Note

Ionization constants of sugars: a predominant factor in the cyanogen-induced phosphorylation of sugars

CH. DEGANI
Isotope Department, The Weizmann Institute of Science, Rehovot (Israel)
(Received November 3rd, 1970; accepted for publication, November 30th, 1970)

Cyanogen-induced phosphorylation has been suggested as a simple method for synthesizing aldose 1-phosphates[1,2]. By this method, phosphorylation of a free sugar with orthophosphate is carried out in aqueous solutions (pH 6.7–8.8) in the presence of cyanogen. Reducing mono- and di-saccharides are readily phosphorylated by this method, whereas polyhydroxy compounds (*e.g.*, glycerol) or non-reducing sugars (*e.g.*, sucrose and trehalose) fail to undergo phosphorylation (see Table I). Furthermore, the presence of a free-hemiacetal hydroxyl group, although necessary, is not sufficient for a successful phosphorylation; thus, 2-deoxy-D-*erythro*-pentose is inactive towards phosphorylation[1] (see Table I).

These experimental facts suggested that phosphorylation of reducing sugars might be controlled by the acidity of the hemiacetal hydroxyl group. In order to find a possible correlation between the pK value of a sugar and its capability to undergo the phosphorylation reaction, the ionization constants of several sugars were measured.

Ionization constants of D-glucose, D-ribose, 2-deoxy-D-*erythro*-pentose, D-arabinose, L-arabinose, D-xylose, and L-xylose were determined by potentiometric titration[3]. The pK values are presented in Table I, together with some literature data. The values obtained by us for pentoses are in good agreement (except in the case of arabinose) with those obtained by the entropy titration procedure[4]. In the case of ribose and glucose, measurements were also made at constant ionic strength ($\mu = 0.1$), and the results were similar to those obtained without maintaining constant ionic strength. As shown in Table I, there is a distinct correlation between the pK values of sugars and their tendency to undergo the phosphorylation reaction. Thus, arabinose, which has a higher pK value than either glucose or xylose, is phosphorylated to a smaller extent (6% yield) than the other two sugars (*ca.* 20% yield). 2-Deoxy-D-*erythro*-pentose, having a still higher pK value, completely fails to undergo phosphorylation[1].

The mechanism suggested for the preferential phosphorylation of the hemiacetal hydroxyl group is shown in Fig. 1.

The cyclic mechanism clarifies the importance attributed to the acidity of the hemiacetal hydroxyl group. The required activation process of the phosphorylation agent cyano(imino)methyl phosphate is accomplished by protonation by means of the

TABLE I

IONIZATION CONSTANTS OF SUGARS IN RELATION TO THEIR REACTIVITY TOWARDS CYANOGEN-INDUCED PHOSPHORYLATION

Sugar	*Temperature (degrees)*	*pK[a], Method of ionization-constant determination*				*Extent (%) of phosphorylation reaction[n]*
		Conductimetry	*Potentiometric titration*	*Thermometric titration*	*Mutarotation studies*	
D-Arabinose	25		12.43 ±0.03	(12.54 ±0.04)[c]		6
	17–18		(12.43)[d]			
L-Arabinose	25		12.41 ±0.03			
D-Xylose	25		12.29 ±0.04	(12.29 ±0.03)[c]		23
L-Xylose	25		12.29 ±0.04			
D-Ribose	25		12.21 ±0.04	(12.22 ±0.04)[c]		20
			12.19 ±0.03[b]			
2-Deoxy-D-*erythro*-pentose			12.65 ±0.04	(12.67 ±0.04)[c]		negative
D-Glucose	25	(12.11)[f]	12.35 ±0.04	(12.46 ±0.05)[c]	(12.34)[h]	23
			12.34 ±0.04[b]		(12.46; 12.17)[i]	
			(12.35 ±0.04)[e]			
		(12.38)[g]	(12.34)[g]			
	23	(12.22)[j]				
	20		(12.09)[k]			
	18		(12.43)[l]		(12.43)[h]	
			(12.24)[m]			
	17–18		(12.18)[d]			
Lactose	25	(11.98)[f]				positive
	23	(12.12)[j]				
	20		(11.99)[k]			
	17–18		(12.22)[d]			
Maltose	25	(11.94)[f]				positive
	23	(12.07)[j]				
	20		(11.93)[k]			
	17–18		(11.74)[d]			
Sucrose	25	(12.51)[f]	(12.62)[d]			negative
	23	(12.7)[j]				
	20		(12.43)[k]			
Glycerol	25	(14.4)[n]				negative
	17–18		(14.15)[d]			

