Carbohydrate Research, 127 (1984) 165–169
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Increase in the acyclic form of sugars in the presence of borate ions, as measured by circular dichroism*

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(Received March 1st, 1983; accepted for publication in revised form, July 2nd, 1983)

The proportion of acyclic forms in a tautomeric equilibrium[1–6] of reducing sugars is of considerable practical interest in static and dynamic sugar–protein interactions, as many chemical and biochemical processes occur through the acyclic forms of these sugars. For instance, in formation of Schiff bases between proteins and sugars, the availability of the carbonyl groups of the acyclic form is a rate-limiting step, and by using reaction conditions conducive to the formation of the acyclic form, an increase in the rate of reaction may thus be anticipated. This is especially true for the reductive amination procedure leading to the preparation of neoglycoproteins[7,8].

This report describes how, by the addition of borate ions to solutions of reducing sugars, the proportion of their acyclic forms may be increased substantially. Further increases in the acyclic forms may also be realized by simultaneously raising the temperature of the borate solutions and by making the solutions more alkaline. Hayward and Angyal[9] had previously reported on the increase in the content of acyclic form of reducing sugars with increasing temperature.

Because of the minute proportions of acyclic forms present ($\leq 0.5\%$ of the total concentration of sugar), we used circular dichroism (c.d.) as the preferred method of measuring the quantities of carbonyl-related forms present in the sugar solutions. This method was also used by others[9–11] and by Hayward and Angyal[12], who demonstrated that the intensity of a weak Cotton effect observed by c.d. spectroscopy is related to the proportion of open-chain forms (aldehydes and ketones) in the tautomeric equilibrium of reducing sugars, and established a symmetry rule for the sugars based on the observations that the sign of $n \rightarrow \pi^*$ circular dichroism band of the carbonyl group of the reducing sugar is dependent on the adjacent chiral center. They also proposed that an approximate calculation of the percentage concentration of the carbonyl form of sugars could be made, provided several assumptions were made.

*NRCC No. 22927.

0008-6215/84/$ 03.00

TABLE I

THE DEPENDENCE OF THE CARBONYL-GROUP[a] CIRCULAR DICHROISM OF REDUCING SUGARS ON BUFFER (0.2M, pH 8.0) AND TEMPERATURE

Sugar	*λ (nm)*	*Configuration at the α-carbon atom*	*Δε × 10³* [b]			
			Phosphate[c]		*Borate*[d]	
			25°	*50°*	*25°*	*50°*
D-Ribose	285	*R*	−1.41[e]	−3.71[e]	−0.333	−1.41
D-Arabinose	288	*S*	+0.386	+1.36	+0.417	+1.48
D-Glucose	290	*R*	−0.012	−0.108	−0.109	−0.424
D-Galactose	290	*R*	−0.174	−0.758	−0.515	−1.516
D-Mannose	292	*S*	+0.109[e]	−0.303[e]	+0.235	+0.834
D-Fructose	275	*S*	+9.70	+29.9	+7.28	+23.4
L-Fucose	290	*S*	+0.058	+0.326	+0.174	+0.652
L-Rhamnose	295	*R*	−0.045[e]	−0.159[e]	−0.326	−0.940
Lactose, pH 8.0	293	*R*	+0.009	−0.039	−0.079	−0.349
Lactose, pH 9.0	293	*R*	−0.006	−0.052	−0.310	−1.02
β-D-Gal*p*-(1→3)-D-Ara	285	*S*	+0.237	+0.849	+0.237	−0.849

[a]The percentage of carbonyl form may be approximated from an estimated, maximum dichroic-extinction coefficient of unity for the pure carbonyl form, using the equation % carbonyl = $\Delta\varepsilon \times 100$. See ref. 12. [b]Values accurate to ±0.01. [c]Sodium phosphate. [d]Sodium tetraborate. [e]A shift of ~7 nm to lower wavelengths was observed.

