Hoagland, V. D., Jr., and Teller, D. C. (1969), *Biochemistry 8*, 594.
Horbett, T. A., and Teller, D. C. (1972), *Anal. Biochem. 45*, 86.
Hummel, B. C. W. (1959), *Can. J. Biochem. Physiol. 37*, 1393.
Inagami, T., and Sturtevant, J. M. (1965), *Biochemistry 4*, 1330.
Jandorf, B. J., Michel, H. O., Schaeffer, N. K., Egan, R., and Summerson, W. H. (1955), *Discuss. Faraday Soc. 20*, 134.
Jansen, E. F., Nutting, M. D. F., Jang, R., and Balls, A. K. (1950), *J. Biol. Chem. 185*, 209.
Johnson, C. H., and Knowles, J. R. (1966), *Biochem. J. 101*, 56.
Kezdy, F. J., and Bender, M. L. (1965), *Biochemistry 4*, 104.
Koshland, D. E., Strumeyer, D. H., and Ray, W. J. (1962), *Brookhaven Symp. Biol. 15*, 10.
Laskowski, M. (1955), *Methods Enzymol. 2*, 8.
Martin, R. B., and Niemann, C. (1958), *J. Amer. Chem. Soc. 80*, 1473.
McDonald, C. E., and Balls, A. K. (1957), *J. Biol. Chem. 227*, 727.
McLaren, A. D., and Finkelstein, P. (1950), *J. Amer. Chem. Soc. 72*, 5423.
Miller, D. D., Horbett, T. A., and Teller, D. C. (1971), *Biochemistry 10*, 4641.
Neet, K. E., and Brydon, S. E. (1970), *Arch. Biochem. Biophys. 136*, 223.
Oosterbaan, R. A., and von Adrichem, M. E. (1958), *Biochim. Biophys. Acta 27*, 423.
Rovery, M., Fabre, C., and Desnuelle, P. (1953), *Biochim. Biophys. Acta 10*, 481.
Sarfare, P. S., Kegeles, G., and Kwon-Rhee, S. J. (1966), *Biochemistry 5*, 1389.
Schoellmann, G., and Shaw, E. (1963), *Biochemistry 2*, 252.
Schonbaum, G. R., Zerner, B., and Bender, M. L. (1961), *J. Biol. Chem. 236*, 2930.
Schwert, G. W., and Kaufman, S. (1951), *J. Biol. Chem. 190*, 807.
Sigler, P. B., Blow, D. M., Matthews, B. W., and Henderson, R. (1968), *J. Mol. Biol. 35*, 143.
Smith, E. L., and Brown, D. M. (1952), *J. Biol. Chem. 195*, 525.
Smith, G. D., and Schachman, H. K. (1971), *Biochemistry 10*, 4576.
Sokolovsky, M., Riordan, J. F., and Vallee, B. L. (1966), *Biochemistry 5*, 3582.
Spackman, D. H., Stein, W. H., and Moore, S. (1958), *Anal. Chem. 30*, 1190.
Stacey, K. A. (1956), Light Scattering in Physical Chemistry, New York, N. Y., Academic Press, p 101.
Steitz, T. A., Henderson, R., and Blow, D. M. (1969), *J. Mol. Biol. 46*, 337.
Teller, D. C., Horbett, T. A., Richards, E. G., and Schachman, H. K. (1969), *Ann. N. Y. Acad. Sci. 164*, 66.
Vaslow, F., and Doherty, D. G. (1953), *J. Amer. Chem. Soc. 75*, 928.
Wilcox, P. E., Kraut, J., Wade, R., and Neurath, H. (1957), *Biochim. Biophys. Acta 24*, 72.
Wootton, J. F., and Hess, G. P. (1962), *J. Amer. Chem. Soc. 84*, 440.
Wright, H. T., Kraut, J., and Wilcox, P. E. (1968), *J. Mol. Biol. 37*, 363.

