Influence of Nonaqueous Solvents on the Conformation of Blood Group Oligosaccharides†

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Abstract: NMR and circular dichroism results in aqueous solution as well as conformational calculations have suggested that the nonreducing terminal sugar residues of blood group oligosaccharides adopt single, well-defined conformations which are determined mainly by nonbonded interactions. As a test of the hypothesis that electrostatic, hydrogen bonding, and hydrophobic effects do not play a decisive role in determining the conformation of the blood group oligosaccharides, we have studied the ^{1}H NMR spectra of a blood group A tetrasaccharide and an H hexasaccharide in Me_2SO and pyridine solution. Since the chemical shifts differ substantially from those in D_2O solution, complete assignments were carried out by 2-D COSY and phase-sensitive HOHAHA techniques. As was found for the case of aqueous solutions, the rotational correlation time of the molecule (τ_c) in pyridine and in Me_2SO depends strongly on temperature so that nuclear Overhauser enhancement (NOE) can be observed for any of these oligosaccharides. The temperature independence of the ratio of the NOE between protons held at fixed distances by the pyranoside ring to NOE between those on different residues implies that the conformation is *not* strongly temperature dependent. The observed NOE's are qualitatively similar to those previously reported in D_2O solution. Comparison of NOE calculated as a function of glycosidic dihedral angles showed that a single molecular conformation was consistent with experimental data on the oligosaccharides. The conformations of the oligosaccharides in Me_2SO solution are essentially identical with those in D_2O, and conformations in pyridine are very similar, consistent with the proposal that nonbonded interactions have a dominant influence in the conformations.

I. Introduction

The conformations of blood group oligosaccharides were first studied by Lemieux et al.[1] who reported carbon and proton NMR spectroscopic assignments and limited nuclear Overhauser effect (NOE) data for several trisaccharide fragments which model the nonreducing terminals of blood group A,B and H oligosaccharides. In conformational energy calculations they employed a method which assumes that only nonbonded energies and the torsional potential about one of the glycosidic dihedral angles (exoanomeric effect) are important. In these calculations which ignore hydrophobic bonding, hydrogen bonding, and other electrostatic effects, a simple Kitaigorodsky form was used for the nonbonded interactions with the parameter set reported by Venkatachalam and Ramachandran.[2] This formulation of the nonbonded interactions had been used in previous studies of carbohydrate conformation by Rao and co-workers.[3] On the basis of these results, Lemieux et al.[1] proposed single conformations for the blood group oligosaccharides which are determined mainly by steric or nonbonded interactions and by torsional potentials. The influence of hydrogen bonding and other electrostatic effects and of hydrophobic bonding were proposed to be unimportant. Since these conclusions rely on a number of poorly tested assumptions, they must be taken as a working hypothesis, but one which is subject to test. It will be recognized by many biophysical chemists that this proposal concerning the conformation of complex oligosaccharides involves principles distinct from those generally accepted regarding the forces responsible for the conformations of polypeptides and polynucleotides, biopolymers for which the importance of hydrogen bonding and hydrophobic effects are well established by numerous experimental and theoretical criteria.

In research previously reported from this laboratory, we have studied blood group oligosaccharides of the A and H type which vary in size from three to ten sugar residues and which were isolated by HPLC from natural sources.[4-7] Quantitative NOE measurements and vacuum UV circular dichroism as a function of temperature were combined with conformational energy calculations using several types of empirical potential energy functions. Our results for blood group A and H oligosaccharides were consistent with single conformations determined mainly by steric nonbonded interactions in agreement with the proposal of Lemieux et al.[1] Specifically for the nonreducing terminal group having the blood group A structure, we conclude that a single conformation is consistent with the NOE data and that Φ and Ψ of the GalNAc(α-1→3) glycosidic bond are such that H1 of GalNAc is closer to H4 of Gal than to H3 of Gal. Conformational energy calculations suggest that there might be other low-energy conformations with H1 closer to H3 which could be distinguished by NOE measurements, but the experimental data imply unambiguously a single conformation. We agree further with the results of Lemieux et al.[1] that steric interactions predominate in determining this conformation but the precise functional form of the nonbonded interaction and the details of the torsional potential (the exoanomeric effect) could not be confirmed.

The conformation of the trimannosyl core of the asparagine linked oligosaccharides of glycoproteins have been studied by Brisson and Carver,[8-10] using the same experimental methods. These authors conclude that the asparagine-linked oligosaccharides also have rigid well-defined conformations. Further studies of the complex and high mannose asparagine-linked glycopeptides[8,11] show that there are certain points of flexibility in the conformation of the asparagine N-linked oligosaccharides mainly concerned with the mannosyl (α-1→6) mannose linkage. But it would appear that there are some similar principles involved in determining the conformations of both the N-linked and the blood group oligosaccharides. While there are some obviously different conformational features between these two structures, there are also some unifying similarities.

If the conformation of an oligosaccharide is determined mainly be steric interactions, or more exactly by the repulsive part of the

† Research supported by NIH Grant GM-31449.

