

Exchangeable Protons as Sources of Long-Range Constraints in Conformational Analysis of G_{A1} by NMR Spectroscopy

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Received November 10, 1995

The three-dimensional (3D) structure of biopolymer is known to play an important role in their biological functions. NMR is an excellent technique for the structural analysis of carbohydrate. The structural information is principally from the measurement of the nuclear Overhauser effect (NOE) which provides constraints on internuclear distances. The second important information comes from the coupling constants which determine the dihedral angles. However, despite their relatively small sizes, carbohydrates generally give very complex proton NMR spectra. The primary reason for this is the poor spectral dispersion of the ring protons, which presents problems due to severe resonance overlap. Furthermore, NMR studies on carbohydrate generally provide very limited NOE data. Since NOE contacts are usually restricted to the interactions between the protons attached to the two carbon atoms at the glycosidic linkage and the other contacts are rare, they are not enough to allow a complete determination of the structure.¹⁻⁶ The number of experimental data available is much smaller than those of proteins.

Gangliosides are sialic acid containing glycosphingolipids, found in the plasma membrane of animal cells, being particularly abundant in the nervous system.^{7,8} Recently interest in asialo- G_{M1} (G_{A1} ; β D-Gal(1-3) β D-GalNAc(1-4) β D-GlcCer, Figure 1) arises from the finding that G_{A1} is more antigenic than most of the gangliosides, and is also capable of eliciting highly specific antibodies. Therefore, there is a growing need to determine the three-dimensional structure of its structure.⁹⁻¹⁵

In this study, we present the supplementary source of the structural informations from the exchangeable protons in G_{A1} . We reported the resonance assignment of the C-linked protons in G_{A1} previously.¹ Unexchanged hydroxyl protons and amide protons in ganglioside are protrude farther from the carbon backbone than the C-linked protons and provides NOE contacts with other protons which can provide additional dis-

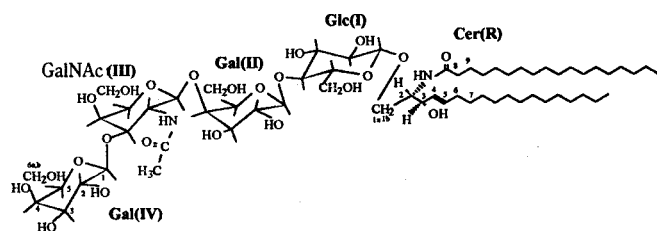


Figure 1. Primary structure of G_{A1} with symbolism and numbering.

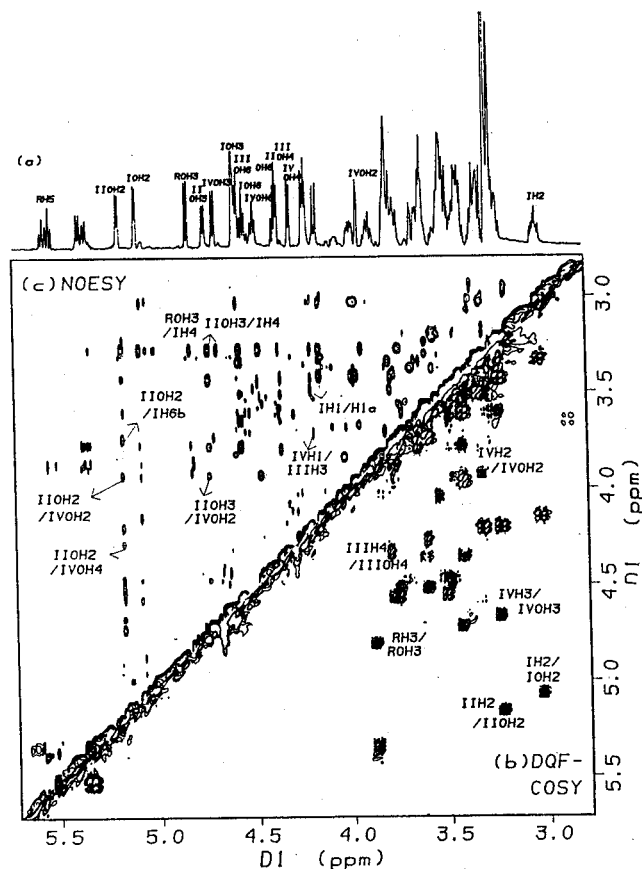


Figure 2. 500 MHz ^1H NMR spectrum of 7 mM G_{A1} in DMSO-d_6 at 303 K in the region of the ring protons and the hydroxyl protons. a) 1D ^1H NMR spectrum of G_{A1} which shows the assignment of the hydroxyl protons. b) Scalar connectivities in DQF-COSY spectrum of G_{A1} is shown in the lower right of the diagonal. c) NOESY spectrum of G_{A1} with a mixing time of 150 msec is shown in the upper left of the diagonal. All spectra were recorded on a Bruker AMX-500 spectrometer in Inter-University Center for Natural Science Research Facilities in Seoul National University and Korea Basic Science Institute and were processed off-line using Felix¹⁶ software on SGI workstation in our laboratory.

tant constraints in structural determination. Also, they can provide important informations about the hydrogen bondings existed in G_{A1} .

