THE EFFECT OF INDIVIDUAL OH-GROUPS IN MONODEOXY-α-D-GLUCOPYRANOSYL PHOSPHATES ON THEIR HYDROLYSIS RATES IN ACID MEDIUM

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2-Deoxy-, 3-deoxy-, 4-deoxy- and 6-deoxy-D-glucopyranose 1-phosphates, labelled with tritium in the corresponding deoxy positions, were prepared and their hydrolysis rates in an acid medium were measured. The reactivity of the above derivatives increases in the following order: α-D-glucopyranosyl phosphate, 6-deoxy-α-D-glucopyranosyl phosphate, 4-deoxy-α-D-glucopyranosyl phosphate, 3-deoxy-α-D-glucopyranosyl phosphate and 2-deoxy-α-D-glucopyranosyl phosphate. The different reactivity of the studied phosphates is explained by the effect of electronic and steric 1,2-gauche interactions in the glucose moiety on the activation energy, required for the formation of an activated complex *via* which the hydrolysis proceeds. Whereas the steric 1,2-gauche interactions affect the ground state of the reacting molecule, electronic interactions stabilise the activated complex.

In our previous communications we investigated the reactivity of uridine diphosphate glucose or its monodeoxyglucose analogues as substrates in transglycosylation reactions[1,2]. It was found that the reactivity decreases in the order UDPG, UDP2dGlc UDP6dGlc, UDP4dGlc and UDP3dGlc. These results are connected with the role of the hydroxyl groups in the transglycosylation reactions in the biosynthesis of $\alpha(1 \rightarrow 4)$glucane or α,α'-trehalose and we intend to compare them with the effect of hydroxyl groups of the glucose moiety in glucosyl phosphate, nucleotide or oligosaccharides on the reactivity of these compounds in substitution reactions which take place at the $C_{(1)}$ atom of glucose. The hydrolysis rate is an important characteristic of these compounds. Holló and coworkers[3] found that 6-deoxy-α-D-glucopyranosyl phosphate is less stable to acids than α-D-glucopyranosyl phosphate. Acidolability of oligosaccharides containing 2-deoxy-D-glucose was observed by Weideman and coworkers[4], as well as by Zemek and coworkers[5]. The aim of our present paper is to investigate how the absence of an individual hydroxyl group in the glucose part of an α-D-glucopyranosyl phosphate* affects the rate of hydrolysis in an acid medium.

* Abbreviations used: Glc-1-P α-D-glucopyranosyl phosphate; 2dGlc-1-P 2-deoxy-α-D-glucopyranosyl phosphate; 3dGlc-1-P 3-deoxy-α-D-glucopyranosyl phosphate; 4dGlc-1-P 4-deoxy-α-D-glucopyranosyl phosphate; 6dGlc-1-P 6-deoxy-α-D-glucopyranosyl phosphate.

EXPERIMENTAL

Materials and Methods

Dipotassium α-D-glucopyranosyl phosphate, used in the experiments, was a Reanal (Budapest) product. The tritiated 2dGlc-1-P, 3dGlc-1-P, 4dGlc-1-P and 6dGlc-1-P were prepared by a modification of the procedure used for the synthesis of non-labelled derivatives[6,7]. The starting [2-^{3}H]-2-deoxy-D-glucose, [3-^{3}H]-3-deoxy-D-glucose, [4-^{3}H]-4-deoxy-D-glucose and [6-^{3}H]--6-deoxy-D-glucose were prepared according to Soukupová and coworkers[8]. Acetylation of the mentioned labelled sugars with acetic anhydride in the presence of anhydrous sodium acetate as catalyst afforded β-peracetyl derivatives of the corresponding hexoses. Phosphorylation of these acetyl derivatives with crystalline H_3PO_4 was carried out following the method described previously[6,7]. The individual fractions were measured on a Packard liquid scintillator 3330, using an SLX-31 scintillation liquid (Tesla, Czechoslovakia). Simultaneously, acid-labile phosphorus was determined in the fractions. Fractions, containing α-anomers (Fig. 1) of the particular D-glucopyranosyl phosphates, were combined, concentrated and obtained in the form of triethylammonium salts (Table I).

