$

permitting the evaluation of V and K_m. With competitive inhibition, *i.e.*, when both substrate and inhibitor are presumably competing for the same

* National Research Council Predoctoral Fellow.

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reactive sites, we may set $K_n = \infty$, transforming equation (1) into

$$\frac{1}{v} = \left(1 + \frac{i}{K_c}\right)\left(\frac{K_m}{V}\right)\left(\frac{1}{s}\right) + \left(\frac{1}{V}\right) \tag{3}$$

From equation (3) it is clear that with competitive inhibition only the slope will be affected, being increased by the factor $(1 + (i/K_c))$ over that obtaining when $i = 0$. Other types of inhibition, *i.e.* non-competitive, "uncompetitive" (6), and "quadratic" (6), may be recognized in the order named by a proportional increase in slope and intercept ($K_c = K_n$), an increase in intercept with no change in slope ($K_c = \infty$), and by apparently unrelated changes in slope and intercept ($K_c \neq K_n$).

TABLE I

Effect of Buffer Concentration upon Kinetics of Hydrolysis of Urea by Urease

Experiment No.	Buffer	Buffer concentration	Slope, m	Intercept, b	$\frac{m}{b}$	K_c
		M				
I	Phosphate	0.030	8.1	1.27	6.4	0.038
		0.056	11.3	1.28	8.8	0.037
		0.109	22.1	1.26	17.5	
II	"	0.056	11.9	1.49	8.0	0.034
		0.109	19.4	1.53	12.7	0.033
		0.161	25.8	1.62	16.0	0.034
		0.267	52.0	1.39	37.6	
		0.380	69.1	1.71	40.5	
III	Maleate	0.16	7.8	1.70	4.6	
		0.32	7.8	1.80	4.3	
		0.53	7.8	2.30	3.4	
	Glycine	0.16	6.8	1.45	4.6	
		0.32	7.0	1.54	4.6	
		0.53	7.2	1.68	4.3	

In the urease-catalyzed hydrolysis of urea in the presence of phosphate it has been observed (Table I, Fig. 1) that the ordinate intercept of the $1/v$ *versus* $1/s$ plot remains essentially unchanged with increasing phosphate concentration, whereas the slope increases markedly, the increase being approximately linear for the lesser phosphate concentrations. Upon extrapolation to zero phosphate concentration a value of 4.5 was obtained for the slope (m), and from the slope-intercept ratio at zero phosphate concentration a value of 0.003 M urea was obtained for the Michaelis constant (K_m) of the urea-urease system at pH 7.0 and 25°.

For a case of competitive inhibition one may obtain from equation (3) the relation

$$m = \left(1 + \frac{i}{K_c}\right)\left(\frac{K_m}{V}\right) \tag{4}$$

and, using the extrapolated value of $m = K_m/V = 4.5$, K_c may be calculated from the relation

$$K_c = \frac{4.5i}{m - 4.5} \tag{5}$$

where i = micromoles of inhibitor per ml. For values of i varying from 30 to 160 micromoles of phosphate per ml. an average value of $K_c = 0.035$ M phosphate at pH 7.0 and 25° was obtained (Table I). That the observed

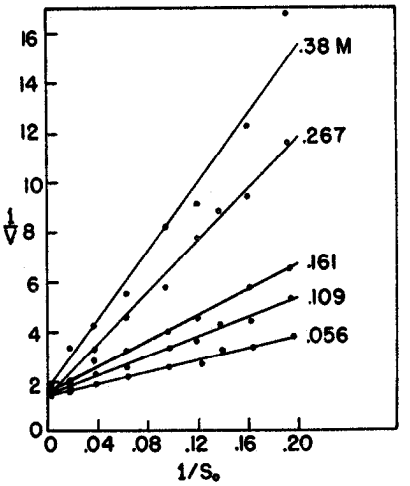

FIG. 1. Effect of phosphate buffer upon the hydrolysis of urea by urease. $1/v$ in (micromoles of ammonia)$^{-1}$ per ml. per minute, $1/s_0$ in (micromoles of urea)$^{-1}$ per ml.

inhibition by phosphate is not simply an effect of ionic strength is shown by the markedly different behavior observed with maleic acid and glycine-carbonate buffers adjusted to pH 7.0 (Table I, Fig. 2). Increasing the maleic acid concentration from 0.16 to 0.53 M caused no change in slope and only a relatively small increase in intercept. The increase in slope and intercept noted with increasing concentration of the glycine-carbonate buffer may or may not be significant, since the observed variations are within the limits of experimental error.

