Composition, Sequence, and Conformation of Polymers and Oligomers of Glucose as Revealed by Carbon-13 Nuclear Magnetic Resonance

Pierre Colson,[1] Harold J. Jennings,* and Ian C. P. Smith*

Contribution from the Division of Biological Sciences, National Research Council of Canada,[2] Ottawa, Canada K1A 0R6. *Received June 25, 1974*

Abstract: The carbon-13 nmr spectra of two cyclodextrins and several linear glucans have been completely assigned. These assignments were made by comparison with the spectra of glucose, some of its specifically O-methylated derivatives, and a number of differently linked glucobioses and glucotrioses. This technique enables the composition, structure, and major sequence of a number of glucans to be determined. Conformational effects are also apparent in some of the spectra. The 1→4, 1→4, and 1→6 linkage sequence of the glucans from *T. mesenterica* and *P. pullulans* give, in addition to an anomeric signal for the 1→6 linkage, two separate anomeric signals for each of the 1→4 linkages. This multiplicity, apparent also in other carbon signals of the spectra, is due to the sensitivity of these carbons to the type of linkage on the neighboring glucose units. Some evidence that conformational effects are involved in this multiplicity is provided by a comparison of the spectra of the 1→4-linked cyclodextrins and linear glucans, where appreciable chemical shift differences are apparent for C1 and C4. These effects are influenced by pH modification and are attributed to differences in rotational isomers at the linked carbons.

Carbon-13 nuclear magnetic resonance provides a very sensitive method for determination of the structure of complex compounds of low to medium molecular weight.[3-5] More recently it has been applied to considerably more complex compounds of biological interest.[6] In the field of carbohydrates where the ^1H nmr spectra are particularly complex and optical rotation data not unambiguous, it has shown enormous potential.[6-23] Our interests in the immunological properties of cellular polysaccharides has led to their detailed study by carbon-13 nmr. The compositions and backbone conformation of a series of polymeric acetamido hexosamine phosphates specific to various serogroups of *Neisseria meningitidis* have been recently determined by this technique.[13] In a preliminary communication, we have reported elucidation of the sequence as well as the composition of a complex glucan from *Tremella mesenterica*.[14] We describe here the details of the method in its application to these and other glucans.

Experimental Section

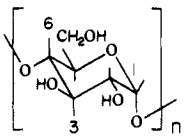
The ^{13}C nmr spectra were obtained on Varian XL-100 (25 MHz) and CFT-20 (20 MHz) spectrometers in sample tubes of outside diameter 12 and 10 mm, respectively. ^1H nmr spectra were obtained on samples in tubes of 5 mm o.d. at 100 MHz (Varian XL-100) or 220 MHz (Varian HR 220, Canadian 220 MHz, NMR Center, Sheridan Park, Ontario). ^{13}C spectra were taken with complete proton decoupling or with coupling using a gated ^1H decoupler sequence (decoupler on–decoupler off–90° pulse–acquire data) to retain nuclear Overhauser enhancements. The solvent deuterium resonance was used as a field-frequency lock and chemical shifts are expressed relative to tetramethylsilane contained in a coaxial sample tube of outside diameter 5 mm.

For comparison with the chemical shifts reported by other workers with respect to other references we use the ^{13}C chemical shifts of glucose equilibrated in deuterium oxide at 32°. The carbohydrates are 100 mg/ml in deuterium oxide, pD 7 (pD = meter reading + 0.4), 32 ± 1°, unless otherwise indicated. Maltose, isomaltose, amylose, and maltotriose were commercial samples used without purification. Methyl-4-*O*-methyl-α-D-glucopyranoside and methyl-6-*O*-methyl-α-D-glucopyranoside were prepared according to Kenner and Richards[24] and Helferich, *et al.*,[25] respectively. Kojibiose and laminaribiose were prepared by the methods of Suzuki and Hehre[26] and Peat, *et al.*,[27] respectively. Pullulan was extracted from *Pullularia pullulans* (de Bary Berkhout) according to Sowa, *et al.*[28] Other compounds were gifts, as indicated in the acknowledgment.

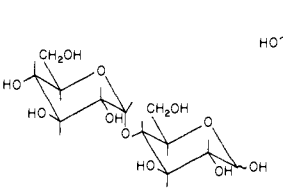
For quantitation of various types of anomeric link by proton nmr several dextrans were permethylated according to Hakamori.[29] Permethylation results in a downfield shift of the anomeric proton resonances. The shifts are sufficiently specific to allow resolution of separate anomeric resonances.[30]

Results and Discussion

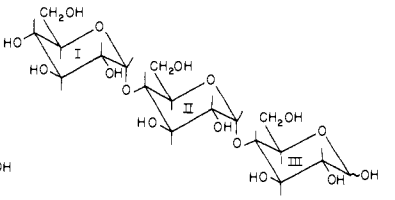
1. Simple Polymers of Glucose. a. Amylose and Cyclodextrins. Amylose and the cyclodextrins are composed of a repeating sequence of glucose units linked α-1→4. While the former is a linear polymer, the latter contains six, seven, or eight monomeric units forming a "super-ring." Our analysis



agrees with that of Usui, *et al.*[21] for β-cyclodextrins but reverses the assignments of the C2 and C3 resonances of amylose and the cyclodextrins given by Takeo, *et al.*[23] Our assignments are in agreement with those made earlier for amylose.[9,14] By reference to our assignments for methyl-4-*O*-methyl-α-D-glucopyranoside, maltose, and maltotriose as well as a series of chlorinated and polyol analogs,[31] the resonance of C2 must be located at a higher field than that of C3. The α-1→4 glucose polymer and oligomers are compared to some related compounds in Table I; the values for maltotriose refer to the central glucosyl moiety of this compound which is similar to those in the homopolymer. The assignments are valuable for later comparison with those of the glucans of mixed linkage (*vide infra*).



