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Membrane surface orientation and motion of glycolipids: ^{2}H NMR investigations

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Abstract - By means of measured quadrupole splittings, dipole-dipole couplings, and lineshape analysis, the degree of motional restriction (ordering) and bilayer orientation of glycolipid head groups can be calculated. From the dependence of the ^{2}H nmr lineshape on multipulse experiments, and from a variety of relaxation time measurements, insight into the rates of motion (10^3-10^{11}s^{-1}) of the carbohydrate species can be gained. Various slow motions (< 10^3 s^{-1}) may be visualized by means of a solid state two-dimensional ^{2}H nmr technique.

INTRODUCTION

Glycolipids constitute a class of biomolecules which is extremely abundant and exhibits enormous structural diversity. In microorganisms glycoglycerolipids (Fig. 1A) can be the major lipid component of the cell membranes, and as such play a critical role in establishing the correct environment for sustaining membrane function (ref. 1). In mammalian systems, glycosphingolipids usually represent minor components of cellular membranes but serve several important roles: cellular recognition, antigenicity, hormone reception, ion and toxin binding (ref. 2). While there has been considerable effort towards the chemical synthesis of glycolipids, it is only relatively recently that there has been attention to physical studies of these lipids. It is hoped that such studies will provide insight into the molecular details of how glycolipids fulfill their diverse functions. A similar motivation has led to numerous studies of phospholipids.

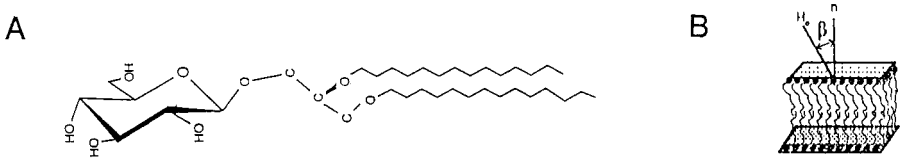

Fig. 1 A) Structure of the glycoglycerolipid β-DTGL.
B) Representation of a lipid bilayer and the orientation of the bilayer normal, **n**, relative to the magnetic field direction $\mathbf{H_o}$.

The realization that the functional roles assumed by glycolipids are intimately related to the carbohydrate residues has spurred interest in probing the head group conformation. This is a difficult task, since the lipids perform their function in the complex and dynamic milieu of a membrane (Fig. 1B) which is composed of lipids, proteins and other biomolecules. A wealth of structural information has been obtained by high resolution nmr and X-ray crystallography of isolated, purified lipid or carbohydrate residues. While such information is useful, its relevance to biological events is doubtful. In addition, such studies cannot provide information about the spacial arrangement of the carbohydrate residues relative to the membrane matrix, a property which may play a critical role in influencing glycolipid function. We have therefore undertaken to study glycolipids in macromolecular complexes such as membranes and bound proteins (e.g. lectin-bound). Our method of choice is solid-state ^{2}H nmr, although other nuclei such as ^{13}C and ^{15}N may be considered.

^{2}H NMR OF GLYCOLIPIDS

The method involves labelling the carbohydrate residue of the glycolipid with deuterium by chemical synthesis or biosynthesis; this may be at one or several sites. The glycolipid is dispersed in water as pure material, or as a mixture with other lipids, to form large multilamellar structures. The system is investigated by solid state nmr which specializes in the observation of spectra with broad lines (as great as 250 kHz for ^{2}H) and may be used on systems which are truly solid or semi-fluid such as liquid crystals (ref. 3). The nmr techniques employed (ref. 4) are quite different from those used for high-resolution studies of small molecules in non-viscous solutions.

Partially averaged quadrupolar and dipolar interactions give insight into the average membrane orientation of the sugar residues and into molecular fluctuations about the average orientation (ref. 5). The rates and types of molecular motion (time scales of 1 - 10^{12} s^{-1}) may be probed by measurements of relaxation times, by lineshape changes in one dimensional multipulse experiments, by spectral simulation, and by two-dimensional spectroscopy (ref. 6,7). Useful reviews of the methodology have been published (ref. 8,9).