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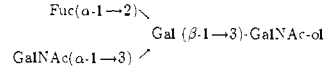
nonbonded interactions, we expect the potential energy barriers surrounding any energy minimum to be high on account of the steeply rising repulsive energy. Therefore, some explicit predictions about the dependence of conformation on temperature and on the nature of the solvent medium are possible. If high-energy barriers surrounding any potential energy minimum are much greater than kT, a change of temperature in the range of 0 to 100 °C is not expected to cause a change in oligosaccharide conformation. This prediction contrasts with the case of peptides and polynucleotides, many of whose conformations are stabilized by specific low-energy bonds such as hydrogen bonds having an enthalpy of stabilization of the order of 2–5 kcal/mol. Although thermal denaturation phenomena are well known for proteins and polynucleotides, they have been shown to be absent in the temperature dependence of CD and of NOE of blood group oligosaccharides.[5,7]

The predominant influence of nonbonded interactions in oligosaccharide conformations has strong implications about the dependence of conformation on the solvent medium. Since hydrophobic forces depend explicitly on the structure of the water and hydrogen bonds are mainly electrostatic, the strength of both types of interaction depends strongly on solvent structure and dielectric constant. In contrast, nonbonded interactions or torsional potentials should not depend so strongly on solvent and we predict that conformations similar to those found in water might be found for blood group oligosaccharides in nonaqueous solvents. The GalNAc anomeric linkage in blood group A oligosaccharides should be an especially sensitive test of this hypothesis. Conformational calculations show that different minima with rather similar energy values exist which can be readily distinguished by NOE measurements.

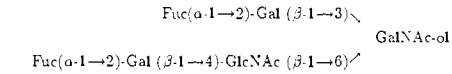
II. Materials and Methods

The oligosaccharide alditols used in this study were isolated from ovarian cyst mucins by HPLC methods previously described.[4,6]

Blood group A tetrasaccharide



Blood group H hexasaccharide



For studies in Me_2SO, about 5 mg of the blood group oligosaccharide was first exchanged in D_2O followed by lyophilization, and the final solution was prepared by dissolving the sample in 0.4 mL of high-purity (99.95 atom % D) Me_2SO-d_6 (Merck Sharp & Dohme). Before recording spectra in pyridine, both samples were first exchanged in D_2O followed by lyophilization; this cycle was repeated once again after exchanging with pyridine-d_5. Final solutions were prepared by dissolving the samples in 0.4 mL of pyridine-d_5 (99.5 atom % D) with small amounts (2.5 and 4% for I and II, respectively) of D_2O. ^1H NMR spectra were recorded at 300 MHz on a Nicolet NT-300 spectrometer equipped with a 293C pulse programmer. Proton spectra were assigned by COSY, 1-D difference decoupling, 1-D and 2-D-homonuclear Hartman Hahn coherence transfer spectroscopy with simulations for spin systems which showed departure from first-order coupling. T_1's were measured by the inversion recovery method and NOE's were measured by the 1-D difference method under steady-state conditions. A 3-s presaturation was used for the irradiated proton and a 1-s delay between successive pulses. Spectra in both solvents were referenced with regard to internal Me_4Si.[12]

Conformational modeling of the oligosaccharides was done with energy calculations using three different sets of empirical potential-energy functions. Following the notation used in an earlier paper,[7] we refer to calculations which used the Lennard-Jones 6–12 potential energy functions parameterized by Momany and Scheraga[13,14] as M&S. We refer to calculations using the parameters of Hopfinger[15,16] as Hop, and we have also used the HSEA method of Lemieux et al.[1] The pyranoside ring conformations were held fixed in the geometry of Arnott and Scott,[17] and the glycosidic dihedral angles were varied in 10° increments. The glycosidic angle Φ is defined by the four atoms O_{ring}–C_1–O_1–C_x and Ψ is defined by C_1–O_1–C_x–C_{x-1} following IUPAC convention in which right-handed rotations correspond to positive dihedral angles. The glycosidic bond angle was 117°.

Because of the large number of degree of freedom involved, not all of the exocyclic dihedral angles could be varied simultaneously with the grid search over the glycosidic angles. Partial searches for the dihedral angles of the hydroxyl and methoxyal groups showed that they had little influence on the energies as a function of the glycosidic dihedral angles. Variation of the amide dihedral angle for the blood group A oligosaccharide showed two minima (χ = 75 and 120°), but both values gave essentially identical results for the variation of the glycosidic angles in agreement with our previous work.[5]

In order to interpret the experimental NOE, the predicted steady-state enhancements and T_1 were calculated for each model conformation by a complete relaxation matrix method used in our earlier work and first suggested by Brisson and Carver.[10,18] The calculated NOE's were then compared with the experimental values. For calculation of the average NOE of an oligosaccharide not having a single conformation, the energies of all conformations below 50 kcal/mol were calculated and the statistical weights were computed using a Boltzmann factor. The cross-relaxation values, σ_{ij}, were calculated for each conformation and multiplied by the statistical weights. Following case II described by Noggle and Schirmer[18] (p 90) for which the internal motions are assumed to be slow compared to τ_c but rapid compared to T_1, the average NOE was then calculated from these averaged σ and ρ using eq 1 of ref 7.

Table I. Experimental Nuclear Overhauser Effect (NOE) Data at 20 and 60 °C for Blood Group A Tetrasaccharide[a]

saturated resonance	observed resonance	NOE (%) in Me_2SO 20 °C	NOE (%) in Me_2SO 60 °C	NOE (%) in pyridine 60 °C
GalNAc H1	GalNAc H2	−17	9	12
	Gal H4	−9	7	9
	Gal H3	−3	2	2
Fuc H1	Fuc H2	−11	7	10
	Gal H2	−13	9	13
	GalNAc H3	−6	3	0
	GalNAc H5	0	0.8	0
Gal H2	Fuc H1	−7	6	11
Fuc H2	Fuc H1	−8	5	8
Gal H4	GalNAc H1	−8	8	12
GalNAc H2	GalNAc H1	n.a.[b]	n.a.[b]	7
GalNAc H3[c]	Fuc H1	−5	5	0

[a] Saturation levels 90–95%; noise level about 1%. [b] n.a. indicates the value is not available because of overlap of resonances. [c] Gal H2 is partially saturated.

