[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE CATHOLIC UNIVERSITY OF AMERICA]

The Molecular Kinetics of the Urea–Urease System. II. The Inhibition by Products[1]

BY J. P. HOARE AND K. J. LAIDLER

Introduction

It has frequently been observed that although at low urea concentrations the initial rate of the urease-catalyzed hydrolysis of urea is proportional to the first power of the urea concentration, the first-order coefficients in a given run decrease markedly with time. This matter was investigated systematically in Part I[1] of this series of papers, where it was pointed out that the drift in constants could be explained qualitatively on the basis of inhibition by products; it was shown in preliminary experiments that there is in fact inhibition by ammonium ions. In a more detailed study of this point we have now been able to show that the inhibition is of the non-competitive type, and that the inhibition by products accounts quantitatively for the drift in first-order coefficients, good constants being obtained when due account is taken of the inhibition in the rate law.

Experimental

The general experimental procedure was as previously described.[1a] Runs were made at one temperature, 20.0°, and a *p*H of 6.6, a phosphate buffer being used. Two inhibition series were run, the initial concentrations of urea being 1.994×10^{-2} mole per liter in one and 19.91×10^{-2} mole per liter in the other. Various concentrations of ammonium ions were introduced in the form of ammonium acetate, which was shown by direct test to have no appreciable influence on the *p*H. The ammonium ion concentration varied from zero to 0.917 mole per liter. Urease solutions were prepared from once-crystallized urease.[2] All water used was distilled from an all-glass apparatus. As in our previous investigation, initial rates were measured in order to avoid the effect of the ammonium ions produced in the reaction.

Results

Representative curves showing amount of reaction *vs.* time for different ammonium ion concentrations are shown in Fig. 1. Initial rates at various inhibitor concentrations are given in Tables I and II.

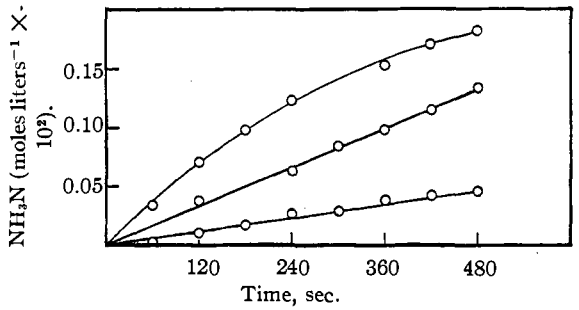

Fig. 1.—Curves showing concentration of ammonia nitrogen produced as a function of time: upper curve, no ammonium acetate; middle curve, 0.120×10^{-2} mole liter^{-1} of ammonium acetate; lower curve, 0.917×10^{-2} mole liter^{-1} of ammonium acetate.

TABLE I

INITIAL RATES AT VARIOUS AMMONIUM ION CONCENTRATIONS

Urea concentration = 1.994×10^{-2} mole liter^{-1}

Initial NH_4^+ concn. moles liter^{-1} $\times 10^2$	Initial rate, mole liter^{-1} sec.$^{-1}$ $\times 10^8$	v_0/v
0	5.35	...
0.120	3.21	1.635
.252	2.31	2.273
.434	1.47	3.571
.605	1.33	3.95
.777	1.12	4.69
.917	1.00	5.25

TABLE II

INITIAL RATES AT VARIOUS AMMONIUM ION CONCENTRATIONS

Urea concentration = 19.91×10^{-2} mole liter^{-1}

Initial NH_4^+ concn. moles liter^{-1} $\times 10^2$	Initial rate, mole liter^{-1} sec.$^{-1}$ $\times 10^8$	v_0/v
0	14.7	...
0.145	9.48	1.555
.216	6.98	2.113
.302	6.18	2.39
.358	4.92	3.00
.429	3.84	3.84
.574	3.06	4.82

Discussion

The Type of Inhibition.—Inhibition of a reaction may be either of the competitive or non-competitive type, and examples of both are known in enzyme systems.[3] In order to distinguish between the two it is necessary to have the kinetic equations for the two types. The rate law for the uninhibited reaction has been seen[1a] to be

$$v_0 = k_0 K c_e c_u/(1 + K c_u)^2 \qquad (5)$$

where c_e and c_u are the concentrations of enzyme and urea, respectively, K (equal to 7.91 liters mole^{-1} at 20°) is the equilibrium constant for complex formation, and k_0 is the rate constant for the decomposition of the complex. If the inhibition is non-competitive, the effect of the inhibitor is simply to reduce the effective enzyme concentration by the factor $1/(1 + K'I)$, where I is the inhibitor concentration and K' is a constant. The rate is thus reduced by this amount, and the rate in the presence of inhibitor is

$$v = \frac{v_0}{1 + K'I} = \frac{k_0 K c_e c_u}{(1 + K c_u)^2(1 + K'I)} \qquad (6)$$

(1) This investigation was carried out under contract N9ONR-91100 with the office of Naval Research, Biological Sciences Division, Biochemistry Branch.

(1a) K. J. Laidler and J. P. Hoare, THIS JOURNAL, **71**, 2699 (1949).

