Wang, T. S.-F., Sedwick, W. D., and Korn, D. (1974), *J. Biol. Chem. 249,* 841.
Weber, K., Pringle, J. R., and Osborn, M. (1972), *Methods Enzymol. 26C,* 1.
Wood, H. N., and Braun, A. C. (1962), *Proc. Natl. Acad. Sci. U.S.A. 48,* 1776.
Yoshida, S., and Cavalieri, L. F. (1971), *Proc. Natl. Acad. Sci. U.S.A. 68,* 200.

Nuclear Magnetic Resonance Studies of the Solution Conformation of Nucleoside Diphosphohexoses and Their Components†

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ABSTRACT: The solution conformations of UDPG, UDPGN, UDPGal, UDPM, UDPGluc, UDPGalc, ADPG, ADPM, GDPG, GDPM, and CDPG and their components Glu-1-P, Gal-1-P, Man-1-P, Gluc-1-P, Galc-1-P, ADP, GDP, UDP, and CDP are studied by high resolution fast Fourier transform nuclear magnetic resonance spectroscopy with iterative computer line shape simulation. The following results were observed. (1) The six-membered ring is in $^4C_1$ chair form with the C(5′)–C(6′) bond in gg ⇄ tg equilibrium for the derivatives of glucose and mannose and gt ⇄ tg for those of galactose. (2) No conformational preference can be detected for C(1′)–O(1′) bond in hexose-1′-P moiety. (3) Chemical shift dependencies for the pyranoid ring protons and their structural and conformational relations are: (a) axial proton is at higher field than equatorial; (b) the shielding effect of a gauche vicinal hydroxyl group is stronger than a trans vicinal; (c) the vicinity of a hydroxyl group located more than three bonds away tends to shift the proton downfield. (4) The conformation of the nucleoside 5′-diphosphate part is [anti, $^2E \rightleftarrows {}^3E$, g′g′ ⇄ g′t′, g″g″ ⇄ g″/t″], with slight variation of each conformation occurring for individual compounds. (5) No significant interactions are detected between the hexose and nucleoside parts in the nucleoside diphosphohexoses, and the hexose and nucleoside components display the same conformational preference as they become integrated to form nucleoside diphosphohexoses.

The derivatives of sugar are important components of biological systems and as such there has been intense effort in the past to unravel the interplay among constitutional, configurational, and conformational aspects of sugar chemistry with a hope of understanding how these molecules are assembled and transformed during cellular processes (Hall, 1964; Horton et al., 1973; Lemieux and Lineback, 1963; Stoddart, 1971). Studies of the aqueous solution conformational dynamics of several sugar derivatives which are also antileukemic agents (Evans and Sarma, 1975; Lee et al., 1975; Wood et al., 1973) have shown that one could advance a conformational rationale for their mechanism of action. In the present paper we attempt to delineate the aqueous solution conformation of known nucleoside diphosphohexoses such as UDPG,[1] UDPGN, UDPGluc, UDPGal, UDPGalac, UDPM, ADPG, ADPM, GDPG, GDPM, and CDPG and their monomeric components. They are cofactors in the biosyntheses of oligosaccharides, polysaccharides, glycoproteins, and glycolipids (Mahler and Cordes, 1971). The present study is undertaken with a hope that investigators in the above area will be able to relate their biological functions vis-a-vis their conformation.

Materials and Methods

The various materials used in the present study are obtained from commercial sources. ^1H nuclear magnetic resonance (NMR) spectra of UDPG, UDPGN, UDPGal, UDPGluc, UDPGalc, UDPM, ADPG, ADPM, GDPG, GDPM, and CDPG (0.1 M, pH 8.0, 30 °C) were recorded at 100, 270, or 300 MHz in the Fourier transform mode. Details of the instrumentation are described elsewhere (Sarma et al., 1973a, Sarma and Mynott, 1972, 1973). ^{31}P NMR spectra of these compounds were recorded at 40.8 MHz to double check the ^1H–^{31}P couplings. Similar experiments for 5′-ADP, 5′-GDP, 5′-UDP, 5′-CDP, Glu-1-P, Gal-1-P, Man-1-P, Gluc-1-P, and Galc-1-P were conducted at pH 8.0 and 5.0 at which the phosphate group is a dianion and a monoanion, respectively. In order to detect the influence of the phosphate at the anomeric position of hexose, α-CH_3-glucose, β-CH_3-glucose, α-CH_3-galactose, β-CH_3-galactose, and α-CH_3-mannose were employed for comparison. Analyses of the spectra were carried out using a LAOCN III computer program. The data thus derived were used to obtain line shape simulation using a program developed in this laboratory. In line shape simulation, the

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[1] Abbreviations used are: UDPG, uridine diphosphoglucose; UDPGN, uridine diphospho-*N*-acetylglucosamine; UDPGluc, uridine diphosphoglucuronic acid; UDPGal, uridine diphosphogalactose; UDPGalc, uridine diphosphogalacturonic acid; UDPM, uridine diphosphomannose; ADPG, adenosine diphosphoglucose; ADPM, adenosine diphosphomannose; GDPG, guanosine diphosphoglucose; GDPM, guanosine diphosphomannose; CDPG, cytidine diphosphoglucose; Glu-1-P, α-glucose 1′-phosphate; Gal-1-P, α-galactose 1′-phosphate; Man-1-P, α-mannose 1′-phosphate; Gluc-1-P, α-glucuronic acid 1′-phosphate; Galc-1-P, α-galacturonic acid 1′-phosphate.

Table II: Chemical Shifts[a] and Coupling Constants[b] of the Nucleoside 5′-Diphosphate Component of the Molecules at 0.1 M, 30 °C