III. Results and Discussion

The complete assignment of the proton NMR spectra for the blood group A tetrasaccharide and the blood group H hexasaccharide shows that the chemical shifts of different protons are influenced differently by the three solvents: pyridine, Me_2SO and D_2O.[12] Particularly note that for the blood group A tetrasaccharide in Me_2SO and in D_2O, H1 of α-fucose is downfield from H1 of α-GalNac, while the order of chemical shifts is reversed in pyridne. While a superficial consideration of these chemical shift differences might lead one to conclude that the oligosaccharide conformations differ among these three solvents, we will demonstrate below that this is not the case.

Nuclear Overhauser data for the blood group A tetrasaccharide are given in Table I for Me_2SO at 20 and at 60 °C and for pyridine at 60 °C. The accuracy and precision of these experimental NOE

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Table II. Experimental Proton Spin-Lattice Relaxation Time (T_1) Data at 20 and 60 °C for Blood Group A Tetrasaccharide in Me_2SO

observed resonance	T_1 (ms) 20 °C	60 °C
Fuc H1	568 (±8)	436 (±15)
GalNAc H1	598 (±20)	450 (±12)
Gal H1	446 (±17)	316 (±10)
Gal H4	n.o.[a]	414 (±11)
GalNAc H2	n.o.[a]	865 (±27)

[a] n.o. indicates the T_1 value is not observed because of overlaps.

Table III. Experimental Nuclear Overhauser Effect (NOE) Data at 15 °C for Blood Group H Hexasaccharide in Pyridine[a]

saturated resonance	observed resonance	NOE (%)
$Fuc^{2,4}$ H1[b]	$Fuc^{2,4}$ H2	−11
	Gal^4 H2	−7
$Fuc^{2,3}$ H1	$Fuc^{2,3}$ H2	−13
	Gal^3 H2	−18
Gal^3 H1	Gal^3 H3	−10
	Gal^3 H5	−10
	GalNAc-ol H3	−12
Gal^4 H1	Gal^4 H3	−11
	Gal^4 H5	−11
	GlcNAc H4	−16
GlcNAc H1	GlcNAc H3	−12
	GlcNAc H5	−8
$Fuc^{2,4}$ H2	$Fuc^{2,4}$ H1	−8
$Fuc^{2,3}$ H2	$Fuc^{2,3}$ H1	−9
Gal^4 H2	$Fuc^{2,4}$ H1	−9
Gal^3 H2	$Fuc^{2,3}$ H1	−12
GlcNAc H5	GlcNAc H1	−9

[a] Noise level about 1%. [b] A superscript at the name of a sugar residue indicates to which position of the adjacent next monosaccharide it is glycosidically linked; e.g., $Fuc^{2,4}$ means Fuc is (α-1→2) linked to Gal which itself is (β-1→4) linked to GlcNAc.

data (about ±1%) have been evaluated previously.[7,12] As in the case of aqueous solution, the rotational correlation times depend strongly on temperature so that in Me_2SO modest negative NOE's are observed at 20 °C and modest positive NOE's are observed at 60 °C. Proton T_1 data for the blood group A tetrasaccharide in Me_2SO at 20 and at 60 °C are given in Table II. For pyridine solution at 20 °C, NOE's were very small and measurable NOE's were observed only at 60 °C. For the blood group H hexasaccharide in pyridine, negative NOE's were observed at 15 °C with smaller effects at higher temperature (see Table III). In spite of the differences in the chemical shifts and absolute values of the NOE's among the D_2O, Me_2SO, and pyridine solutions, the ratios of the NOE's do not differ greatly. It will be shown that a detailed interpretation of the data can be based on the assumption that only the rotational correlation times of the oligosaccharides change among the solvents and that the conformations change very little or not at all.

The calculation of the NOE for a model conformation using the full relaxation method is sensitive not only to the distances between protons of the model but also to the rotational correlation time of the molecule.[7-10] In our earlier work on the blood group A tetrasaccharide in aqueous solution, the values of τ_c used in the model calculations were adjusted so that calculated carbon T_1's and values of NOE between protons which were not very sensitive to model geometry agree with the experimental values. Unfortunately, no carbon T_1 data for any of these oligosaccharides in pyridine or Me_2SO are available, so τ_c was adjusted to reproduce experimental values of NOE between protons on the same pyranoside ring such as at fucose H2 on saturation of fucose H1 and that at GalNAc H2 on saturation of GalNAc H1. The estimated τ_c value for the blood group A tetrasaccharide was approximately 0.4 ns at 60 °C in both pyridine and Me_2SO and about 0.9 ns at 20 °C in Me_2SO. As a result of the very great sensitivity of the calculated NOE to the choice of τ_c, the experimental NOE data were taken as ratios of NOE at an inter-ring proton to an intra-ring proton.[8-10]

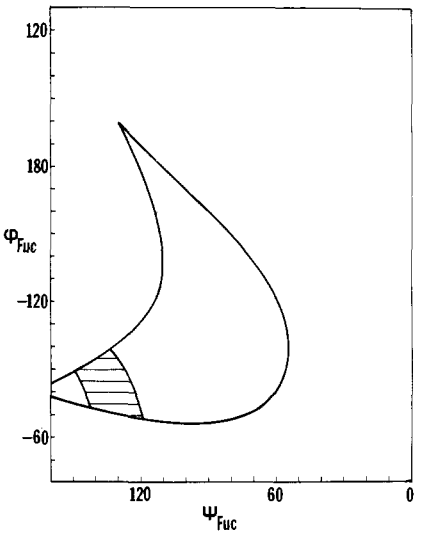

Figure 1. Regions of the conformational map for the Fuc(α-1→2) linkage of R6 in which computed NOE's agree within experimental error of the values in Table I, for Me_2SO solvent. The large enclosed region is that in which the ratio of the NOE at Gal H2 to that at Fuc H2 on saturation of Fuc H1 agrees with experiment, and the smaller lined region is that in which the computed NOE values of GalNAc H3 and H5 on saturation of Fuc H1 also agree with experimental values.