(2) J. B. Sumner, *J. Biol. Chem.*, **69**, 435 (1926).

(3) W. D. McElroy, *Quarterly Rev. Biology*, **22**, 25 (1947).

In competitive inhibition the inhibitor competes with the substrate molecules for a place on the active sites of the enzyme molecule. Reaction is assumed to occur when a urea molecule is adsorbed on site 1 and a water molecule on site 2, and it will first be supposed that the inhibitor can be adsorbed only on sites of type 1. If θ_1 is the fraction of sites 1 covered by urea, and θ_1' the fraction covered by inhibitor, then

$$\theta_1/(1 - \theta_1 - \theta_1') = Kc_u \quad (7)$$

and

$$\theta_1'/(1 - \theta_1 - \theta_1') = K'I \quad (8)$$

These equations give rise to

$$\theta_1 = Kc_u/(1 + Kc_u + K'I) \quad (9)$$

while θ_2, the fraction of sites 2 covered by urea, is similarly given by

$$\theta_2 = Kc_u/(1 + Kc_u) \quad (10)$$

The rate of reaction, equal to $k_0c_e\,\theta_1(1 - \theta_2)$, is therefore

$$v = \frac{k_0Kc_ec_u}{(1 + Kc_u + K'I)(1 + Kc_u)} = \frac{v_0(1 + Kc_u)}{1 + Kc_u + K'I} \quad (11)$$

A second possibility with competitive inhibition is that the ammonium ions are adsorbed on both sites of the urea molecule. If this is the case

$$\theta_1 = \theta_2 = Kc_u/(1 + Kc_u + K'I) \quad (12)$$

and the rate of reaction, equal to $k_0c_e\theta_1(1 - \theta_2 - \theta_2')$, is therefore

$$v = \frac{k_0Kc_ec_u}{(1 + Kc_u + K'I)^2} = \frac{v_0(1 + Kc_u)^2}{(1 + Kc_u + K'I)^2} \quad (13)$$

Three possible kinetic equations, (6), (11) and (13) have thus been developed, and the data may be tested with respect to each of them. If either (6) or (11) is obeyed, a plot of v_0/v against I should be linear. In the former case (non-competitive inhibition) the intercept on the v_0/v axis will be unity, and the slope will be K'; both slope and intercept will be independent of c_u. In the second case (competitive inhibition on sites of type 1) the intercept is again unity, but the slope is $K'/(1 + Kc_u)$ and will decrease with increasing substrate concentration. Equation (13) does not give rise to a linear plot, and the intercept on the v_0/v axis and the slope at any given value of I varies with c_u.

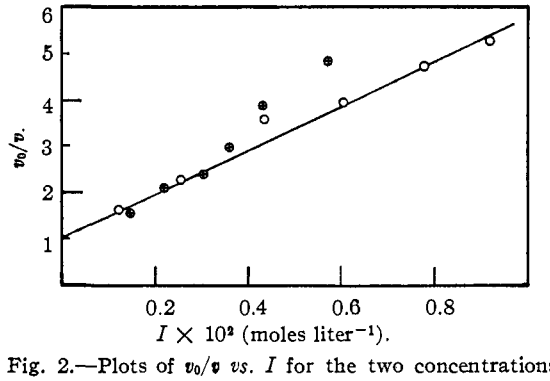

Fig. 2.—Plots of v_0/v *vs.* I for the two concentrations of substrate: open circles, $c_u = 1.994 \times 10^{-2}$ mole liter^{-1}; crossed circles, $c_u = 19.91 \times 10^{-2}$ mole liter^{-1}. The straight line is of unit intercept and slope equal to 475 liters mole^{-1}.

Figure 2 shows a plot of v_0/v *vs.* I for the two substrate concentrations used. It is seen that the low-concentration points all fall about a straight line of unit intercept. The points for the high substrate concentration initially fall about the same line, but deviate at higher inhibitor concentrations. However, this deviation is in the opposite direction from what is expected for competitive inhibition, and we conclude that the inhibition must therefore be of the non-competitive type. The deviations indicate, however, that the simple treatment leading to eq. (6) is not entirely adequate. The value of K' equal to the slope, is 475 liters mole.$^{-1}$

The Complete Rate Equation.—If the proposed inhibition law is the correct one, it should be possible to calculate satisfactory first-order rate constants for an uninhibited run. Let a be the initial concentration of urea and x that at time t; then the concentration of ammonium ion produced is $2(a - x)$. Equation (6) may therefore be written as

$$-\frac{dx}{dt} = \frac{k'x}{\{1 + 2K'(a - x)\}(1 + Kx)^2} \quad (14)$$

k' being equal to k_0Kc_e. This equation integrates to

$$k' = \frac{1}{t}\left\{(1 + 2K'a)\ln\frac{a}{x} + (2K - 2K' + 4KK'a)(a - x) + \left(\frac{K^2 - 4KK' + 2K'^2a}{2}\right)(a^2 - x^2) - \frac{2K'^2}{3}(a^3 - x^3)\right\} \quad (15)$$