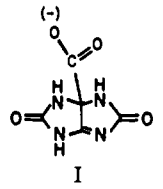
Uricase Reaction Intermediate. Mechanism of Borate and Hydroxide Ion Catalysis†

O. M. Pitts and D. G. Priest*

ABSTRACT: It has been previously shown that an intermediate is produced during the uricase reaction which absorbs strongly in the ultraviolet (uv) region where uricase is typically assayed (293 nm). A thorough knowledge of the kinetics of this intermediate is mandatory for detailed spectrophotometric study of the uricase reaction. The decay rate of this compound has been shown by others to be enhanced by borate and hydroxide ions. The present studies show that this decay rate is not influenced by changes in oxygen or uricase concentration. Kinetic studies with both borate and hydroxide ion indicate that they act catalytically by independent, parallel processes. Both catalysts show saturation behavior over the concentration range studied but only if the temperature is above 23°. Activation parameters for the separate processes are reported. An empirical rate equation has been obtained and a partial mechanism proposed for each catalyst. It is further shown that the previously reported substrate inhibition with urate was due to the accumulation of intermediate and is not an inherent property of the enzymic catalysis.

Transitory intermediates have been reported to occur during the uricase, urate:oxygen oxidoreductase (EC 1.7.3.3), catalyzed oxidation of urate (Praetorius, 1948; Mahler *et al.*, 1956). One of these intermediates absorbs strongly in the region 270–330 nm and thus may interfere with the commonly used spectrophotometric assay at 293 nm (Priest and Pitts, 1972). Structure I has been proposed for this intermediate, 1-carboxy-2,4,5,8-tetraazabicyclo[3.3.0]octa-4-ene-3,7-dione (Mahler *et al.*, 1956). Borate and hydroxide ions enhance the decay of this intermediate to stable products (Praetorius, 1948). Detailed kinetic studies of the uricase mechanism of action necessitate a thorough prior understanding of the

† From the Department of Biochemistry, the Medical University of South Carolina, Charleston, South Carolina 29401. *Received November 27, 1972.* Financial assistance was provided in part by a grant from the Research Corporation and a General Research Support grant (RRO-5420) from the National Institutes of Health.



I

behavior of this intermediate. This paper describes studies concerning the components of both the intermediate decay and enzyme-catalyzed reactions, the catalytic behavior of borate and hydroxide ions on the intermediate decay reaction, and the temperature perturbation of the intermediate decay mechanism. Mechanisms are proposed which account for all of the results obtained.

Experimental Procedures

Uricase was obtained from Worthington Biochemicals, Sigma, and Boehringer-Mannheim Corp. The Boehringer-Mannheim preparation (4.5 units/mg) was used for the majority of these studies without further purification.

Uric acid was obtained from Calbiochem. Three-milliliter samples of 100 μM urate in glycine or borate buffers were routinely used. An ϵ_{290}^{1cm} of 1.22×10^4 for urate (Kalckar, 1947) was used throughout. Oxygen–nitrogen mixtures were obtained from Maloney Distributing Co., Charleston, S. C., and used to equilibrate reaction mixtures in Thunberg cuvets (Priest and Fisher, 1969). A Beckman Acta III spectrophotometer was used in the double beam mode for these studies. The thermostated cell chamber was maintained at the proper temperature by circulating water. A Medspec mass spectrometer was used in the studies of O_2 uptake and CO_2 evolution.

To follow the decay reaction, the intermediate was routinely generated *in situ* by the addition of 100 μl of uricase containing 0.1 mg/ml to 3.0 ml of 100 μM urate in 3.0-ml cuvets. After 30 sec a sufficient volume of a 1% KCN solution was added to stop the enzymatic production of the intermediate compound (Baum *et al.*, 1956). The decay was followed at 302 nm at temperatures from 23 to 46°. Modifications of this general procedure are shown where appropriate.

Results

Several investigators (Mahler *et al.*, 1956; Cannelakis and Cohen, 1955) have studied reaction intermediates produced during the uricase-catalyzed oxidation of urate. Based on symmetry considerations from isotope exchange studies and a pK_a in the carboxylic acid region of 4.5 the primary compound was deduced to be compound I. The proposed structure assumes an oxidation by O_2 during the course of the enzyme-catalyzed reaction. Release of CO_2 during the intermediate breakdown was suggested by Klemperer based on observations using manometric techniques (Klemperer, 1945). Using a mass spectral technique, we have confirmed the uptake of O_2 during the enzyme-catalyzed reaction and the evolution of CO_2 during the decay of the intermediate (Figure 1). Following enzymatic generation of approximately 15 μM intermediate, KCN was added to block any further increase in the intermediate concentration. The detector was set to monitor both O_2 and CO_2 partial pressures simultaneously. It can be seen in Figure 1 that O_2 utilization ceased upon addition of KCN. Thus, while O_2 is utilized during the enzymatic production of the intermediate, the decay reaction is independent of O_2. On the other hand CO_2 continues to be evolved after complete inhibition of the intermediate production.