MALTOSE (α, 1→4)



MALTOTRIOSE (α, 1→4; α, 1→4)

The C1, C3, C4, and C6 resonances of all compounds in Table I can be assigned without ambiguity by considering the effect of methylation on the chemical shifts of the contiguous and neighboring carbon atoms (downfield shifts of

Table I. Assignment of α-1→4 Polymers and Related Compounds

	Glucose α-anomer pD = 7	Methyl-4-*O*-methyl-α-D-glucopyranoside pD = 14[b]	Maltotriose pD = 7	Amylose pD = 14	Amylose pD = 7[c]	Cyclodex-6 pD = 14	Cyclodex-6 pD = 7	Cyclodex-7 pD = 14	Cyclodex-7 pD = 7
C1	93.1	101.1	101.0	102.9	100.9	103.3	102.5	104.0	102.9
C2	72.5[a]	72.8	72.7	73.8	72.7	73.8	72.8	74.3	72.9
C3	73.8	76.1	74.5	75.4	74.5	75.3	74.4	75.1	74.1
C4	70.7	83.0	78.2	80.6	78.4	82.9	82.3	83.0	82.2
C5	72.5[a]	72.1	72.4	72.6	72.4	73.2	73.1	73.1	73.1
C6	61.7	61.4	61.8	62.0	61.8	61.8	61.5	61.7	61.4

[a] In contrast to the data of Perlin, *et al.*,[36] obtained at 55°, where the C2 and C5 resonances are separated by 0.3 ppm, we obtained no substantial separation (less than 0.05 ppm) at either 32 or 55° with a spectral window of 1.25 KHz and 8K and 4K data points, respectively. [b] The chemical shifts of methyl carbons on C4 and C1 are 61.1 and 56.2 ppm, respectively. [c] Data from ref 9 were assigned to specific carbons using arguments described in the text.

Table II. Assignments of α-1→6 Glucans and Related Compounds

	Methyl-6-*O*-methyl-α-D-glucopyranoside[a] pD = 7[e]	Isomaltose[b] pD = 7	Panose[d] pD = 7	α-1→6 glucan pD = 14	α-1→6 glucan pD = 7[e]
C1	100.8	99.25 (93.4)	101.0 (99.3)	99.4	99.0
C2	72.6	73.25 (73.0)	73.0 (73.0)	73.1	72.5
C3	74.5	74.3 (74.3)	74.3	75.4	74.5
C4	71.6[e]	70.8 or 71.3 (70.8 or 71.3)	70.8	71.8	71.3[e]
C5	71.2[e]	72.7 (71.3)	71.2	71.1	70.7[e]
C6	72.6	61.8[c] (67.0)	67.2	66.8	66.7

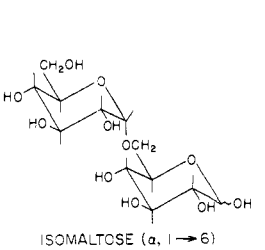
[a] The assignments of methyl carbons on C6 and C1 are 59.8 and 56.4 ppm, respectively. [b] The chemical shifts in parentheses refer to the carbons of the reducing unit with the α configuration, while the others refer to those of the nonreducing unit. [c] The resonance at 63.8 ppm previously assigned[14] to a C6 of isomaltose was due to an impurity. [d] The chemical shifts of panose given here are those of the central glucosyl unit. The C1 and C2 resonances of the nonreducing end unit are also given to show the influence of the α-1→6 linkage. [e] Assignments which may be reversed.

8–11 and less than 2 ppm, respectively), and the unique chemical shift of an unlinked hydroxymethyl group. At pD 7 a very close agreement exists between the chemical shifts of the central glucosyl moiety of maltotriose and those of amylose. On the other hand, the chemical shifts of C1 and C4 of the cyclic oligomers differ substantially from those of the linear compounds; the chemical shifts of the unlinked carbons are very similar in both the cyclic and linear compounds. The latter suggests that the characteristic C1 and C4 chemical shifts are due to differences in rotamer populations at the linked carbon atoms rather than to differences in the conformations of the hexapyranose rings. In the case of maltotriose and amylose considerable mobility is allowed about the C1–O and O–C4 bonds, whereas the cyclic nature of the cyclodextrins permits at most slight oscillations in the dihedral angles at the linkage position, and the dominant rotamers are dictated by geometric constraints. These conformationally determined chemical shifts may be of some analytical value if a better calibration of the dependence can be made.

