On the conformation of UDP-Glc, a sugar nucleotide †

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Received (in Cambridge, UK) 29th November 1999, Accepted 6th January 2000

UDP-Glc [uridine 5′- (α-D-glucopyranosyl pyrophosphate)] is present in a folded conformation, at least in part, in a number of solvents and *in vacuo* based on differential reactivity studies of its hydroxy groups, NOE effects, and *ab initio* calculations.

The sugar nucleotides are an important group of molecules about which relatively little conformational information exists. There is evidence for both extended and folded forms. The evidence for an extended structure is: a) a crystal structure for UDP-Glc [uridine 5′-(α-D-glucopyranosyl pyrophosphate)];[1] b) proton solution NMR studies for ADP-Glc (the adenosine analog) and UDP-Glc;[2] and c) ^{13}C NMR studies for UDP-Glc using lanthanide-induced shifts.[3] On the other hand, the evidence for a folded structure was until recently: a) a crystal structure for cytidine-5′-diphosphocholine;[4] b) kinetic evidence showing that the rates of hydrogenation,[5] hydroxylaminolysis,[5] and mercuration[6] of the uracil residue of UDP-Glc are slow as compared with the corresponding rates for UMP or UDP; c) studies showing that the addition of urea produces large changes in the optical rotations of sugar nucleotide solutions;[7] d) less compelling but suggestive evidence comes from considerations of which portions of these molecules are essential and non-essential for enzyme activity.[8] Finally, NAD^+ exists in two crystal forms, one extended and one folded.[9]

Two papers appeared in 1999 which give further evidence for a folded conformation. Petrová *et al.*[10] made extensive calculations while Kim *et al.*[11] reached similar conclusions on the basis of differential reactivity and more limited computations. We expand here our combined experimental and theoretical approach and present evidence for a folded conformation of UDP-Glc, differing from a previous proposal,[12] based on the reactivity of its hydroxy groups toward acylation, new NMR evidence, and on calculations of minimum energy structures.

Results and discussion

Selective reactivity

UDP-Glc, as the sodium salt, reacted with 2 equivalents of benzoic, phthalic, isatoic, and *N*-methylisatoic anhydrides in aqueous dioxane (1 : 3) to yield, principally, a one-to-one mixture of UDP-Glc monoacylated at the 2′ and 3′ positions. (See Fig. 1 for the numbering conventions.) Table 1 reports the ^{1}H NMR data for these products from reaction with isatoic anhydride. These mixtures of monoacylated products are produced in about 50% yield using 2 equivalents of acylating reagent. Polyacylated products are also formed. But of much greater interest is the formation of products in which the glucose residue is acylated. Here we find an unusual pattern of hydroxy group reactivity which has led to our proposal for the conformation of UDP-Glc. Thus, the examination of NMR spectra of crude mixtures of similar reactions using tosyl chloride, benzoyl chloride, and acetic anhydride showed mixtures of the 2′, 3′, 4″, and 6″ derivatives. In no case were the 2″ and 3″ positions acylated, although these positions react under forcing conditions.[13] Table 1 shows the NMR data for the reactions with acetic anhydride.

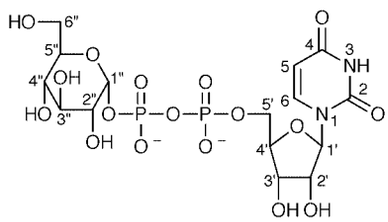

Fig. 1 The numbering convention for UDP-Glc.

The sites of acylation in partially derivatized UDP-Glc mixtures are determined by examining the chemical shifts, the splitting patterns, and the integration values. Acylation produces a chemical shift of about 1 ppm on the carbon-bound proton 3 bonds distant and about 0.1 ppm on the proton 4 bonds distant from the acyl group. Thus, by comparison with UDP-Glc itself and the completely acylated species (Table 1), it is easy to assign the underivatized positions in the mixture. It is more difficult to be certain of the exact composition of the mixtures; this is done by identifying the sets of resonances of equal integration value, which is possible if the sets differ sufficiently from each other in abundance. On this basis we find that the mixtures resulting from reaction with two or five equivalents of acetic anhydride consist largely of four components: two triacetylated molecules: 2′,3′,4″-tri-*O*-acetylUDP-Glc and 2′,3′,6″-tri-*O*-acetylUDP-Glc in about 20% yield for the reaction with two equivalents of acetic anhydride and about 30% for the reaction with five equivalents. The remainder consists of the diacetylated molecules: 2′(3′),4″-di-*O*-acetylUDP-Glc and 2′(3′),6″-di-*O*-acetylUDP-Glc in 80% yield for the 2 equivalent reaction and 70% yield for the 5 equivalent case, where the parentheses indicate that the diacetylated compounds are equilibrating mixtures of the 2′- and 3′-derivatives.

