Carbohydrate Research, 36 (1974) 231–232

Note

Acidities of the anomeric D-glucopyranosyl phosphates*

EDWARD J. BEHRMAN
Department of Biochemistry, The Ohio State University, Columbus, Ohio 43210 (U. S. A).
(Received March 29th, 1974; accepted May 1st, 1974)

Prihar and Behrman[1] showed that five pairs of anomeric glycosyl phosphates were separable by paper electrophoresis at pH values in the vicinity of the second dissociation constants. No separation was observed at higher or lower pH values. These results suggested[2] that the anomeric pairs differ slightly in their pKa_2 values, thus leading to differences in the fraction ionized and hence to different net mobilities at pH values in the vicinity of the pKa_2. It was further observed that the β-anomer migrated more rapidly in each case, from which result it was concluded that the β-anomer was the more acidic. This conclusion also rationalizes the observed separations of several pairs of anomeric glycosyl phosphates by anion-exchange chromatography. In each case for which separation was observed, the α-anomer migrated the more rapidly[3]. The only previous report I have found of the measurement of pKa'_2 values for a pair of anomeric glycosyl phosphates is the study by O'Brien[4] on the 2-acetamido-2-deoxy-D-glucopyranosyl phosphates. Although he reported identical values, his successful separation of the anomers by anion-exchange chromatography suggests differences in the dissociation constants.

TABLE I

ACIDITIES OF THE ANOMERIC D-GLUCOPYRANOSYL PHOSPHATES

Anomer	pKa'_2	pKa_2[a]
alpha	6.22 ±0.04	6.46 ±0.04
beta	6.00 ±0.02	6.24 ±0.02

[a] pKa values were calculated by using the ionic-strength correction term, $3AI^{1/2}/1+I^{1/2}$, given by Ashby *et al.*[5]. A control titration of the α-anomer in the presence of cyclohexylamine hydrochloride (which was present during the titration of the β-anomer) gave $pKa_2 = 6.47$.

The results reported here (Table I) confirm our supposition concerning the relative acidities of the anomeric phosphates. The pKa_2 value, 6.46, for α-D-gluco-

---

*Supported in part by NIH Grant GM-20002. I thank G. Piazza for his help in this investigation.

pyranosyl phosphate is in reasonable agreement with the highly precise value, 6.50, determined by Ashby *et al.*[6] and by Trevelyan *et al.*[7].

EXPERIMENTAL

α-D-Glucopyranosyl dipotassium phosphate dihydrate was obtained from the Sigma Chemical Co., $[\alpha]_D^{25}$ +76.9 ±1° (*c* 7, water) lit.[8] $[\alpha]_D^{25}$ +78.5°. *β*-D-Glucopyranosyl phosphate was prepared by the method of MacDonald[9]. The di(cyclohexylammonium) salt crystallized as the trihydrate; $[\alpha]_D^{25}$ +7.8 ±1° (*c* 5, water). Calc. for $C_{18}H_{39}N_2O_9P \cdot 3H_2O$: C, 43.68; H, 8.69. Found: C, 43.79; H, 8.85 (Het-Chem. Co.). The trihydrate has been previously reported[1], $[\alpha]_D^{25}$ +4.1°, as well as the anhydrous salt[9], $[\alpha]_D^{24}$ +5.9°, the salt containing one molecule each of water and ethanol[10], $[\alpha]_D^{26}$ +7.3°, and the monohydrate[11], $[\alpha]_D^{20}$ +5.4°.

Determinations of pKa were made according to the potentiometric procedure outlined by Albert and Serjeant[12] by using 104mM solutions of the glucose phosphates in water at 25°. The titrant was 104mM hydrochloric acid. Measurements of pH were made by using a Beckman Research pH meter equipped with an A. H. Thomas combination electrode, pH 0–12, 0–75°, type 4858-Q15, Ag–AgCl reference.

REFERENCES

1 H. S. Prihar and E. J. Behrman, *Biochemistry*, 12 (1973) 997.
2 G. Zweig and J. R. Whitaker, *Paper Chromatography and Electrophoresis*, Academic Press, New York, 1967, Vol. 1, p. 5.
3 D. L. MacDonald, *Carbohyd. Res.*, 6 (1968) 376 and references therein.
4 P. J. O'Brien, *Biochim. Biophys. Acta*, 86 (1964) 628.
5 J. H. Ashby, E. M. Crook, and S. P. Datta, *Biochem. J.*, 56 (1954) 205.
6 J. H. Ashby, H. B. Clarke, E. M. Crook, and S. P. Datta, *Biochem. J.*, 59 (1955) 203.
7 W. E. Trevelyan, P. F. E. Mann, and J. S. Harrison, *Arch. Biochem. Biophys.*, 39 (1952) 419.
8 M. L. Wolfrom and D. E. Pletcher, *J. Amer. Chem. Soc.*, 63 (1941) 1050.
9 D. L. MacDonald, *Carbohyd. Res.*, 3 (1966) 117.
10 E. W. Putman and W. Z. Hassid, *J. Amer. Chem. Soc.*, 79 (1957) 5057.
11 L. V. Volkova, L. L. Danilov, and R. P. Evstigneeva, *Carbohyd. Res.*, 32 (1974) 165.
12 A. Albert and E. P. Serjeant, *The Determination of Ionization Constants*, Chapman and Hall, London, 1971, Chapter 2.