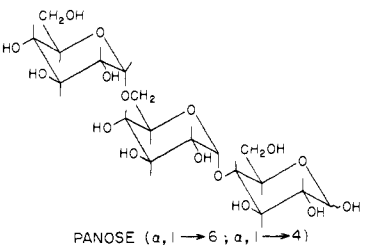
It is well known[32,33] that pH strongly influences the ionic structure of carbohydrates; the effect on the ^{13}C chemical shifts of amylose has been reported by Dorman and Roberts.[9] In the monomers, carbons 2, 3, and 6 are the most sensitive to pH change. Comparison of the data for the α-1→4 linked glucose oligomers at pD 7 and 14 shows that the two most shielded resonances (C5 and C6) of each compound are insensitive to change of pD. The insensitivity of the C6 resonance (which is unequivocally assignable by virtue of its chemical shift and multiplicity in the ^1H-coupled spectrum) to either inductive or conformational changes induced by pH is not very surprising due to the fact that in α-1→4 glucans the hydroxyl group at C6 cannot stabilize an oxyanion through hydrogen bonding from the hydroxyl group at C4.[32] Confirmation of the other resonance as that of C5 comes from the expectation that it should be less sensitive than C2 to ionization of hydroxyl groups. This also explains the reversal of the C2 and C5 resonance on variation of pD from 14 to 7.

This study of the α-1→4 glucans demonstrates that the ^{13}C chemical shifts are indicative of conformational and ionization effects; a detailed interpretation must await the study of rigid model compounds of known conformation.

b. The (α-1→6) Glucans. Comparison of the C1 chemical shifts of these glucans with those of amylose or the cyclodextrins under similar conditions reveals an upfield displacement of about 2 ppm due to involvement in a α-1→6 rather than a α-1→4 linkage. As pointed out earlier,[14] this conclusion also follows from comparison of the C1 chemical shifts of maltose (α-1→4), isomaltose (α-1→6), maltotriose (α-1→4; α1-→4), and panose (α-1→4; α-1→6). In Table II the most significant resonances of isomaltose and panose[34] are shown in order to help assignment of the α-1→6 glucan resonances. Methyl-6-O-methyl-α-D-glucopyranos-



ISOMALTOSE (α, 1→6)



PANOSE (α, 1→6 ; α, 1→4)

Table III. Assignments of 1 → 3 Glucans and Related Compounds

	3-*O*-Me-D-glucose[a]	α-1→3 glucobiose (nigerose) pD = 7	α-1→3 glucan pD = 14	β-1→3 glucobiose (laminaribiose) pD = 7	β-1→3 glucan (laminarin) pD = 14	pD = 7	$(\Delta\delta)_d$[b] pD = 7	$(\Delta\delta)_p$ pD = 14
C_1'		100.2	101.3	104.0	104.7	103.8	−3.4	−3.4
$C_1\alpha$	93.4	93.4		93.1				
$C_1\beta$	97.3	97.1		96.8				
C_2'		72.9	72.2	74.6	74.9	74.4	−2.5	−2.7
$C_2\alpha$	72.4	72.4		72.1				
$C_2\beta$	75.0	74.1		74.9				
C_3'		74.1	83.2	76.7	88.0	85.5	−4.9	−4.8
$C_3\alpha$	84.0	80.9		83.6				
$C_3\beta$	86.5	83.4		85.8				
C_4'		71.2	71.7	70.7	69.9	69.3	1.3	1.8
$C_4\alpha$	70.4	70.6		69.3				
$C_4\beta$	70.2	70.5		69.3				
C_5'		72.9	73.7	76.7	77.8	76.8	−4.2 or −4.7	−4.1
$C_5\alpha$	72.6	72.9 or 72.4		72.3				
$C_5\beta$	77.0	76.7		77.1				
C_6'		61.7	62.2	62	62.5	61.9	−0.4	−0.3
$C_6\alpha$	62.0	61.6		62				
$C_6\beta$	62.0	61.4		62				

[a] Reference 34. The methyl resonance on C3 is situated at 61.2 and 60.9 ppm for the α and the β anomer, respectively. [b] $(\Delta\delta)_d$ and $(\Delta\delta)_p$ give the chemical shift differences between anomeric units of the disaccharides and polymers, respectively. A positive difference indicates that the resonance in the α-anomeric unit occurs at lower magnetic field (larger chemical shift from $(CH_3)_4Si$). For $\Delta(\delta)_d$, the difference was taken for the reducing moiety, subtracting the value for the β anomer of the reducing unit of the β-linked disaccharide from that of the α anomer of the reducing unit of the α-linked disaccharide, since this represents most closely the situation in the homopolymers.

ide was also assigned because it is the most simple glucose derivative related to the α-1→6 glucan. The C6 resonance of this glucan is deshielded by 4.9 ppm relative to that of amylose due to the formation of the α-1→6 linkage, whereas the C4 resonance is shielded by 7.1 ppm due to the absence of the α-1→4 linkage. It is noteworthy that methylation and addition of another glucopyranose ring influence to different extents the chemical shift of the carbon atom on which the substitution has been made: for linear compounds about 10–13 and 5–8 ppm, respectively (see Tables I and II). An uncertainty remains with respect to the relative chemical shifts of C4 and C5 in the α-1→6 glucan and methyl 6-O-methyl-α-D-glucopyranoside. According to the coupled spectrum of the methylglycoside and the expected influence of pD on carbohydrates (see also Table I), the position of the lines must be as described in Table II. The influence of pD on the chemical shifts of all the α-1→6 glucan carbons seems to be less than on those of amylose, especially for C1 and C4.