It is not surprising that UDP-Glc reacts with acylating reagents preferentially at the 2′- and 3′-hydroxy groups based on pK_a considerations[14] and transesterification data.[15] However, in considering the reactivity of the glucose residue, the observed reactivity order (6″,4″ > 2″,3″) is not what is expected from most of the data cited by Haines,[16] which predict a greater reactivity for the 2″- and 3″-hydroxy groups than for the

† Atomic coordinates are available as supplementary data. For direct electronic access see http://www.rsc.org/suppdata/p2/a9/a909389c.

DOI: 10.1039/a909389c

Table 1 ^{1}H NMR chemical shifts

	1′	2′	3′	4′	5′	5	6
UDPG[a]	6.002(d)	4.403	4.378	4.306	4.271, 4.222	5.991(d)	7.964(d)
UDPG-Ac_6[b]	6.10(d)	5.36–5.41		4.44	4.05, 4.25	5.93(d)	7.92(d)
UDPG-$Ac_{(2)}$[c]	5.95, 6.05	5.20–5.30	5.35–5.40	4.5	4.10–4.30	5.8–5.9	7.8–7.9
UDPG-$Ac_{(5)}$[d]	6.05, 6.10	5.20–5.30	5.35–5.40	4.5	4.10–4.30	5.85–5.90	7.8–8.0
UDPG-I[e] 2′	6.09	5.4–5.6	4.0–4.3	4.0–4.3	4.0–4.3	5.85(d)	Obscured
3′	6.04	4.5–4.7	5.4–5.6	4.5–4.7	4.0–4.3	5.88(d)	Obscured

	1″	2″	3″	4″	5″	6″	-$COCH_3$
UDPG[a]	5.621	3.556	3.793	3.483	3.915	3.799, 3.875	—
UDPG-Ac_6[b]	5.68(d)	4.95	5.36–5.41	5.04(dd)	4.25–4.32	4.15, 4.30	1.90–2.10(6s)
UDPG-$Ac_{(2)}$[c]	5.5–5.6	3.4–3.5	3.8	3.35–3.40(dd) 4.75(dd)(minor)	4.0–4.3	3.85–4.00	1.90–2.10(4s)
UDPG-$Ac_{(5)}$[d]	5.5–5.6	3.4–3.5	3.8	3.4(dd)(minor) 4.8(dd)	4.0–4.3	3.85–4.00	1.90–2.10(4s)
UDPG-I[e] 2′	5.4–5.7	3.3–3.4	3.6–3.8	3.3–3.4	3.6–3.8	3.6–3.8	—
3′	5.4–5.7	3.3–3.4	3.6–3.8	3.3–3.4	3.6–3.8	3.6–3.8	—
Glucose-1-P-I[f]	5.59	4.84	4.03	3.50	3.88	3.6–3.8	—

[a] Lee and Sarma[2] in D_2O. [b] In DMF, see ref. 13 for data in DMSO. [c] Mixture resulting from reaction with 2 equiv. Ac_2O in DMF. See Experimental section. [d] Mixture resulting from reaction with 5 equiv. Ac_2O in DMF. See Experimental section. [e] 2′- and 3′-monoisatoyl UDP-Glc in D_2O. See Experimental section. For a COSY spectrum, see ref. 19, p. 58. [f] 2-*O*-Isatoyl-α-D-glucopyranosyl phosphate in D_2O. See Experimental section. For a COSY spectrum, see ref. 19, p. 59.