The c.d. coefficients ($\Delta\varepsilon$) of various reducing sugars at a concentration of 0.2M in phosphate buffer (0.2M, pH 8.0) shown in Table I, agree well, within the accuracy of the method, with those obtained by Hayward and Angyal[12] in pure water; D-ribose, D-mannose, and L-fucose being the only exceptions. The previously deduced symmetry rule was also confirmed in all of our results obtained with monosaccharides and disaccharides. Changes in temperature and buffer anion were made and, from the data in Table I, the variation of $\Delta\varepsilon$ with temperature in both phosphate and borate buffer indicated a general increase in the content of carbonyl form of the sugars on raising the temperature from 25 to 50°. This phenomenon was previously observed[12] for sugars in aqueous solution using c.d. spectroscopy and on D-fructose using ^{13}C-n.m.r.[13], and is probably due to the alleviation of steric interactions at higher temperatures. At constant temperature, with the exception of D-ribose and D-fructose, a further substantial increase in the acyclic form of the sugars was also achieved by changing the buffer from phosphate to borate. Presumably the acyclic forms of these sugars are stabilized by the formation of borate complexes.

The effects of boric acid and borate ions on carbohydrates[14–18] have been studied extensively, and the nature of some of the cyclic complexes formed has

been established by ^{11}B-, ^{1}H-, and ^{13}C-n.m.r. spectroscopy[19,20]. Pertinent to our study is that, from observed changes in optical rotation, it was deduced[18] that cyclic complexes are formed between borate ion and reducing sugars, which causes a shift in the equilibrium state. However, the possibility of the contribution of acyclic-sugar borate complexes in the equilibrium mixtures was not mentioned by these authors.

In the aldohexose series, the $\Delta\varepsilon$ value determined in phosphate buffer varied in the order D-glucose < D-mannose < D-galactose. This order also remained unchanged in borate buffer. However, the change of buffer at 25° resulted in an increase of open-chain forms of 9.1× for D-glucose, 2.9× for D-galactose, and 2.2× for D-mannose. This order of increase in the presence of borate anions (namely, D-glucose > D-galactose > D-mannose) was supported by a recent study[21] by pH measurements depicting the first and second stability-constants of various polyols and related aldoses with borate ions. This study presented evidence that the order of stability of aldoses with borate anions was D-glucose > D-galactose > D-mannose. With the knowledge that free-energy differences are small for the acyclic forms, that the more-stable rotamers are those in the planar, zigzag conformation, and that 1,3-parallel interactions increase free-energy by forming a sickle conformation, it is possible to deduce that acyclic D-glucose would probably be the least stable of the three isomers in aqueous solution[22,23], but the most stable in the presence of complexing borate ions. On the other hand, the increase of open-chain forms resulting from changing the buffer at 50° was lower and for each sugar more nearly equal (namely, 2.4×). A plausible explanation for this phenomenon is that at higher temperatures the barrier of energy of rotation of each of the sugars becomes equivalent.

For the two 6-deoxyaldohexoses, both L-fucose and L-rhamnose experienced an increase in $\Delta\varepsilon$ on changing the buffer from phosphate to borate at both 25 and 50°, the $\Delta\varepsilon$ value of L-rhamnose in borate being double that of L-fucose. As the reverse situation occurs in phosphate buffer, it may be concluded that the acyclic form of L-rhamnose has a higher borate-binding capacity than the acyclic form of L-fucose. Interestingly the effect of borate on the c.d. absorption of the 6-deoxyaldohexoses is greater than that exhibited by their related aldohexoses (D-galactose and D-mannose), indicating that the primary hydroxyl group on C-6 is not a requirement for the observed influence of borate ion on the acyclic forms of these sugars.

Of the two pentoses investigated, D-ribose would be expected to give a larger increase of $\Delta\varepsilon$ in the presence of borate ions than D-arabinose on the basis of its supplementary 1,3-parallel interaction in the acyclic form. However, whereas borate ions had essentially no effect on D-arabinose, or on its corresponding disaccharide β-D-Gal*p*-(1→3)-D-Ara, they caused an unexpected and at present inexplicable decrease in the $\Delta\varepsilon$ value of D-ribose. D-Ribose also proved unusual in that it was one of the sugars having a higher carbonyl content in 0.1M phosphate buffer at 25° than it did in aqueous solution.