[a]Values in parentheses are collected from the literature. [b]Measurements were performed at constant ionic strength (μ = 0.1). [c]Ref. 4. [d]Ref. 6. [e]Ref. 7, μ = 0.05. [f]Ref. 8. [g]Ref. 9. [h]Ref. 10, μ = 0.05. [i]Ref. 11. The first value is for the α-D-anomer and the second for the β-D-anomer. [j]Ref. 12. [k]Ref. 18. [l]Ref. 3.

relatively acidic hydrogen of the hemiacetal hydroxyl group. Protonation of the imino nitrogen atom makes it electron deficient and thus causes increasing electron-withdrawal from the adjacent phosphorus atom which favours a simultaneous nucleophilic attack by the hemiacetal oxygen atom.

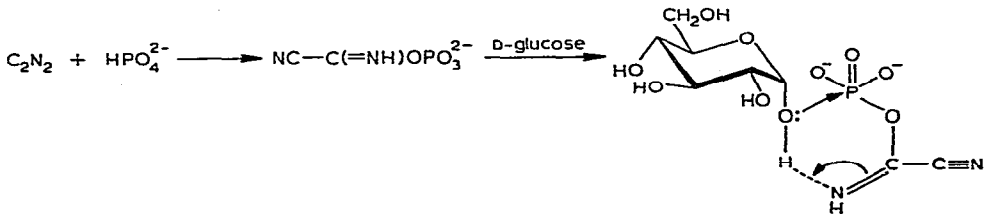

Fig. 1. Mechanism for cyanogen-induced phosphorylation of a hemiacetal group of a sugar.

The acidity of the hemiacetal group is explained by the combined inductive effects of the ring oxygen atom and the hydroxyl groups which are both electron-attracting and exhibit negative inductive effects ($-I$). The remarkably high pK value of 2-deoxy-D-*erythro*-pentose, relative to that of D-ribose, is apparently due to the absence in the former compound of an hydroxyl group at the position α to the hemiacetal group. A similar effect on the ionization of an acidic group exerted by an α-hydroxyl group is found in the case of propionic and lactic acids. The former acid, which lacks an α-hydroxyl group, has a pK value of 4.87, whereas the latter has a pK value of 3.86[5a].

EXPERIMENTAL

D-Arabinose, L-arabinose, D-ribose (Mann), L-xylose (Calbiochem, B Grade), and 2-deoxy-D-*erythro*-pentose (Sigma) were used without further purification. D-Glucose (British Drug House) and commercial D-xylose (Eastman–Kodak) were purified according to Isbell and Wade[16].

Ionization constant measurements. — All pH measurements were made with a Radiometer pH meter TTTla equipped with a scale expander and a special Radiometer glass-electrode GK 2025B for the region above pH 12. An accuracy of ± 0.005 pH unit was achieved. The pH meter was calibrated with the following buffer solutions: (*1*) Beckman buffer solution, pH 10; and (*2*) 10mM trisodium phosphate buffer [5b] [Na_2HPO_4 (1.419 g) + 100 ml of 0.1M sodium hydroxide in 1 litre of water], pH 11.72. Titrations were carried out in a thermostated beaker (± 0.1)°. A slow stream of argon was continuously bubbled through the titrated solution. This provided efficient stirring as well as an inert atmosphere, which was essential for preventing the presence of carbon dioxide and for avoiding the oxidation of sugars under basic conditions. Care was taken to exclude atmospheric carbon dioxide from all solutions, which were made up from ion-free, degassed water.