Table 2 Torsional angles, degrees

	C3′–C4′–C5′–O5′	C4′–C5′–O5′–P	C5′–O5′–P–O_{PP}	O5′–P–O_{PP}–P	P–O_{PP}–P–O1″	O_{PP}–P–O1″–C1″
AM1	−57.0	155.3	−77.1	81.7	70.0	−37.2
6-31G*	−58.1	132.7	−67.5	101.0	82.9	−69.8
"C"[a]	49.1	−168.7	74.4	34	23.5	85.0
"C, opt"	50.7	−168.0	68.8	43.7	38.0	70.0
"Uup"[a]	−60.9	−73.7	82.6	72.9	−28.9	160.2
"Uup, opt"	−62.8	−77.6	90.9	80.2	−14.4	138.1
Crystal[b]	56.5	−151.1	76.2	93.5	−164.9	−72.6

[a] From coordinates supplied by Professor Imberty.[10] [b] From the data in ref. 1.

4″-group, although we note that these relative reactivities are strongly dependent on the reaction conditions.[17,18] In any case, under our conditions, we find that α-D-glucopyranosyl phosphate is acylated preferentially at the 2-OH group (Table 1), as shown by disappearance of the 2-H resonance at δ 3.5 and appearance of a new resonance at δ 4.8. This experiment also provides evidence that the diminished reactivity of the 2″- and 3″-OH groups in UDP-Glc is a true kinetic phenomenon and not the result of equilibration to the more stable product since no acyl migration was observed. Indeed, one can calculate from the data of ref. 15 that at 60 °C in pyridine even the relatively fast migration of an acetyl group from the 2′-OH to the 3′-OH (ribose) has a half-life of the order of 20 h and that at 20 °C, the 2′-*O*-*p*-anisoyl group isomerizes to the 3′-derivative only about 5% in 180 h.

This diminished reactivity of the 2″- and 3″-hydroxy groups in UDP-Glc is consistent with a folded conformation in which these hydroxy groups are partially protected from reaction by the uracil ring. These considerations led, in 1997, to the proposal of a folded conformation by one of us in ref. 19 (p. 70).

This model is consistent with all of the data so far discussed. The low rates of addition or substitution at the 5 and 6 positions of uracil[5,6] are due to shielding by the glucose residue. The uracil residue is in its expected *anti*-conformation. The 2′- and 3′-hydroxy groups of ribose and the 6″- and 4″- hydroxy groups of glucose are unshielded, whereas the 3″- and particularly the 2″-hydroxy groups of glucose are partially shielded by the uracil residue consistent with the observed reactivity pattern for acylation. This model is also consistent with the failure to find changes in the chemical shift and coupling data in proton and ^{13}C NMR spectra[2,3] (as compared with glucose-1-phosphate and UMP) as the distances among the parts of UDP-Glc are too great.

Results of calculations

We have obtained confirmation of a structure of this kind by energy minimization calculations at several levels, beginning at the semiempirical and up to RHF/6-31G*. Table 2 gives the critical torsional angles at two levels of calculation. Also shown for comparison in Table 2 are data from two of the low energy forms of ref. 10 as well as data from the crystal structure.

Fig. 2a shows the structure obtained at the 6-31G* level. This conformation may be described as follows: the glucose residue is $^4C_1$ and the uridine portion *anti*. The uracil moiety is aligned vertically over the essentially flat ribose residue (2′-*endo*). Viewed in this way, with the uracil as the first and ribose as the second and third determinants of a "torsional angle", the glucose residue as the fourth torsional component is placed so as to form, approximately, a −130° "torsional angle". The plane of the glucose residue is displaced about 30° away from that of the uracil ring.

Table 3 lists the distances between the 5- and 6-hydrogens of the uracil residue and the 1″-, 2″-, and 3″-hydrogens of the glucose residue.

The solvation energy as calculated by the Onsager reaction field model is very small and thus the structure is almost independent of the relative permittivity of the solvent.

