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KINETICS OF THE ACID HYDROLYSIS OF URIDINE DIPHOSPHATE GLUCOSE AND 3-N-METHYLURIDINE DIPHOSPHATE GLUCOSE

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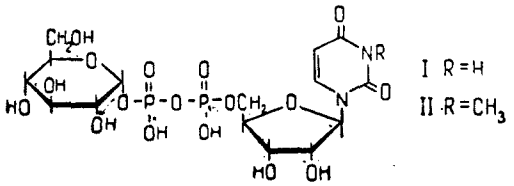
Nucleoside diphosphate sugars occupy a key position in carbohydrate metabolism. In recent years there have been numerous investigations of the biochemical reactions of these compounds (for reviews, see [1, 2]) and of the biochemical and chemical properties of their analogs [3]. It has long been known that these compounds are unstable in an acid medium [4], but there is no more detailed information on the kinetics of their acid hydrolysis in the literature. This makes it more difficult to select the experimental conditions for studying certain transformations of the nucleoside diphosphate sugars.

Consequently, we have investigated the influence of the pH and the temperature on the rate of hydrolysis of uridine diphosphate glucose (I) and its analog with a modified heterocyclic nucleus, 3-N-methyluridine diphosphate glucose (II) [5].



The acid hydrolysis of substance (I) takes place with the formation of glucose and uridine 5'-diphosphate, which is then gradually converted into uridine 5'-phosphate and phosphoric acid [4]. In view of this, it is best to follow the kinetics by means of a colorimetric determination of the reducing sugar. However, it is essential that the conditions of the determination should be fairly mild and that further hydrolysis of (I) should not take place during the determination. After a series of preliminary experiments, we have found that the method of determining a reducing sugar proposed by Park and Johnson [6] satisfies these requirements.

The dependence of the rate of hydrolysis of (I) on the pH was studied at 50° C in the pH range 2.60–3.52, giving the following results:

pH	2.60	2.70	2.85	3.10	3.52
$k \cdot 10^4$ (min^{-1})	14.6	11.2	7.7	4.3	1.5

The logarithm of the rate constant was a linear function of the pH; it is described satisfactorily by the equation

$$\log k\,(\min^{-1}) = -0.26 - \mathrm{pH}. \quad (1)$$

A study of the dependence of the rate of hydrolysis of (I) on the temperature (pH 2.70) gave the following figures:

Temperature, °C	45	50	55
$k \cdot 10^4$ (min^{-1})	4.1	11.2	26.7

In the Arrhenius coordinates, the results obtained fall satisfactorily on a straight line described by the equation

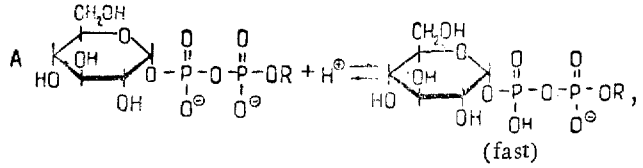
$$\log k\,(\min^{-1}) = 22.75 - \frac{8300}{T}. \quad (2)$$

The slope of the line corresponds to an activation energy of 38 kcal/mole. By combining Eqs. (1) and (2), we obtain Eq. (3) for the rate of hydrolysis of (I) as functions of the pH and the temperature

$$\log k\,(\min^{-1}) = 25.45 - \mathrm{pH} - \frac{8300}{T}. \quad (3)$$

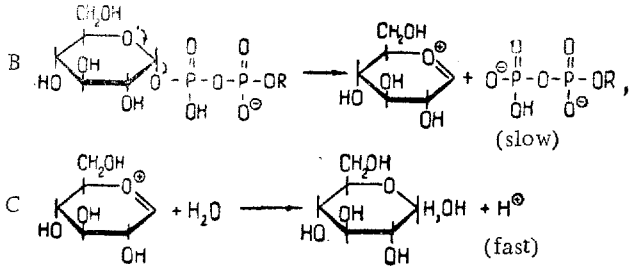
By using Eq. (3) it is possible to evaluate the stability of (I) under given pH and temperature conditions. Thus, at pH 2.70 and 20° C [the usual conditions for the ion-exchange chromatography of (I)], $k \simeq 2.5 \cdot 10^{-6}\,min^{-1}$, which corresponds to a half-decomposition period of approximately 20 days.