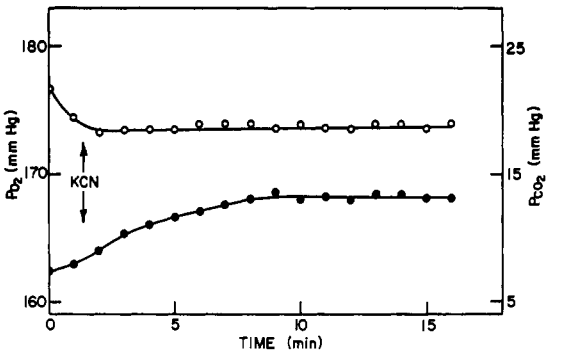


FIGURE 1: Study of changes in P_{O_2} (O) and P_{CO_2} (●) during intermediate decay reaction. Urate solutions (100 μM) were prepared in 0.1 M phosphate buffer at pH 6.5 and 23°. At time zero, 30 μl of uricase (2.0 mg/ml) was added; 100 μl of 1% KCN was added at the time shown to the 3.0-ml reaction cell.

By structural analogy, it was assumed that CN^- interacted with the O_2 site on the enzyme, thus preventing binding at this site. This assumption does not preclude the possibility that the intermediate species could interact with the enzyme at a position other than the O_2 site. Should this occur, the enzyme could affect the intermediate decay reaction. Results shown in Figure 2, however, indicate that such a mechanism is not operable. The intermediate was generated enzymatically, KCN added, and the first-order decrease in ultraviolet (uv) absorbance at 302 nm used to follow intermediate decay. It can be seen that the half-life of 87 sec in aqueous solution was totally independent of the KCN-inhibited enzyme concentration. To test further the possibility that O_2 participates in intermediate breakdown, reaction cells were flushed thoroughly with 100% O_2 and experiments similar to those described in Figure 2 were conducted. Figure 3 indicates that no enhancement of the rate of intermediate breakdown is observed nor is the order of the reaction altered. It thus appears that during the enzyme-catalyzed process (reaction 1) urate is oxidized by O_2 producing a species consistent with that proposed by Mahler (1963). This compound subsequently decays by a first-order process which is independent of O_2 or uricase. Cannelakis and Cohen (1955) have shown that the final products of this decay reaction can vary depending upon the absence (reaction 2) or presence (reaction 3) of borate.

Reaction 2 is reportedly enhanced by hydroxide ion (Prae-

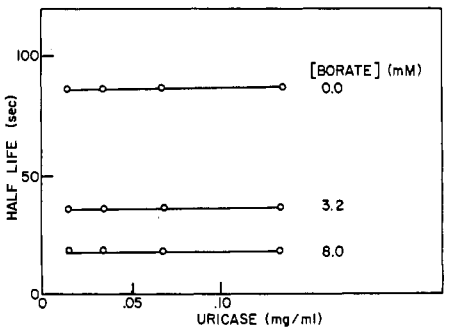


FIGURE 2: Effect of uricase concentration on intermediate half-life. Intermediate was generated at pH 9.0 with uricase concentrations from 0.01 to 0.13 mg/ml; 50 μl of a 1% KCN solution, sufficient to completely block urate disappearance, was added with adequate borate to give the final concentration shown. The uv decrease at 302 nm was monitored at 23°.

