THE CONFORMATION OF SOME PYRANOSE MOIETIES IN THE MOLECULES OF α-D-HEXOPYRANOSE-1-PHOSPHATES, PURINE AND PYRIMIDINE 5'-(α-D-PYRANOSYL PYROPHOSPHATES), AND MUCOPOLYSACCHARIDES

Konoshin Onodera and Shigehiro Hirano
Laboratory of Biochemistry, Department of Agricultural Chemistry
Kyoto University, Kyoto, Japan

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Purine and pyrimidine 5'-(α-D-pyranosyl pyrophosphates) ("sugar nucleotides") are well known as glycosyl donors in the biosynthesis of numerous glycosides, including oligo- and polysaccharides. In order to elucidate the conformational changes during the glycosyl transfer to mucopolysaccharides, it is of significance to examine the conformation of pyranose moities in the molecules of sugar nucleotides as well as in the molecules of oligo- and polysaccharides. Some typical results of our experiments are discussed in the present paper on the basis of nuclear magnetic resonance(nmr) spectral analysis. The presence of ^{C1} conformation is first found in α-D-mannopyranose moieties of α-D-mannopyranose-1-phosphate(I) and guanosine 5'-(α-D-mannopyranosyl pyrophosphate)(II) and in 2-acetamido-2-deoxy-D-hexopyranose moieties of uridine 5'-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate)(III), chondroitin 4- and 6-sulphates, desulphated keratosulphate(HIRANO *et al.*, 1961), and a tetrasaccharide which was prepared from hyaluronic acid(HIRANO *et al.*, 1962). In addition to this, a conformational inversion is newly found in a part of 2-acetamido-2-deoxy-D-galactopyranose moiety in the molecule of chondroitin.

MATERIALS AND METHOD

II was a gift from Professor K. Ogata at our Department, and uridine 5'-(α-D-glucopyranosyl pyrophosphate) was supplied by courtesy of Dr. E. Ohmura, Takeda Research Laboratory, Osaka. The other sugar nucleotides examined were products of the Sigma Chemical Co., U.S.A. Chondroitin 6-sulphate was a product of the Seikagaku Kogyo Co., Tokyo, and was kindly supplied by Dr. T. Furuhashi. All other samples used in the present study were prepared in our laboratory by conventional methods. All nmr spectra were recorded at 60 Mc with a Varian A-60 spectrometer as described in a foot note in TABLE I.

RESULTS AND DISCUSSION

α-D-Hexopyranose-1-phosphates

H-1 signals of α-D-hexopyranose-1-phosphates appear at δ 5.34-5.68 ppm as quartet due to the spin-spin couplings of H-1 with both H-2 and P as shown in TABLE I. In the nmr spectrum of I, H-1 signal appears at δ 5.34 ppm as quartet with $J_{1,2}$ 1.5 cps and $J_{1,P}$ 8.5 cps. The α-D-mannopyranose moiety involves two axial C-H bonds at C-1 and 2 in 1C conformation, and the projected angle between the both C-H bonds is estimated at ca. 180°, according to the Karplus equation(KARPLUS, 1959). Therefore, the small value (ca. 1.0 cps) of $J_{1,2}$ does not support 1C conformation but strongly support C1 conformation for I. Furthermore, the full acetylation of I was carried out, and the nmr spectrum reveals the presence of O-acetate-methyl signals in one axial and three equatorial orientations in the pyranose moiety. The observation fully supports C1 conformation for I.

Purine and Pyrimidine 5'-(α-D-Pyranosyl Pyrophosphates)

H-1' signals in D-ribofuranose moieties of nucleotides

TABLE I

H-1' Signals of D-Hexopyranose and D-Ribofuranose Moieties in the Molecules of Some α-D-Hexopyranose-1-phosphates, Sugar Nucleotides, and Nucleotides[a]

Compound	D-Hexopyranose			D-Ribofuranose	
	H-1'	$J_{1',2'}$	$J_{1',P}$	H-1'	$J_{1',2'}$
α-D-Mannopyranose-1-phosphate[b]	5.34q	1.5	8.5	---	---
α-D-Glucopyranose-1-phosphate[c]	5.40q	3.0	7.0	---	---
α-D-Galactopyranose-1-phosphate[d]	5.68q	ca. 1.0	7.0	---	---
Guanosine 5'-(α-D-mannopyranosyl pyrophosphate)[e]	5.61q	ca. 1.0	7.5	5.90d	5.0
Uridine 5'-(α-D-glucopyranosyl pyrophosphate)[f]	5.61q	3.0	7.0	5.98d	4.0
Uridine 5'-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate)[d]	5.62q	3.0	7.0	5.98d	4.0
Uridine 5'-(α-D-glucopyranosyl-uronic acid pyrophosphate)[d]	5.62q	3.0	7.0	5.98d	4.0
Guanosine 5'-monophosphate[d]	---	---	---	5.91d	5.0
Uridine 5'-monophosphate[d]	---	---	---	5.98d	4.0