Hydrolysis

a) α-D-*Glucopyranosyl phosphate*: A mixture of 1 mg of Glc-1-P (di-K salt) and 1 ml of 0·005M--H_2SO_4 was kept at 38°C and 100 µl aliquots were withdrawn at appropriate intervals. The hydrolysis was followed colorimetrically at 750 nm by molybdenum reagent[9], using a Spekol (Carl Zeiss, Jena) instrument.

b) *Deoxy-α-D-glucopyranosyl phosphate*: Aliquots (10 µl) were taken at appropriate time intervals from reaction mixtures, containing 0·1 mg of triethylammonium salt of 2dGlc-1-P, 3dGlc-1-P, 4dGlc-1-P, or 6dGlc-1-P (labelled in the corresponding positions) in 0·1 ml of 0·005M--H_2SO_4, neutralized with aqueous ammonia solution and chromatographed on a paper Whatman 3MM. After 16 hours' developing in an ethyl acetate–2-propanol–water mixture (12 : 4 : 1) the deoxy hexoses, liberated by hydrolysis, were separated whereas their phosphates remained at the start. The areas at the start, corresponding to the deoxy derivatives of α-D-glucopyranosyl phosphates, were cut off and their radioactivity was measured on a Packard 3330 instrument, using the scintillation liquid SLX-31.

TABLE I

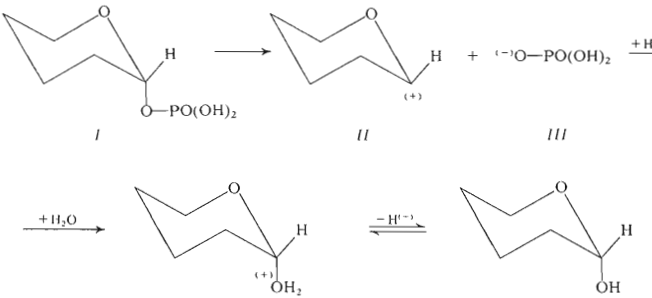
Physical Constants and Hydrolysis Rates of α-D-Glucopyranosyl Phosphate and Its Deoxy Analogues

Compound	Labelled	$[\alpha]_D$	$[\alpha]_{Dlit}$	Specific activity of phosphate µCi mmol^{-1}	Starting specific activity µCi mmol^{-1}	Hydrolysis rate $k \cdot 10^3$ min^{-1}
Glc-1-P	—	78·0	78·0	—	—	7·10
2dGlc-1-P	[2-^{3}H]	48·6	45·9	0·0003	0·0004	61·72
3dGlc-1-P	[3-^{3}H]	53·8	61·9	0·00017	0·00022	38·38
4dGlc-1-P	[4-^{3}H]	69·3	7·25	0·0005	0·0007	23·92
6dGlc-1-P	[6-^{3}H]	74·8	71·8	0·00023	0·00032	9·72

The kinetics of hydrolysis of Glc-1-P and its monodeoxyglucose analogues were measured and the rate constants, which were evaluated graphically (Fig. 2), are given in Table I.

RESULTS AND DISCUSSION

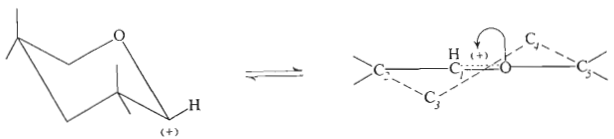
The hydrolysis of α-D-glucopyranosyl phosphate in the region pH 1–8 proceeds *via* two concurrent reactions: either *via* a monoanion with cleavage of the P—O bond (S_N2-mechanism) or *via* a neutral form with cleavage of the C—O bond (S_N1-mechanism), the latter path being preferred in the region of pH 2 (Scheme 1) (ref.[10]).