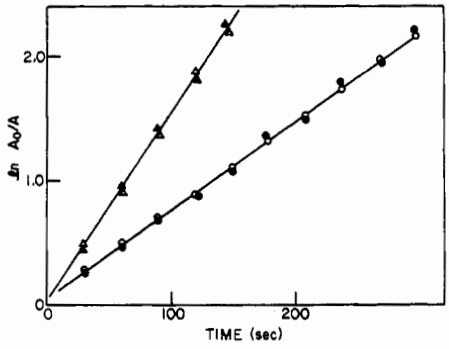
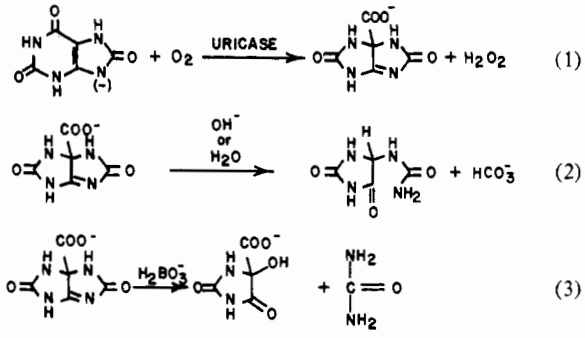


FIGURE 3: The effect of oxygen tension upon the apparent first-order rate constant for the intermediate decay reaction. Urate solutions were equilibrated with 100% O_2 in sealed Thunberg cuvets. Intermediate was generated typically to a level of 15 μM. The various conditions are identified by (○) 0.0 mM borate–atmospheric oxygen, (●) 0.0 mM borate–100% oxygen, (△) 1.6 mM borate–atmospheric oxygen, and (▲) 1.6 mM borate–100% oxygen.



torius, 1948; Mahler *et al.*, 1956). While borate was presumed to function catalytically (reaction 3) it remained unclear whether hydroxide ion functioned stoichiometrically or catalytically in this hydrolysis reaction. Figure 4 shows that the decay reaction follows first-order kinetics over the entire time course. This is true over a broad range of hydroxide ion concentrations even at or below those equivalent to the intermediate ($\simeq$15 μM). Since hydroxide ion shows a potent stimulatory effect we conclude that its function is quite likely catalytic.

Prior to the studies on the mechanism by which borate catalyzes breakdown of the intermediate, it was established that its presence does not create a dependence on enzyme or O_2 concentration (Figures 2 and 3). That the reaction is

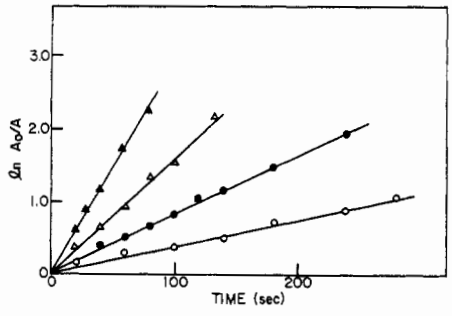


FIGURE 4: First-order decay of intermediate as a function of hydroxide ion concentration. Conditions were as in Figure 2 except (○) 1.0 × 10^{-6} M, (●) 10.0 × 10^{-6} M, (△) 40.0 × 10^{-6} M, and (▲) 100.0 × 10^{-6} M hydroxide ion concentrations were used.

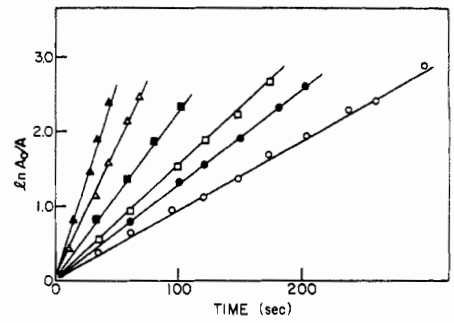


FIGURE 5: First-order decay of intermediate as a function of borate concentration at 23 and 36°. Conditions were as in Figure 2 except (○) 0.16 mM and 23°, (□) 1.6 mM and 23°, (△) 8.0 mM and 23°, (●) 0.16 mM and 36°, (■) 1.6 mM and 36°, and (▲) 8.0 mM and 36° borate concentrations and temperatur es were used.

strictly first order in the presence of a range of borate concentrations can be seen in Figure 5. It is thus concluded that both borate and hydroxide ion function catalytically and do not alter the stoichiometry of the reaction.

