^1H, ^1H-{^{31}P}, ^{31}P, AND ^{31}P-{^1H} FAST FOURIER TRANSFORM NMR STUDY OF THE SOLUTION CONFORMATION OF THE COFACTORS INVOLVED IN GLYCOGEN SYNTHESIS: ADENOSINEDIPHOSPHOGLUCOSE AND URIDINEDIPHOSPHOGLUCOSE

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1. Introduction

Adenosinediphosphoglucose (ADPG, I) and uridinediphosphoglucose (UDPG, II) (scheme) are important cofactors involved in polysaccharide synthesis. By the use of ^1H, ^1H-{^{31}P}, ^{31}P and ^{31}P-{^1H}* NMR studies and computer simulations, we have unravelled the dynamic three-dimensional solution conformation of these compounds.

2. Materials and methods

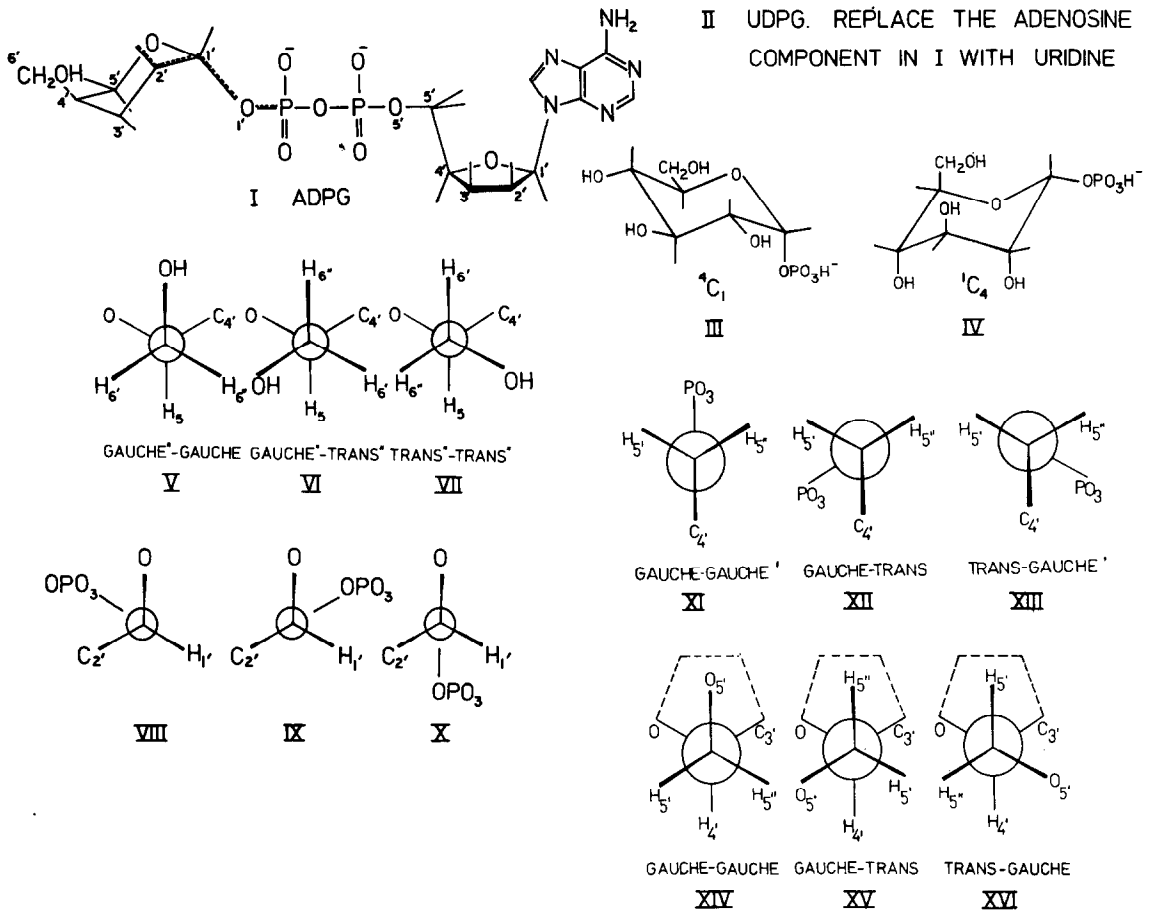
Spectra of the component monomeric units ADP, UDP and α-glucose-1′-phosphate and the cofactors ADPG and UDPG (commercial products) 0.1 M, 30°C in D_2O were obtained using a 220 MHz, continuous wave and a 100 MHz fast Fourier transform system. In the 100 MHz system, spectra were taken in both ^1H and ^1H-{^{31}P} modes. ^{31}P and ^{31}P-{^1H} spectra were recorded in the Fourier mode at a frequency of 40.48 MHz. Details of instrumentation are described elsewhere [1, 2]. The pH of the solution for ADP, UDP and α-glucose-1′-phosphate were respectively 5.2, 5.1 and 5.0 and that for ADPG and UDPG were 8.0. These pH values were selected such that ADP and UDP would remain as diphosphate dianion and α-glucose-1′-phosphate as monophosphate monoanion. This situation is most nearly compatible with the ionic state of the phosphates in ADPG and UDPG at biological pH values. Spectra were analysed using the computer program LAME. In figs. 1 and 2 we illustrate the experimental and computer simulated ^1H and ^{31}P spectra of ADPG. The phosphorus hydrogen couplings for the ribose region ($J_{5'P}$, $J_{5''P}$ and $J_{4'P}$) and for the glucose region ($J_{1'P}$ and $J_{2'P}$) are obtained from both ^1H and ^{31}P spectra. The data for the various protons are compiled in the table.