It is noteworthy that with the maleic acid and glycine-carbonate buffers a slope-intercept ratio is obtained which is in good agreement with the ratio of 4.5 obtained from the phosphate data upon extrapolation to zero phosphate concentration, and one may conclude that the true Michaelis constant of the urea-urease system at pH 7.0 and 25° is approximately 0.003 M urea.

It is clear that the higher values for K_m reported previously (7) are a consequence of the hitherto unrecognized competitive inhibitory action of phosphate in the urease-catalyzed hydrolysis of urea. On the basis of computed values for K_m and K_c it appears that at pH 7.0 and 25° the inhibitory quotient of phosphate in the urea-urease system (K_c/K_m) is approximately 12.

Preliminary experiments with N-butylurea, N-*tert*-butylurea, and N-methylurea have indicated that these substances exert an inhibitory action in the urea-urease system, their effectiveness being in the order named. Present information does not permit definition of the nature of their in-

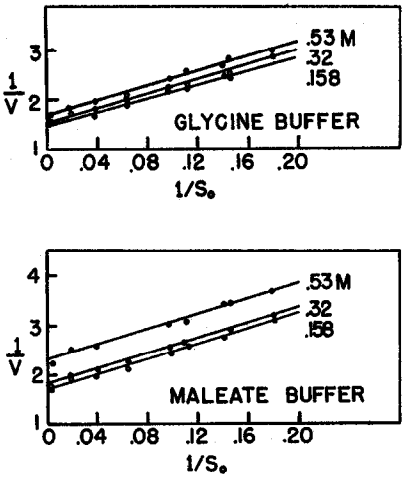

FIG. 2. Effect of maleate and glycine-carbonate buffers upon the hydrolysis of urea by urease. $1/v$ in (micromoles of ammonia)$^{-1}$ per ml. per min. $1/s_0$ in (micromoles of urea)$^{-1}$ per ml.

hibitory action, but it does appear that the action is not one of simple competition as was observed in the case of phosphate.

EXPERIMENTAL

The procedure used for the determination of urease activity and for the study of the kinetics of the hydrolysis of urea by urease at pH 7.0 and 25° has been described (7). In order to avoid complications arising from the dependence of the specific activity of urease upon the apparent absolute enzyme concentration (7) solutions of thrice recrystallized urease were prepared containing approximately 1 γ of protein N per ml., stabilized with hydrogen sulfide (7). These enzyme solutions were 0.01 M in the buffer and were allowed to stand at 25° for 5 hours before use.

The phosphate buffers were prepared from recrystallized dipotassium hydrogen phosphate and potassium dihydrogen phosphate, the maleate buffers by the addition of solid reagent grade sodium hydroxide to a solution of recrystallized maleic acid, and the glycine buffers by the addition of recrystallized sodium carbonate to a solution of recrystallized glycine. In every case, irrespective of the concentration of the buffer, the pH of the solutions was 7.0 at 25°.

A reaction time of 3 minutes was used in all of the experiments herein reported. Control experiments with maleic acid buffers in which the reaction time was varied between 2 and 5 minutes showed only the usual experimental variation. A least squares treatment, in which the data were weighed proportionally to the reaction velocities, was used in computing the slopes and intercepts of the $1/v$ *versus* $1/s$ plots.

SUMMARY

The urease-catalyzed hydrolysis of urea has been found to be competitively inhibited by phosphate at pH 7.0 and 25°. The Michaelis constant of the urea-urease system has been found to be approximately 0.003 M urea and the comparable constant defining the phosphate-urease system 0.035 M phosphate.

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