c. The (1→3) Glucans. The α- and β-1→3 glucans provide an excellent example of the configurational sensitivity of the ^{13}C resonances. Their formulas and ^{13}C nmr spectra are shown in Figure 1. Assignments were made by reference to the data for the corresponding disaccharides, nigerose (α-1→3) and laminaribiose (β-1→3) (Table III). The nigerose and laminaribiose resonances were assigned themselves by comparison with the corresponding methylglycosides[21,35,36] and 3-*O*-methylglucose[35] for reducing and nonreducing end units, respectively. Our assignments are in agreement with the data of Usui, *et al.*,[21] except that the C2 resonance of the reducing end α anomer of nigerose must be at 72.4 and not 71.3 ppm by reference to 3-*O*-methyl-α-D-glucose. It can be seen again here that O-methylation on a given carbon causes a larger downfield shift than does linkage of another glucopyranose ring on the same carbon. The assignment of the resonances in the β-1→3 glucan is done by comparing the data with those of the β-anomeric reducing end unit of laminaribiose at the same pD, remembering that linkage formation does not significantly affect the resonances of neighboring carbons (upfield shift equal to or less than 1 ppm on a nearest neighbor carbon). This comparison is invalid for C1 because this carbon is linked in the polymer and free in the monomer, but its position is unique in any case. The influence of pD is greatest on C3 in the polymer, as it was on C4 in amylose (see Table I). The C6 in the α-1→6 glucan did not behave similarly with respect to pD because of its exocyclic position. Thus, it appears that the resonances of carbon atoms involved in linkages (other than C6 or C1) are very sensitive to pD change. This can be due to modification of ionization behavior and/or conformation, which are not easily separated. Taking into account again the effect of substitution on the nearest neighbor carbon

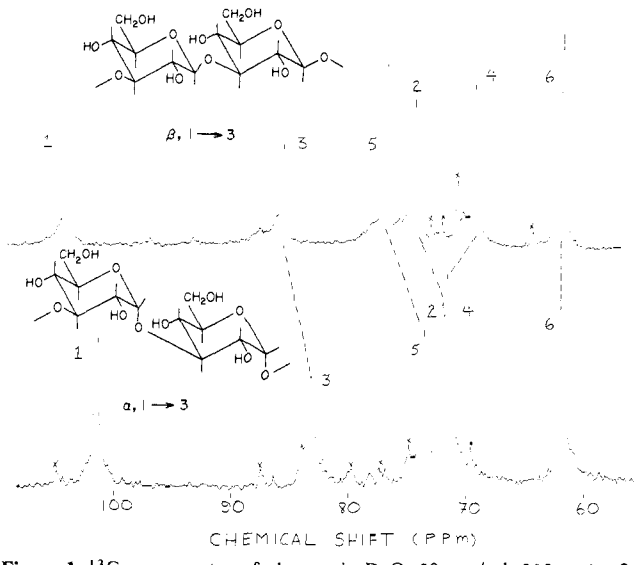


Figure 1. ^{13}C nmr spectra of glucans in D_2O, 50 mg/ml, 32°: α-1→3, pD 14, 111,000 transients; β-1→3, pD 7, 129,000 transients, pulse angle 90°, cycle time 0.4 sec. Both spectra were obtained with spectral widths of 5 kHz and stored in 4K computer data points. The resonances marked X are extraneous due to impurities and/or products of degradation.

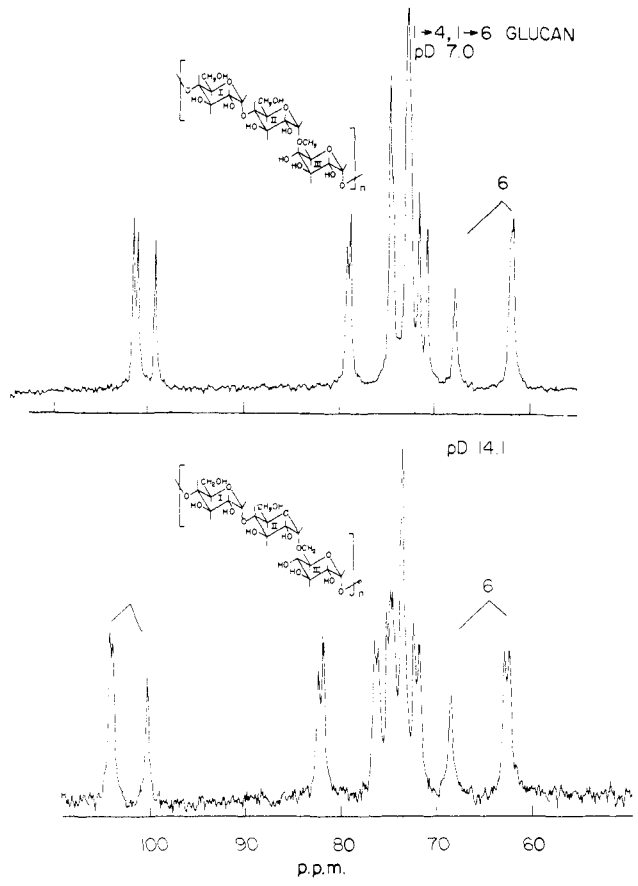


Figure 2. ^{13}C nmr spectra of the glucan from *Tremella mesenterica*, 100 mg/ml in D_2O, 32°, pD = 7.0 (48,000 transients) and pD = 14.1 (138,000 transients), spectral width 5 kHz, 4K data points, cycle time 0.4 sec, pulse angle 55°. Area measurements were made on spectra taken with spectral windows of 2.5 kHz taken with pulse angle and cycle time of 60° and 1.2 sec, respectively.

