Binding of Sugars to DNA. An NMR Study of D-Fructose

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The major tautomers of D-fructose in water are the β-pyranose and β-furanose forms, the former predominating. NMR studies have shown that when aqueous DNA is added, there is a shift in the β-pyranose ⇌ β-furanose equilibrium, the latter form being favoured. It is suggested that this is caused by weak, preferential binding of the β-furanose form to DNA. This is supported by computer modelling, which shows that for the former, only two hydrogen bonds can be formed between sugar O–H groups and phosphate units, whereas three good bonds can be formed with the latter.

Studies of the O–^{1}H resonances of the anomeric groups of the two forms confirm this change in equilibrium. In addition this feature for the β-furanose form shifts to low-field without significant broadening. This indicates that strong hydrogen bonding to DNA occurs, but that the equilibrium is very rapid giving fast averaging of the NMR signals.

Continuing our spectroscopic studies of the O–^{1}H resonances of simple sugars in aqueous solution,[1–5] and of the interactions of polycations with aqueous DNA,[6] we have looked for changes in the NMR spectra of aqueous monosaccharides in the presence and absence of duplex DNA (calf-thymus). In addition to the C–^{1}H and ^{13}C resonances, we selected the O–^{1}H proton resonances because they are more sensitive to changes in solvation or hydrogen bonding than are the C–^{1}H or ^{13}C resonances. Generally, the O–H protons of sugars are in fast exchange with water protons so that they cannot be detected directly by NMR spectroscopy. However, we have shown that by working at low temperatures, with careful pH control, well resolved resonances for the O–H protons can be detected. The anomeric protons, being more acidic, are shifted down-field from the remainder and are most readily identified.[1–5]

We know of no previous studies of possible interactions between monosaccharides and DNA anions. It is known that reducing sugars (having aldehyde groups in their open structures) slowly degrade DNA, which may imply some degree of binding. However, Cu^{II} ions are also implicated in these reactions.[7,8] No degradation was observed in the present study. Several monosaccharides have major biological roles, especially in metabolism, and it therefore seemed important to determine whether or not there was any significant interaction between them and DNA. In our initial studies, we decided to compare the behaviour of glucose and fructose in the presence of DNA.

Results and Discussion

At 5 °C (selected because the O–^{1}H resonances are relatively well resolved) aqueous D-fructose comprises *ca.* 80% β-D-fructopyranose (F_p) and 20% β-D-fructofuranose (F_f) (Fig. 1). (We disregard the presence of a low concentration of the α_f form, which was not monitored.)

The proton resonances for aqueous solutions of D-glucose were unaffected by DNA, apart from a small degree of broadening caused by increases in viscosity. However, for D-fructose, the feature for the anomeric proton resonance assigned to F_f increased in intensity and that due to F_p decreased. (Because of the difficulties in obtaining differential width changes, we have used changes in the ratios of the areas rather than absolute intensity changes in the derivations given in Table 1.) Small shifts in the peaks were observed for the F_f anomeric resonance, but not for the F_p resonance.

These results suggest that there is significant binding between DNA and F_f but not F_p. We argue that when the F_f form is

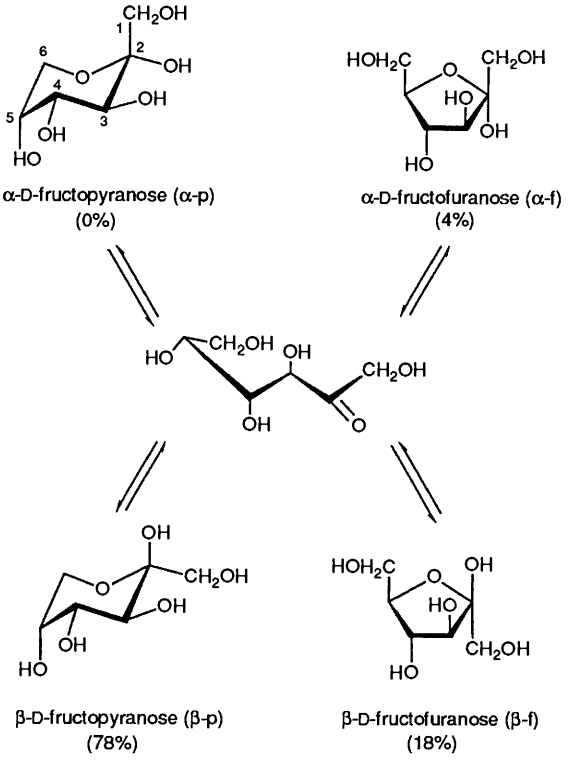

Fig. 1 Tautomeric equilibria of D-fructose (20%) in water at 0 °C

attached to DNA it is effectively removed from participating in equilibrium (1). This selectively reduces $[F_f]$ and hence

$$F_f \overset{K_1}{\rightleftharpoons} F_p \qquad (1)$$

equilibrium (1) moves to the left. We envisage two limiting cases: in one, the reactions for the binding equilibrium (2) are

$$(F_f)_{free} + DNA \overset{K_2}{\rightleftharpoons} (F_f)_{bound} \qquad (2)$$

slow. In that case, two separate features should exist, that for $(F_f)_{free}$ being in its normal position, and that for $(F_f)_{bound}$ being shifted down-field. However, the latter should be an extremely broad line because of the relative immobility of the DNA, and

