MAGNETIC RESONANCE STUDIES OF THE ANOMERIC DISTRIBUTION AND MANGANESE BINDING PROPERTIES OF FRUCTOSE PHOSPHATES

Stephen J. Benkovic

Department of Chemistry, Pennsylvania State University, University Park, Pennsylvania 16802

and

James L. Engle and Albert S. Mildvan

The Institute for Cancer Research, Fox Chase, Philadelphia, Pennsylvania 19111

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SUMMARY

The anomeric distributions of the furanose forms of fructose-6-P and fructose-1,6-diP have been found by ^{13}C NMR to be 20 ± 4% α and 80 ± 10% β. The dissociation constants of the Mn complexes of fructose-1,6-diP and of a configurationally stable cis-1,6-diP analog are similar to those of various Mn-monophosphate complexes and are therefore inconsistent with chelation of the Mn by the cis-diphosphoryl moieties. Thus the affinity for metal is an unsatisfactory method for diagnosis of anomeric equilibria.

As shown for a number of simple ketoses ^{13}C NMR provides a direct means of elucidating anomeric distribution in aqueous solution (1, 2). The present paper extends these techniques to the phosphorylated ketoses, fructose-6-P and fructose-1,6-diP and examines the Mn binding properties of compounds of known anomeric configuration in order to determine whether the neighboring phosphate groups, such as those of α-fructose-1,6-diP can chelate a metal ion. The present studies are also essential for the determination of the anomeric specificity of enzymes which utilize fructose phosphates (3).

MATERIALS

The sodium salts of fructose-6-P, fructose-1,6-diP, and (2,3)diphosphoglyc acid were purchased from Sigma Chemical Company.

The tetra sodium salts of cis-2,5-bis(hydroxymethyl)tetrahydrofuran diphosphate and the mixture of 1,6-sorbitol and mannitol-diP ("hexitol diP") were synthesized as previously described (4).

METHODS

The natural abundance ^{13}C NMR spectra of aqueous solutions of fructose-diP (pH 7.2) and fructose-6-P (pH 7.8) and of the CS_2 standard were obtained at 25.1 MHz with the Varian XL-100-15 NMR spectrometer while field-frequency locked on an external standard of deuterated acetone at 15.4 MHz, and broad band "noise' decoupling of the protons at 100 MHz. The samples consisted of concentrated sugar solutions (1 to 3 M) or neat CS_2 in a sample tube 10 mm in diameter which was placed inside a 12 mm outer tube. The outer tube contained a shell of deuteroacetone for locking. Spectra were accumulated and integrated with the use of the Fabtitek Model 1074 instrument computer. The integrations were checked by cutting and weighing the spectral peaks. Chemical shifts were calculated from the CS_2 standard.

Mn binding studies were carried out by EPR (5) and by measurements of the longitudinal proton relaxation rate of water at 24.3 MHz as previously described (6).

RESULTS AND DISCUSSION

Anomeric Equilibria of Fructose Phosphates

Fig. 1 shows the ^{13}C NMR spectra in the region of the anomeric carbon atoms of fructose-6-P, fructose-1,6-diP, and fructose for comparison. The chemical shifts and integrated areas are summarized in Table I. With fructose, two peaks were resolved with chemical shifts corresponding to the β furanose and β pyranose forms (Table I) (1, 2). These chemical shifts had been previously assigned on the basis of NMR studies of several fructosides and pyranosides of independently established structure (1, 2). Additional peaks detected by pulsed Fourier transform NMR and assigned to minor components (2) were not

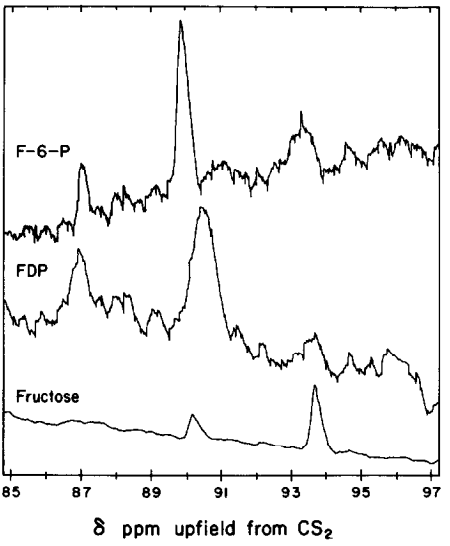

FIG. 1. Natural abundance ^{13}C NMR spectra of the anomeric carbon atoms of equilibrated solutions of fructose and its phosphates. F-6-P: 1 M disodium fructose-6-phosphate, pH 7.8, 256 scans; FDP: 1 M tetrasodium fructose-1,6-diphosphate, pH 7.1, 512 scans; and 3 M fructose, 64 scans. The solutions all contained 0.3 mM EDTA and were run at 29° and at 25.1 MHz with noise decoupling of the protons as described in the text. The chemical shifts, assignments, and integrated intensities are given in Table I.