Our approach to the interpretation of the experimental NOE for the blood group A tetrasaccharide in Me_2SO is similar to that used previously for the D_2O data in which the NOE values were divided into three groups.[7] In the first group were those whose calculated values depend primarily on the glycosidic angles of GalNAc such as the computed ratio of NOE at Gal H3 or H4 or GalNAc H2 on saturation of GalNAc H1. The second group consisted of those NOE's which depend primarily on the glycosidic dihedral angles of fucose and a third group whose NOE's depend on both glycosidic angles. For the first group of NOE data, the conformations of the fucosyl and galactosyl linkages were held constant while a two-dimensional map of the computed NOE ratios of Gal H3 and of Gal H4 to GalNAc H2 on irradiation of GalNAc H1 as a function of the GalNAc glycosidic angles was generated at 10° intervals. The resulting maps show that the regions of conformational space in which the computed values are within experimental error of the data of Table I for the blood group A tetrasaccharide in Me_2SO at both 20 and at 60 °C were essentially identical with those for the D_2O data, (see Figure 3 of ref 7).

The second group of NOE's for the blood group A tetrasaccharide in Me_2SO in Table I depend mainly on the value of the glycosidic dihedral angles of fucose. In this group, the two-dimensional map of the computed NOE ratios of Gal H2 to fucose H2 on saturation of fucose H1 was calculated and compared to the experimental values in Table I. The region consistent with the experimental value is shown in Figure 1 by the large enclosed region which is similar to that in Figure 4 of Bush et al.[7] shifted to the left and up about 20° because the experimental NOE ratio of this pair is larger than in D_2O.

The third group of NOE's includes those on GalNAc H3 and GalNAc H5 on saturation of fucose H1 which depend on the glycosidic dihedral angles of both fucose and GalNAc and thus do not lend themselves to simple two-dimensional mapping. All the conformations within the regions obtained from the NOE maps described above were considered in the NOE calculation of the third group. The only conformations of the tetrasaccharide giving calculated NOE in this group consistent with experiment were $\Phi_{GalNAc} = 60 \pm 10°$ and $\Psi_{GalNAc} = -160 \pm 10°$, and $\Psi_{Fuc} = 130 \pm 10°$ and $\Phi_{Fuc} = -80 \pm 10°$. The computed proton T_1 values for Fuc H1 and GalNAc H1 of the above-proposed conformation were consistent with the experimental values in Table II within ±20 ms. Thus we conclude that the blood group A tetrasaccharide in Me_2SO at both 20 and at 60 °C exists in a single conformation consistent with the experimental NOE data and that conformation

Table IV. Conformations of Blood Group A Tetrasaccharide and Blood Group H Hexasaccharide Fragments Consistent with Observed NOE[a] and T_1[b]

A. Blood Group A Fragment, Fuc(α-1→2)[GalNAc(α-1→3)]Gal-β-O-Me

solvent	temp, °C	Φ_{Fuc}	Ψ_{Fuc}	Φ_{GalNAc}	Ψ_{GalNAc}
D_2O[c]	24, 60	−70 (±10)	130	50	−160
Me_2SO	20, 60	−80 (±10)	130 (±10)	60 (±10)	−160 (±10)
pyridine	60	−80 (±10)	100 (±10)	60 (±10)	−160 (±10)

B. Blood Group H Hexasaccharide Fragments

solvent	temp, °C	Φ_{Fuc}	Ψ_{Fuc}	Φ_{Gal}	Ψ_{Gal}
a. 3-Arm: Fuc(α-1→2)Gal-β-O-Me					
D_2O[d]	5, 70	−150 (±10)	100 (±10)		
		−70 (±10)	130 (±10)		
pyridine	60	−150 (±10)	100 (±10)		
		−80 (±10)	120 (±10)		
b. 6-Arm: Fuc(α-1→2)Gal(β-1→4)GlcNAc-β-O-Me					
D_2O[d]	5, 70	−80 (±10)	140 (±10)	−60 (±10)	120 (±10)
pyridine	60	−70 (±10)	140 (±10)	−70 (±10)	120 (±10)

[a] See Tables I and III for list of observed NOE's. [b] See Table II for observed T_1's. [c] From Bush et al.[7] [d] From Rao et al.[5]

is essentially the same as that in the aqueous environment.[7]

The data of Table I for the blood group A tetrasaccharide in pyridine show that the NOE ratios of Gal H4 to GalNAc H2 and of Gal H3 to GalNAc H2 on saturation of GalNAc H1 and that of Gal H2 to Fuc H2 on saturation of Fuc H1 are very similary to those in Me_2SO. Therefore the 2-D maps of regions of glycosidic angles for the NOE in the first and second groups are essentially identical with those in Me_2SO. However, in contrast to the data in Me_2SO and D_2O solvents, there is no observable NOE at GalNAc H3 and H5 on saturation of Fuc H1. Therefore, the region of the conformational map consistent with the experimental NOE is much larger and the conformation could not be further restricted by the third group of NOE's.