The observed dependence of the rate of hydrolysis of (I) on the pH and the temperature corresponds to the following reaction mechanism:



The increase in the rate of the reaction with a decrease in the pH is connected with the increase in the concentration of the monoanion of (I)—the product of the equilibrium stage A. This increase can be regarded as proportional to the concentration of hydrogen ions at $[n^+] \ll K_a$, where K_a is the ionization constant of the monoanion of (I).

The high activation energy for the hydrolysis of (I) (38 kcal/mole) is comparable with the activation energies for the hydrolysis of α-D-glucose-1-phosphate (31.0 kcal/mole) [7] and of glycosides (32.0—35.1 kcal/mole) [8]. A monomolecular mechanism has been shown strictly for these reactions; it may be concluded by analogy that the hydrolysis of the monoanion of (I) apparently takes place by a monomolecular mechanism, i.e., via stages B and C. This mechanism is also shown by the positive activation entropy for the hydrolysis of (I) (at 50° C, ΔS = +38 cal/deg · mole).



We have also compared the rates of hydrolysis of substances (I) and (II) as functions of the temperature. It was found that within the limits of experimental error the rates of hydrolysis of these compounds were the same and, thus, the nature of the heterocyclic base does not appreciably affect the rate of hydrolysis of the glycosidic bond in a nucleoside diphosphate sugar.

Experimental

Determination of the reducing sugar [6]. A modification of the method of Park and Johnson [6] was used. Reagents: A) solution of 100 mg of $K_3[Fe(CN)_6]$ in 200 ml of water; B) solution of 1.15 g of anhydrous sodium carbonate in 200 ml of water; C) solution of 150 mg of ferric sulfate nonahydrate and 50 mg of sodium dodecyl sulfate in 200 ml of 0.05 N sulfuric acid.

Determination: to 3 ml of a solution containing 0—50 γ of reducing sugar were added 1 ml of solution B and 1 ml of solution A, and the mixture was heated in the boiling water bath for 20 min, cooled in a cold water bath for 5 min, and treated with 5 ml of solution C, after which the optical density at 690 mμ was measured in a 2-cm cell against water. The measurement was performed not more than 15 min after the addition of solution C.

The calibration curve corresponded to the graph Y = 39.69 X − 3.67, where Y is the amount of glucose and X is the optical density at 690 mμ. The correlation coefficient r = 0.9987 at N = 10.

Kinetic experiments. Each of five 20-ml test tubes was charged with 0.3 ml of a 0.005 M solution of (I) or (II) and 0.03 ml of a 0.02 M hydrogen phthalate—hydrochloric acid buffer solution with pH 2.40—4.10. The test tubes were tightly closed and were kept in a thermostat at the given temperature. After predetermined intervals of time the contents of each tube were treated with 0.45 ml of a 0.02 M solution of sodium carbonate, and were diluted with water to 3 ml, and the reducing sugar was determined.

In the measurement of a series of samples, a solution neutralized immediately after the mixing of the reagents was used as the reference sample. To achieve complete hydrolysis, 0.3 ml of a solution of (I) or (II) was treated with 0.03 ml of 0.01 N hydrochloric acid, kept at 100° C for 15 min, and neutralized with 0.15 ml of a 0.02 N solution of sodium carbonate.

The pseudo-first-order hydrolysis rate constants were determined from the slope of the line expressing $\ln(D_\infty - D)$ (where D is the optical density at 690 mμ) as a function of the time.

To check the pH, a mixture of 0.5 ml of a solution of (I) or (II) and 0.5 ml of buffer was kept in the thermostat for 15 min and the pH was measured on the LPU-01 potentiometer adjusted with a solution of potassium hydrogen phthalate at the corresponding temperature.

Conclusions

1. The rate of acid hydrolysis of uridine diphosphate glucose as functions of the pH and the temperature is expressed by the equation

$$\log k\,(\text{min}^{-1}) = 25.45 - \text{pH} - \frac{8300}{T}.$$

2. The expression for the rate constant as functions of the pH and the temperature that has been obtained is in harmony with the idea that the acid hydrolysis of uridine diphosphate glucose takes place as a monomolecular substitution in its monoanion.

3. The replacement of the uridine residue by a 3-N-methyluridine residue in the molecule of a nucleoside diphosphate sugar does not lead to an appreciable change in the rate of acid hydrolysis.

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