MOLECULAR CONFORMATION, ORIENTATION AND ORDER

Fig. 2A shows a series of ^{2}H nmr spectra of a glycolipid, 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-sn-glycerol (β-DTGL) multiply labelled in the carbohydrate moiety. At 25°C the lipid is in the lamellar gel state where molecular motion is slow and of small amplitude (high order). As a result, the spectra are broad and relatively featureless. However, they reveal that there is still a significant degree of molecular motion since the total spectral width is < 100 kHz whereas a completely immobile residue would have a spectral width of ca. 250 kHz. Raising the temperature to 52°C induces a transition to a less ordered state, known commonly as the biologically relevant liquid-crystalline state, where the molecules fluctuate about the bilayer normal. Individual lineshape patterns can be seen for each deuteron on the glucose ring. The separation between the pairs of peaks gives the quadrupolar splitting and reflects the degree to which molecular fluctuations about the bilayer normal are damped, that is the degree of ordering of the molecular fragment. Since the glucose ring is essentially rigid, all positions on the ring experience the same degree of order. The different quadrupolar splittings indicate that each labelled site has a particular orientation with respect to the axis of rapid molecular motion, taken to be the normal to the bilayer surface (Fig. 1B). Analysis of the relative magnitudes of the splittings for each labelled site in the molecule yields the location of the motional axis within the molecular frame and as a result the average orientation of the carbohydrate residue relative to the bilayer plane (ref. 10).

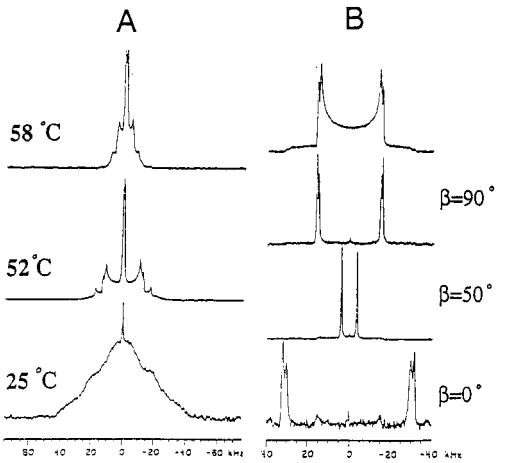

Fig. 2 A) Temperature dependence of ^{2}H nmr spectra of β-DTGL.
B) ^{2}H nmr spectra of β-DTGL labelled at the glycerol C3 position: top - aqueous dispersion (unoriented); bottom three spectra - bilayers oriented between glass plates at indicated angles of the bilayer normal relative to the magnetic field direction.

Further heating to 58°C results in a spectrum (Fig. 2A top) in which the peak separations are approximately half of those seen at 52°C. The spectral changes arise from a second thermal structural transition from a lamellar to an hexagonal state in which the lipids organize into tubes of water surrounded by an annulus of lipid with headgroups directed inward. Rapid diffusion of the lipid molecules about the cylinder axis averages the quadrupolar splittings to approximately half their values in the lamellar structures.

Labelling the glycoglycerolipid β-DTGL in the glycerol region (C3) provides information on the interfacial region of the bilayer (Fig. 2B). In general, spin interactions such as ^{2}H-^{2}H and ^{1}H-^{2}H dipolar coupling are not discernable in the spectra from aqueous lipid dispersions. However, hydrated multibilayers may be oriented between glass plates and the system made to resemble a single-crystal sample. ^{2}H nmr spectra of such samples consist of two resonances for each deuteron (Fig. 2B), with fine structure from which the ^{2}H-^{2}H dipolar couplings can be determined (ref. 5). Using the quadrupolar splittings and the dipolar couplings for the glycerol C3 position, the orientation and ordering of the glycerol C2-C3 fragment may be calculated and a conformation about the glycosidic linkage of the glucosylglycerolipid deduced (Fig. 3A,B).