Compounds	pH	$\delta_{1'}$	$\delta_{2'}$	$\delta_{3'}$	$\delta_{4'}$	$\delta_{5'}$	$\delta_{5''}$	δ_2 or δ_5	δ_8 or δ_6	$J_{1'2'}$	$J_{2'3'}$	$J_{3'4'}$	$J_{4'5'}$	$J_{4'5''}$	Σ'^c	$J_{5'5''}$	$J_{5'P}$	$J_{5''P}$	Σ''^d	$J_{4'P}$
ADP	8	2.917	1.561	1.441	1.214	1.099	1.048	4.954	5.294	5.0	5.2	4.5	2.6	3.4	6.0	−12.8	5.5	5.2	10.7	1.8
ADP	5	2.913	1.559	1.378	1.226	1.088	1.054	4.971	5.274	5.5	5.2	3.8	3.5	2.5	6.0	−12.8	5.0	5.0	10.0	2.2
GDP	8	2.726	1.517	1.420	1.156	1.067	1.029		4.926	5.0	5.1	4.8	2.5	3.5	6.0	−12.8	5.8	5.8	11.6	2.0
GDP	5	2.742	1.565	1.358	1.182	1.048	1.040		4.925	5.7	5.2	3.6	3.5	3.5	7.0	−12.0	4.7	4.7	9.4	1.8
UDP	8	2.777	1.202	1.256	1.077	1.054	1.034	2.783	4.803	4.0	5.2	5.1	2.8	3.0	5.8	−11.9	5.5	5.5	11.0	2.6
UDP	5	2.803	1.206	1.186	1.093	1.048	1.003	2.777	4.768	4.4	5.3	5.0	2.1	3.1	5.2	−11.9	4.5	4.5	9.0	2.5
CDP	8	2.785	1.140	1.239	1.079	1.070	1.061	2.936	4.797	3.6	5.2	5.6	3.1	3.1	6.2	−11.9	5.4	5.4	10.8	2.6
CDP	5	2.795	1.147	1.183	1.105	1.094	1.016	2.979	4.828	3.6	5.2	5.2	2.9	2.9	5.8	−11.9	5.1	5.1	10.2	2.6
UDPG	8	2.802	1.203	1.178	1.106	1.071	1.022	2.791	4.764	4.9	5.2	4.4	2.6	3.2	5.8	−12.0	4.5	5.7	10.2	1.8
UDPGN	8	2.795	1.187	1.174	1.099	1.060	1.004	2.785	4.764	4.9	5.4	4.4	2.6	3.2	5.8	−12.0	4.5	5.7	10.2	1.8
UDPGluc	8	2.801	1.197	1.175	1.099	1.054	1.004	2.787	4.761	4.9	5.2	4.4	2.6	3.2	5.8	−12.0	4.5	5.7	10.2	1.8
UDPGal	8	2.802	1.202	1.174	1.104	1.069	1.020	2.789	4.763	4.9	5.2	4.4	2.6	3.2	5.8	−12.0	4.5	5.7	10.2	1.8
UDPGalc	8	2.801	1.190	1.168	1.097	1.044	0.993	2.786	4.753	4.9	5.2	4.4	2.6	3.2	5.8	−12.0	4.5	5.7	10.2	1.8
UDPM	8	2.804	1.199	1.176	1.108	1.065	1.016	2.789	4.770	4.9	5.2	4.4	2.6	3.2	5.8	−12.0	4.5	5.7	10.2	1.8
ADPG	8	2.930	1.579	1.369	1.231	1.092	1.056	4.982	5.278	5.8	5.2	3.7	3.7	2.5	6.2	−10.0	5.1	5.1	10.2	2.4
ADPM	8	2.931	1.573	1.354	1.228	1.081	1.045	4.995	5.285	5.8	5.2	3.7	3.8	2.6	6.4	−10.0	5.4	5.4	10.8	2.0
GDPG	8	2.738	1.562	1.347	1.182	1.059	1.049		4.906	5.6	5.2	3.8	3.5	3.5	7.0	−12.0	5.2	5.2	10.4	2.0
GDPM	8	2.713	1.668	1.428	1.272	1.135	1.128		4.914	5.8	5.2	3.4	3.7	3.7	7.4	−12.0	5.4	5.4	10.8	2.0
CDPG	8	2.813	1.136	1.174	1.102	1.100	1.030	2.944	4.766	3.9	5.2	5.2	2.9	2.9	5.8	−11.9	5.1	5.1	10.2	2.6

[a] The chemical shifts (ppm) are measured from the internal reference, tetramethylammonium chloride (TMA). [b] The coupling constants (Hz) are checked by computer line shape simulation. [c] $\Sigma' = J_{4'5'} + J_{4'5''}$. [d] $\Sigma'' \simeq J_{5'P} + J_{5''P}$

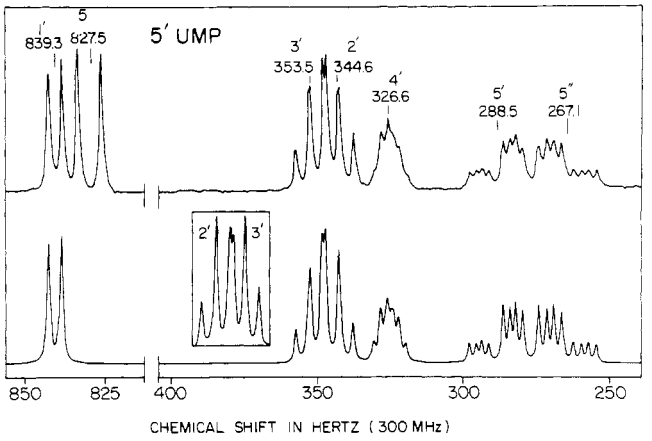
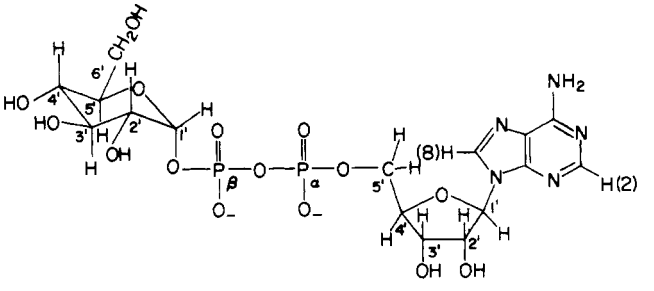


FIGURE 1: The 300-MHz ^{1}H NMR spectrum of 5′-UMP, pD 5.4, 27 °C (top), and the corresponding computer simulation (bottom). The chemical shifts are given in hertz from internal reference tetramethylammonium chloride (TMA). Details are in the text.

hexose phosphate is treated as an ABCDEFMX system and the nucleoside phosphate part as an ABCDEMX system. The normal 7 spin LAOCN III program was modified to accommodate an 8 spin system. Assignments of the 1′ and 2′ resonance for the hexose part are obvious from inspection of ^{1}H and ^{1}H-{^{31}P} spectra. The assignment of the rest of the resonances for the hexose part was made from extensive computer simulations in which from all the possible combinations of assignments a unique set of parameters were derived which generate a simulation identical with the experimental one. The method of assignment employed in the present study primarily utilizes the fact that the ratio of coupling constant (J_{ij}) to chemical difference ($\Delta\delta_{ij}$) of the protons studied is such that the second-order perturbation enables assignment of a unique set of parameters to generate a spectrum which fits the original. Virtual coupling will show up if such second-order effects are strong enough (large $J_{ij}/\Delta\delta_{ij}$). However, if the second-order perturbation is weak resulting from small J_{ij}/Δ_{ij} ratio, the present method of assignment cannot be employed. The 300-MHz ^{1}H NMR spectrum of 5′-UMP, pH 5.0, shown in Figure 1, simply and dramatically illustrates the principle behind our method of assignments. The simulation, bottom of Figure 1, contains an insert of the 2′ and 3′ regions with reverse assignment of these protons. The wrong intensity in the insert indicates that such assignment is incorrect. The derived assignments for the present set of molecules in the form of chemical shifts and coupling constants are summarized in Tables I[2] and II, and the data derived are entirely in accord with those expected of hexose derivatives (Kotowycz and Lemieux, 1973; Angyal, 1968, 1969; Lemieux and Stevens, 1966; Rudrum and Shaw, 1965). Figures 2–5 show some samples of experimental NMR spectra together with the corresponding simulated ones. Despite the excellent agreement between the experimentally observed and calculated spectra, it should be emphasized that there could be an error of as high as 0.4 Hz in J values and chemical shifts (at the employed frequency) in those domains of the spectra where extensive overlap occurs between resonances. In order to minimize the error all spectra were obtained using 16K transform and were recorded noise free using a scale of 2.5 Hz/cm. The numbering of the molecule is shown in the structure of ADPG (I).