To obtain information with regard to the mechanism by which these two catalysts act, an empirical rate equation was developed. To decide whether borate and hydroxide ions act independently or in a cooperative manner, experiments were performed in which hydroxide ion concentration was varied over a wide range in the presence of borate. Figure 6 shows that plots of the apparent first-order rate constant, k_{app}, *vs.* hydroxide ion concentration at several borate concentrations are parallel. Thus, rate equation terms containing the product of borate and hydroxide concentration are precluded. Considering the first-order disappearance of the intermediate, the simplest form of an empirical rate equation in the presence of both hydroxide and borate is given by

$$\frac{-d[I]}{dt} = k_{app}[I] \tag{4}$$

$$k_{app} = (k_0 + k_{OH}[OH^-] + k_B[\text{borate}]) \tag{5}$$

It was desirable to determine whether or not the catalytic constants k_{OH} and k_B were individual rate constants or accumulations of rate constants. The temperature perturbation of these constants was thus examined over the range 23–46°. Since the hydroxide-catalyzed reaction could be examined independently of borate, this system was tested first. Figure 7 shows that plots of k_{app} *vs.* hydroxide ion concentration become nonlinear at higher temperatures. These hydroxide ion concentrations have been corrected for the change in the ionization constant of water with temperature. The saturation kinetics with hydroxide ion concentration can be described by the introduction of an additional empirical constant.

$$\frac{-d[I]}{dt} = (k_0 + k_{OH}[OH^-])[I] \tag{6}$$

$$k_{OH} = \frac{k_1}{1 + k_2[OH^-]} \tag{7}$$

It can be noted that the reciprocal form of eq 7 is linear. Such linear reciprocal plots were used to estimate the empirical constants, k_1 and k_2, shown in Table I.

TABLE I: Catalytic Constant Assignments for Empirical Rate Equation 10.

Temp (°C)	k_0	k_1	k_2	k_3	k_4
23	0.002	500	12,500	3.70	10.0
36	0.005	2300	50,600	6.25	60.0
46	0.010	4350	61,000	9.50	114.0

When borate was added to the system at constant pH, similar nonlinear behavior was observed at the higher temperatures. The results presented in Figure 8 are calculated in terms of total added borate concentration. When borate anion concentration was corrected for the temperature dependence of the ionization constant, similar but not as pronounced nonlinearity was observed. The results shown in Figure 8 can be fit by the empirical relationship shown in eq 8 and 9. A

$$\frac{-\mathrm{d[I]}}{\mathrm{d}t} = \left(k_0 + \frac{k_1[\mathrm{OH^-}]}{1 + k_2[\mathrm{OH^-}]} + k_B[\mathrm{borate}]\right)[\mathrm{I}] \quad (8)$$

$$k_B = \frac{k_3}{1 + k_4[\mathrm{borate}]} \quad (9)$$

linear reciprocal relationship was again used to estimate the constants, k_3 and k_4 (Table I).

The following empirical rate equation (10) will describe the behavior of the intermediate decay over all conditions of borate and hydroxide ion concentration and temperature studied.

$$\frac{-\mathrm{d[I]}}{\mathrm{d}t} = \left(k_0 + \frac{k_1[\mathrm{OH^-}]}{1 + k_2[\mathrm{OH^-}]} + \frac{k_3[\mathrm{borate}]}{1 + k_4[\mathrm{borate}]}\right)[\mathrm{I}] \quad (10)$$

All lines in Figures 7 and 8 were calculated using eq 10 and the constants shown in Table I.

Temperature perturbation data allowed estimation of activation parameters for each of the three processes: (1) the borate and hydroxide ion independent reaction, (2) the borate-catalyzed reaction, and (3) the hydroxide ion catalyzed reaction. Linear Arrhenius plots were obtained in all cases. Values for free-energy, enthalpy, and entropy of activation are presented in Table II.

Hydroxide and borate independent estimates were obtained from extrapolations of k_{app} *vs.* hydroxide ion concentration to zero hydroxide ion concentration. Care was taken to obtain estimates of the activation parameters for each of the three processes which were independent of catalyst concentration.

TABLE II: Activation Parameters for Intermediate Breakdown at 23°.