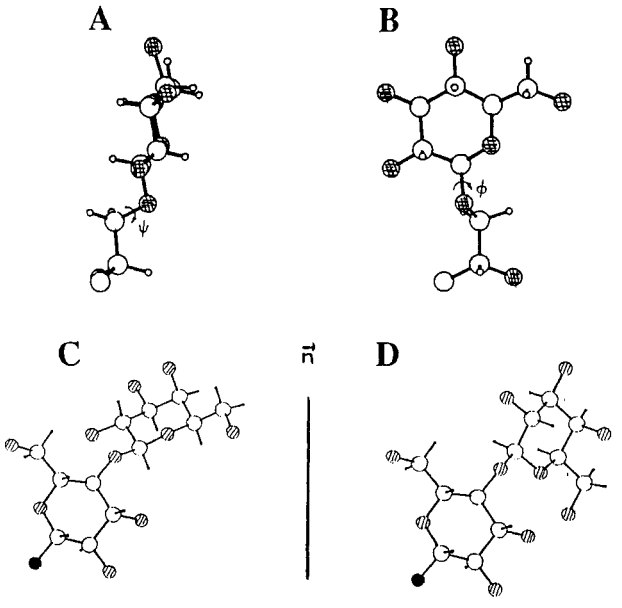

Fig. 3 A) and B) Orientation of the glucopyransosyl-glycerol segment of β-DTGL relative to the bilayer surface as determined by ^{2}H nmr.
Conformation of a lactosyl residue.
C) Determined by X-ray crystallography of lactose.
D) ^{2}H nmr of lactolipid in multilamellar dispersion.

Fig.3 (D) shows the bilayer headgroup orientation and conformation, determined by ^{2}H nmr methods, of a liquid crystalline glycolipid with a lactosyl moiety as headgroup (ref. 11). Fig. 3C shows the conformation of lactose as determined from X-ray diffraction data for crystalline lactose and high resolution nmr studies of methyl lactoside. The conformational differences between the disaccharide residue in solution and in a membrane environment suggest that the presence of the membrane surface may strongly influence headgroup conformation. If this is so, one might speculate that the membrane surface influences the process of molecular recognition.

In the case of the lactolipid, the headgroup order parameter was about 0.52. Since the order parameter for a lipid molecule as whole in liquid-crystalline bilayers is generally 0.6 - 0.7 the results for the lactolipid suggest that motion about the disaccharide-glycerol linkage is of small amplitude - it is not very flexible.

MOLECULAR DYNAMICS

Membranes are not static assemblies at the molecular level but represent a complex matrix in which constituent molecules can have internal segmental motions (conformational isomerization), overall molecular motion and collective motions involving the entire membrane. Such motions occur over a large range of time scales (1s - < 10^{-12} s). In order to come to a quantitative description of membrane systems, in particular surface carbohydrate, it is essential to consider both the structural aspects (conformation, orientation, and order) and the rates and types of molecular motion that are associated with the structural description. ^{2}H nmr provides a powerful method of enquiring into molecular motions on time scales which cover the entire range expected in membrane systems.

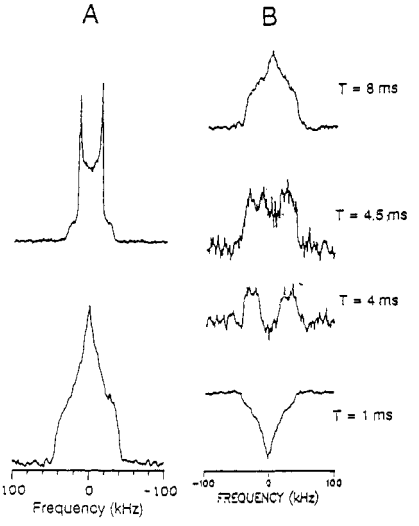

Fig. 4 A) ^{2}H nmr spectra of β-DTGL labelled at the glycerol C3 position: top 52°C (liquid crystalline phase); bottom 25°C (gel phase).
B) Partially-relaxed ^{2}H nmr spectra (inversion recovery experiment) of β-DTGL labelled at the C3 position, 25°C: note relaxation rate is not uniform throughout the entire spectrum.