I. ADPG

Results and Discussion

(*A*) *Conformation of the Pyranoid Ring.* The conformation of the pyranoid ring is comparable to that of cyclohex-

[2] See paragraph at end of paper regarding supplementary material.

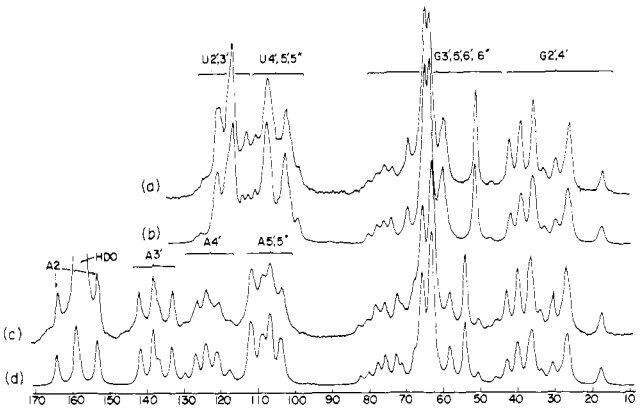


FIGURE 2: The 100-MHz ^{1}H NMR spectra of uridine diphosphoglucose (a) and adenosine diphosphoglucose (c) with corresponding simulations (b) and (d) underneath. The proton peaks of glucose moiety are on the right-hand side, and those of the ribose of the nucleoside moiety are on the left. The chemical shift in Hz is measured from the internal reference tetramethylammonium chloride (TMA). The peaks of the H(1')'s and the base protons are not shown.

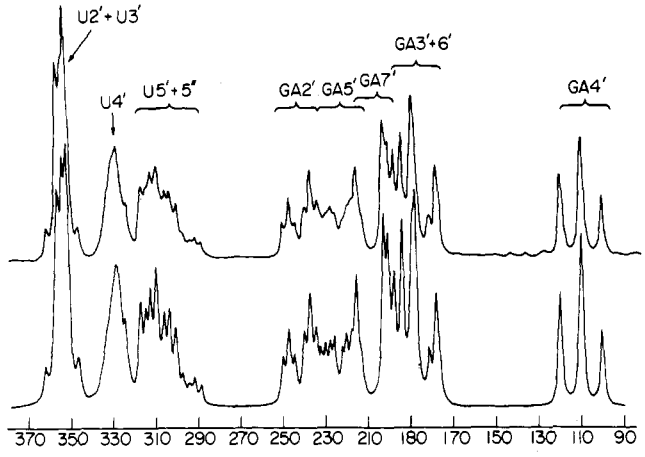
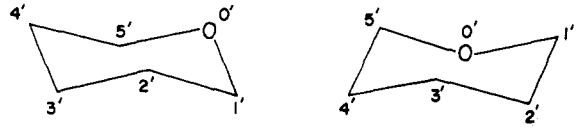


FIGURE 3: The 300-MHz ^{1}H NMR spectrum of uridine diphospho(*N*-acetyl)glucosamine (top) and the corresponding simulation. The chemical shift in Hz is measured from the internal reference tetramethylammonium chloride (TMA). The peaks of H(1')'s, acetyl and base protons are not shown.

ane and its derivatives which are known to exist as various equilibrating chair forms (Romers et al., 1969). Stoddart (1971) has concluded that for the hexoses the $^4C_1$(II) and $^1C_4$(III) chair forms are the most stable ones among the



II, $^4C_1$ III, $^1C_4$

several that occur in the pseudorotational itinerary. There is a wealth of NMR, crystal, and theoretical data (Kotowycz and Lemieux, 1973; Angyal, 1968, 1969; Lemieux and Stevens, 1966; Rudrum and Shaw, 1965; Hall, 1964; Jeffrey and Rosenstein, 1964) that have shown that hexoses show preference to exist in the $^4C_1$ conformation. The magnitude of the vicinal coupling data for the 21 hexose derivatives investigated in this report (Table I) clearly indicates that they show an overwhelming preference to exist in the $^4C_1$ conformation. Later we present the four bond H(2')-P(β) coupling data to substantiate this point. The data (Table I) do not enable us to state whether these molecules exist exclusively in $^4C_1$ form or as an equilibrium system of $^4C_1 \rightleftarrows {}^1C_4$ where the $^1C_4$ population is very small ($\simeq$10%). This ambiguity results from the fact that one does not know precisely the limiting values of J in the pure $^4C_1$ and $^1C_4$ conformers. One may explore the manifestation of this equilibrium situation in the magnitude of the sum of a few coupling constants. Thus, in β-CH_3-galactose H(1') and H(2') are diaxial and H(4') and H(5') are diequatorial in the $^4C_1$ conformer; however, in the $^1C_4$ form they respectively become diequatorial and diaxial. A consequence of this is that irrespective of the position of the equilibrium, $J_{1'2'} + J_{4'5'}$ should

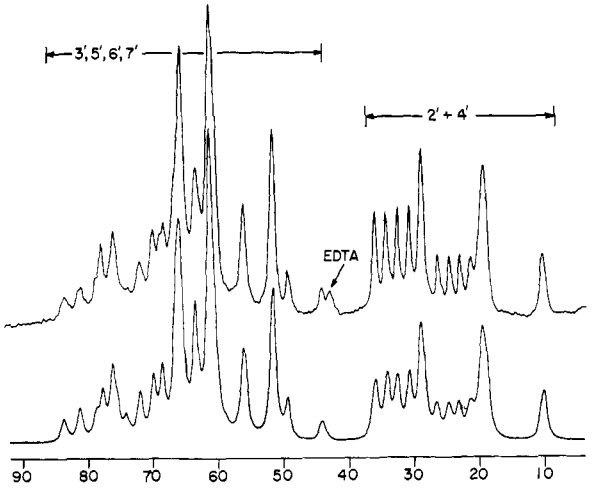


FIGURE 4: The 100-MHz ^{1}H NMR spectrum of α-glucose 1-phosphate at pH 8 (top) and the corresponding simulation. The rest of the details are as in Figure 2.