* The abbreviation ^1H-{^{31}P} etc. stands for ^1H NMR studies done under conditions in which ^{31}P nuclei were decoupled.

3. Results and discussion

3.1. *Conformation of the α-D-glucose moiety of ADPG, UDPG and α-glucose-1′-phosphate*

A D-glucose moiety may exist in either of two mutually interconvertible chair conformations [3] designated $^4C_1$ and $^1C_4$ (III, IV) (scheme). The coupling constant data for the glucose components of α-D-glucose-1′-phosphate, ADPG and UDPG (table 1) are consistent with a transdiaxial relationship for the H(2′)–H(3′), H(3′)–H(4′) and H(4′4)–H(5′) pairs and a gauche



Scheme.

orientation for H(1′)–H(2′) and thus show that each glucose shows a strong bias for the $^4C_1$ form (III) in which the bulky CH_2OH and hydroxyl groups are equitorial and the 1′-phosphate is axial. (Any reasonable estimate of the populations suggests $^4C_1 : {}^1C_4 \simeq 10:1$.) This preponderance of $^4C_1$ is not unexpected in view of the unfavorable 1,3-diaxial interactions and 'anomeric effect' [4] experienced in the $^1C_4$ conformer.

3.2. *The conformation of the exocyclic–CH_2OH group of the glucose moiety of ADPG, UDPG and α-glucose-1′-phosphate*

The magnitude of the sum $J_{5'6'} + J_{5'6''}$ should give information regading the rotational preferences about the C(5′)–C(6′) bond (V–VII). It is reasonable to use the equations developed for computing the rotational preferences of the $-CH_2OH$ group of a ribose [5–7] to determine the extent of contributions from conformations such as V, VI, and VII to the time average observable conformation of the CH_2OH group of the glucose moiety. Computations of the percentage populations from $J_{5'6'} + J_{5'6''}$ using the expressions in references [5–7] indicates a bias (60%) for the 'gauche–gauche' (V) conformer in each glucose moieties.

3.3. *The conformation of the α-1′-phosphate group of ADPG, UDPG and α-D-glucose-1′-phosphate*

Depending upon the C(1′)–O(1′) torsion angle the phosphate may assume the orientations relative to