a Nmr spectra were recorded at 60 Mc with a Varian A-60 spectrometer at its normal operating temperature. Chemical shifts were shown on δ scale in parts per million(ppm) down field displacement from 2,2-dimethylsilapentane-5-sulphonate as an internal standard in D_2O and spin-spin coupling constants were shown in cycles per second(cps). For α-D-hexopyranose-1-phosphates, the position numbers 1' and 2' are taken to mean 1 and 2, respectively; d, doublet; q, quartet.

b Cyclohexylammonium salt.

c Potassium salt.

d Sodium salt.

e Barium salt.

f Lithium salt.

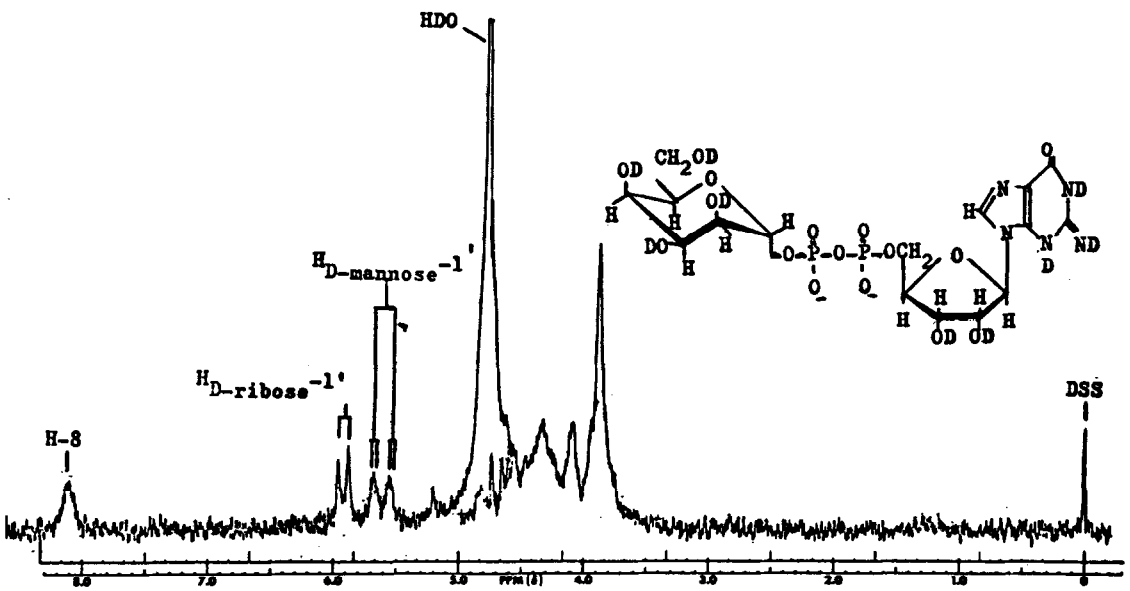

FIG. I
The nmr spectrum of guanosine 5'-(α-D-mannopyranosyl pyrophosphate) recorded at 60 Mc in D_2O.

and sugar nucleotides appear at δ 5.91-5.98 ppm as doublet with $J_{1',2'}$ 4.0-5.0 cps. H-1' signal in α-D-hexopyranose moieties of sugar nucleotides appears at δ 5.34-5.68 ppm as quartet with $J_{1',2'}$ 1.0-3.0 cps and $J_{1',P}$ 7.0-8.5 cps. The difference of chemical shifts makes it possible to assign H-1' signals of both monosaccharide moieties for the conformational analysis on the basis of the spin-spin coupling constants of H-1' and 2'. FIG. I shows the nmr spectrum of II. H-1' signal in D-mannopyranose moiety appears at δ 5.61 ppm as quartet with $J_{1',2'}$ *ca.* 1.0 cps and $J_{1',P}$ 7.5 cps, and that of D-ribofuranose moiety appears at δ 5.90 ppm as doublet with $J_{1',2'}$ 5.0 cps. The small value(*ca.* 1.0 cps) of $J_{1',2'}$ supports *Cl* conformation for α-D-mannopyranose moiety of II. Recently, we observed a conformational inversion caused by certain aglycons with the molecules of α-D-mannopyranosides, and we stated that the conformation and size of substituents