Since the NOE data for the blood group A tetrasaccharide in pyridine do not imply any one single conformation, some additional assumption is necessary for their interpretation. Since single conformations were found for this oligosaccharide in D_2O and Me_2SO, we will assume the same for pyridine and further that the conformational energy calculations give the correct locations of the potential energy minima if not the precise values of their conformational energy. The selected distinct energy minima calculated by three different methods (M&S, Hop, and HSEA) which were described in Materials and Methods above, are listed in Table III of Bush et al.[7] F1, F2, F3, and F4 represent, in ascending order of energy, the four minima calculated by the M&S method, A1 to A6 are the six minima calculated by the Hop method, and K1 to K3 are the three minima found by the HSEA method. Among the tabulated minima, the glycosidic dihedral angles of $\Psi_{GalNAc} = -160 \pm 10°$ and $\Phi_{GalNAc} = 60 \pm 10°$ are the only ones for which values of NOE at Gal H3 and H4 on saturation of GalNAc H1 agree with experiment, suggesting that the conformation of this glycosidic linkage is the same in pyridine as it is in D_2O and Me_2SO. Regarding the conformation of the fucosyl linkage, for the minima A2 and A3 of Table III of Bush et al.,[7] the calculated NOE's are not consistent with the measured values of the ratio of NOE at Gal H2 to Fuc H2 on saturation of Fuc H1. Of the remaining conformations, F4, A6, and K2 must be eliminated because the NOE's at GalNAc H3 and H5 on saturation of Fuc H1 are too large to agree with experimental observation. Therefore, we conclude that the fucosyl dihedral angles of the blood group A tetrasaccharide in pyridine are $\Phi_{Fuc} = -90 \pm 10°$ and $\Psi_{Fuc} = 100 \pm 10°$. While these values are not very different from those determined in Me_2SO and in D_2O, they are distinguishable within the experimental error of the NOE data. The conformations of the blood group A tetrasaccharide in three different solvents are compared in Table IV. The alternative interpretation of the NOE data, that there is no single conformation for this oligosaccharide in pyridine but rather several conformations in equilibrium, will be discussed below.

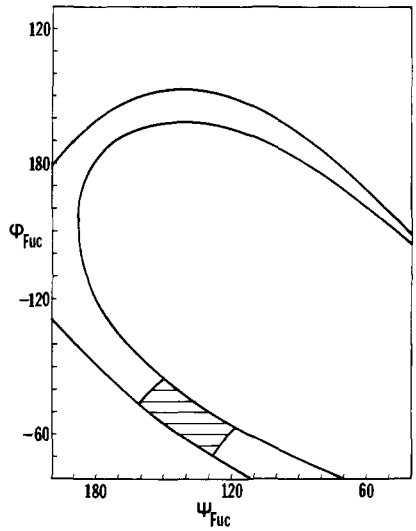

Figure 2. Regions of the conformational map for the Fuc(α-1→2) linkage of the 6-arm of difucohexasaccharide in which computed NOE's agree within experimental error of the values in Table III. The large area of conformational space in the strip is that in which the ratio of the NOE at Gal H2 to that at Fuc H2 on saturation of Fuc H1 agrees with experiment. The smaller lined region is the area of overlap of the low-energy conformations and the large strip.

The nuclear Overhauser effect data for the blood group H hexasaccharide structure in pyridine at 15 °C (Table III) are qualitatively similar to those reported earlier for this same hexasaccharide in aqueous solution.[5] Therefore, we anticipate that the conformation of this oligosaccharide in pyridine should be similar to that proposed in D_2O at 5 and 70 °C. For detailed calculations of NOE of model conformations, a value of τ_c, the rotational correlation time, is needed. In the absence of carbon T_1 data, it was necessary to rely on intra-ring NOE's between fucose H1 and H2 and between galactose H1 and H3 and H5. The τ_c value deduced by comparison of computed and experimental NOE's is 0.95 ± 0.1 ns. The values of τ_c deduced from the intra-ring NOE's of the galactose and fucose rings differ by about 0.1 ns, a difference which is easily detectable in the NOE. This ambiguity is avoided by taking ratios of inter- to intra-ring NOE's in making the maps.

For the 6-arm of the hexasaccharide, which has the type II blood group H sequence, the computed NOE at Gal H2 on saturation of Fuc H1, or that at Fuc H1 on saturation of Gal H2, is sensitive to the conformation of the fucosidic linkage, and that at GlcNAc H4 on saturation of Gal H1 is strongly dependent on the conformation of the type II galactosyl linkage. The other NOE values in Table III are not sensitive to the conformation. Figure 2 shows a 2-D map of the regions of fucosyl linkage conformation for which the calculated NOE agree with the experimental data given in Table III. A large area of conformational space in the strip is the region of agreement for the ratio of NOE at Gal H2 to that at Fuc H2 on saturation of Fuc H1. Since these NOE data are not adequate to completely define a single conformation for this glycosidic linkage, it is necessary to resort to conformational energy calculations.

Since single conformations were found to be adequate to explain the NOE data for the blood group A oligosaccharides, we adopt the working hypothesis that single conformations account for the data on the blood group H hexasaccharide. The validity of this assumption will be examined in more detail below. The conformational energy of the model fragments, Fuc(α-1→2)Gal-β-OMe for the 3-arm and Fuc(α-1→2)Gal(β-1→4)GlcNAc-β-OMe for the 6-arm, calculated with the three different methods described above, are given in Table V. While only one or two energy minima within 5 kcal/mol of the global minimum are found for the methods of M&S and HSEA, four or five are found for the Hop method. This results from differences in the nonbonded interaction in the three methods; the atoms in Hop are smaller than they are in M&S and HSEA.[7] The area of overlap of the

Table V. Selected Distinct Energy Minima for the Blood Group H Hexasaccharide Fragments Calculated by Three Methods[a]

conformer	conformational angles Φ_{Fuc}	Ψ_{Fuc}	Φ_{Gal}	Ψ_{Gal}	rel energy (kcal/mol)
A. 6-Arm: Fuc(α-1→2)Gal(β-1→4)GlcNAc-β-O-Me					
a. HSEA					
1	70	140	−70	120	0.0
b. M&S					
1	−90	150	−60	110	0.0
c. Hop					
1	−130	170	50	130	0.0
2	−50	180	−100	−60	1.5
3	−70	130	−70	110	1.8
4	−50	180	−100	120	2.1
5	−50	150	−30	100	2.6
B. 3-Arm: Fuc(α-1→2)Gal-β-O-Me					
a. HSEA					
A″	−70	130			0.0
b. M&S					
A′	−80	120			0.0
D′	−150	100			2.1
c. Hop					
A	−70	110			0.6
B	−70	40			0.0
C	−80	150			0.4
D	−160	110			0.6

[a] Only the minima with relative energies ≤5 kcal/mol are listed.