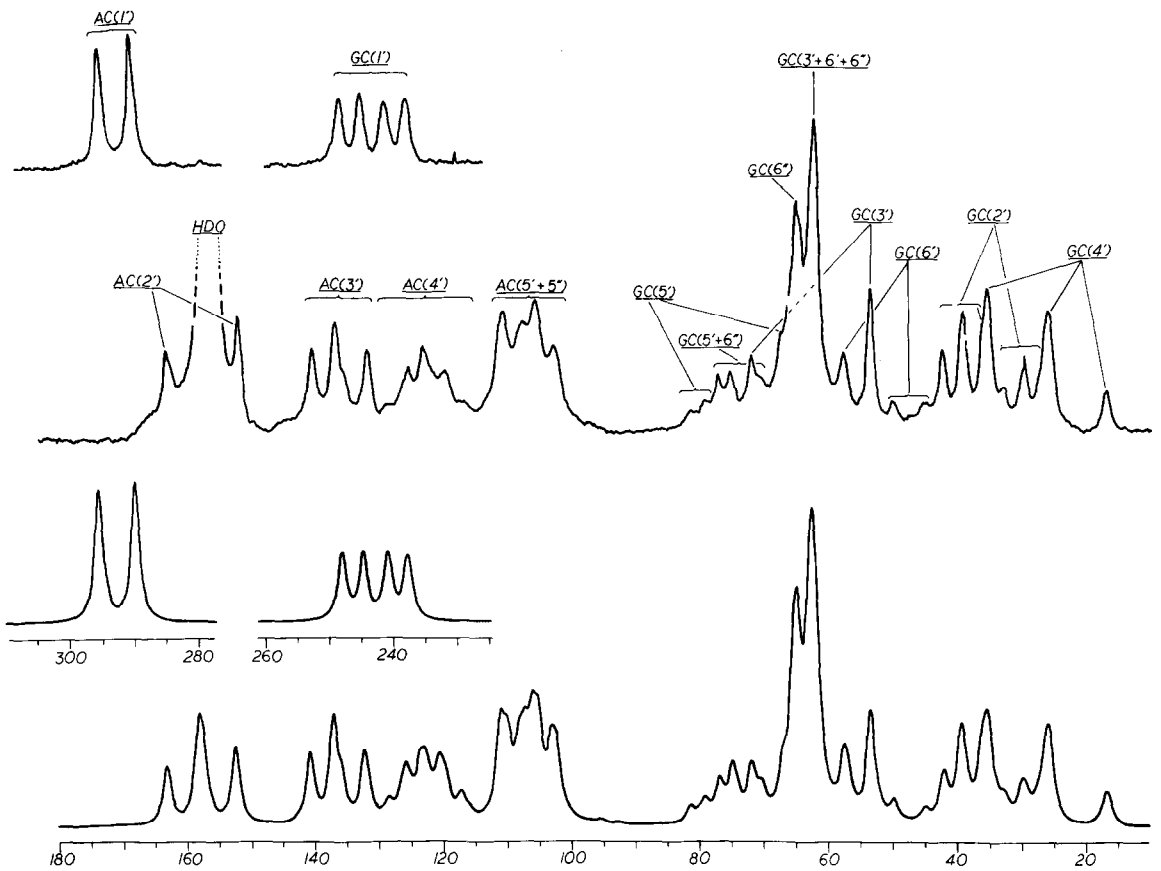


Fig. 1. ^{1}H NMR spectrum (100 MHz) of the ribose and glucose of ADPG. (top) taken using a 16K Fourier transform. The ribose region is labelled *A* and glucose region *G*. Concentration 0.1 M, pH 8.0. The chemical shifts are expressed in Hz upfield from tetramethyl-ammonium chloride; Bottom part shows the computer simulated spectra. Simulations were carried out separately for the glucose and ribose part and were combined by a separate computer program. The agreement between the observed (top) and simulated (bottom) spectra is excellent.

the pyranose ring depicted by VIII, IX and X. The magnitudes of $^3J_{1'P}$ and $^4J_{2'P}$ enable one to estimate the conformer distribution. Thus VIII can be eliminated as a significant contributer since the observed $^3J_{1'P}$ values in each instance are near to the expected gauche H–P coupling in H–C–O–P fragments [8]. Furthermore the $^4J_{2'P}$ values (2.7–3.1 Hz) are compatible only with the (virtually) exclusive occurrence of an in-plane W relationship [5–11] for the H(2')–C(2')–C(1')–O(1')-P fragment which occurs *only in IX coupled with $^4C_1$ but is absent in any other of the six possible combinations of ring-puckering and C(1')–O(1') rotamers* ($^1C_4$–VIII, $^4C_1$–VIII etc.). Thus the 'small' $^3J_{1'P}$ ($\simeq$ 7 Hz) and 'large' $J_{2'P}$ ($\simeq$ 3 Hz) is consistent only with a predominance of the single conformer $^4C_1$–VIII.