Table 1 NMR data for the interaction between fructose and aqueous DNA

Resonance	K_1	ΔI^a	% f_{bound}	$K'_2{}^b$
$(O-H)_f$	4.1	0.04_0	5	0.22
$(O-H)_p$	4.0	0.04_0	5	0.25
$(C-H)_{av}{}^c$	4.3	0.04_6	5	0.18
$(^{13}C)_{av}{}^c$	4.1	0.03_5	4	0.21

[a] ΔI is the relative change in the intensity of the resonance in the presence of DNA. [b] $K'_2 = K_2 \times$ [DNA]. [c] Average values for p and f forms.

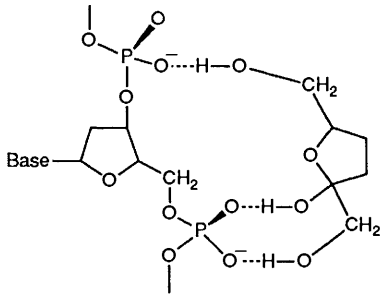

Fig. 2 Possible hydrogen bonding sites (- - -) of β-D-fructofuranose with the phosphate groups of DNA

hence might not be detectable. The sum of the areas under the $(F_f)_{free} + (F_p)$ peaks would be reduced. In the alternative limiting case, (2) is a rapid equilibrium in fast exchange. Then a weighted mean shifted peak for F_f would be obtained, with no marked broadening. The latter situation is that observed. We stress that using NMR spectroscopy we detect $(F_f)_{free} + (F_f)_{bound}$, but that K_1 is governed by $[(F_f)_{free}]$.

Qualitatively, the results establish that only the F_f form binds significantly to DNA and that the complex is in rapid equilibrium with free sugar. To treat the results quantitatively, we have adopted the following procedure, in order to eliminate fortuitous broadening and instrumental artifacts. We set $[F_f] + [F_p] = 1$, *i.e.* we normalise the separate experiments. Then we normalise the individual results by using $[F_f]/([F_f] + [F_p])$ and $[F_p]/([F_f] + [F_p])$. The results, given in Table 1, are reasonably self-consistent and show that *ca.* 5% of the total furanose is bound to DNA and 95% is free, in the concentration range used. Hence, ignoring activities, we can estimate approximate equilibrium constants, which are also given in Table 1. Very similar changes were observed for selected C–^{1}H NMR features for the F_f and F_p forms. No shifts or broadening were detected, but a similar switch from the F_p to the F_f structure was observed. In limited studies of selected ^{13}C resonances, a similar shift in equilibrium (1) was detected.

The small shift of the F_f resonance is the weighted average of the chemical shifts for bound and free β_f O–^{1}H resonances. Since the measured shift is *ca.* 0.03 ppm under our conditions, and since *ca.* 0.25 of the F_f form is bound and 0.75 free, the calculated shift for the bound form relative to the free is *ca.* 0.12 ppm, to low field. According to our postulate for the bound species (Fig. 2) this proton is hydrogen bonded to a P–O oxygen. The results suggest that this bonding is considerably stronger than the average sugar O–H • • • water hydrogen bonds, as expected.[9]

In order to check that this result really is a consequence of the presence of three relatively strong hydrogen bonds we have studied the O–^{1}H NMR spectra for aqueous β-furanose in the presence of an excess of $(MeO)_2PO_2^-$ anions. These ions had no effect on equilibrium (1).

We conclude that binding to DNA requires a geometry that orients at least three O–H protons towards anionic phosphate oxygen ligands, giving three strong bonds. Even so, the DNA complex has a low life-time. We suggest that a suitable choice of oligosaccharides may reveal more significant bonding.

Comparison with Polyamine Interactions.—Polyamines such as spermine are fully *N*-protonated at pH 7 (**1**). Hence they

$$H_3\overset{+}{N}(CH_2)_3NH_2{}^+(CH_2)_4NH_2{}^+(CH_2)_3NH_3{}^+$$

1

interact very strongly with naked aqueous duplex DNA. We,[6] and others [10] have used ^{1}H NMR techniques to probe the extent to which these polyammonium cations (PACs) are strongly bonded to DNA or loosely bonded. If the former were the case, as is usually supposed, the bound species would be immobile whilst bonded. In slow exchange, this species would give a very broad ^{1}H resonance. Since the association constant is large, the experiment would show a major overall broadening. In fact, there is almost no broadening other than that induced by viscosity increases. Hence we conclude that there is mainly loose coulombic binding, the PACs moving rapidly around and along the DNA strands.[6] This work was extended and supported by the use of a spin-labelled PAC, whose EPR spectrum was unaffected by DNA.

Thus, it seems that, surprisingly, there is a difference between F_f and the PACs despite the positive charges of the latter. Nevertheless, we stress that, without equilibrium (1), evidence for any complexation would have been very small and indeed, non-existent using C–^{1}H or ^{13}C resonances. We are currently using IR spectroscopy to probe this situation further.

Acknowledgements

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