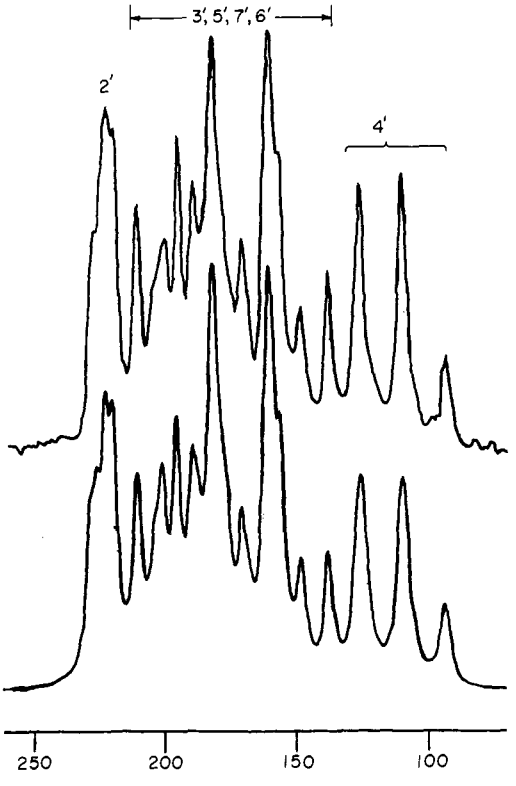


FIGURE 5: The 220-MHz ^{1}H NMR spectrum of α-mannose 1-phosphate at pH (top) and the corresponding simulation. The rest of the details are as in Figure 2.

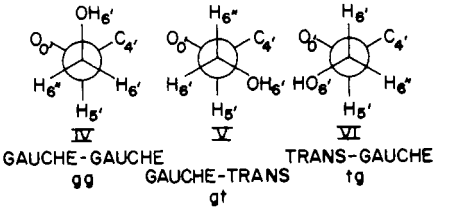
have a constant value. The observed value for this sum is 9.2 Hz. Similarly in the case of α-CH_3-mannose, irrespective of the position of the equilibrium, the sum $J_{1'2'} + J_{4'5'}$ should be constant and the observed value is 11.9 Hz. In fact, if dihedral angle relationship is the only major factor influencing J values in hexoses, the observed sum for $J_{1'2'} + J_{4'5'}$ should be the same for β-CH_3-galactose and α-CH_3-mannose, irrespective of the position of the $^4C_1 \rightleftarrows {}^1C_4$ equilibrium in the two hexose derivatives. The fact that this is not the case indicates that the values of coupling constants are influenced by sterically dependent electronegativity effects (Stoddart, 1971) as well as due to the extent of pucker, i.e., flattening of the ring. The use of the sum of appropriate coupling constant to determine the degree of pucker has been discussed elsewhere (Altona and Sundaralingam, 1973; Evans and Sarma, 1974a). Further qualitative insight into the flattening of the pyranoid ring can be obtained by computing the approximate dihedral angles from some form of Karplus equations[3] such as those of Abraham et al. (1962a,b), Lemieux et al. (1962), or Altona and Sundaralingam (1973). The derived dihedral angles showed that in most cases they significantly deviate from the normal values for the gauche (60°) and trans (180°) conformations. This is again an indication of the flattening of the ring which may result from (1) intramolecular interaction of the atoms and the groups as in cyclohexane and its derivatives (Romers et al., 1969; Davies and Hassel, 1963), and (2) the facts that C–O bond (1.42 Å) is 10% shorter than C–C bond (1.54 Å) in the ring and the endocyclic C–O–C angle (112–114°) is usually larger than the tetrahedral angle (109.5°) (Jeffrey and Rosenstein, 1964; Sundaralingam, 1968). In addition to coupling constants, the chemical shifts of the pyranoid ring protons (Table I) also reveal some structural and conformational dependencies. Firstly, as observed in cyclohexane (Anet, 1962) and in several pyranoids (Lemieux et al., 1958; Lemieux and Stevens, 1966), the chemical shift of an equatorial proton is at a lower field than that of a corresponding axial proton. Secondly, a gauche vicinal hydroxyl (or electron-rich) groups shields the proton to a greater extent than does the trans vicinal one. The H(3′) of galactose and the H(3′) of mannose are at lower field than those of glucose. The same situation exists for the H(2′) of α-glucose and α-galactose with respect to their corresponding β anomers. Chemical shift data on 3′,5′-cAMP, 3′,5′-cUMP, 2′-deoxy-3′,5′-cAMP and 2′-deoxy-3′,5′-cTMP (Lee and Sarma, 1976) further indicate the same conclusion. Thirdly, the presence of a hydroxyl (or electron-rich) group which is spatially *near* but more than three bonds separated from the proton tends to shift the proton downfield in the series of compounds examined. Thus, H(2′) of galactose and H(4′) of mannose are at lower field than those of glucose. The H(3′) and H(5′) of α-glucose and α-galactose (probably α-mannose, too) are at lower field than those of the corresponding β anomers. Also, the H(3′) and H(5′) of Glu-1-P, Gal-1-P, and Man-1-P are at lower field than those of α-CH_3-glucose, α-CH_3-galactose, and α-CH_3-mannose, respectively. Similar electronegative effects have been reported on 8-Br-5′-AMP, 8-methio-5′-AMP, 8-aza-5′-AMP, 8-aza-5′-GMP as well as in formycin 5′-monophosphate (Sarma et al., 1974; Lee et al., 1975, C. H. Lee, R. H. Sarma, N. Yathindra, and M. Sundaralingam, unpublished data).

Table III: Conformations of the Segments in Nucleoside Diphosphohexoses and Their Components at 0.1 M, 30 °C.

Compounds	pH	Hexose Moiety: Hexose Ring[a]	gg[b]	g⁺ + g⁻[b]	φH(1′)-P(β)[c]	Nucleoside Moiety: ²E[b]	g′g′[b]	g″g″[b]
α-CH_3-Glu		$^4C_1$	61					
α-CH_3-Gal		$^4C_1$	13					
α-CH_3-Man		$^4C_1$	56					
β-CH_3-Glu		$^4C_1$	56					
β-CH_3-Gal		$^4C_1$	17					
α-Glu-1-P	8	$^4C_1$	61	75	38			
α-Glu-1-P	5	$^4C_1$	70	76	39			
α-Gal-1-P	8	$^4C_1$	13	75	38			
α-Gal-1-P	5	$^4C_1$	13	76	39			
α-Man-1-P	8	$^4C_1$	57	69	33			
α-Man-1-P	5	$^4C_1$	65	72	36			
α-Gluc-1-P	8	$^4C_1$		74	37			
α-Gluc-1-P	5	$^4C_1$		76	40			
α-Galc-1-P	8	$^4C_1$		75	38			
α-Galc-1-P	5	$^4C_1$		76	40			
ADP	8					50	79	69
ADP	5					55	79	72
GDP	8					50	79	64
GDP	5					57	69	75
UDP	8					40	81	67
UDP	5					44	88	77
CDP	8					36	77	68
CDP	5					36	81	71
UDPG	8	$^4C_1$	74	76	40	49	81	71
UDPGN	8	$^4C_1$	74	75	39	49	81	71
UDPGluc	8	$^4C_1$		76	39	49	81	71
UDPGal	8	$^4C_1$	13	76	39	49	81	71
UDPGalc	8	$^4C_1$		76	39	49	81	71
UDPM	8	$^4C_1$	56	73	37	49	81	71
ADPG	8	$^4C_1$	69	76	39	58	77	71
ADPM	8	$^4C_1$	56	73	37	58	75	68
GDPG	8	$^4C_1$	71	76	39	56	69	70
GDPM	8	$^4C_1$	56	73	37	58	65	68
CDPG	8	$^4C_1$	74	76	40	39	81	71

[a] The six-membered ring is in flattened $^4C_1$ chair form. [b] The conformations are expressed as percent populations. [c] The dihedral angle φH(1′)P(β) is in degrees.