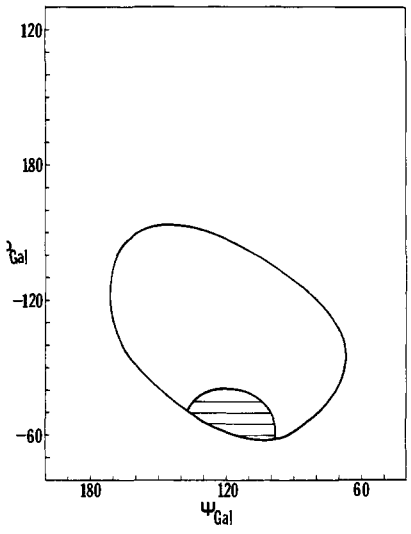

Figure 3. Regions of the conformational map for the Gal(β-1→4) linkage of difucohexasaccharide. The large enclosed circular area is the region in which the calculated NOE ratio at GlcNAc H4 to that at Gal H3 on saturation of Gal H1 agrees within experimental error with the values in Table III. The smaller lined region is the area of overlap of the low-energy conformations and the circular area.

low-energy conformations and those for which the calculated NOE agree with experimental data is lined in Figure 2. Thus, under the assumption of a single conformation and that the three empirical energy methods do not overlook any low-energy conformations, we conclude that the conformational dihedral angles of the fucosyl linkage are $\Psi_{Fuc} = 140 \pm 10°$ and $\Phi_{Fuc} = -70 \pm 10°$.

For the conformation of the Gal(β-1→4)GlcNAc linkage, the map in Figure 3 shows the region of agreement for the ratio of NOE at GlcNAc H4 to that at Gal H3 on saturation of Gal H1 as a large enclosed circular area. Under the assumption that a single conformation dominates the NOE, the smaller lined region indicates the overlap between the low-energy conformations and those for which NOE agrees with experiment. This analysis of the NOE data then leads to the conclusion that the conformation of the type II H terminal fragment of the 6-arm of the H hexasaccharide has $\Psi_{Gal} = 120 \pm 10°$ and $\Phi_{Gal} = -70 \pm 10°$ and is very similar to that deduced by Rao et al.[5] in D_2O at 5 °C and also at 70 °C. A comparison of the results in these two solvents is given in Table IV.

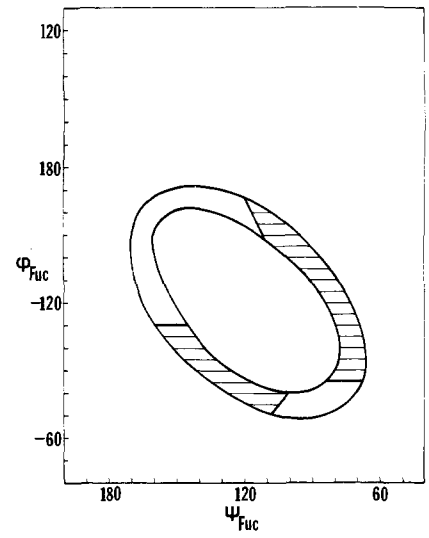

Figure 4. Regions of the conformational map for the Fuc(α-1→2) linkage of the 3-arm of difucohexasaccharide. The full circle is the computed region in which the ratio of NOE at Gal H2 to that at Fuc H2 on saturation of Fuc H1 agrees with the values in Table III. In the smaller lined regions the calculated conformational energies are also within 5 kcal/mol of the global minimum.

An analysis of the NOE data for the fucosyl linkage of the 3-arm similar to that for the 6-arm can also be carried out, but the results do not indicate unambiguously that a single conformation is responsible for the observed NOE. In Figure 4 the full circle is the region in which the ratio of the NOE at Gal H2 to that at Fuc H2 on saturation of Fuc H1 of the 3-arm agrees within experimental error with the values in Table III. In the smaller lined areas of Figure 4, the calculated conformational energies are also within 5 kcal/mol of the global minima of the three methods. Two minima in Table V fall in or very close to the lined regions. One of them corresponds to the conformation A, A′, or A″ from different methods and the other to D or D′. It is not clear in either D_2O or in pyridine whether a single conformation or an equilibrium of two or more conformations exists in solution. But the fact that the ratios of NOE at Gal H2 to that at Fuc H2 in both D_2O at 5 and at 70 °C and in pyridine at 15 °C are similar suggests that the conformations of the 3-arm in both solvents are similar.