Figure 2 and Table 1 shows the resonance assignment of the G_{A1} in 100% DMSO-d_6 at 303 K referenced to TMS. Notice that all the resonances of the hydroxyl and the amide protons are assigned and the chemical shift from the C-linked protons remain unchanged when they are compared to those values in $\text{DMSO-D}_2\text{O}$ (98:2%).¹ Table 2 shows the coupling constants and temperature coefficients of the amide and hydroxyl protons in G_{A1} . A reduction in temperature susceptibility (ppb/deg) has been commonly accepted as an indicator of reduced interaction with solvent, due to the intramolecular hydrogen bonding.¹⁷⁻²⁰ A small temperature coefficient is observed for the IOH3 proton (hydroxyl proton attached to the third carbon of the ring D) and the IVOH2 proton. This should correspond to a transfer of electron density from the OH bond as a result of hydrogen bonding with

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Table 1. Complete Assignment of ^1H resonances of G_{A1} dissolved in DMSO-d_6 at 500 MHz and 303 K, referenced to TMS

Residue		1	2	3	4	5	6(a;b)	CH_2	CH_3
D-Glc (I)	H	4.157	3.037	3.323	3.285	3.285	3.610 3.742		
	OH		5.086	4.586			4.537		
D-Gal (II)	H	4.209	3.233	3.436	3.789	3.489	3.419 3.626		
	OH		5.175	4.737			4.372		
D-GalNAc (III)	H	4.580	3.769	3.653	3.789	3.347	3.503 3.521		1.828
	OH		^c 7.463		4.358		4.575		
D-Gal (IV)	H	4.217	3.324	3.248	3.615	3.317	3.484 3.519		
	OH		3.937	4.687	4.292		4.481		
Cer (R)	H	^a 3.424	3.769	3.882	3.355	5.533	1.933	1.234	0.852
	OH	^b 3.975	^c 7.449	4.825	^d 1.270	^e 2.021	^f 1.448		

^aH1a, ^bH1b, ^cNH, ^dCeramide H7, ^eCeramide H8, ^fCeramide H9.

Table 2. Temperature Coefficients^a (ppb/deg) and Coupling Constants^b (Hz) for Amide and Hydroxyl Protons of G_{A1} in DMSO-d_6

	OH2	OH3	OH4	OH6	NH
D-Glc (I)	-8.77 (3.4)	-4.35 (<2)		-7.76 (5.9)	
D-Gal (II)	-9.99 (4.6)	-9.37 (5.4)		-7.96 (4.6)	
D-GalNAc (III)			-11.78 (4.3)	-7.66 (11.6)	-6.15 (5.57)
D-Gal (IV)	-5.55 (<2)	-11.73 (5.6)	-9.05 (4.5)	-7.37 (5.5)	
Cer (R)		-8.53 (5.6)			-8.40 (8.35)

^aCalculated slope from the linear least squares analysis of eight data points from 293 K to 328 K. ^bDigital resolution is 0.26 Hz/point.

the other atoms as electron donors. Also, the vanishingly small $^3J_{\text{H3,IOH3}}$, $^3J_{\text{IVH2,IVOH2}}$ coupling constants as shown in Table 2 indicate that these OH protons are involved in strong hydrogen bondings.²¹⁻²³ According to the molecular orbital calculation, the potential energy of H-C-O-H has three minima which occur at the dihedral angles of 80, 180, and 300°.^{24,25} For the dihedral angle of H-C-O-H at 80°, the observed and calculated coupling constant is about 0.2 Hz while the configuration at 180° has a coupling constant larger than 10 Hz. If the interconversion between these three states is rapid,

Table 3. Interresidue NOE contacts of G_{A1} observed in the NOESY and the ROESY spectra with a mixing time of 150 msec, at 303 K

between C-linked protons	between C-linked protons and exchangeable protons	between exchangeable protons
IH1 / H1a	IOH3 / IIH1	IIOH2 / IVOH2
IH1 / H1b	IH6b / IIOH2	IIOH2 / IVOH4
IIH3 / IVH1	IH4 / IIOH3	IIOH3 / IVOH2
	IH4 / ROH3	IIOH6 / IVOH3
	IIINH / IVH1	IIOH3 / IIINH
	IIINH / IVH3	IOH2 / RNH
	IH1 / RNH	