(*B*) *Conformation of the C(5′)–C(6′) Bond of the Hexose Unit.* Among the three energy minimum conformers about the C(5′)–C(6′) bond (IV, V, and VI), Hall and associates (1969) have suggested that in glucose derivatives, rotamer IV, gg is present exclusively. Lemieux and Stevens (1965) and Holland et al. (1967) suggested an equilibrium between rotamers IV (gg) and tg (VI) for glucose derivatives. By selective deuteration of the H(6′) and H(6″) protons their assignments were unambiguously established and it was shown that in a glucose derivative the forms IV and VI contribute significantly to the rotamer population (Horton et al. 1973).

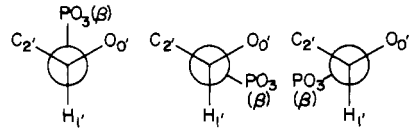


The NMR data of the various derivatives of glucose and mannose (Table I) reveal the tendency that the lower field geminal proton H(6″) couples with H(5′) at a smaller cou-

[3] In order to stress that the dihedral angles computed are only useful in arriving at some generalized qualitative conclusions, we have not reported the computed dihedral angle data in Table I. Hall (1964) in the addendum part of his paper has drawn attention to this.

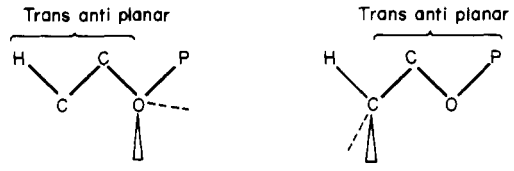
pling constant (1.2–2.4 Hz), while the higher field proton H(6′) couples at a larger value (5.4–6.6 Hz). Using the empirical rules of chemical shift dependence (see previous section), the magnitude of the coupling constants of concern, as well as the repulsion between OH(6′) and the equatorial OH(4′) groups in gt (V) conformation, the geminal protons can be assigned such that conformers IV (gg) and VI (tg) predominate in solution. As a result, the H(6′) proton appears at a higher field. Computed values (Table III) indicate 56–74% population of gg (IV) conformation assuming J_t = 11.7 Hz and J_g = 2.0 Hz. The assignments of the H(6′) and H(6″) protons derived here are in agreement with those reported by Horton et al. (1973). Even though we conclude that both gg and tg conformers are present significantly for glucose derivatives, it should be pointed out that between the two forms, there is in general a preference for gg orientation which is the essence of the original contention of Hall and associates (1969). In the case of galactose derivatives, the geminal protons are magnetically equivalent. However, the magnitude of the coupling constants strongly suggests that gt (V) and tg (VI) are the major conformers present (gg $\simeq$ 13–15%, Table III). Again, the small population of gg (IV) conformer is attributed to the repulsion between the OH(6′) and the axial OH(4′).

(*C*) *Conformation Along the C(1′)–O(1′) Bond.* The Newman projections of the three most stable conformers along the C(1′)–O(1′) bond are shown in VII, VIII, and IX.



VII, trans VIII, gauche$^+$ IX, gauche$^-$

Only a time-averaged population distribution of the trans (VII) and gauche (VIII + IX) conformation states can be determined since vicinal coupling cannot distinguish between conformers VIII and IX. Comparison of the H(3′) and H(5′) chemical shifts of Glu-1-P, Gal-1-P, and Man-1-P with those of the corresponding 1′ methylated analogues indicates that the phosphate shifts the H(5′) downfield to a greater extent than it shifts the H(3′) probably suggesting the existence of trans (VII) conformation and the preference for gauche$^+$ (VIII) conformer over gauche$^-$ (IX). The population of conformer VII (trans) in glucose and galactose derivatives is computed to be 25% (Table III) from $J_{1'P}$ coupling (7.2 Hz) based on the values of $^3J_{^1H-^{31}P,180°}$ (22.9 Hz) and $^3J_{^1H-^{31}P,60°}$ (2.1 Hz) (Hall and Malcolm, 1972a, 1968; Donaldson and Hall, 1972). In the case of mannose derivatives ($J_{1'P}$ = 7.7–8.6 Hz), an increase of 5% (Table III) in the trans conformation is apparently resulting from the absence of the interactions between the phosphate and the OH(2′) group. However, considering the remarkable steric hindrance between the phosphate group and H(3′) and H(5′), the C(1′)–O(1′) bond might exist exclusively in the gauche$^+$ (VIII) and gauche$^-$ (IX) forms with dihedral angles ϕH(1′)P(β) around 33–40° (Table III) computed from the Karplus equation $^3J_{HP}$ = 18.1 cos^2 ϕ_{HP} − 4.8 cos ϕO_{HP} (Lee and Sarma, 1976). Evidence supporting an accurate indisputable C(1′)–O(1′) conformation remains unattainable at present. Even though the two gauche conformations VIII and IX cannot be differentiated by vicinal coupling, after consideration of the steric and electronic interactions occurring between phosphate and OH(2′) group in the gauche$^-$ (IX) conformation, the 1′ phosphorylated glucose and galactose seem to exhibit slight preference for the gauche$^+$ (VIII) conformer. For the four-bond $^4J_{^1H-^{31}P}$ coupling, Hall and Malcom (1968, 1972b) have reported a value of 2.5–2.7 Hz when either pair of alternate bonds in the four-bond linkage are trans anti-planar as the heavy drawing shown in X and XI. In other words,

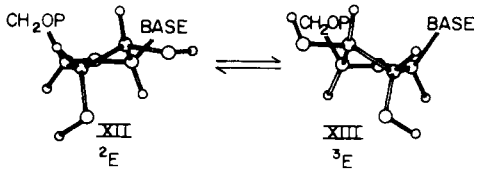


X XI

there are six cases (three for each of X and XI) which may show such magnitude of four-bond coupling. In the case of 1′-phosphoglucose and galactose, when the 1′-phosphate is a monoanion, the $^4J_{^1H-^{31}P}$ is 2.0–2.8 Hz. This is explainable in terms of the existence of a trans anti-planar conformation for H(2′)–C(2′) and C(1′)–O(1′) bonds. This once more proves the preference for $^4C_1$ conformation for the pyranoid ring in these derivatives of glucose and galactose. In 1′-phosphomannose, the H(2′)–C(2′)–C(1′)–O(1′) trans anti-planar conformation cannot exist in the $^4C_1$ chain form so that there is only little chance for the molecule to show any detectable value of the $^4J_{H-P}$ four-bond coupling. As shown in Table I, ionization of the 1′-phosphate tends to reduce the $^1H-^{31}P$ four-bond coupling probably due to the induced change of the electron distribution in the four bonds involved.