NMR Evidence

Our initial conventional NOE experiments in D_2O revealed only those interactions between the 5- and 6-hydrogens of the

Table 3 Calculated hydrogen–hydrogen distances, Å

	Hydrogen pairs					
	6–1″	6–2″	6–3″	5–1″	5–2″	5–3″
AM1	3.77	4.42	5.11	4.51	3.62	3.71
6-31G*	5.00	5.99	5.93	6.25	6.34	6.44
"C"[a]	5.00	4.65	3.63	5.49	4.20	4.36
"C, opt"	5.19	5.53	4.85	5.53	4.95	5.08
"Uup"[a]	9.85	9.87	7.12	11.92	11.63	8.81
"Uup, opt"	10.71	11.94	9.47	13.01	14.06	11.41

[a] From coordinates supplied by Professor Imberty.[10]

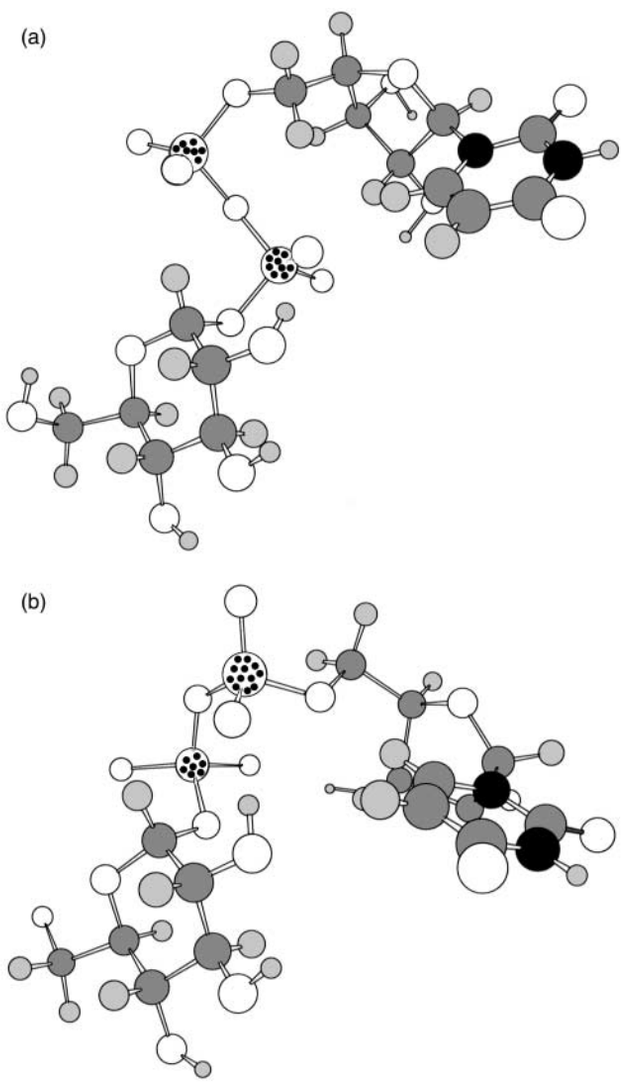

Fig. 2 The 6-31G* (a) and C, opt (b) minimum energy structures for UDP-Glc.

uracil portion and the ribose protons expected for the *anti*-conformation; no interactions were observed with the glucose protons. However, upon using the DPFGSE pulse sequence[20] in DMSO-d_6, we observed the very weak enhancements summarized in Table 4. Two representative spectra are shown in Fig. 3. The DPFGSE pulse sequence is a one-dimensional NOE difference technique developed by Shaka and colleagues which is notable for its smooth baseline allowing very weak NOE effects to be observed. We have ruled out effects due to spin diffusion by observing a linear build-up of NOE enhancement (with extrapolation to zero) with an increase in mixing time for protons 5″, 4″, 3″, and 2″ upon irradiation of proton 5, although the intensities for 4″ are very small.