one would observe only an average coupling constant. Therefore, freely rotating OH groups show large $^3J_{\text{CH,OH}}$ coupling constant and a very small $^3J_{\text{CH,OH}}$ coupling constant should result from the rigid H-C-O-H configuration by a stable intramolecular hydrogen bonding. Table 3 shows the interresidue NOE contacts observed in NOESY and ROESY spectra with a mixing time of 150 msec. In order to avoid spin diffusion problems the data set with a mixing time of 150 msec among the data sets acquired with the mixing times of 150, 250, and 350 msec was used in this table. IOH3 shows a strong IOH3/IIH1 interresidue NOE and this contact points to a hydrogen bonding between the IOH3 proton and the oxygen atom in $\beta\text{D-Gal}(1-4)\beta\text{D-Glc}$ glycosidic linkage. IVOH2 proton shows the interresidue NOE contacts with IIOH2 and IIOH3 protons and this suggests that IVOH2 may have a strong hydrogen bonding with the oxygen atom either in IIOH2 or in IIOH3 hydroxyl groups.

G_{M1} and G_{D1a} which have polar sialic acids have strong intramolecular hydrogen bondings between the NH proton in GalNAc and the carboxyl group in sialic acid and are stabilized energetically.^{21,26} G_{A1} does not have sialic acid but has two NH protons in GalNAc and ceramide. As reported in the previous paper, one in GalNAc exchanges more slowly with deuterium than the other NH proton in ceramide and this NH proton was considered to be involved in intramolecular hydrogen bonding.¹ Surprisingly, NH in GalNAc does not have a small temperature coefficient as shown in Table 2 and this suggests that NH in GalNAc does not form a strong hydrogen bonding. This NH proton shows many interresidue NOE contacts with the protons in sequential residues such as IIOH3, IVH1 and IVH3. NOE contacts between the hydroxyl protons such as IIOH2/IVOH2, IIOH3/IVOH2, IIOH2/IVOH4 and IIOH6/IVOH3 are very important in tertiary folding of G_{A1} . These NOE contacts between the exchangeable protons provide the long range constraints in structural determination of G_{A1} . We can conclude that ring IV should be stacked underneath the galactose(II) ring, establishing a hydrogen bonding between the hydroxyl oxygens of ring II and the IVOH2 proton. And also acetamide group in GalNAc (III) appears to be surrounded by ring II and ring IV. Therefore, G_{A1} does not have a linear structure but have a folded structure even though it does not have sialic acid which forms a stable hydrogen bonding in most of the gangliosides. Based on these experimental data, calculation of the three

dimensional structure of G_{AI} will be carried out and this study will lead to a better understanding of molecular basis of its structure.

Acknowledgment. This work was financially supported by Korea Science and Engineering Foundation (94-1400-04-01-3).

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An Empirical Net Atomic Charge Calculation Method of Ligand-Metal Ion Complex

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Received November 14, 1995

An accurate description of electrostatic potentials is crucial importance in connection with the study of intermolecular interactions, especially, between polar molecules. For calculating the reliable electrostatic potentials, both quantum mechanical¹⁻⁸ and empirical point charge models⁹⁻¹⁴ were proposed by several workers. For large molecules, empirical models are adequate for the point charge calculation because of the limitations in the computing power which is necessary for large scale quantum mechanical calculations. Therefore, several empirical point charge calculation methods have been proposed for the point charge calculation of large molecules, *i.e.* proteins, nucleic acids, and zeolites, etc. Most of the methods were developed based on the *Electronegativity Equalization* (EE) concept.¹⁵⁻¹⁸

In the EE based methods, the *equalization* occurs by transferring electron partially through covalent bonds. As a consequence of the physical basis of the EE concept, these methods can be applied for the molecules which have covalent bonds only. Whereas, the bonds formed between ligands and metal ions are neither covalent bond nor pure non-bond. In the ligand-metal ion bonds, there are some partial electron transfer between contacting atoms and the degree of the electron transfer depends on there distance. Therefore, one can neither apply the EE method to the ligand-metal ion bond nor put fixed integer net atomic charge on the ion. In the computer simulation of metal ion bound system, since the distance between the ion and the ligand atom changes at every evolution step, it is physically unrealistic to use fixed charges for the ion and ligand atoms. Since the electrostatic interaction energy contributes dominantly to the total interaction energy between polar molecules, even if the change in the net atomic charge is small, the total binding energy changes considerably. Therefore, it is indispensable to describe the net atomic charges of the ion and the ligand atoms as a function of the ligand atoms-ion distances.

In this work, an empirical point charge calculation method for the ligand-ion complex was developed based on the *Partial Equalization of Orbital Electronegativity* (PEOE) method proposed by Gasteiger *et al.*¹⁹ The degree of the partial electron transfer between the ion and the ligand atom was expressed as a function of the ligand atom-ion distance. As a model compound, Na-A zeolite was introduced. In the PEOE method,¹⁹⁻²⁴ the magnitude of the fractional charge transfer $dq^{(n)}$ between covalent bonding atomic pair *A-B* was described as

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