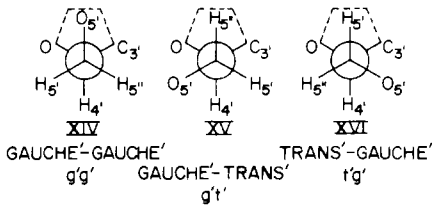
(*D*) *Sugar–Base Torsional Angle.* To determine the glycosidic conformation, the Mn(II) binding (Chan and Nelson, 1969; Evans and Sarma, 1974a,b) studies were carried out for ADPG and ADP. Results indicate that ADPG and ADP preferentially exist in the anti conformation since the peak broadening of H(8) is much greater than that of H(2). This is consistent with the results of studies on 5′-AMP and 3′-AMP by Chan and Nelson (1969), Evans and Sarma (1974b), Schweizer et al. (1968), and Danyluk and Hruska (1968). It is a reasonable assumption that the base in ADPM preferentially exists in the anti conformation, too. In the case of GDP, UDP, CDP, and their hexose derivatives, no direct studies have been conducted in an attempt to investigate this aspect. However, it can probably be assumed that the anti conformation is the preferred one in these molecules based on the following reasons: (1) the available data reported on guanosine, 5′-GMP (Danyluk and Hruska, 1968; Schweizer and Robins, 1973; Thewalt et al., 1970; Murayama et al., 1969), and pyrimidine compounds (Schleich et al., 1972; Frank, 1968; Hruska et al., 1970; Yathindra and Sundaralingam, 1973); (2) the existing evidence (Table I and II) and the chemical shifts and coupling constants of the nucleoside moiety are little perturbed by the pyrophosphate and/or the hexose part; (3) the chemical shifts of H(2′) and H(3′) of the ribose unit show no distinct change as those of the compounds with accessibility of syn conformation (Sarma et al., 1974; Lee et al., 1975; Schweizer et al., 1971; Lee et al., unpublished).

(*E*) *Conformation of the Ribofuranose Ring.* The ribofuranose conformation is described as an equilibrium state between two major conformers, 2′-endo (or ^{2}E, XII) and 3′-endo (or ^{3}E, XIII) which interconvert via the pathway of pseudorotation. According to the pseudorotation treatment (Altona and Sundaralingam, 1973), the percent ^{2}E popula-



tion is about $10J_{1'2'}$. The data in Table III indicate that purine compounds reveal a slight preference for the ^2E conformation (50–58%), but pyrimidine compounds exhibit preference for the ^3E conformation (^2E 36–49%). This trend is essentially similar to that shown by the nucleosides and mononucleotides. Protonation or esterification of the pyrophosphate increases the ^2E population slightly. It is also apparent that the ribose ring conformation essentially remains constant in the nucleoside diphosphohexoses consisting of the same nucleoside moiety but a different hexose. In addition, the magnitude of the $J_{2'3'}$ value in the ribose throughout the nucleotides and their hexose derivatives is nearly constant (5.2 Hz). This suggests that the ring pucker is uniformly constant (Altona and Sundaralingam, 1973) in spite of the presence of the hexose moiety.

(F) Conformation Along the C(4′)-C(5′) Bond of the Nucleoside Part. The time-averaged conformation of the C(4′)-C(5′) bond is expressed as the percent population of the three energy-minimal conformers g′g′ (XIV), g′t′ (XV), and t′g′ (XVI). The absolute assignment of the 5′ geminal

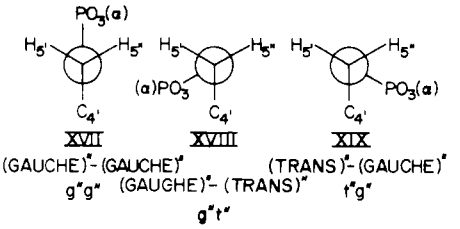


protons at this moment is not unambiguously possible because of complications imposed by the environment and small chemical shift differences. However, the populations of g′g′ and g′t′ + t′g′ (or g′/t′) can be computed using the equations[4] proposed elsewhere (Hruska et al., 1973; Wood et al., 1973a,b):

$$g'g'\,\% = \frac{13.7 - \Sigma}{9.7} \times 100$$

where $\Sigma = J_{4'5''} + J_{4'5'''}$; and g′/t′ % = 100 − g′g′ %. The pyrimidine compounds show generally invariable and slightly higher g′g′ (81%, Table III) population than the purine analogues (g′g′, 65–80%). The data also show that the g′g′ conformation in guanosine compounds is less stable than that in the adenine series. This is probably a reflection of stronger repulsion between guanine and the exocyclic backbone linkage.

(G) Conformation Along the C(5′)-O(5′) Bond of the Nucleoside Part. The three energy-minimal conformers along the C(5′)-O(5′) bond are depicted in Newman projections g″g″ (XVII), g″t″ (XVIII), and t″g″ (XIX). For

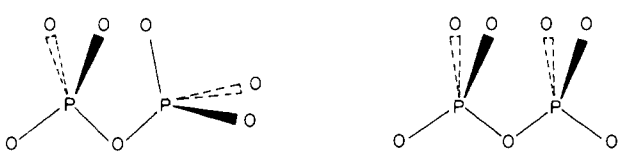


similar reasons as described in previous paragraphs, the time-averaged conformation is expressed in g″g″% and g″g″ + t″g″ (or g″/t″)% computed from the equation[4] (Hruska et al., 1973; Wood et al., 1973a,b):

$$g''g''\,\% = \frac{25.9 - \Sigma'}{20.9} \times 100$$

where $\Sigma' = J_{5'P} + J_{5''P}$ and g″/t″ % = 100 − g″g″ %. The nucleoside diphosphohexoses show constant g″g″ population (70%, Table III) except for ADPM and GDPM (67%). Even though the difference is slight, it does reflect the influence of the axial OH(2′) in the mannose moiety on the C(5′)-O(5′) conformation of the ribose unit. The 5′-diphosphates (Table III) show somewhat discrete distribution of the g″g″ population (64–76%) depending upon the pH conditions of the samples, i.e., lower g″g″ at pH 8, higher g″g″ at pH 5. Compared to 5′-mononucleotides (g″g″, 74–78%) (Davies and Danyluk, 1974), these pyrophosphate compounds display a reduced existence in the g″g″ state (except GDP and UDP at pH 5). It should be noted that these observed changes in the values of g″g″ populations as one goes from the monophosphates to diphosphates may not be true changes in populations because they may as well originate from the changes in electronegativity and inductive effects on Σ″ as the system changes from the monophosphate to diphosphate. The conclusion that in the nucleoside diphosphates and the corresponding nucleoside diphosphohexoses examined the C(4′)-C(5′) and C(5′)-O(5′) bonds preferentially exist in g′g′ and g″g″ conformations (Table III) is further supported by the observed magnitude of the four-bond coupling (Table II) between $^1H(4')$ and $^{31}P(5')$; a discussion on this subject has appeared elsewhere (Sarma et al., 1973b).