In DMSO, irradiation of the 5-proton of uracil leads to

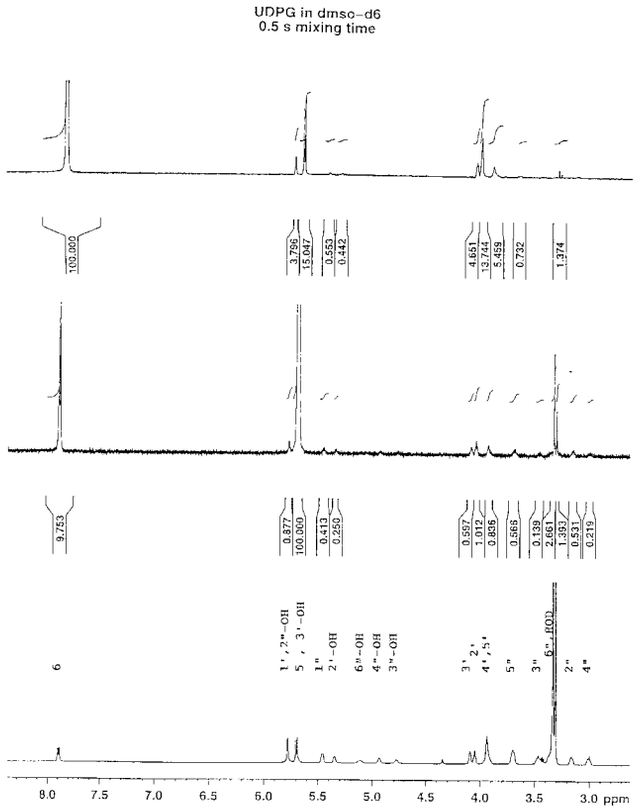

Fig. 3 DPFGSE-NOE spectra at 27 °C. The lowest figure is the normal ^{1}H spectrum. In the upper spectrum, proton 6 was irradiated; in the middle, proton 5. In this sample, the 3′-OH proton overlaps proton 5. In other samples, with slightly different water content, the 3′-OH is upfield of the 5-proton by about 0.1 ppm. Enhancement of the same glucose protons was observed in these samples in a ROESY experiment thus removing any question of interference from the 3′-OH proton.

enhancements of all of the carbon-bound protons of the glucose residue with the possible exception of the 6″ protons, which are hard to observe as they are very close to the HDO resonance. Irradiation of the 6-proton of uracil leads to enhancements of both 1″- and 5″-hydrogens (glucose).

In D_2O the resonances of 5-H and 1′-H are too close to allow separate irradiation of 5. Irradiation of this pair shows enhancement of the 1″-resonance. These two resonances are, however, well-separated in DMSO and irradiation of 1′-proton in DMSO shows no effect on the glucose protons. Therefore, the enhancement of 1″ seen in D_2O upon irradiation of the 5,1″ pair is most likely due to an NOE from 5.

We examined the effects of concentration of UDP-Glc on the NOE enhancements in order to test the possibility of intermolecular effects. DPFGSE-NOE spectra were acquired at 8 mg ml^{-1} (512 scans), 4 mg ml^{-1} (2048 scans), and 2 mg ml^{-1} (8192 scans) while irradiating H-5 (uracil). There was no change in the pattern of enhancements. However, we observed a uniform decrease in the intensities of all the enhancements upon dilution. We do not think that this is an intermolecular effect because the same percent decrease (about 50% at the lowest concentration) is observed for the intensity of H-6 (uracil) as for the ribose and glucose protons. We think it unlikely that the H-5/H-6 interaction can be other than intramolecular and so we tentatively attribute the overall decrease to a viscosity effect on the correlation time especially since we are probably close to the crossover point in the η_{max} *vs.* $\omega\tau_c$ plot.

These NOE experiments provide good evidence on an independent basis from the reactivity studies and the calculational results that UDP-Glc exists, at least in part, in both DMSO and D_2O in a folded conformation. We cannot argue from these NMR results anything about the proportion of

Table 4 NOE enhancements[a,b,c]

Irradiated Proton	Solvent	Proton Number										
		Ribose					Glucose					
		1′	2′	3′	4′	5′	1″	2″	3″	4″	5″	6″
U-5	DMSO-d_6	0.9	1	0.6	0.8		0.4	0.5	0.1	0.2	0.6	[d]
U-5 + R-1′	D_2O	—	1.4		0.9	—	0.3	—	[e]	—	—	[e]
U-6	DMSO-d_6	3	12	4	5		—	—	—	—	0.6	[d]
U-6	D_2O	3	4			—	—	—	—	—	—	—
R-1′	DMSO-d_6	—	5.5		4.5		—	—	—	—	—	[d]

[a] The NOE's are positive for experiments in D_2O, negative for those in DMSO-d_6. [b] Mixing time, 500 ms. In DMSO, the intensity of the irradiated resonance is about 30% larger at zero mixing time (T_1 = *ca.* 2 s) so that the enhancements are reduced by this amount on that basis. The corresponding reductions in D_2O are about 25%. [c] Concentrations here and in Fig. 3 are 8–10 mg ml^{-1}, acquisition time 1 h, 512 scans. [d] Obscured by HDO. [e] Less than 0.03.

folded molecules in the population, but the agreement with the predictions based on the reactivity studies and the calculational data argues for considerable significance.