3.4. *The conformation of the C(5')–O(5') bonds of the ribose region of ADP, UDP, ADPG and UDPG*

The torsional isomers constrained to the C(5')–O(5') bond are: *gauche'–gauche'* (XI), *gauche'–trans' (XII)* and *trans'–gauche'* (XIII). In earlier work we have demonstrated how estimates of the conformational distribution about the C(5')–O(5') bond of a nucleotide moiety can be obtained from the magnitude of the sum $(\Sigma')\ ^3J_{5'P(\alpha)} + {}^3J_{5''P(\alpha)}$ [5–7, 9–11]. The Σ' values for ADP, UDP, UDPG and ADPG lie in the range 9.6–10.4 Hz which translates into a 75–80% bias for

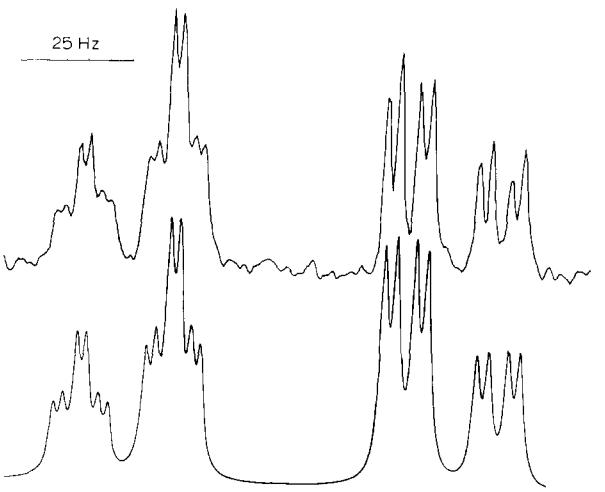


Fig. 2. ^{31}P NMR spectrum (top) of ADPG under the same conditions as above (40.48 MHz) taken using a 4K Fourier Transform. Computer simulated spectrum is shown at the bottom. The group of resonances on the left hand side are from the ^{31}P atom attached to the ribose part; those on the right hand side from the ^{31}P atom attached to the glucose part.

the gauche′–gauche′ rotamer, the predominant form observed in crystalline nucleotides [12, 13].

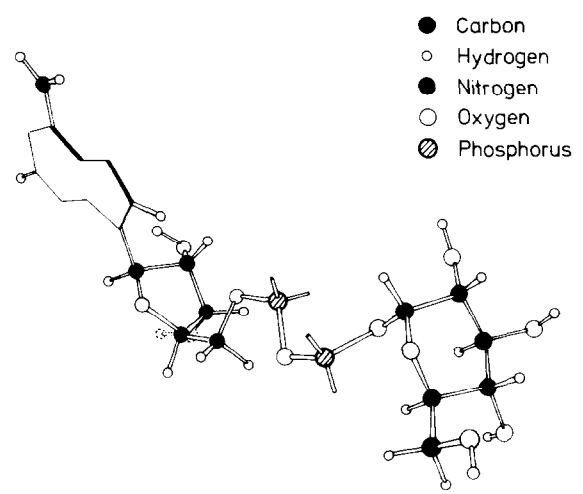


Fig. 3. Three dimensional preferred solution conformation of ADPG.

3.5. *The conformation of the C(4′)–C(5′) bonds of the ribose region of ADP, ADPG, UDP and UDPG*

In a similar fashion the population distribution of the conformers constrained to the C(4′)–C(5′) bond (XIV–XVI) can be evaluated from the experimental sum $J_{4'5'} + J_{4'5''}$ (Σ) using equations developed in references [5–7]. In ADP and ADPG the estimated populations of XIV are 65–70%; in UDP and UDPG, about 80%. These trends are consistent with the observed preferences for XIV in crystalline 5′-nucleotides [12, 13] and with our observations that the uracil base stabilizes XIV (slightly) relative to the adenine base [7].

3.6. *Conformation of the ribose moiety*

Evidence has been presented [2, 14, 15] that the furanose ring is best discussed as a dynamic equilibrium between various puckered forms. Recently a refinement of the model has been presented by Altona and Sundaralingam [16] who discuss the ring in terms of an N($C_{3'}$ *endo*) ↔ S($C_{2'}$ endo) equilibrium). Using their procedure and assuming an average ring pucker of 38° and an average angle of rotation of 162°, we have computed the S populations for ADP (65%), ADPG (65%), UDP (55%) and UDPG (55%). Thus in each instance significant contributions from both N and S are indicated with perhaps a slight bias for the latter.