I *II* *III* *IV* *V*

SCHEME 1

The rate determining step in the hydrolysis of phosphates is formation of the oxoniocarbonium intermediate *II* and its stabilisation which is accompanied by a conformational change (Scheme 2). The change of $^4C_1$ conformation of the intermediate



$^4C_1$ $^4H_3$

SCHEME 2

II into the $^4H_3$ conformation, which involves the path through an energy barrier, depends on steric "1,2-gauche" and electronic interactions. The steric 1,2-gauche interactions between vicinal hydroxyl groups in the *syn*-clinal conformations of the

carbons $C_{(2)}$, $C_{(3)}$, $C_{(4)}$ and $C_{(5)}$ in glucopyranose tend to stabilise the starting glucosyl phosphate. These interactions become weaker during the conformational change of the intermediate *II* as a result of opening the dihedral angle between the corresponding bonds. The energy, required for this, also increases the activation energy of the hydrolysis. The electronic interactions, caused by the inductive effects of the hydroxyl groups in the vicinity of the pyranose $C_{(1)}$ atom, tend to destabilise the intermediate *II* because they increase the positive charge on the $C_{(1)}$ atom in glucose; this manifests itself by a higher activation energy of hydrolysis.

The effects of the electronic and steric interactions in molecules of the individual glucosyl phosphates are additive quantities and their sum affects the activation energy required for the formation of the intermediate *II*. As seen from our results, the rate of the acid hydrolysis of α-D-glucopyranosyl phosphates decreases in the following order:

$$2dGlc\text{-}1\text{-}P > 3dGlc\text{-}1\text{-}P > 4dGlc\text{-}1\text{-}P > 6dGlc\text{-}1\text{-}P > Glc\text{-}1\text{-}P\,.$$

The difference in the reactivity of the phosphates Glc-1-P, 6dGlc-1-P and 4dGl-1-P can be explained by different steric 1,2-gauche interactions, since the electronic interactions in these compounds are the same (the presence of OH groups on $C_{(2)}$ and $C_{(3)}$). Whereas in the case of Glc-1-P the activation energy of hydrolysis is

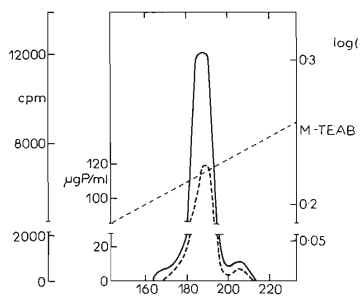


FIG. 1

Separation of the Mixture obtained by Phosphorylation of [6-^{3}H]-1,2,3,4-Tetra-O-acetyl--6-deoxy-β-D-glucose

——— Radioactivity, ····· phosphorus content.

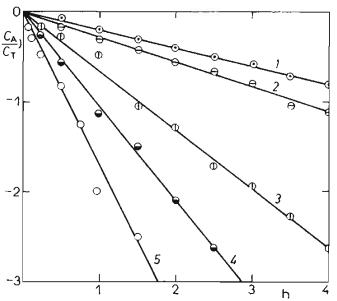


FIG. 2

Hydrolysis of 1-Phosphates of Monodeoxy-α-D-glucopyranoses at 38°C in 0·005M--H_2SO_4: Graphical Evaluation of the Results

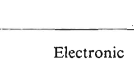
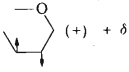
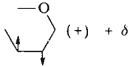
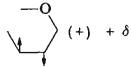
1 Glc-1-P, *2* 6dGlc-1-P, *3* 4dGlc-1-P, *4* 3dGlc-1-P, *5* 2dGlc-1-P.

increased by three 1,2-gauche interactions (between $C_{(2)}$—OH and $C_{(3)}$—OH, between $C_{(3)}$—OH and $C_{(4)}$—OH, and between $C_{(4)}$—OH and $C_{(5)}$—CH_2OH), in the more reactive 6dGlc-1-P this activation energy is increased only by two 1,2-interactions (between $C_{(2)}$—OH and $C_{(3)}$—OH and between $C_{(3)}$—OH and $C_{(4)}$—OH), and in 4dGlc-1-P by only one 1,2-interaction (between $C_{(2)}$—OH and $C_{(3)}$-OH) (Table II).