In the case of the simple glycolipid β-DTGL, the ordering for the glucosyl and glycerol residue was 0.45 and 0.65, respectively, indicating some motion about the glycosidic linkage, a structural fluctuation. The ^{2}H nmr spectrum of β-DTGL in the ordered gel state (Fig. 4A bottom) has a lineshape which indicates that molecular motion has lower symmetry than that present in the liquid crystalline phase (Fig. 4A top). The low temperature spectrum suggests that there is interconversion between the gauche(-), gauche(+) and trans conformations about the glycerol C2-C3 bond, and that there is no detectable rotation of the molecule as a whole. The rate of this motion may be determined by measurement of longitudinal relaxation times. In these studies the ^{2}H nuclear spin population is perturbed from equilibrium and the rate of return to equilibrium (relaxation rate) is measured. In membrane systems the relaxation rate depends on the rate of molecular motion, and on the orientation of the membrane bilayer normal relative to the magnetic field direction. The details of the angular dependence can be a signature of the type of motion occurring, and are reflected in the changes in the ^{2}H nmr lineshape (Fig 4B). Simulation of the experimental results using rotameric jumps about the glycerol C2-C3 bond confirmed the identity of the segemental motion (ref. 6).

As β-DTGL is heated from 25°C toward the liquid crystalline phase (52°C) the ^{2}H nmr spectrum not only changes shape but also there is a significant loss in intensity at 45°C which is fully recovered above 52°C (Fig. 5). This spectral behaviour indicates that on heating a molecular motion is activated and that its rate increases from $<10^3$ s^{-1} to $> 10^6$ s^{-1}. This motion is reorientation of the lipid about its long molecular axis.

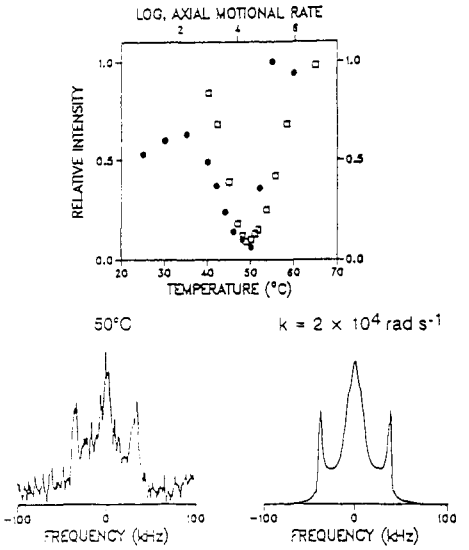

Fig.5 Top: temperature dependence of the ^2H nmr spectral intensity for β-DTGL labelled at the C3 position: (o) experimental, ( ) simulated. From ref. 6.
Bottom - ^2H nmr spectrum at 40°C: left, experiment; right, simulated.

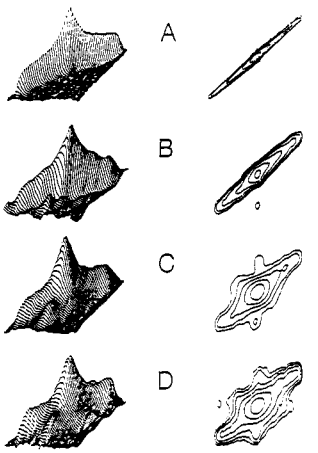

Fig. 6 ^2H 2D-exchange nmr spectra(left) and contour plots (right) of β-DTGL labelled at the glycerol C3 position:

(A) 25°C , t_{mix}=1 ms;
(B) 35°C, t_{mix}=0.5 ms;
(C) 35°C, t_{mix}=2 ms;
(D) 40°C, t_{mix}=2 ms.