atom (1 ppm upfield shift) and the influence of pD on the spectra of nigerose and the α-1→3 glucan, the C5, C2, and C4 resonances of the α polymer can be unambiguously positioned at 73.7, 72.2, and 71.7 ppm, respectively. The relatively high sensitivity of the C6 resonance of laminarin to a pD change can be interpreted on the basis of intramolecular hydrogen bonding,[32] in contrast to the behavior of amylose (*vide supra*). The two last columns of Table III give respectively the differences of all carbon chemical shifts between the α- and β-disaccharides, on the one hand, and between the (α-1→3) and the (β-1→3) glucans, on the other hand. They show that the differences in ^{13}C chemical shifts between the two disaccharides give a good picture of the differences existing between their related polymers.

2. Polymers of Glucose with Mixed Linkages. The elucidation of the composition, sequence, and conformation of glucans with mixed linkages will be developed from the full assignment of disaccharide and homoglucan resonances just presented.

a. *Tremella mesenterica* and *Pullularia pullulans*. Chemical evidence[37] has indicated that the glucans from *T. mesenterica* and *P. pullulans* contain two kinds of linkage (α-1→4 and α-1→6) in an approximate ratio of 2:1. Recently, Jennings and Smith[14] confirmed the relative composition of the *T. mesenterica* glucan and obtained its sequence by ^{13}C nmr spectroscopy. Sequence information came from the sensitivity of some resonances to the nature of linkages to the next glucosyl moieties. By comparison of the glucan spectra with those of amylose (α-1→4 glucan), the α-1→6 glucan, maltotriose, and panose a complete assignment of glucan resonances can be made. In addition, the very close similarity of the ^{13}C spectra of the glucans from *T. mesenterica* and *P. pullulans* reveals that the sequences are essentially the same (*i.e.*, repetitive sequence: . . . α-1→4, α-1→4, α-1→6 . . .).[14]

Table IV. Assignment of the (α-1→4; α-1→6) Glucans

Assignment[a]	Glucan *Tremella mesenterica* b	c	d	Pullulan[c]
C1 (1→4)	103.8	101.6	101.3	101.4
	103.3	101.0	100.9	101.0
C1 (1→6)	99.6	99.3	99.0	99.2
C2 (1→4)	74.6	72.7	72.7	72.8
	74.1	72.7	72.7	72.8
C2 (1→6)	73.9	72.7	72.5	72.8
C3 (1→4)	75.9[e]	74.5	74.5[e]	74.6[e]
C3 (1→6)	75.5[e]	74.5	74.3[e]	74.3[e]
C4 (1→4)	81.7	79.2	79.0	79.0
	81.3	78.8	78.7	78.7
C4 (1→6)	71.7[f]	71.6[f]	71.5[f]	71.6[f]
C5 (1→4)	72.9	72.7	72.5	72.8
C5 (1→6)	71.2[f]	70.8[f]	70.7[f]	70.7[f]
C6 (1→4)	61.3	62.0	61.8	62.0
	61.8	61.8	61.6	61.7
C6 (1→6)	68.0	67.8	67.8	67.8

[a] C1 (1→4) indicates a C1 involved in a (α-1→4) link. [b] pD = 14; 32°. [c] pD = 7; 32°. [d] pD = 7; 85°. [e] Interchangeable peaks due to coincidence of these two resonances in homoglucans. [f] Uncertainty concerning the relative positions of C4 and C5 carbons.

The ^{13}C nmr spectra of the glucan from *T. mesenterica* are shown in Figure 2; assignments are presented in Table IV. The resonances due to C1 fall into two distinct groups: two resonances of approximately equal intensity at 101.6 and 101.0 ppm, and a resonance of intensity comparable to each of the former at 99.3 ppm. Comparison with the data for maltose and maltotriose shows that the group at 101.6 and 101.0 must be due to C1 linked α-1→4. The doublet is due to the sensitivity of a C1 in an α-1→4 link to the presence of a glycosidic link on the C4 of the same glucosyl moiety. Such a sensitivity is demonstrated by comparing the C1 chemical shifts of residues I and II of maltotriose (101.0 and 100.7 ppm, respectively). The equal intensities of the resonances at 101.6 and 101.0 ppm indicate that a C1 linked α-1→4 experiences equal probability of the C4 of the same residue being linked or unlinked (which implies that the C6 of this residue is linked since there are only two types of link possible). The single resonance at 99.3 ppm is clearly due to C1 of an α-1→6 link by comparison with the data for isomaltose, panose, and the α-1→6 glucan. The absence of a splitting of this resonance implies that the C1 of an α-1→6 link experiences only one chemical environment in the polymer. The relative areas of the resonances due to α-1→4 and α-1→6 links are 2.2:1.0, in agreement with the chemical data.[37] The splitting of the C1 resonance of the α-1→4 link and the absence of a splitting of the C1 resonance of the α-1→6 implies that the sequence of the polymer is 1→4, 1→4, 1→6. This is confirmed by consideration of the C4 resonances in an α-1→4 link and the C6 resonances of an α-1→4 linked glucosyl residue, *vide infra*.