Comparison with the results of ref. 10

Imberty *et al.*[10] have published a conformational study on two sugar nucleotides in which they perform RHF/6-31G* *ab initio* calculations (using Gaussian 94) on α-D-glucopyranosyl hydrogenphosphate and β-L-fucopyranosyl hydrogenphosphate and use the results to generate a force field parametrization for AMBER in order to calculate conformations for the sugar nucleotides using CICADA. Their results show six low energy families of conformers. Most are stabilized by intramolecular H-bonding. They report that the conformers differ from one another in energy by less than 3 kcal mol^{-1}. Their subsequent molecular dynamics simulations show that there is an interconversion between the more extended and more folded families on a timescale of nanoseconds.

Our calculational study looked instead for a single most stable configuration. We performed a series of geometry optimization calculations starting from the previous optimized configuration and increasing the basis set each time: AM1 (using MOPAC), RHF/STO-3G, RHF/3-21G, RHF/6-21G, RHF/6-31G, and RHF/6-31G* (using Gaussian 94). Our preferred conformation (Fig. 2), like the majority of Imberty's conformers, is significantly folded. Distances relevant to our NMR results are presented in Table 3. Our optimized conformer is at least 10 kcal mol^{-1} more stable at the 6-31G* level than any of the structures found by Imberty *et al.*[10] This may be due to the fact that they optimized only fragments at the 6-31G* level and that the families of low energy conformers were found using parametrization.

We therefore carried out geometrical optimizations on two of Imberty's structures in order to make a meaningful comparison with our own work. The optimization process can be pictured as follows: we start with a relatively low energy structure, that is, within a well on the energy diagram. The process looks downwards and seeks the minimum energy but only within that well. Upon optimizing the geometry of Imberty's conformation "C", we reduced its energy by about 28 kcal mol^{-1} to a new conformation, "C, opt". This is the lowest energy structure that we have found. Table 5 compares the energies of the lowest energy structure obtained by our calculational procedure (6-31G*), two of the structures from ref. 10 (those with uridine in the *anti*-conformation), and also the energies obtained by optimizing the geometries of "C" and "Uup". We see that the optimized conformers are of the order of 30 kcal mol^{-1} more stable than the structures given in ref. 10 and that "C, opt" is about 11 kcal mol^{-1} more stable than "6-31G*". However, these are structures calculated in the absence of solvent. Taking an average hydrogen bond to be worth about 4 kcal mol^{-1}, it only takes about seven hydrogen bonds from solvent to make up a 30 kcal

Table 5 Energies at the 6-31G* level

	Hartrees	Δ/kcal mol^{-1}
AM1	−2644.04092636	59.9
6-31G*	−2644.11823085	11.5
C	−2644.09214090	27.8
C, opt	−2644.13650962	0
Uup	−2644.05677725	50
Uup, opt	−2644.11612838	12.8

difference. Viewed in this way, the energy differences among the structures given in Table 5 are small and may be considered as conformers of nearly the same energy content.

In considering the fit between the calculated structures and the experimental data presented here, the relevant facts are: a) the relative unreactivity of the 3″- and 2″-OH groups of the glucose portion of UDP-Glc toward acylation in aqueous dioxane solution or DMF and b) the NOE enhancements from H-6 and H-5 of uracil toward both the ribose and glucose protons in DMSO and D_2O. Two of the calculated low energy structures fit these data: "C, opt" and "6-31G*". Both of these have uridine in the *anti*-conformation required by the NOE's from H-5 and H-6 to the glucose and ribose protons. "Uup", the only other conformer of ref. 10 to have the *anti*-conformation, has H-5/H-6 to glucose distances too great for NOE contacts.