The enhancement of the acyclic form of the reducing end-group D-glucose re-

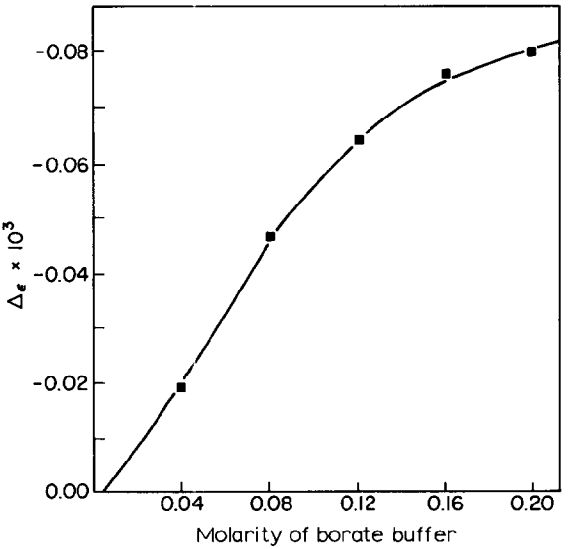

Fig. 1. The effect of changing the molarity of borate ion on the $\Delta\varepsilon_{290}$ value of lactose (0.2M, pH 8.0, 25°). Ion molarities were maintained at 0.2M by replacement of borate ions by phosphate ions.

sidue of lactose was similar to that observed with D-glucose itself, and, as in the case of D-glucose, a fourfold increase in $\Delta\varepsilon$ was observed when the temperature of the borate solution was raised from 25 to 50°. In addition, by changing the alkalinity of the borate solution from pH 8.0 to 9.0 at both 25 and 50°, a further, significant increase in the open-chain form of lactose was realized. This evidence is consistent with the results of recent ^{13}C-n.m.r. studies[13], from which it was postulated that the proportion of carbonyl forms in aqueous solutions of some hexoses and ketoses increases with increasing alkalinity. The $\Delta\varepsilon$ variation for solutions of lactose upon increasing the molarity of borate buffer is shown in Fig. 1. This result indicated that the percentage of the open-chain form of lactose increased rapidly with increasing molarity of borate, the rate of increase beginning to slow down considerably at 0.12M borate.

Finally, as it is possible that alkaline pH and borate ions might be responsible for a Lobry de Bruyn–Alberda van Ekenstein transformation[24–27], whereby D-glucose would mainly be transformed into D-mannose and D-fructose, an experiment was performed to establish that this process had not occurred to an observable extent. To rule out this possibility, a solution of D-glucose was deliberately contaminated with 1% (w/w) of D-fructose at a final concentration of 0.2M of the sugars in both buffers at pH 8.0 and 25°. The $\Delta\varepsilon$ values observed were positive in the phosphate buffer and negative in the borate buffer. A simple, linear combination* of the $\Delta\varepsilon$ values of both sugars would predict such results, because D-fructose has its chiral center adjacent to the carbonyl group in the (*S*) configuration, which gives

*$\Delta\varepsilon_{obs} = (0.99)\,\Delta\varepsilon_{Glc} + (0.01)\,\Delta\varepsilon_{Fru}$.

a positive $\Delta\varepsilon$ values, whereas D-glucose, having the (*R*) configuration at C-2, has a weak, negative value.

EXPERIMENTAL

Materials and procedures. — The reducing sugars were the purest grade from Pfanstiehl, Waukegan, Illinois and were used as received, except for D-mannose and D-ribose, which were recrystallized according to published procedures[28,29]. The buffered solutions were allowed to equilibrate at room temperature for 24 h before the experiments were started. The c.d. spectra were measured on thermostated solutions in a calibrated spectropolarimeter[30] (Cary–Varian, model 61). Quartz cells having path lengths of 1 and 10 cm were used. Carbohydrate concentrations were 0.2M, unless noted otherwise.

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