The nature of the reorientation of β-DTGL about its long axis may be probed using two-dimensional solid state nmr. It is possible when molecular motion is slow (< 10^3 s^{-1}) to examine the ^2H quadrupolar splitting at one particular time, and then a short time later. Depending on the rate and nature of the molecular motion, the splitting may change between the two observation times. In simple cases, a change in splitting corresponds to a change in the angle the C-^2H bond makes with the magnetic field direction - it is possible to measure directly the angle through which a molecule has jumped. If there is no frequency change a diagonal two-dimensional spectrum is observed, whereas frequency changes during the internal lead to off-diagonal peaks or ridges which reveal the frequency correlations. Figure 6 illustrates what happens as β-DTGL is heated from the gel state to the liquid crystalline state (ref. 7). At low temperature the spectrum shows no frequency changes. As the temperature is elevated to 40°C cross-ridges reveal that motion has occurred. Spectral simulations indicate that the lipid reorients about its long axis, most likely via large angle jumps and not by small steps (rotational diffusion). Thus, for β-DTGL the picture that emerges is of a lipid which undergoes fast (10^{10} s^{-1}) isomerization about the glycerol C2-C3 bond and a slow rotation about the molecular long axis. It is now possible to extend these approaches to examine conformational equilibria about the glycosidic linkages of the carbohydrate head groups.

In membranes, molecules are free to diffuse within the plane of the membrane surface. Such motion is believed to be important for proper membrane function and antibody binding (ref. 12). In the case of lipid vesicles such motion will change the value of the ^2H quadrupolar splitting and these changes can be monitored by two-dimensional ^2H nmr. Fig. 7 demonstrates the effect for a phospholipid labelled at the acyl chain terminus (CD_3). If the molecules are allowed only a short time (about 10 ms) to diffuse, only a diagonal spectrum is observed; at long times (100 ms) the molecules have completely changed positions, as evidenced by large cross-peak patterns (Fig. 7 bottom). At intermediate times (Fig. 7 top and middle) the development of the exchange spectra may be followed. It is possible by monitoring the development of the cross-peaks at several times to determine the rate of lateral diffusion. While such experiments are technically difficult, one might speculate that they offer a means of probing molecular complexes in membrane systems.

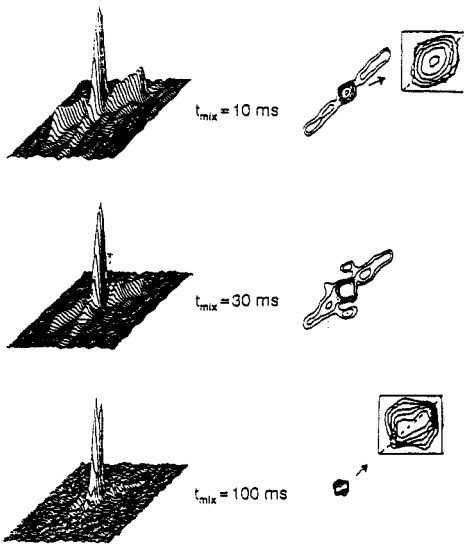

Fig. 7 2D absorption mode exchange spectra and corresponding contour plots of a multilamellar dispersion of perdeuteriated DPPC at 51°C for different mixing times. For t_{mix}=100 ms, dashed line of the inset represents where the diagonal would occur. The total spectral width in both dimensions is ± 25 kHz.

CONCLUSIONS

We have demonstrated the utility of ^2H nmr to probe conformational, orientational and dynamic aspects of glycolipids in systems which model the situation in biological membranes. With the elegant synthetic and semisynthetic methodology being developed today it should be possible to investigate more complex glycolipids which are of significant biological importance. While the technical difficulties are great, solid state nmr studies offer the potential to investigate macromolecular complexes such as lectin-bound membrane glycolipid and carbohydrate-antibody complexes.

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