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resolved in the present continuous wave NMR study. In the cases of both fructose-1,6-diP and fructose-6-P, a major furanose component (80 ± 10%) with a chemical shift corresponding to the β furanose form and a minor furanose component (20 ± 4%) with a chemical shift corresponding to the α furanose form (2) were observed. The upfield peak, appearing prominently in the spectrum of fructose-6-P is probably due to contamination of the sample by glucose-6-P which was shown by direct assay to be ∿30%. Since fructose-1,6-diP in aqueous solution does not contain a measurable amount of the keto isomer (7), the above percentages represent the total equilibrium distribution of fructose-1,6-diP. These results are in reasonable agreement with those recently obtained for

TABLE I

^{13}C Chemical shifts[a] and relative amounts of fructose and fructose phosphates

	α		β		Other		$\frac{\alpha}{\alpha + \beta}$
	δ (ppm)	Amount (%)	δ (ppm)	Amount (%)	δ (ppm)	Amount (%)	
Fructose-6-Phosphate	87.0	15 ± 3	89.9	60 ± 3	93.4	25 ± 4[b]	0.20 ± 0.04
Fructose-1,6-diphosphate	87.0	19 ± 2	90.6	70 ± 7	93.6	11 ± 1[c]	0.21 ± 0.03
Fructose	87.0	< 3	90.2	27 ± 3	93.7	73 ± 7	< 0.04

[a] Expressed in ppm upfield from CS_2

[b] Pyranose forms resulting from 30% glucose-6-phosphate contamination.

[c] Pyranose forms resulting from partial hydrolysis (11).

fructose-1,6-diP from ^{31}P measurements (8) although the latter are probably less accurate owing to overlap of the ^{31}P resonances of the α- and β-anomers. Fructose-6-P apparently exists in equilibrium with up to 5% as the keto form (7) so that the ratio of α:β:keto configuration is calculated at 76:19:5.

The anomeric equilibrium ratios (Table I) were calculated for the $\alpha \rightleftharpoons \beta$ equilibrium of the furanoses from the areas under the respective resonances. Since noise decoupling was employed in these studies, these equilibrium ratios are only an approximation because of the possible inequality of the Overhauser enhancements produced by decoupling. However because the anomeric carbon atoms are in similar chemical environments and are not directly bonded to protons, such differences are small and probably within the experimental errors in the measured areas. The view is supported by our finding that varying the intensity of the decoupling field had no detectable effect on the relative

areas under the peaks in the fructose spectrum. It is also noted that the solutions of sugar phosphates employed were at molar concentrations; consequently the reported equilibrium ratios might be concentration dependent. Unfortunately, it is not experimentally feasible to extend the continuous wave NMR method to more dilute solutions.

TABLE II

Dissociation Constants (K_d) and Enhancements (ε_a) of Binary Mn Complexes[a]

Complex	K_d (mM)	ε_a
Mn-Cis-2,5-bis(hydroxymethyl) tetrahydrofuran diphosphate	1.5 ± 0.2	1.53 ± 0.09
Mn-hexitol-diphosphate	3.0 ± 0.4	1.55 ± 0.05
Mn-FDP[b]	2.7 ± 0.5	1.7 ± 0.2
Mn-2,3-diPGA	0.8 ± 0.2	1.5 ± 0.2
Mn-ADP[c]	0.10 ± 0.02	1.7 ± 0.1
Mn-ATP[c]	0.014 ± 0.006	1.7 ± 0.1
Mn-PO_3OH[d]	4.7 ± 0.6	1.12 ± 0.02
Mn-PO_3F[d]	1.8 ± 0.7	0.99 ± 0.04
Mn-PEP[c]	1.9 ± 0.3	1.15 ± 0.04
Mn-AMP[e]	4.9 ± 0.3	-

[a] Conditions: pH = 7.5, μ = 0.1 (KCl, NaCl or tetramethyl ammonium Cl), T = 24 ± .10.

[b] Reference (9)

[c] Reference (12)

[d] Reference (13)

[e] Reference (14)

Mn Binding Properties of Diphosphate Compounds

In the minor α anomer of fructose diphosphate, the two phosphate groups are cis with respect to the furanose ring rendering possible the bidentate chelation of cations such as Mn^{2+}. Such a possibility had been suggested as a means of determining anomeric distributions (9). Chelate complexes of this type would be of biochemical significance in that they would permit divalent cations to shift equilibria to the cis forms of sugar diphosphates in vitro and in vivo. A chelate complex of Ca^{2+} between phosphate groups on separate protein molecules has been proposed as an essential intermediate in muscle contraction (10). The binding parameters of the Mn complexes of several diphosphate and monophosphate complexes determined in the present and in previous studies are summarized in Table II. From Table II it is apparent that the diphosphate compounds, even when constrained to be cis, fail to show the high affinity characteristic of chelate complexes such as Mn-ADP and Mn-ATP. The enhancement factor ε which depends on the tumbling time of the complex increases with molecular weight of the complex but is independent of the extent of chelation (Table II). It is therefore concluded that the repulsion of separate phosphate groups on the same molecule cannot be overcome by chelation of Mn^{2+} and that the metal binding affinities and enhancement properties of diphosphates are not useful in determining anomeric structures.

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