Reaction Medium	$\Delta G^{\ddagger}$ (kcal/mol)	$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta S^{\ddagger}$ (eu)
Water	20.9	13.5	−25.2
Hydroxide	19.8	10.8	−31.0
Borate	19.7	2.5	−58.0

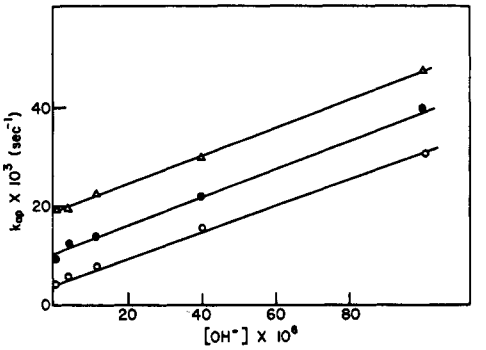


FIGURE 6: Apparent first-order rate constant as a function of borate and hydroxide ion concentration. Borate concentrations were (○) 0.0 mM, (●) 1.6 mM, and (△) 3.2 mM.

Concentration dependence was observed at low catalyst concentrations, but essentially no change was detected above 3.0×10^{-5} M hydroxide ion and 1.6×10^{-3} M borate.

Because the accuracy of the spectral determination of the rate of urate decrease (reaction 1) at 293 nm depends upon the relative accumulation of intermediate in the reaction mixture, the observation that borate exhibits saturation behavior raised questions as to the interpretation of the uricase kinetics taken under conditions in which it was assumed that borate concentration was sufficiently high to instantaneously degrade the intermediate (reaction 3). It was of special interest to reinvestigate the conditions under which the enzyme velocity would be expected to be relatively high. Baum *et al.* (1956) have reported substrate inhibition for urate with pig liver uricase. In the urate concentration region where such behavior is exhibited, initial velocities would be expected to be relatively high for the same enzyme concentration were it not for the substrate inhibition. Figure 9 shows initial velocities of urate decrease as determined at 293 nm. The conditions used were similar to those used by Baum *et al.* (1956) and these results are essentially identical. At urate concentrations higher than 100 μM an apparent decrease in initial velocity as reflected by a decrease in the rate of absorbance change is observed. However, since the intermediate exhibits significant absorbance at 293 nm (Priest and Pitts, 1972), the apparent substrate inhibition could be due to a relative accumulation of this species as urate concentration is increased. Thus, initial velocities were obtained at 320 nm, a wavelength at which the intermediate but not urate absorbs significantly. At concentrations of urate greater than 100 μM there was a low but detect-

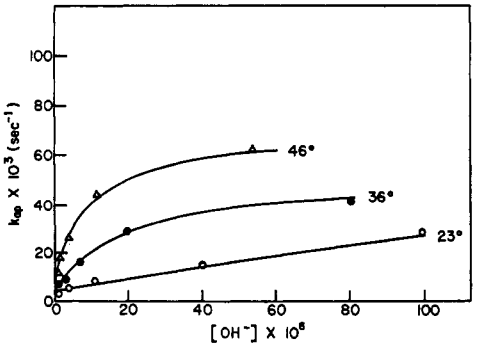


FIGURE 7: Apparent first-order rate constant as a function of hydroxide ion concentration and temperature. Lines were calculated using eq 10 and the constants shown in Table I.

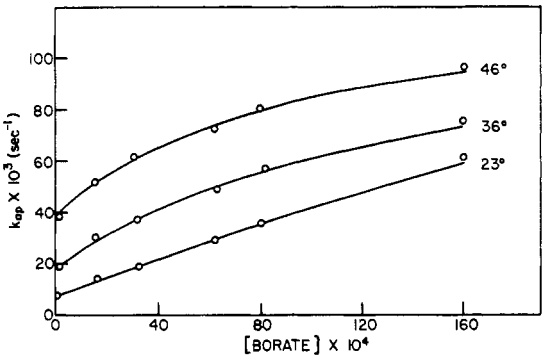


FIGURE 8: Apparent first-order rate constant as a function of borate concentration and temperature at pH 9.0. Lines were calculated from eq 10 and the constants shown in Table I.

able increase in absorbance at 320 nm. This increase appeared to be roughly proportional to the urate concentration present, which suggested interference from the intermediate at higher urate concentration. It was thus desirable to obtain velocity measurements which did not rely on the assumption that intermediate was instantaneously removed. One approach is to measure the accumulation of the intermediate itself. It has been shown previously (Priest and Pitts, 1972) that the wavelength of maximal absorbance difference for the urate–intermediate pair is 312 nm. Figure 9 shows that linearity is maintained between reciprocal velocity and reciprocal urate concentration up to 1000 μM when initial velocities are accessed at 312 nm. It is thus concluded that the substrate inhibition observed with urate using pig liver uricase is due to interference by intermediate and is not a property of the enzymic reaction (reaction 1).