Titrations were carried out with potassium hydroxide, which is preferable to sodium hydroxide in potentiometric titrations, because a higher ratio of potassium to hydrogen ions can be permitted before accuracy is affected[5c].

For pH measurements, solutions (total volume, 2 ml) were prepared by mixing four different amounts of 0.1M potassium hydroxide with a stock solution of a sugar. All measurements were performed at least twice, and each sugar was titrated at three different molar concentrations (0.1, 0.06, 0.04).

Calculations of the ionization of sugars (K_s) were made according to the following equation[3]:

$$K_s = A \cdot f_{OH^-} \cdot a_{H^+}/(C_{glucose} - A), \qquad \text{where,}$$
$$A = \{C_{KOH} - [K_{H_2O}/(f_{OH^-} \cdot a_{H^+})]\}.$$

C_{KOH} and $C_{glucose}$ are the molar concentrations of potassium hydroxide and D-glucose respectively; K_{H_2O} is the ionic product of water at 25° (1.01×10^{-14}, Ref. 5*d*); a_{H^+} is the potentiometrically measured, hydrogen-ion activity; and f_{OH^-} is the activity coefficient of the hydroxyl ion determined by measuring the pH of a pure solution of potassium hydroxide of known concentration[3] (C_{KOH}). Calculations were performed by using an Olivetti Programma 101 computer.

ACKNOWLEDGMENTS

The author thanks Professor M. Halmann for helpful discussions, and Mr. K. Lifschitz for competent technical assistance. This work was supported by the Israel Academy of Sciences and Humanities.

REFERENCES

1 M. HALMANN, R. A. SANCHEZ, AND L. E. ORGEL, *J. Org. Chem.*, 34 (1969) 3702.
2 CH. DEGANI AND M. HALMANN, *J. Chem. Soc.*, (1971) in press.
3 J. THAMSEN, *Acta Chem. Scand.*, 6 (1952) 270.
4 R. M. IZATT, J. H. RYTTING, L. D. HANSEN, AND J. J. CHRISTENSEN, *J. Amer. Chem. Soc.*, 88 (1966) 2641.
5 A. ALBERT AND E. P. SERJEANT, *Ionization Constants*, Wiley, 1962, (*a*) p. 124; (*b*) p. 19; (*c*) p. 17; (*d*) p. 171.
6 L. MICHAELIS AND P. RONA, *Biochem. Z.*, 49 (1913) 232.
7 G. GUILLOT AND P. RUMPE, *Compt. Rend.*, 259 (1964) 4064.
8 P. HIRSCH AND R. SCHLAGS, *Z. Physik Chem. Abt.*, A 141 (1929) 387.
9 C. A. BUNTON AND H. CHAIMOVICH, *J. Amer. Chem. Soc.*, 88 (1966) 4082.
10 G. KILDE AND W. F. K. WYNNE-JONES, *Trans. Faraday Soc.*, 49 (1953) 243.
11 J. M. LOS AND L. B. SIMPSON, *Rec. Trav. Chim.*, 76 (1957) 267.
12 A. E. STEARN, *J. Phys. Chem.*, 35 (1931) 2226.
13 S. Z. IVANOV AND E. S. LIGIN, *Zh. Prikl. Khim.* (Leningrad), 41 (1968) 2722.
14 H. T. S. BRITTON, *J. Chem. Soc.*, (1925) 1896.
15 P. BALLINGER AND F. A. LONG, *J. Amer. Chem. Soc.*, 82 (1960) 795.
16 H. S. ISBELL AND C. W. R. WADE, *J. Res. Nat. Bur. Stand.*, 71A (1967) 137.