The resonances at 79.2 and 78.8 ppm (pD 7.0) of the glucan from *T. mesenterica* are attributable to C4 in an α-1→4 link by comparison with the data for amylose (78.4 ppm), the central residue of maltotriose (78.2 ppm), and the reducing residue of maltose (78.3 and 78.1 ppm for the α and β anomers, respectively). The C4 resonance is deshielded by approximately 8 ppm relative to its position in glucose on formation of the α-1→4 glycosidic link. The presence of two resonances for C4 is due to the sensitivity at this position to the nature of the link at C1. A comparable

Table V. Assignment of Dextran B-742 with Respect to Its Components[a]

α-1→4 glucan (amylose)		α-1→6 glucan		α-1→3 glucan		Dextran B-742	
δ, ppm	Assignment	δ, ppm	Assignment	δ, ppm	Assignment	δ, ppm	Assignment
100.9	C1			101.3	C1	101.3	C1 (1→4)
						100.5	C1 (1→3)
		99.0	C1			99.1	C1 (1→6)
				83.2	C3		
						81.9	C3 (1→3)
78.4	C4						
74.5	C3	74.5	C3			74.2	C3 (1→6); C3 (1→4)
				73.7	C5		
						73.0	C5 (1→3); C2 (1→4)
72.7	C2						
		72.5	C2				
72.4	C5						
				72.2	C2		
				71.7	C4		
		71.3	C4			71.3	C4 (1→3); C2 (1→3)[b]
						71.1	C4 (1→6); C2 (1→6)[b]
		70.7	C5			70.7	C5 (1→6)
		66.7	C6			66.8	C6 (1→6)
61.8	C6			62.2	C6	61.7	C6 (1→3; 1→4)

[a] All compounds at pD 7 except the α-1→3 glucan, which is at pD = 14. [b] Uncertainty concerning the relative assignments.

sensitivity is seen in maltotriose where C4 of residue II has a chemical shift of 78.2 ppm, and C4 of residue III has chemical shifts of 78.6 and 78.4 ppm corresponding to the α and β anomers, respectively, at the reducing end. Thus, a C4 in an α-1→4 link experiences roughly equal probability that the C1 of the same glucosyl residue is linked α-1→4 or α-1→6. This implies that the polymer sequence is 1→4, 1→4, 1→6.

Further confirmation of the sequence comes from consideration of the C6 resonances. The group at 62.0 and 61.8 ppm (pD = 7.0) is assigned to an unlinked C6 (of a residue necessarily linked α-1→4) by comparison with the value for amylose (61.8 ppm). The splitting of this C6 resonance must be due to the possibility that C1 of that residue can be linked α-1→4 or α-1→6. Such a sensitivity is implied by comparable splittings of the unlinked C6 resonances of maltotriose and panose. The resonance at 67.8 ppm (pD = 7.0) is attributable to a C6 linked α-1→6 by comparison with the values for the α-1→6 glucan (66.7 ppm) and the linked C6 of panose (67.2 ppm). The relative areas of the two groups of peaks confirm the ratio of 1→4 to 1→6 links as 2:1. The relative intensities of the two resonances due to the linked C6's implies that they experience roughly equal probabilities that the C1 of the same glucosyl moiety is linked 1→4 or 1→6. This implies once more that the sequence of the polymer is 1→4, 1→4, 1→6.

The remaining resonances in the intermediate field range can be assigned by comparison with the data for amylose and the α-1→6 glucan, taking into account the influence of pD on each carbon. The ambiguity between the C4 and C5 resonances of the α-1→6 glucan cannot be definitively resolved.

Some experiments were done at higher temperature with the glucan from *T. mesenterica*. As seen in Table IV, no significant change of the chemical shifts is induced up to 85° and the splitting of the C1 and C4 resonances of the α-1→4 linked moieties is still present. Therefore, it must be assumed either that long-range through-bond effects are the principal sources of this splitting or that a great potential energy barrier has to be overcome in order to modify appreciably the conformational preferences manifest at ambient temperature.

At pD 14, the ^{13}C nmr spectrum of *T. mesenterica* is better resolved than at pD 7 and is assigned in Table IV, taking into account the pD dependence of its different constitutive glycosidic units. The insensitivity to pD change of some chemical shifts, such as those of C1 and C4 of α-1→6 linked units, C5 and C6 of any unit, are again manifest. The other carbons are more or less deshielded by a pD increase similar to their behavior in the homoglucans (Tables I and II).

Chemical analysis of a similar glucan from *P. pullulans* has indicated the presence of 6% α-1→3 linkages.[28] The ^1H nmr spectrum (100 MHz) of the permethylated polysaccharide contains no evidence for α-1→3 links and yields a ratio of α-1→4 to α-1→6 links of approximately 2:1. A similar conclusion follows from the ^{13}C nmr spectra.[38] Taking into account the signal-to-noise ratios of both the ^1H and ^{13}C nmr spectra, we estimate that the proportion of α-1→3 links is less than 3%.

b. Dextran B-742. The composition of this dextran was determined by chemical analysis (periodate oxidation) as 57% of α-1→6, 26% of α-1→3, and 17% of α-1→2 and/or α-1→4 linkages.[39] The absence of resonances in the regions around 97 and 77 ppm enables us to conclude that the polymer contains no significant quantity of α-1→2 linked glucose units. The α-1→4 linkage is suggested by a shoulder on the left of the most downfield resonance (101.3 ppm in the C1 region of Figure 3), whereas no peak characteristic of this kind of C4 appears at about 78.5 ppm. Moreover, the four remaining resonances of the α-1→4 glucosyl unit cannot be identified due to overlap with those of glucosyl units in different types of linkages.