In Table III are shown values of x corresponding to various intervals of time for a complete run carried out at 20° and *p*H 6.6, and in the last column are given values of k' calculated with $K = 7.91$ liters mole^{-1} and $K' = 475$ liters mole.$^{-1}$ The values of k' are seen to show no significant drift, in contrast to the ordinary first-order constants $k = (1/t)\log a/x$, shown in the third column, which show a very marked drift. It therefore, appears that inhibition by products offers an explanation of the drifting rate coeffi-

TABLE III

SPECIFIC RATE CONSTANTS USING THE INHIBITION LAW

t, sec.	x, mole liter^{-1} $\times 10^2$	k, sec.$^{-1}$ $\times 10^4$	k', sec.$^{-1}$ $\times 10^4$
0	7.478	...	...
780	6.883	1.163	1.01
900	6.801	1.051	1.07
1050	6.749	0.975	1.06
1200	6.691	.985	1.06
1350	6.645	.878	1.05
1500	6.554	.823	1.15
1800	6.444	.826	1.17

cients found in this reaction, and that the method of initial rates must be used unless it is desired to go through the somewhat elaborate treatment of this inhibition.

Summary

1. The inhibition of the urease-catalyzed hydrolysis of urea by the product ammonium ions has been investigated, by the addition of various amounts of the ions and the measurement of initial rates. The inhibition is found to be of the non-competitive type.

2. The complete rate law, taking account of the inhibition by products, has been integrated, and it is found that a satisfactorily constant first-order coefficient is obtained. The inhibition is therefore adequate to account for the drifting first-order constants that have generally been found.

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The Molecular Kinetics of the Urea–Urease System. III. Heats and Entropies of Complex Formation and Reaction[1]

BY K. J. LAIDLER AND J. P. HOARE

Introduction

In a previous paper[1a] (Part I) it was suggested that the kinetics of the urease-catalyzed hydrolysis of urea could best be interpreted on the basis of a model involving the formation of a complex consisting of the enzyme, the substrate, and a water molecule. The mechanism may be represented as

$$\text{Urea} + \text{urease} + H_2O \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \text{urea–urease–}H_2O$$

$$\text{Urea–urease–}H_2O \xrightarrow{k_1} \text{urease} + \text{products}$$

where k_1, k_{-1} and k_0 are the rate constants. The resulting rate equation is

$$v = \frac{k_0 K_1 c_s c_e}{(1 + K_1 c_s)(1 + K_2 c_s)} \qquad (1)$$

where c_s and c_e are the initial concentrations of substrate and enzyme, and K_1 and K_2 are constants. If the intermediate complex can be assumed to be in equilibrium with the reactants, *i. e.*, if $k_2 \ll k_{-1}$, K_1 is the equilibrium constant k_1/k_{-1}, while K_2 is the analogous constant for the process

$$\text{Urea–urease–}H_2O + \text{urea} \rightleftarrows \text{urea–urease–urea} + H_2O$$

It was found that the best agreement with the data was obtained with $K_1 = K_2 = K$, so that the rate equation reduces to

$$v = \frac{k_0 K c_s c_e}{(1 + K c_s)^2} \qquad (2)$$

In the present paper kinetic data at four temperatures are analyzed to give, on the basis of the above mechanism, heats and entropies of reaction corresponding to the formation of the enzyme-substrate complex, and heats and entropies of activation for the decomposition of the complex. This work was undertaken with the object of obtaining thermodynamic and pseudo-thermodynamic data which may help to throw some light on the mechanism of enzyme action.

(1) Abstracted from a dissertation submitted by J. P. Hoare in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry at the Catholic University of America, June, 1949.

(1a) K. J. Laidler and J. P. Hoare, THIS JOURNAL, **71**, 2699 (1949).

Experimental

Runs were made according to the procedure described in Parts I and II[2] at the temperatures 20, 30, 40 and 50°; at the latter temperature the rate of deactivation of the urease is measurable, but it was verified that this was not sufficiently great to affect the over-all rate of hydrolysis to a significant extent. Duplicate runs were carried out in phosphate buffers at *p*H 6.6 and 6.2. In addition to the enzyme-catalyzed reaction, the acid-catalyzed hydrolysis was investigated at the temperatures 62, 70, 85 and 100°, using 0.5 *N* hydrochloric acid.

Results and Discussion

The Acid-Catalyzed Reaction.—The first-order rate constants k for the acid-catalyzed reaction were calculated using the ordinary rate equation $k = (1/t) \ln [a/(a - x)]$, where a is the initial substrate concentration and x is that after time t. The constancy of the k's at a given temperature as shown in Table I indicates that the

TABLE I

TYPICAL FIRST-ORDER RATE CONSTANTS FOR THE ACID-CATALYZED REACTION (T = 70°)

Time, sec.	$k \times 10^5$, sec.$^{-1}$
75,300	0.144
89,700	.146
99,000	.123
184,800	.142
259,200	.133
323,100	.147

TABLE II

RATE CONSTANTS FOR THE ACID-CATALYZED REACTION

Temp., °C.	$k \times 10^7$, sec.$^{-1}$
62.0	7.38
70.0	13.9
85.0	65.2
100.0	359.0

(2) J. P. Hoare and K. J. Laidler, *ibid.*, **72**, 2487 (1950).