TABLE II

Chemical Shifts of N-Acetate-Methyl Signals in the Molecules of Some Compounds Involving 2-Acetamido-2-deoxy-D-hexopyranoses

Compound	Solvent	N-COMe(ppm)
Methyl 2-acetamido-2-deoxy-α-D-galactopyranoside	D_2O	2.02
Methyl 2-acetamido-6-O-acetyl-2-deoxy-α-D-galactopyranoside 4-sulphate(Ba salt)*	D_2O	2.02
Methyl 2-acetamido-6-O-acetyl-2-deoxy-3,6-di-O-methylsulphonyl-α-D-galactopyranoside*	$CDCl_3$**	2.02
Methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-methylsulphonyl-α-D-glucopyranoside	$CDCl_3$**	2.07
2-Acetamido-2-deoxy-D-mannopyranose	D_2O	2.11
Phenyl 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-α-D-mannopyranoside*	$CDCl_3$**	2.10
Phenyl 2-acetamido-2-deoxy-α-D-mannopyranoside*	D_2O	2.11
Chondroitin methyl ester, prepared from chondroitin 6-sulphate	D_2O	2.00, 2.10
Chondroitin 4-sulphate(Ca salt)	D_2O	2.03
Chondroitin 6-sulphate(Ca salt)	D_2O	2.00
Desulphated Keratosulphate	D_2O	2.03
D-GlcAβ1-3D-GalNAcβ1-4D-GlcAβ1-3D-GalNAc(a tetrasaccharide, prepared from hyaluronic acid)	D_2O	2.01
Uridine 5'-(2-acetamido-2-deoxy-α-D-glucopyranosyl pyrophosphate)	D_2O	2.07

* The details of the preparation of these new compounds will be reported elsewhere.

** Tetramethylsilane was used as an internal standard in $CDCl_3$.

in axial orientation might be an influential factor of conformational inversion(ONODERA et al., 1966). In spite of the relatively large aglycon, the conformational inversion is not found in α-D-mannopyranose moiety of II.

From the examination of chemical shifts of N-acetate-methyl signals in axial and equatorial orientations with the use of a number of N-acetyl derivatives of D-hexopyranoses, it is found that the N-acetate-methyl signal in axial orientation appears at δ 2.10-2.15 ppm and that in equatorial orientation at δ 2.00-2.07 ppm.* Some of the results are shown in TABLE II. The empirical rule is found to be applicable to the conformational analysis of 2-acetamido-2-deoxy-α-D-glucopyranose moiety in the molecule of III. The N-acetate-methyl signal appears at δ 2.07 ppm. This indicates the presence of N-acetyl group in equatorial orientation, which supports C1 conformation for III.

Mucopolysaccharides

H-1 signals are difficult to assign in the nmr spectra of oligo- and polysaccharides. Sometimes, the signals can not be distinguished from the other ring hydrogen-signals. But the conformational analysis with the use of N-acetate-methyl signal is found to be applicable to 2-acetamido-2-deoxy-D-hexopyranose moieties of mucopolysaccharides. As shown in TABLE II, N-acetate-methyl signals in equatorial orientation appear at 2.00-2.03 ppm for 2-acetamido-2-deoxy-D-hexopyranose moieties in the polymers of chondroitin 4-and 6-sulphates, desulphated keratosulphate, and a tetrasaccharide which was prepared from hyaluronic acid. On the other hand, two N-acetate-methyl signals appear at δ 2.00 and 2.10 ppm in chondroitin. This srongly indicates the presence of N-acetyl groups in both axial and equatorial orientations

* The details will be reported elsewhere.

in the molecule of chondroitin. The ratio of the area of both equatorial and axial peaks is ca. 3:2. The inversion mechanism is under investigation on the basis of the conformation of whole polymer in solution.

These results strongly indicate the presence of conformational changes in the course of the biosynthesis of chondroitin-sulphates if biochemical sulphation proceeds on the molecule of chondroitin. The full acetylation of α-D-hexopyranose-1-phosphates and sugar nucleotides as well as mucopolysaccharides is now in progress in our laboratory in order to analyze the complete conformation in solution on the basis of O-acetate-methyl signals in axial and equatorial orientations(HALL, 1964). A more detailed account of the studies will be reported elsewhere.

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