These two structures, although differing greatly in the torsional angles around the pyrophosphate linkage, have strikingly similar orientations of the relatively rigid uridine and glucose portions toward each other (Fig. 2).

We agree with Imberty *et al.*[10] that the molecule is flexible. This is shown most convincingly by the NOE enhancements not only of H-1″, H-2″, and H-3″ when H-5 is irradiated (as predicted by the conformers shown in Fig. 2) but also enhancements of H-4″, -5″, and -6″. Examination of a CPK model of UDP-Glc suggests that rotation about the O-1″–P bond is sterically unhindered and can bring successively the protons at the 5- and 6-positions of the uracil residue within NOE distance of all of the carbon-bound protons of the glucose residue. The conformation is also solvent dependent as shown by the NOE differences observed in DMSO and D_2O (Table 4). Note that our reactivity studies were carried out in other solvents, but the point is that there is good evidence for a folded conformation in every solvent we have dealt with (DMSO, D_2O, aqueous dioxane, DMF) and *in vacuo* for the calculational work.

Although Neuhaus and Williamson[21] advise against quantitative interpretations in a flexible molecule such as this, calculation of the inter-hydrogen distances (ref. 21, p. 108) gives numbers in the same range as those shown in Table 3 for our structure.

Other variables that should be explicitly considered in future work include solvent and the counter ions, as has already been noted by Petrová *et al.*[10] Our calculations show, for example, that protonation of the phosphate groups leads to an extended rather than a folded structure.

See Anh *et al.*[22] for a good discussion and comparison between results obtained by semi-empirical and *ab initio* methods. The differences between the results of Petrová *et al.*[10] and those presented here are hardly surprising on this basis.

We point out that there are also a number of reports of sugar nucleotides bound to proteins in various conformations.[23]

Preliminary work suggests similar structures for a number of other sugar nucleotides.[24]

Experimental

TLC was performed on Silica Gel 60F254 (Aldrich) on aluminum plates with UV detection using *n*-propanol–NH_4OH–H_2O, 6:3:1 as solvent. Column chromatographies were performed on DEAE Sephadex A-25, Dowex 50W (50X8-200), Sephadex G-10 (Pharmacia Fine Chemicals) or Bio-Gel P2 (BioRad) using 12 × 2.5, 18 × 1.8 and 44 × 3 cm columns. UV absorption of eluates were monitored by a Single Path Monitor UV-1 (at 280 nm) and a Linear 1200 (Pharmacia Fine Chemicals) recorder. Evaporations were carried out by using a Rotavapor R110 (Buchi) under reduced pressure (water pump). Microanalyses were performed by Quantitative Technologies, Inc., Whitehouse, N.J. ^{1}H NMR spectra were recorded at 500 MHz (Bruker AM-500) or at 600 MHz (Bruker DMX 600) for the DPFGSE-NOE spectra. ^{13}C NMR spectra were recorded at 125.8 MHz (Bruker AM-500) and ^{31}P spectra at 121.5 MHz (Bruker MSL-300). DPFGSE-NOE experiments were recorded with mixing times ranging from 0.1 to 1 s and a recycle time of 6 seconds to allow for relaxation. Usually a 50 ms gaussian shaped 180 degree pulse was used to selectively excite the resonance signal of interest. In a few cases a 100 ms gaussian shaped pulse was used when another resonance signal was near the one of interest. Except when we were examining the effect of mixing times on the NOE enhancement, the selected peak was arbitrarily assigned a value of 100% to measure the NOE buildup. It should be noted that the observed NOE's were positive when the UDPG sample was dissolved in D_2O and negative when it was dissolved in DMSO-d_6. A phase sensitive, States-TPPI, ROESY 2-D experiment was acquired using a 250 ms spin-lock pulse whose field strength was 2500 Hz. The data were processed to give a 1 K by 1 K real data set. Phthalic anhydride was purified by fusion and followed by solidification with stirring in a hydrocarbon solvent. All other reagents were reagent grade and were used without further purification.

