## SHORT COMMUNICATIONS

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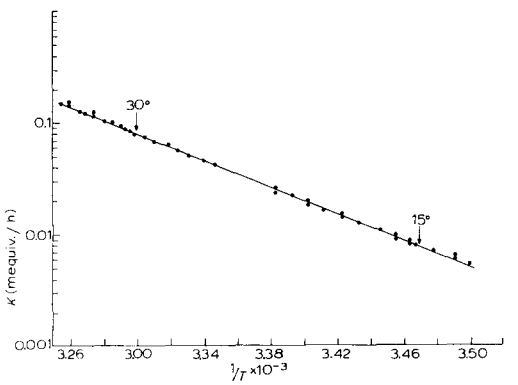
### Temperature effects on the acid hydrolysis of glucose 1-phosphate and urease hydrolysis of urea

Evidence that water and aqueous solutions undergo thermal anomalies at or near 15, 30, 45, and 60° has recently been reviewed by DROST-HANSEN[1]. Much of this evidence has been questioned by FALK AND KELL[2] and by CLAUSSEN[3]. Many biological systems show growth discontinuities near these temperatures[1,4,5] and it has been suggested that these anomalies are due to the water anomalies. Since cause and effect relationships are hard to establish in complex biological systems, simpler reactions were studied.

Water is an integral part of hydrolysis reactions, and if the structure of water changes at specific temperatures, it might be expected that the rate of hydrolysis would be discontinuous at these temperatures. The acid hydrolysis of glucose 1-phosphate (Glc-1-*P*) and the urease hydrolysis of urea were studied. Previous studies of these hydrolysis reactions did not show thermal anomalies[6–11]. The large temperature intervals used in their studies, commonly 5° or larger, would prevent finding any anomalous behavior. The results reported here do not show thermal discontinuities where temperature intervals of about 1° were used.

α-D-Glucose 1-phosphate hydrolyzes under acid conditions with a water molecule reacting with the carbon atom releasing phosphate. The reaction was found to obey first order kinetics. The released phosphate was determined by a modification of the procedure of MARTIN AND DOTY[12]. Phosphate was determined at 720 mμ.

The experiments were conducted in a multi-temperature bath[13], with a temperature control of approx. 0.05°. The Glc-1-*P* was obtained from Sigma Chemical Co. and was at least 98% pure. The reaction vessels contained 0.335 M $H_2SO_4$ and 1.2 mg of Glc-1-*P* in a volume of 10 ml. At the end of either 4 or 8 h, depending upon the tem-



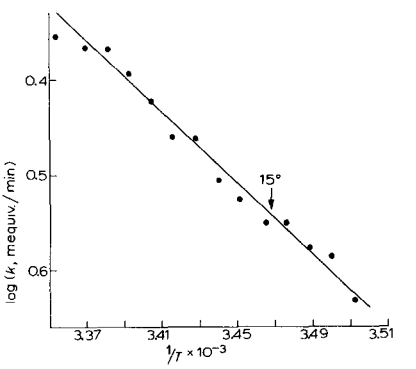




Fig. 1. The effect of temperature on the acid catalyzed hydrolysis of glucose 1-phosphate. $E_a$ = 27.4 ± 0.8 kcal/mole.

Fig. 2. The effect of temperature on the urease hydrolysis of urea. $E_a$ = 8.0 kcal/mole.

perature, the reaction was stopped by adding 5 ml of the reaction solution to a mixture of alcohol–benzene.

Fig. 1 shows the results for temperatures in ranges around both 15 and 30°. Duplicate determinations were made at each temperature, and only one point is shown where the results were identical. Ten separate experiments were conducted in the 30° range and five in the 15° range. All results were similar to those shown in Fig. 1. The activation energy for Glc-1-*P* hydrolysis was 27.4 ± 0.8 kcal/mole at the 99% confidence level. This value agrees well with the value of OSBORN AND WHALLEY[8] of 27.8 kcal/mole.

Urease (type III glycerol, 75 S.U./ml) was obtained from Sigma Chemical Co. All other chemicals were analytical grade. The urea concentration (0.07 M) and urease concentration (0.74 S.U./ml) gave a maximum reaction velocity at all temperatures studied[14]. The concentration of the Tris–sulphuric acid buffer in the reaction solution was 0.2 M. The pH optimum for the reaction is from 6 to 7 (ref. 15) which is outside the buffering range of Tris[16]. Our results also show a pH optimum from pH 6 to 7, but they show a more rapid drop in activity as pH increases beyond 7 than did the results of FISHBEIN, WINTER AND DAVIDSON[15]. When experiments are conducted in the optimum pH range, the pH changes during hydrolysis (due to inefficiency of buffer in this range) can be large enough to move the reaction away from the pH optimum, thus affecting the results. Even though Tris is not a good buffer for this system it has commonly been used to characterize the reaction. Thus, in spite of the above limitations, Tris was used in this study.