Baum *et al.* (1956) reported an apparent K_m of 19.5×10^{-6} M for urate in the presence of atmospheric oxygen. Figure 9 shows that not only are the high urate concentration results affected by the intermediate but also the low linear portion of the curve is as well. Using the 312-nm assay, a K_m of 50×10^{-6} M

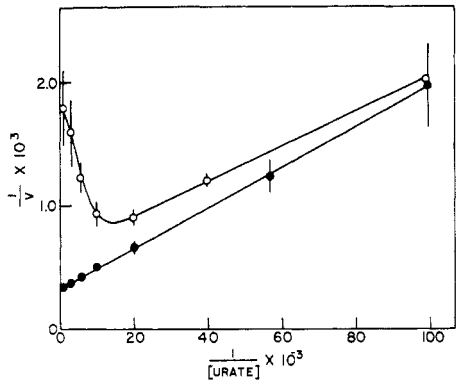


FIGURE 9: Double reciprocal plots for urate determined at 293 and 312 nm. Initial velocities at 293 nm (○) were obtained in the presence of 5.0 mM borate and those at 312 nm (●) in 0.1 M Tricine (*N*-tris(hydroxymethyl)methylglycine). Reactions were initiated by the addition of 5 μg of uricase in a 50-μl volume to 3.0-ml cells at pH 8.0 and 27°. Assays using greater than 100 μM urate were transferred to 0.1-cm path-length cells for recording of absorbance at 293 nm. Velocities were calculated as molecular activities assuming an approximate molecular weight for uricase of 127,000 (Fish, W. W., Pitts, O. M., and Priest, D. G., unpublished results). Error bars represent standard deviations for six separate determinations.

is estimated. Caution should be used in a direct comparison of the K_m values since a somewhat different oxygen solubility may occur in the Tricine buffer used for the 312-nm assay.

Discussion

For purposes of clarity, the uricase reaction scheme has been separated into two separate functional processes: first, the enzyme-catalyzed reaction (reaction 1) and secondly the subsequent decay of the unstable intermediate produced during the enzymatic reaction, catalyzed by hydroxide ion (reaction 2) or borate (reaction 3). Several points were unclear with regard to the separation of the enzyme reaction from the intermediate decay reaction (Mahler, 1963). It has been established that O_2 is consumed during the enzymatic reaction but not during the subsequent intermediate decay reaction. It has been confirmed (Klemperer, 1945) that CO_2 is produced during the intermediate decay reaction in aqueous alkaline solutions. When the decay reaction is conducted in the presence of borate, the stable products have been previously shown to be mainly alloxanate and urea, and in aqueous alkaline solutions the major product of the decay is allantoin (Cannelakis and Cohen, 1955).

Others have shown through radioisotope experiments that a transient, symmetrical intermediate precedes the formation of allantoin in the aqueous alkaline case. In the presence of borate, on the other hand, urate labeled in the C-8 position gives rise to the labeling of urea (Mahler *et al.*, 1956; Cannelakis and Cohen, 1955). The intermediate generated during the enzymatic reaction has identical uv absorbance properties whether this reaction occurs in aqueous, alkaline, or borate-containing solutions (Priest and Pitts, 1972). This strongly suggests that the same intermediate is produced regardless of the medium in which the enzymatic reaction is conducted. On the basis of symmetry and ionization characteristics this intermediate has been suggested to be compound I.

Temperature perturbation studies showed a large decrease in activation enthalpy when borate catalyzed the decay of the intermediate (Table II). This decrease was of a magnitude not unusual for enzyme-catalyzed reactions; however, the concentration of CN^--inhibited uricase had no effect on the rate of the intermediate decay reaction. That hydroxide ion acts catalytically has been deduced from a lack of deviation from first-order kinetics at low hydroxide ion concentrations.