Typical procedure for a monoacylated UDP-Glc using isatoic anhydride: 2′- and 3′-monoisatoyl UDP-Glc

UDP-Glc (45 mg, sodium salt) was dissolved in 1 ml of water, and adjusted to pH ~9.3 with 2 M NaOH. Isatoic anhydride (25 mg, 2.5 eq.) dissolved in 3 ml dioxane was added and stirred for an hour at room temperature. After UDP-Glc disappeared (TLC), the reaction mixture was applied to a P-2 column, and the peak first eluted with water was collected, and lyophilized to dryness. The resulting solid was dissolved in 3 ml of water, and applied to a DEAE Sephadex column (HCO_3^- form). Products were eluted with a linear gradient of ammonium bicarbonate buffer (0.01 M and 1 M, 250 ml each). A peak that corresponded to the product in TLC was collected, and lyophilized to dryness. The solid obtained was dissolved in water (2 ml), applied to a P-2 column, collected, and lyophilized to give a white solid. ^{1}H NMR (500 MHz, D_2O), see Table 1. ^{13}C NMR; (125.8 MHz, D_2O, external dioxane reference), C-5 (104.0, 103.6), C-6 (142.8, 142.4), C-1′ (88.6), C-2′ (73.3, 76.2), C-3′ (73.8, 69.4), C-4′ (84.1, 82.7), C-5′ (66.2, 65.5), C-1″ (96.4), C-2″ (72.5), C-3″ (73.8), C-4″ (70.1), C-5″ (73.8), C-6″ (61.3), aromatic-C (179.6, 168.5, 167.0, 151.2, 136.1, 132.3). Fluorescence emission maximum; 420 nm (excitation at 332 nm). Anal. Calcd. for $C_{22}H_{27}O_{18}N_3P_2 \cdot 2NH_4^+ \cdot 2H_2O$: C, 34.97; H, 5.20; N, 9.27. Found C, 34.72; H, 5.25; N, 9.67%.

Similar procedures were followed for *N*-methylisatoic, benzoic, and phthalic anhydrides.

Acylation of α-D-glucopyranosyl phosphate with isatoic anhydride

α-D-Glucose-1-phosphate (60 mg) was dissolved in 1 ml of water, and the pH of the aqueous solution was adjusted to ~9.3. To the solution, isatoic anhydride (80 mg) (2 eq.) dissolved in dioxane (3 ml) was added slowly at room temperature. The reaction mixture was stirred for an hour, and then applied to a G-10 column. An UV-active fluorescent compound was collected, and lyophilized to give a white solid (2′-*O*-isatoyl-α-D-glucopyranosyl phosphate). ^{1}H NMR (500 MHz, D_2O): See Table 1.

Typical reaction of UDP-Glc with acetic anhydride in DMF

To a DMF solution (2 ml) containing UDP-Glc (tetrabutylammonium salt, 25 mg), acetic anhydride (7.5 μl, 3.3 eq.), pyridine (0.5 ml), and DMAP (<1 mg) were added, and stirred for 2 hours at room temperature under N_2 atmosphere. NMR data are reported in Table 1.

Calculational procedures

To analyze these structures, wavefunction calculations were done using the Austin Model #1 (AM1) Hamiltonian of a standard semi-empirical molecular orbital program, MOPAC. MOPAC uses four valence orbitals per atom plus an appropriate core–core potential. The atomic parameters are optimized so that calculated results reproduce successfully the heats of formation, geometry, dipole moment, and ionization potentials of a number of molecules containing each of the elements studied here. No intrinsic parameters of AM1 were altered for our calculations. Because the semi-empirical method is successful in predicting the stability and properties of hundreds of compounds with bonding patterns similar to those studied here, it is reasonable to use this method in this context. As a further check on the semiempirical results, we performed *ab initio* Hartree–Fock calculations, using GAUSSIAN with the STO-3G, 3-21G, 6-31G and 6-31G* basis sets. The optimized MOPAC configuration was used as a starting point for the GAUSSIAN optimization process. The STO-3G basis results, while unreliable themselves, provided a starting point for the split valence basis sets of 3-21G and 6-31G (which allow orbitals to change size), and the polarized basis set of 6-31G* (which also allows the orbitals to move off the atom centers). See ref. 22 for leading references.

Acknowledgements

We are indebted to Professor V. N. Shibaev for keeping us up to date with the literature, to Professor Imberty for providing unpublished data, and to the referees and Professors G. E. Means and M. P. Foster for useful suggestions.

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Paper a909389c