Ammonium production was monitored using a Beckman 39137 cationic electrode[17]. The results are easily obtained and reproducible. The Tris buffer does interfere with the electrode, but if care is taken to maintain a precise buffer concentration, reproducible and reliable results can be obtained. If the system is not adequately buffered, the pH changes can cause erroneous results because the electrode is sensitive to hydrogen. A standard curve was made using ammonium sulphate–Tris standards.

The urease which was stored at 0° was diluted in water and aged for at least 4 h before use. The aging characteristics of the urease may vary from purchase to purchase and need to be checked. The solutions were allowed to reach temperature equilibrium in a water bath (± 0.01°) before the reaction was initiated.

Under the conditions used in these experiments Michaelis–Menten kinetics were applicable.

Fig. 2 shows an Arrhenius plot for the urease hydrolysis of urea at pH 8 in 0.2 M Tris. The activation energy is 8.0 kcal/mole. The activation energy at pH 7 was within experimental error of this value. The large variation in activation energy is probably due to the various batches of urease used. Similar variations were found by LYNN[18]. This activation energy is in reasonable agreement with the value of KISTIAKOWSKY AND LUMRY[9] but not with that of WALL AND LAIDLER[11] or LYNN AND YANKWICH[10]. LYNN AND YANKWICH[10] suggest several reasons that may account for the various reported activation energies, the most important of which may be the variations in the urease used. The above activation energy is an average of 10 separate experiments, all of which had plots similar to Fig. 2. LYNN[18] reported some Arrhenius plots with very different activation energies at his high and low temperatures with no temperature response in between. None of these characteristics were observed in this work.

In both Figs. 1 and 2 there is no suggestion of anomalous temperature changes.

In a few of the initial experiments, with both systems, anomalous temperature changes were found. These nonlinear results occurred only occasionally and were not reproducible. Linear results were always obtained when the analytical techniques and temperature control were adequate.

The results reported here agree with the conclusion of KISTIAKOWSKY AND LUMRY[9]. Why MASSEY[19] got such pronounced changes at specific temperatures in the fumarase system is not known, unless it was due to thermally induced reversible protein structural changes as suggested by MASSEY, CURTI AND GANTHER[20]. Recent papers by DYSON AND NOLTMANN[21] and by KAYNE AND SUELTER[22] suggest similar changes. If water does undergo structural changes at specific temperatures it is not reflected in these reactions. It is possible that rate constants do not reflect water properties since the reaction with water may not be the rate-limiting process.

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1 W. DROST-HANSEN, *Advan. Chem. Ser.*, 67 (1967) 70.
2 M. FALK AND G. S. KELL, *Science*, 154 (1966) 1013.
3 W. F. CLAUSSEN, *Science*, 156 (1967) 1226.
4 C. B. DAVEY, R. J. MILLER AND L. A. NELSON, *J. Bacteriol.*, 91 (1966) 1827.
5 R. J. MILLER AND C. B. DAVEY, *Soil Sci. Soc. Am. Proc.*, 31 (1967) 286.
6 M. J. COHN, *J. Biol. Chem.*, 180 (1949) 771.
7 P. W. C. BARNARD, C. A. BUNTON, D. R. LLEWELLY, K. G. OLOHAM, L. B. SILVER AND C. A. VERNON, *Chem. Ind. London*, (1955) 760.
8 A. R. OSBORN AND E. WHALLEY, *Can. J. Chem.*, 39 (1961) 597.
9 G. B. KISTIAKOWSKY AND R. J. LUMRY, *Am. Chem. Soc.*, 71 (1949) 2006.
10 K. R. LYNN AND P. E. YANKWICH, *Biochim. Biophys. Acta*, 56 (1962) 512.
11 M. WALL AND K. J. LAIDLER, *Arch. Biochem. Biophys.*, 43 (1953) 299.
12 J. B. MARTIN AND D. M. DOTY, *Anal. Chem.*, 21 (1946) 965.
13 A. R. OVERMAN AND R. J. MILLER, *Anal. Biochem.*, 19 (1967) 195.
14 K. D. GIBSON, *Biochim. Biophys. Acta*, 10 (1953) 221.
15 W. N. FISHBEIN, T. S. WINTER AND J. D. DAVIDSON, *J. Biol. Chem.*, 240 (1965) 2402.
16 N. E. GOOD, G. D. WINGET, W. WINTER, T. N. CONNALLY, S. IZAWA AND R. M. M. SINGH, *Biochemistry*, 5 (1966) 467.
17 S. A. KATZ AND J. A. COWAN, *Biochim. Biophys. Acta*, 107 (1965) 605.
18 K. R. LYNN, *Biochim. Biophys. Acta*, 146 (1967) 205.
19 V. MASSEY, *Biochem. J.*, 52 (1953) 72.
20 V. MASSEY, B. CURTI AND H. J. GANTHER, *J. Biol. Chem.*, 241 (1966) 2347.
21 J. E. D. DYSON AND E. A. NOLTMANN, *J. Biol. Chem.*, 243 (1968) 1401.
22 K. J. KAYNE AND C. H. SUELTER, *Biochemistry*, 7 (1968) 1678.

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