The saturation kinetics observed with the hydroxide ion and borate-catalyzed reactions can be obtained from the following sequence of events if the steady-state approximation is applied with respect to the secondary intermediate, I′,

$$\text{I} + \text{catalyst} \underset{k'}{\overset{k}{\rightleftharpoons}} \text{I}' + \text{catalyst} \qquad (11)$$

$$\text{I}' \xrightarrow{k''} \text{products} \qquad (12)$$

concentration. Such a reaction sequence that regenerates catalyst prior to formation of final stable products yields rate equation 13. This equation has the same form as empirical

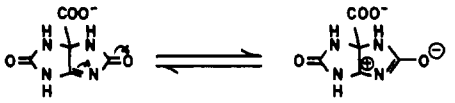
$$\frac{-\text{d[I]}}{dt} = \frac{k''k[\text{catalyst}]}{k'' + k'[\text{catalyst}]}[\text{I}] \qquad (13)$$

equations 6 and 8.

In summary, proposed catalytic mechanisms for the intermediate decay reaction should be consistent with the following

characteristics. (1) When water or hydroxide ion is the catalyst, carbon dioxide and allantoin are produced but oxygen is not reduced. An unstable and symmetrical intermediate is traversed prior to the formation of allantoin. (2) When borate is the catalyst carbon dioxide is not produced nor is oxygen reduced. The products alloxanate and urea maintain the asymmetry contained in the starting material, urate. (3) The enzyme, uricase, participates in the formation of the primary intermediate but not in the subsequent intermediate decay reaction regardless of the presence of the secondary catalyst. (4) Hydroxide ion does not participate cooperatively in the borate-catalyzed reaction but rather acts independently of borate. (5) With borate and hydroxide ion as catalysts, saturation kinetics are obtained which can be explained by a reaction scheme involving regeneration of catalyst and formation of a secondary unstable intermediate prior to the formation of stable products.

Since the two catalysts show similar kinetic behavior (although different catalytic efficiencies) but act by parallel processes, it is reasonable to assume the same site of primary attack on the intermediate by both catalysts. The most likely site of attack is the relatively electropositive bridgehead carbon opposite the carboxyl group.



Attack at this point by borate would give rise to a complex which would be more stable with respect to maintenance of asymmetry than would the hydroxyl complex. The much greater lowering of activation entropy for the borate case supports a bidentate type complex which could provide stability with respect to loss of the carboxyl function. This possibility does not exist with hydroxide ion. Both catalysts can be hydrolytically removed prior to the formation of final products. An alternative to the kinetic mechanism suggested (reactions 11 and 12) which would satisfy the observed kinetics is a rapid formation of a catalyst–intermediate complex such that essentially no primary intermediate (compound I) is present under saturating conditions of catalyst. Such a mechanism is not favored because no shift in the uv spectrum of the intermediate was observed regardless of the presence or absence of catalyst. The trapping and identification of all intermediates, however, appears to be the only completely satisfactory approach to ascertaining unequivocally the catalytic mechanisms.

Several mechanisms have been proposed to explain the substrate inhibition observed with uricase. This report indicates that the observed substrate inhibition is most probably due to uv interference though accumulation of the primary intermediate under conditions which had previously been assumed to cause instantaneous removal (*i.e.*, high borate concentration). Reciprocal plots determined on the basis of initial intermediate production are linear over the range of urate concentration studied. Furthermore, kinetic constants, obtained by others from linear regions of reciprocal plots determined at 293 nm, are not in agreement with those reported here. While the substrate inhibition mechanism has been dealt with here, further kinetic studies utilizing the appearence of intermediate or some other unambiguous assay will be necessary to determine the enzymic mechanism of the uricase reaction.

References

Baum, H. M., Hubscher, G., and Mahler, H. R. (1956), *Biochim. Biophys. Acta 22*, 514.

Cannelakis, E. S., and Cohen, P. P. (1955), *J. Biol. Chem. 213*, 385.

Kalckar, H. M. (1947), *J. Biol. Chem. 167*, 429.

Kelmperer, F. W. (1945), *J. Biol. Chem. 160*, 111.

Mahler, H. R. (1963), *Enzymes 8*, 285.

Mahler, H. R., Baum, H. M., and Hubscher, G. (1956), *Science 124*, 705.

Praetorius, E. (1948), *Biochim. Biophys. Acta 2*, 602.

Priest, D. G., and Fisher, J. R. (1969), *Eur. J. Biochem. 10*, 439.

Priest, D. G., and Pitts, O. M. (1972), *Anal. Biochem. 50*, 195.