The slower hydrolysis of Glc-1-P, 6dGlc-1-P, 4dGlc-1-P and 3dGlc-1-P relative to 2dGlc-1-P can be explained by a primary inductive effect caused by the presence of the hydroxyl group on the $C_{(2)}$ atom of the glucose part of the molecule. The lower hydrolysis rate found for 4dGlc-1-P (or 6dGlc-1-P or Glc-1-P) as compared with the rate for 3dGlc-1-P is explained by a weaker secondary inductive effect caused by the hydroxyl group on the $C_{(3)}$ atom. In both 3dGlc-1-P and 4dGlc-1-P there is only one 1,2-gauche interaction (Table II).

TABLE II

Interactions in Derivatives of α-D-Glucopyranosyl Phosphate

Compound	Steric 1,2-gauche	Electronic
Glc-1-P	$C_{(2)} \rightarrow$ OH a $C_{(3)} \rightarrow$ OH $C_{(3)} \rightarrow$ OH a $C_{(4)} \rightarrow$ OH $C_{(4)} \rightarrow$ OH a $C_{(5)} \rightarrow CH_2OH$	 (+) + δ⁺ + δδ⁺
[2-^{3}H] 2dbGlc-1-P	$C_{(3)} \rightarrow$ OH a $C_{(4)} \rightarrow$ OH $C_{(4)} \rightarrow$ OH a $C_{(5)} \rightarrow CH_2OH$	 (+) + δ⁺ + δδ⁺
[3-^{3}H] 3dbGlc-1-P	$C_{(4)} \rightarrow$ OH a $C_{(3)} \rightarrow CH_2OH$	 (+) + δ⁺ + δδ⁺
[4-^{3}H] 4dbGlc-1-P	$C_{(2)} \rightarrow$ OH a $C_{(5)} \rightarrow$ OH	 (+) + δ⁺
[6-^{3}H] 6dbGlc-1-P	$C_{(2)} \rightarrow$ OH a $C_{(3)} \rightarrow$ OH $C_{(3)} \rightarrow$ OH a $C_{(4)} \rightarrow$ OH	 (+) + δδ⁺

It follows from the observed hydrolysis rates of the studied α-D-glucopyranosyl phosphates that electronic interactions affect the reactivity of the phosphates more than the steric interactions do. This explains the not very marked differences between the reactivity of Glc-1-P, 6dGlc-1-P or 4dGlc-1-P on the one hand and 3dGlc-1-P or 2dGlc-1-P on the other hand.

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REFERENCES

1. Zemek J., Kučár Š., Bauer Š.: Eur. J. Biochem. *40*, 195 (1973).
2. Zemek J., Strmeň J., Kučár Š., Bauer Š.: Eur. J. Biochem. *64*, 283 (1976).
3. Holló J., László E., Hoschke Á.: *Plant*, α-1,4-*Glucan Phosphorylase*, p. 93. Akadémia Kiadó, Budapest 1971.
4. Weideman G., Fischer W.: Z. Physiol. Chem. *342*, 238 (1965).
5. Zemek J., Bauer Š.: Biochim. Biophys. Acta *264*, 393 (1972).
6. Shibayev V. N., Kusov J. J., Kučár Š., Kochetkov N. K.: Izv. Akad. Nauk SSSR Ser. Khim. *1973*, 430.
7. Shibayev V. N., Kusov J. J., Kučár Š., Kochetkov N. K.: Izv. Akad. Nauk SSSR Ser. Khim. *1973*, 922.
8. Soukupová V., Černý M., Vereš K.: VII. Intern. Symp. on Carbohydrate Chem., Bratislava 1974, Abstract p. 50.
9. Lowry O. H., Lopez J. A.: J. Biol. Chem. *162*, 421 (1946).
10. Bunton C. A., Llewelyn D. R., Oldham K. G., Vernon C. A.: J. Chem. Soc. *1958*, 3588.

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