Taking into account that a pD decrease causes an upfield shift of about 1 ppm for the carbons involved in a linkage, the resonances at 100.5 and 81.9 ppm of the dextran can be certainly assigned to the C1 and C3 carbons involved in the α-1→3 linkage. The chemical shift of the unlinked C3 of the dextran has to be very similar to that in the α-1→6 and α-1→4 linked homoglucans and is positioned at 74.2 ppm. The resonances at 99.1 and 66.8 ppm are due to the carbons involved in the α-1→6 linkage, and the 61.7 ppm resonance is typical of the unlinked C6 of α-1→3 and α-1→4 glucose units.

From reference to our experiments with cellobiose–maltose mixtures,[38] it can be inferred that the percentage of the α-1→4 linkage has to be less than 10%. The measurement of the peak areas of the anomeric resonances in ^{13}C and ^1H nmr spectra (on the native polysaccharide and its permethylated derivative, respectively) confirms this assumption: by ^1H nmr, 63% of α-1→6, 32% of α-1→3, and 5% of α-1→4; by ^{13}C nmr, 57% of α-1→6, 34% of α-1→3, and 9%

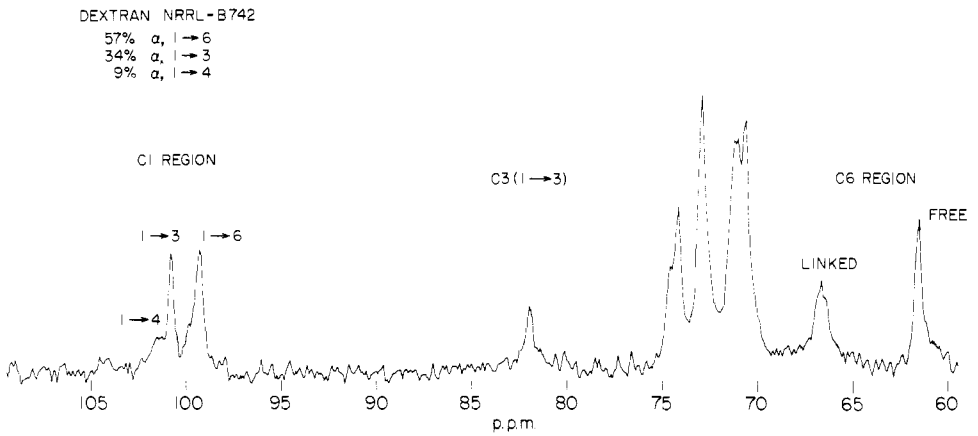


Figure 3. ^{13}C nmr spectra of the dextran B-742, 70 mg/ml, 32°, pD 7.0, 144,000 transients, spectral width 5 KHz with 4K data points, cycle time 0.4 sec, pulse angle 50°.

of α-1→4. In the ^1H nmr spectrum of the permethylated compound the H1 resonances of each type of linkage are well-resolved, whereas in the ^{13}C nmr spectrum (Figure 3) that of C1 of the 1→4 link is a shoulder on a resonance. Thus, in this case, the quantitative aspect of the ^1H nmr is more reliable. Comparison of the areas of ^{13}C resonances due to the C6 carbons is unjustified due to the possibly different spin–lattice relaxation times (T_1) and nuclear Overhauser enhancements of linked and unlinked carbons; under the conditions used to obtain the spectra, the resonances due to carbons with longer T_1's could be partially saturated. The ratio of the peak intensities corresponding to the C3 resonances indicates 69% of α-1→6 plus α-1→4 linkage and 31% of α-1→3 linkage.

The remaining carbon resonances to assign are those due to C2, C4, and C5. The C4 resonances of α-1→3 and α-1→6 subunits are positioned as expected at 71.3 and 71.1 ppm, respectively, and the C5 (α-1→6) resonances at 70.7 ppm. The other carbons, in the homoglucans, have chemical shifts between 73.0 and 72.0 ppm, taking into account that the α-1→3 glucan was assigned at pD = 14. Thus, the resonance at 73.0 ppm is logically assigned to a C5 of a glucose moiety in a α-1→3 linkage. It is particularly important to notice that no strong resonance appears in the C2 region of the dextran spectrum at the value expected for C2 from the α-1→6 homoglucan spectra (72.0 ppm). We can therefore exclude as unlikely for this dextran a sequence with large blocks of α-1→6 linked units. Moreover, the rather small number of resonances in such a complex dextran suggests that it has an overall repetitive sequence 1→6, 1→6, 1→3, with occasional 1→4 linkages randomly distributed. The argument for the above sequence is more tentative than in the case of the glucans from *T. mesenterica* and *P. pullulans* due to the particularly broad lines obtained with this dextran. We have previously seen that the long-range effects of linkage give only small resonance displacements (0.5-ppm maximum). There is also the possibility of a partially branched sequence, but we have no evidence to substantiate this.