3.7. *The sugar-base torsion angle*

The spectra of ADP and ADPG were recorded in the absence and presence of Mn(II) ions as described by Chan and Nelson [18] and Sarma and Myott [19, 20]. It was found that the paramagnetic species selectively broadens the adenine C(8)H resonance in ADP and ADPG indicating that in both of these compounds, the base is preferentially oriented in the *anti* conformation. No attempt was made to determine whether the uracil base in UDPG and UDP is *syn* or *anti*; however, it is likely to be *anti* as in UMP [17].

3.8. *General comments*

In sections (3.1–3.7) we have evaluated the conformational preferences of various fragments of ADPG as well as UDPG. From this information we have constructed a three dimensional model for ADPG with these preferred orientations (fig. 3). Although no coupling constant data is available here to define the conformational preferences of the P–O bond which

Table 1

Parameter Compounds	Ribose protons				Glucose protons		
	ADP	ADPG	UDP**	UDPG**	α-G-1'P	ADPG	UDPG
pH	5.2	8.0	5.1	8.0	5.0	8.0	8.0
δ1'	291.0	293.0	281.0	281.3	231.2	243.2	242.0
δ2'	155.2	157.9	122.3	121.9	36.4	35.6	35.6
δ3'	137.0	136.9	121.4	121.3	57.1	61.4	59.1
δ4'	122.1	123.2	112.5	112.8	27.2	27.6	28.1
δ5'	107.1	109.3	107.2	109.3	67.0	72.4	71.3
δ5''	106.6	105.0	103.1	104.7	Na	Na	Na
δ6'	Na†	Na	Na	Na	65.8	67.4	68.1
δ6''	Na	Na	Na	Na	59.2	57.1	59.2
$J_{1'2'}$	5.6	5.8	4.2	4.5	3.5	3.4	3.4
$J_{2'3'}$	5.1	5.0	5.3	5.0	9.8	9.8	9.9
$J_{3'4'}$	3.8	3.8	4.8	4.7	9.4	9.4	9.3
$J_{4'5'}$	Na	Na	Na	Na	9.9	10.1	9.9
$J_{4'5''} + J_{4'5'}$	6.3	6.0	4.9	5.2	Na	Na	Na
$J_{5'5''}$	−10.0	−10.0	−11.9	−12.1	Na	Na	Na
$J_{5'6'} + J_{5'6''}$	Na	Na	Na	Na	6.9	7.0	6.8
$J_{6'6''}$	Na	Na	Na	Na	−12.1	−12.4	−12.1
$J_{1'P}$	Na	Na	Na	Na	6.9	7.0	7.3
$J_{2'P}$	Na	Na	Na	Na	2.7	2.8	3.1
$J_{4'P(\alpha)}$††	1.2	2.1	1.3	1.0	Na	Na	Na
$J_{5'P} + J_{5''P(\alpha)}$	10.4	10.4	9.6	9.8	Na	Na	Na

* Chemical shifts are in Hz upfield from tetramethyl-ammonium chloride.
** Chemical shifts accurate only to ±1 Hz and coupling constants to only ±0.3 Hz.
† Not applicable.
†† Cannot be determined accurately due to broadening.

define the *relative* orientation of the monophosphate fragments, we have presented the pyrophosphate as the staggered (anti) arrangement of the phosphate oxygens which is favored in biological and inorganic pyrophosphates [12]. This arrangement of the pyrophosphate gives the molecule an extended geometry with a large separation between the glucose and adenosine (uridine) moieties. Inspection of the chemical shift data for the glucose fragments of ADPG and UDPG show but slight differences between corresponding protons and support our contention that the molecules are extended since in any folded arrangement which places the base in proximity to the glucose the influence of the ring current fields (large in adenine, small in uracil [21]) should be reflected in characteristic glucose shifts for hydrogens. A further point is made clear when comparing the conformations (J values) of ADP and ADPG, UDP, and UDPG and α-D-glucose-1'-phosphate with UDPG and ADPG is that the monomeric units essentially maintain their conformational integrity in going from monomer to dimer, perhaps of a further manifestation of the extended nature of the dinucleotide.

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