(H) Conformation of the Pyrophosphate Bridge. One of the difficult problems encountered in the present study is the determination of the conformation of the pyrophosphate bridge. The four bonds composing the pyrophosphate linkage are O(5′)-P(α), P(α)-O(αβ), O(αβ)-P(β), and P(β)-O(1′). These presumably possess rotational freedom and thus rotate in such a manner that the linkage assumes the most stable conformations. In his work with "polyphosphate", Sundaralingam (1969) has concluded that the staggered (or anti) conformation (XX) is preferred rather than the eclipsed (or cis) conformation (XXI). In rotating a



XX, staggered XXI, eclipsed

[4] The equations proposed (Hruska et al., 1973; Wood et al., 1973a,b) for estimating g′g′ and g″g″ populations are: g′g′% = [(13 − Σ)/10]100, where $\Sigma = J_{4'5'} + J_{4'5''}$; and g″g″% = [(24 − Σ′)/18]100, where $\Sigma' = J_{5'P} + J_{5''P}$. The former is revised as such g′g′% = (13.7 − Σ)/9.7, based on $^3J_{180°HH}$ = 11.7 Hz and $^3J_{60°HH}$ = 2.0 Hz, resulting from the Karplus equation proposed by Altona and Sundaralingam (1973); the latter is revised as such g″g″% = [(25 − Σ′)/20.8]100 by adapting the data $^3J_{180°HH}$ = 22.9 and $^3J_{60°HP}$ = 2.1 Hz (Donaldson and Hall, 1972). Strictly, the equations for calculating g″g″ should be modified to include the electronegativity and inductive effects in the diphospho compounds. This is at present not possible because a large number of data on rigid diphospho compounds are not known. However, it is unlikely that such modifications would affect the populations any more than 5–10%.

P-O bond, three possible anti conformation states may result. Consequently, there will be 27 possible conformations for the P(β)-O($\alpha\beta$)-P(α)-O(5′) linkage in 5′-dinucleotides and 81 possible combinations of the conformation of the O(1′)-P(β)-O($\alpha\beta$)-P(α)-O(5′) linkage in the nucleoside diphosphohexoses. The number of the theoretically possible conformations, indeed, indicates the vast variety of forms in which these biomolecules can exist. Distinction among them cannot be made by NMR methods at present.

(*I*) *The Overall Conformation of Nucleoside Diphosphohexoses.* Information about the overall conformation of nucleoside diphosphohexoses can be obtained by comparing the chemical shift data for the individual components vis-a-vis the integrated nucleoside diphosphohexoses. In examining the data in Tables I and II, it is observed that there is no significant change in chemical shifts in both moieties as the molecules unite to form one entity. The only exception is the H(1′) (0.11–0.13 ppm downfield shift) of the hexose unit. This could be attributed to the deshielding effect of the pyrophosphate linkage. In other words, the intramolecular interaction between the two parts of the molecules is not strong enough to show any considerable change in chemical shift. Therefore, it is reasonable to propose that in aqueous solution, the nucleoside diphosphohexoses exist in states of equilibria among various linear forms in which the component units maintain basically the same conformation as the monomers itself. The pyranose moiety is in [gg* $\rightleftarrows$ tg, $^4C_1$, trans $\rightleftarrows$ gauche*] for glucose and mannose derivatives (Table III) and [gt $\rightleftarrows$ tg, $^4C_1$, trans $\rightleftarrows$ gauche*] for galactose derivatives; the nucleotide part is in [anti, $^2E^* \rightleftarrows {}^3E$, g′g′* $\rightleftarrows$ g′/t′, g″g″* $\rightleftarrows$ g″/t″] for purine compounds and [anti, $^2E \rightleftarrows {}^3E^*$, g′g′* $\rightleftarrows$ g′/t′, g″g″* $\rightleftarrows$ g″/t″] for pyrimidine series. The asterisk indicates the preference in the equilibrium. This is somewhat similar to the situation occurring with coenzyme A and its analogues (Lee and Sarma, 1975) in which the interaction between the adenosine part and the linear segment is also weak.

Supplementary Material Available

Table I, listing of chemical shifts and coupling constants (1). Ordering information is given on any current masthead page.

References

Abraham, R. J., Hall, L. D., Hough, L., and McLauchlan, K. A. (1962a), *Chem. Ind. (London)*, 213.

Abraham, R. J., Hall, L. D., Hough, L., and McLauchlan, K. A. (1962b) *J. Chem. Soc.*, 3699.

Altona, C., and Sundaralingam, M. (1973), *J. Am. Chem. Soc. 95*, 2333.

Anet, F. A. L. (1962), *J. Am. Chem. Soc. 84*, 1053.

Angyal, S. J. (1968), *Aust. J. Chem. 21*, 2737.

Angyal, S. J. (1969), *Angew. Chem., Int. Ed. Engl. 8*, 157.

Chan, S. I., and Nelson, J. H. (1969), *J. Am. Chem. Soc. 91*, 168.

Danyluk, S. S., and Hruska, F. E. (1968), *Biochemistry 7*, 1038.

Davies, D. B., and Danyluk, S. S. (1974), *Biochemistry 13*, 4417.

Davies, M., and Hassel, O. (1963), *Acta Chem. Scand. 17*, 1181.

Donaldson, B., and Hall, L. D. (1972), *Can. J. Chem. 50*, 2111.

Evans, F. E., and Sarma, R. H. (1974a), *J. Biol. Chem. 249*, 4754.

Evans, F. E., and Sarma, R. H. (1974b), *FEBS Lett. 41*, 253.

Evans, F. E., and Sarma, R. H. (1975), *Cancer Res. 35*, 1458.

Frank, G. W. (1968), Ph. D. Thesis, University of New York, Buffalo.

Hall, L. D., (1964), *Adv. Carbohyd. Chem. 19*, 51.

Hall, L. D., and Malcolm, R. B. (1968), *Chem. Ind. (London)*, 92.

Hall, L. D., and Malcolm, R. B. (1972a), *Can. J. Chem. 50*, 2092.

Hall, L. D., and Malcolm, R. B. (1972b), *Can. J. Chem. 50*, 2102.

Hall, L. D., Manville, J. F., and Bhacca, N. S. (1969), *Can. J. Chem. 47*, 1.

Holland, C. V., Horton, D., Miller, M. J., and Bhacca, N. S. (1967), *J. Org. Chem. 32*, 3077.

Horton, D., Durette, P. L., and Wander, J. D. (1973), *Ann. N.Y. Acad. Sci. 222*, 885.

Hruska, F. E., Grey, A. A., and Smith, I. C. P. (1970), *J. Am. Chem. Soc. 92*, 4088.

Hruska, F. E., Wood, D. J., Mynott, R. J., and Sarma, R. H. (1973), *FEBS Lett. 31*, 153.

Jeffrey, G. A., and Rosenstein, R. D. (1964), *Adv. Carbohyd. Chem. 19*, 7.

Kotowycz, G., and Lemieux, R. U. (1973), *Chem. Rev. 73*, 669.

Lee, C. H., Evans, F. E., and Sarma, R. H. (1975), *J. Biol. Chem. 250*, 1290.

Lee, C. H., and Sarma, R. H. (1975), *J. Am. Chem. Soc. 97*, 1225.

Lee, C. H., and Sarma, R. H. (1976), *J. Am. Chem. Soc.* (in press).