Because we could find evidence for single rigid conformations for the blood group A tetrasaccharide in D_2O and in Me_2SO, we have assumed that a single conformation is predominant in the interpretation of all of the NOE data on the two blood group oligosaccharides. While energy calculations can be readily reconciled with this assumption for the type II H and the A tetrasaccharide, this is not the only possible interpretation of the experimental data. On the contrary, Lipkind et al. and Tvaroska and Perez[19-22] have studied the conformations of some different oligosaccharides and interpreted NOE and vicinal carbon–hydrogen coupling constants differently. They assumed that there should be an equilibrium among several different conformations which were assumed to be at the conformational energy minima from energy calculations. We have seen here and in our previous results that the relative energies of the minima, and hence their statistical weights, are quite sensitive to the choice of empirical energy parameters used in the calculation. If one could compute

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Table VI. Results of Average NOE Calculations

saturated resonance	observed resonances	relative NOE: experimental D_2O^a	experimental Me_2SO^b	experimental pyridine	calculated average M&S	calculated average Hop	calculated average HSEA
	A. Blood Group A Fragment: Fuc(α-1→2)[GalNAc(α-1→3)]Gal-β-OMe						
Fuc H1	Gal H2/Fuc H2	0.8	1.3	1.3	1.03	1.05	1.13
	GalNAc H3/Fuc H2	0.4	0.4	0.0	0.02	0.05	0.07
	GalNAc H5/Fuc H2	0.2	0.1	0.0	0.01	0.03	0.04
GalNAc H1	Gal H4/GalNAc H2	0.6	0.8	0.7	0.25	0.56	0.63
	Gal H3/GalNAc H2	0.2	0.2	0.2	0.50	0.11	0.13
	B. Blood Group H Hexasaccharide Fragments						
	a. 3-Arm: Fuc(α-1→2)Gal-β-O-Me						
Fuc H1	Gal H2/Fuc H2	1.2		1.4	1.20	0.83	1.15
	b. 6-Arm: Fuc(α-1→2)-Gal(β-1→4)GlcNAc-β-O-Me						
Fuc H1	Gal H2/Fuc H2	1.2		0.6	0.79	0.44	0.60
Gal H1	Gal H5/Gal H3	n.o.[c]		1.0	1.19	1.20	1.18
	GlcNAc H4/Gal H3	n.o.[c]		1.4	0.99	0.15	1.07

[a] At 24 °C. [b] At 60 °C. [c] Not observed because of overlaps.

reliable statistical weights, it is obvious that the assumption of a conformational equilibrium could be used to deduce single conformations as well. While the discrepancies among the calculated conformational energies with the three methods used in this work indicate that none of them is capable of giving reliable statistical weights, it is at least possible to test whether any better agreement with experiment can be achieved with this alternative interpretation of the NOE data.

The results of average NOE calculations for both the blood group A tetrasaccharide and for the blood group H hexasaccharide employing the three energy functions described in Materials and Methods above are summarized in Table VI. For the blood group A structure, the average NOE's calculated from HSEA functions were nearly the same as those calculated from the lowest energy conformation, K1 in the notation of Bush et al.[7] The contribution of conformation K1 is greater than that of K2 even though the energies are similar since K1 is a broader energy minimum on the potential surface. Since the conformation F1, the lowest energy conformation, dominates in the calculation with the M&S method, with a minor contribution only from F2, the average NOE's do not agree even qualitatively with experiment. While several conformations contributed to the average NOE in the calculation by the Hop method, the global minimum at A1 dominated so that the results are similar to those reported for the single conformation A1. For the blood group H hexasaccharide it was also found that one or a very few conformations dominate in the average NOE calculations. Thus it appears that the statistical average NOE calculations do not generally yield better agreement with experiment and may be in substantial conflict as a result of flaws in the empirical energy functions. In principle the statistical average method should be the best method for interpretation of these experiments but only if accurate energy functions which give the correct statistical weights are available.

It is of interest that changing the temperature from 60 to 10 °C in the statistical average NOE calculations without changing the rotational correlation time for tumbling of the oligosaccharide in solution caused essentially no change in the calculated average NOE values. This result is in agreement with our observations concerning temperature dependence of the conformation of these blood group oligosaccharides.[5]

IV. Conclusions

Although the relative chemical shifts of the protons of the blood group oligosaccharides differ greatly among the solvents D_2O, Me_2SO, and pyridine, we deduce from the similar ratios of NOE that the conformations are not greatly different. In fact, our detailed analysis indicates that only the conformation of the fucosidic linkage shows any solvent dependence and that its variation among the three solvents is only slightly beyond experimental error. In spite of the fact that conformational energy calculations which use different empirical potential energy functions lead to conflicting predictions for the conformation of the GalNAc glycosidic linkage as a result of several energy minima, the NOE data indicate that a single conformation exists for this linkage which is not altered by more than ±10° by changes in either solvent or temperature. The conformation of the fucosidic linkage of the blood group A tetrasaccharide is the same within experimental error (ca. 10°) in D_2O and in Me_2SO and Ψ_{Fuc} differs by only about 30° in pyridine. The conformation of the type II H oligosaccharide fragment is nearly same within experimental error in pyridine and D_2O.

The very weak dependence of the conformation of these blood group oligosaccharides on solvent and temperature contrasts with the much greater sensitivity of the conformations of peptides and nucleic acids. The dependence of the conformations of proteins and DNA on solvent composition and their thermal denaturation properties have been the subject of extensive studies in biophysical chemistry over the past 25 years. It is our interpretation that the different behavior of complex carbohydrates results from the difference in the physical forces which determine the conformation of these three classes of biopolymers. Hydrogen bonding plays an important role in stabilizing the conformation of double helical nucleic acids as well as of certain conformations of peptides such as the α helix and β sheet and perhaps certain of the turn or loop conformations. Hydrophobic forces, which depend strongly on solvent structure, are important in stabilizing nucleic acid conformations as well as the tertiary and quaternary structures of globular proteins.