Conclusion

The analysis of the ^{13}C nmr spectra of substituted glucose derivatives and di- and trisaccharides related to dextrans and glucans has demonstrated that the methylation of a hydroxyl group of a glucose unit always produces a larger downfield shift of the carbon bearing this hydroxyl group than the binding of another glucose ring to the same group. This fact was illustrated with each kind of linked glucose compound studied. The sensitivity of a glucose carbon resonance to a pD change is related to its ability to participate in intramolecular hydrogen bonding. Moreover, the resonances of carbons, other than anomeric or exocyclic, involved in α linkages are very sensitive to pD changes.

The assignment of the ^{13}C nmr spectra of polymers of glucose is relatively easy by reference to their related compounds, permitting analysis of their composition and sequence without chemical alteration. Conformational effects are also revealed in the ^{13}C spectra of disaccharides (kojibiose), homoglucans (chemical shift differences betwcen linear α-1→4 glucan, *i.e.*, amylose, and cyclic α-1→4 oligomers, *i.e.*, cyclodextrins), and glucans with mixed linkage. In these latter compounds, the conformational effects are revealed either by small chemical shift differences relative to homoglucans or by small splittings of resonances. The splittings mainly appear at the carbon resonances involved in glycosidic linkages whereas chemical shift differences (relative to monomer) can be manifest in the resonances of other carbons. In some cases the splittings enable a determination of the sequence of the glucan. Carbon-13 nmr spectroscopy is far more sensitive to a given sequence than ^1H nmr. The accuracy with which composition and sequence can be determined depends mainly on the time required to obtain a high signal-to-noise ratio with signal averaging.

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Structure and Stereochemistry of Nucleic Acid Components and Their Reaction Products. III.[1a] Crystal Structure of the Potassium Salt of *N*-(Purin-6-ylcarbamoyl)-L-threonine. Possible Role of Hypermodified Bases Adjacent to Anticodon in Codon–Anticodon Interaction

R. Parthasarathy,*[1b] Jean M. Ohrt,[1b] and Girish B. Chheda[1c]

Contribution from the Center for Crystallographic Research and General Clinical Research Center, Roswell Park Memorial Institute, Buffalo, New York 14203. *Received May 25, 1974*

Abstract: The crystal structure of the potassium salt of *N*-(purin-6-ylcarbamoyl)-L-threonine, a hypermodified base adjacent to anticodons in tRNAs responding to the codons beginning with adenine, has been accurately determined using X-ray diffraction techniques and refined to an R of 0.057 for 1894 reflections. The crystals are monoclinic, space group $P2_1$, with cell constants $a = 15.289$ (2) Å, $b = 6.410$ (1) Å, $c = 8.815$ (1) Å, $\beta = 105.9°$, and $Z = 2$. The hydrogen atoms were located from electron-density difference maps; they indicate a possible coexistence of N(9)–H and N(7)–H tautomers in the crystal. The N^6 substituent is distal (*trans*) to the imidazole ring, forming an intramolecular hydrogen bond N(threonine)—H - - -N(1) of adenine. This conformation of the N^6 substituent is typical of ureidopurines and blocks the two sites N(6)–H and N(1) of adenine that are normally utilized for complementary base pairing in the double helical regions of nucleic acids; the internal hydrogen bonding further enhances the shielding of N(1). This blocking of N(6)–H and N(1) may be important in enhancing the single-stranded conformation of the anticodon loops of tRNA and in preventing the modified adenosine adjacent to the anticodon from taking part directly in codon–anticodon interaction through the complementary base pairing. The conjugated ureidopurine moiety is stacked in a head-to-tail fashion in planes 3.2 Å apart. The C^α and C^β atoms of threonine are nearly in the plane through the ureidopurine moiety; the bulky carboxyl and methyl groups are pointing in opposite directions from this plane and do not interfere with the close stacking. All polar hydrogens take part in hydrogen bonding; in addition, there are two short C—H - - -O contracts. There is little self association of amino acid or nucleic acid moieties through hydrogen bonding; any interaction by way of hydrogen bonding or stacking between these two moieties is minimal. The four water molecules, exhibiting variable hydrogen bonding geometries, are linked to one another and to the threonine and adenosine residues. The K^+ ion has a sixfold coordination. the torsion angles (χ) about the C^α–C^β bond for O^γ and C^γ atoms with respect to N are respectively 54.5 and −67.1°; this conformation of the threonine side chain is different from those observed previously. The carboxyl group is twisted with respect to the nitrogen about the C^α–C' bond by $\Psi = 154.2°$, similar to what is commonly found in amino acids.

The ureidopurine derivative *N*-(purin-6-ylcarbamoyl)-L-threonine (PCT) was isolated and characterized from the total tRNA of yeast.[2] Subsequently, it was shown that PCT occupies a position adjacent to the 3′ end of the anticodon in several tRNAs which respond to the codons beginning with adenine (A).[3] Other such nucleic acid bases as N^6-(Δ^2-isopentenyl)adenine (IPA), its 2-methylthio analog (2-MT-IPA), etc., have been found in an analogous position in several tRNAs whose codons begin with uracil (U).[4] As a part of the macromolecule, these components appear to be involved in acceptor as well as transfer activity of tRNA[5] while as free bases some of them act as cytokinins.[6] Although PCT did not show any such cytokinin activity when assayed using tobacco and soyabean systems, some of its analogs did exhibit excellent activity.[7] In order to understand the role of these hypermodified components in tRNA and also to learn about the stereochemical requirements for cytokinin activity, we undertook X-ray crystallographic and