Lemieux, R. U., Kullnig, R. K., Bernstein, H. J., and Schneider, W. G. (1958), *J. Am. Chem. Soc. 80*, 6098.

Lemieux, R. U., and Lineback, D. R. (1963), *Annu. Rev. Biochem. 32*, 155.

Lemieux, R. U., and Stevens, J. D. (1966), *Can. J. Chem. 44*, 249.

Lemieux, R. U., Stevens, J. D., and Fraser, R. R. (1962), *Can. J. Chem. 40*, 1958.

Lemieux, R. U., and Stevens, J. D. (1965), *Can. J. Chem. 43*, 2059.

Mahler, H. R., and Cordes, E. H. (1971), Biological Chemistry, 2nd ed, New York, N.Y., Harper and Row, pp 384, 550.

Murayama, W., Nagashima, N., and Shimuzu, Y., (1969), *Acta Crystallog., Sect. B 25*, 2236.

Romers, C., Altona, C., Buys, H. R., and Havinga, E. (1969) *Top. Stereochem. 4*, 39.

Rudrum, M., and Shaw, D. F. (1965), *J. Chem. Soc.*, 52.

Sarma, R. H., Lee, C. H., Evans, F. E., Yathindra, N., and Sundaralingam, M., (1974), *J. Am. Chem. Soc. 96*, 7337.

Sarma, R. H., and Mynott, R. J. (1972), *Org. Magn. Reson. 4*, 577.

Sarma, R. H., and Mynott, R. J. (1973), *J. Am. Chem. Soc. 95*, 1641.

Sarma, R. H., Mynott, R. J., Hruska, F. E., and Wood, D. J., (1973a), *Can. J. Chem. 51*, 1843.

Sarma, R. H., Mynott, R. J., Wood, D. J., and Hruska, F. E. (1973b), *J. Am. Chem. Soc. 95*, 6457.

Schleich, T., Blackburn, B. J., Lapper, R. D., and Smith, I. C. P. (1972), *Biochemistry 11*, 137.

Schweizer, M. P., Broon, A. D., Ts'o, P. O. P., and Hollis,

D. P. (1968), *J. Am. Chem. Soc. 90,* 1042.
Schweizer, M. P., and Robins, R. K. (1973) Proceedings of the Fifth Jerusalem Symposium on Quantum Chemistry and Biochemistry, Bergman, E. D., and Pullman, B., Ed., Jerusalem, Israel Academy of Sciences and Humanities, pp 329–434.
Schweizer, M. P., Witkowski, J. T., and Robins, R. K. (1971), *J. Am. Chem. Soc. 93,* 277.
Stoddart, J. F. (1971), Sterochemistry of Carbohydrates, New York, N.Y., Wiley-Interscience.
Sundaralingam, M. (1968), *Biopolymers 6,* 189.
Sundaralingam, M. (1969), *Biopolymers 7,* 821.
Thewalt, U., Bugg, C. E., and Marsh, R. E. (1970), *Acta Crystallogr., Sect. B 26,* 1089.
Wood, D. J., Hruska, F. E., Mynott, R. J., and Sarma, R. H. (1973a), *Can. J. Chem. 51,* 2571.
Wood, D. J., Hruska, F. E., Mynott, R. J., and Sarma, R. H. (1973b), *FEBS Lett. 34,* 323.
Yathindra, N., and Sundaralingam, M. (1973), *Biopolymers 12,* 2261.

Binding of Human Fibroblast Interferon to Concanavalin A-Agarose. Involvement of Carbohydrate Recognition and Hydrophobic Interaction†

Mary W. Davey, Eugene Sulkowski, and William A. Carter*

ABSTRACT: Human fibroblast interferon binds to a concanavalin A-agarose (Con A-Sepharose) equilibrated with methyl α-D-mannopyranoside, or levan; in contrast, it is only partially retarded on a similar column equilibrated with ethylene glycol. Interferon does not bind, however, to a lectin column equilibrated with both methyl α-D-mannopyranoside and ethylene glycol. Thus, a hydrophobic interaction between fibroblast interferon and the immobilized lectin seems to account for a large portion of the binding forces involved. Other hydrophobic solutes, such as dioxane, 1,2-propanediol, and tetraethylammonium chloride, were found equally or more efficient than ethylene glycol in displacing interferon from the lectin column. The elution pattern of interferon from a concanavalin A-agarose (Con A-Sepharose) column, at a constant ethylene glycol concentration and with an increasing mannoside concentration, reveals the existence of four distinct interferon components. The selective adsorption to, and elution from, a concanavalin A-agarose (Con A-Sepharose) column resulted in about a 3000-fold purification of human fibroblast interferon and complete recovery of activity. The specific activity of the partially purified interferon preparation is about 5×10^7 units per mg of protein. The chromatographic behavior of human leukocyte interferon is remarkable in that it does not bind to concanavalin A-agarose at all indicating the absence of carbohydrate moieties recognizable by the lectin, or if present, their masked status. When concanavalin A was coupled to an agarose matrix (cyanogen bromide activated) at pH 8.0 and 6.0 human fibroblast interferon bound to both lectin-agarose adsorbents and could be recovered with methyl α-D-mannopyranoside. Concanavalin A, immobilized directly on agarose matrix at pH 8.0 and 6.0, thus displays only carbohydrate recognition toward interferon. By contrast, unless a hydrophobic solute was included in the solvent containing methyl mannoside, human fibroblast interferon could not be recovered from concanavalin A-agarose coupled at pH 9.0. When concanavalin A was immobilized via molecular arms, in tetrameric as well as dimeric forms, the binding of interferon again occurred exclusively through carbohydrate recognition. Thus, the hydrophobic interaction can be eliminated by appropriate immobilization of the lectin, and then adsorbed glycoproteins, as exemplified here by interferon, can be recovered readily with methyl mannoside alone.

The understanding of forces involved in lectin–glycoprotein recognition is of immediate importance for the judicious use of lectins in the studies of cell membrane topography (Noonan and Burger, 1973; Cuatrecasas, 1973; Penhoet et al., 1974) and their application as solid phase affinity chromatography adsorbents (Cuatrecasas and Tell, 1973; Bessler and Goldstein, 1973). These forces may encompass—a priori—both carbohydrate–protein (lectin) and protein–protein (glycoprotein–lectin) interactions.

The pioneering studies of Goldstein and his coworkers (1974) first established the structural requirements for a carbohydrate to be recognized by concanavalin A. The protein–protein interactions are still largely unexplored despite the growing list of reports on the use of lectins, concanavalin A in particular, as chromatographic adsorbents (Norden and O'Brien, 1974; Rush et al., 1974; Gurd and Mahler, 1974). It is apparent, however, that only successful purification procedures are reported, the recovery of a glycoprotein from the lectin column being taken as the immediate measure of achievement. The failure to recover a glycoprotein with a simple monosaccharide is either reported as a successful immobilization (Sulkowski and Laskowski, 1974) or otherwise becomes an unheralded personal experience.

In our previous report on the binding and elution of human fibroblast interferon from a concanavalin A-agarose column it was established that bound interferon could not

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