Our conclusion that conformation is determined mainly by nonbonded interactions should be valid independent of the soundness of our assumption that single conformations dominate. Even if the correct interpretation of our NOE data involves an average over many conformations, the approximate independence of the NOE ratios on temperature and solvent means that the statistical weights do not change greatly. The evidence for our second conclusion, that a single conformation or a single group of closely related conformations dominates, is less strongly supported by the data. For the blood group A oligosaccharides in D_2O and in Me_2SO the experimental NOE data themselves argue for the interpretation of a single conformation.[7] For the blood group A structure in pyridine and for the blood group H type II structure, the NOE data plus a reasonable interpretation of the energy calculations lead to the conclusion that a single conformation is responsible for the observations. Since only one small region of low conformational energy is consistent with the NOE results, the conformational energy calculations for all three methods used would have to be seriously in error to alter our conclusions.

We wish to emphasize that these results apply to a limited group of blood group oligosaccharides and the generality of our conclusions, especially concerning charged oligosaccharides containing sialic or uronic acids or sulfated and phosphorylated sugars, remains to be established. While our evidence for the rigidity of

the galNAc (α-1→3) linkage is strong, that for the Fuc (α-1→2) is less so and there must be some oligosaccharides, especially those with internal (1→6) linkages which show substantially greater flexibility. The Man (α-1→6) linkage in the asparagine linked glycopeptides has been extensively studied and, although the evidence has received conflicting interpretations, it is generally agreed that conformational isomerism of the (α-1→6) linkage is important.[8,11] Furthermore, some other oligosaccharides such as maltose and cellobiose have been studied by methods similar to those used in this work, and different conclusions regarding conformational flexibility were reached.[19-22] It is not clear whether the different conclusions reached by these workers arise solely from differing assumptions in the interpretation of the data or whether there are real differences in the nature of the conformational flexibility between maltose and cellobiose and the blood group oligosaccharides. Furthermore, the extent of rigidity or lack of flexibility which we wish to imply for oligosaccharides and polysaccharides is not so great as that for globular proteins which are stabilized by interactions among residues separated by many chemical bonds. Since the nonbonded interactions responsible for the conformations of blood group oligosaccharides are mostly between atoms separated by only a few chemical bonds, there must be some small amount of relative motion between adjacent carbohydrate residues. For a polysaccharide this motion propagated over many residues leads to the observation of a stiff random coil in which segmental motion is readily observed. Thus, for high molecular weight polysaccharides, well-resolved NMR spectra are generally observed in which the line widths do not depend on molecular weight above a certain value. It is the local stiffness rather than the segmental motion which is the subject of this paper.

While the conformations of blood group oligosaccharides do not depend strongly on solvent and temperature, the observed NOE's of oligosaccharides do vary as a result of the change in the rotational correlation time. The simplest interpretation of the dependence of rotational correlation time on temperature and solvent is that it is determined by the bulk viscosity. Since τ_c of a rigid sphere is linearly related to the ratio η/T, plots of τ_c values obtained from the NOE data for both blood group oligosaccharides should be linear if this interpretation is correct. Such plots for the three solvents, D_2O, Me_2SO, and pyridine, at various temperatures give reasonably straight lines, implying that the NOE dependence of the rotational correlation time can be explained by the bulk viscosity of the solvent.

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Solid-State Gallium-69 and Gallium-71 Nuclear Magnetic Resonance Spectroscopic Studies of Gallium Analogue Zeolites and Related Systems†

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Abstract: We have obtained solid-state 11.7-T ^{69}Ga and ^{71}Ga nuclear magnetic resonance (NMR) spectra of a series of gallosilicates (gallium analogues of zeolites Na–X, Na–Y, Na–sodalite, and Na–natrolite). From the apparent ^{69}Ga and ^{71}Ga chemical shifts, values of the nuclear quadrupole coupling constants (e^2qQ/h) and the true, isotropic chemical shifts (δ_i) have been deduced for the framework, tetrahedral, $Ga(OSi)_4$ sites. The e^2qQ/h values are in the range 1.9–2.7 MHz for ^{69}Ga and 1.2–1.7 MHz for ^{71}Ga. The isotropic chemical shifts are all in the range 171–186 ppm downfield from an external standard of 1 M $Ga(NO_3)_3$. Our results indicate that measurement of the apparent (second-order shifted) chemical shifts for *pairs* of isotopes of nonintegral spin quadrupolar nuclei in solids at one magnetic field strength is a useful new approach for determination of both isotropic chemical shifts and nuclear quadrupole coupling constant values.

There has recently been considerable interest in the isomorphous replacement of Al in the framework structure of zeolites.[1-4] For example, gallium-replaced zeolites possess chemical properties different from their aluminum analogues, and offer the possibility of new catalytic activity.[3,4] Among the framework nuclei of gallosilicates (Si, Ga, and O), only the ^{29}Si nucleus has been extensively studied using nuclear magnetic resonance (NMR) methods.[5,6] The ^{29}Si "magic-angle" sample-spinning (MASS) NMR spectra of these systems can exhibit up to five resonances, depending on the number of next-nearest-neighbor galliums.[5] As a result, ^{29}Si MASS NMR techniques can be used to determine the composition of the gallosilicate framework,[5] in much the same manner as with (aluminum-containing) zeolites, and other framework aluminosilicates. More recently, ^{17}O NMR studies of a few gallosilicates have identified the two chemically distinct oxygen sites, Ga–O–Si and Si–O–Si, and have made some interpretation of the bonding of these oxygens.[7]

Expansion of NMR methods to investigation of gallium nuclei in gallosilicates is in a much earlier stage of development.[4,8] However, a fully multinuclear NMR approach should provide useful structural information, as has already been obtained with the case of ^{17}O NMR.[7] In this paper, we report the first com-

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