NMR Diffusion, a Method for Studies of Dynamics and Mesophase Structure of Membrane Lipids *

GÖRAN LINDBLOM

Division of Physical Chemistry 2, University of Lund, Chemical Centre, P.O. Box 740, S-220 07 Lund 7, Sweden

The pulsed NMR diffusion technique provides a convenient method for studies of translational diffusion in aggregated systems of lipids. The main advantage with this method is that the various diffusion coefficients (lipid, water and ions) are *directly* measured without using probe or labelled molecules. Furthermore the diffusion time can be varied from milliseconds to seconds so that restricted diffusion within lipid aggregates can be determined. This can be utilized to extract information about the aggregate structure of different liquid crystalline systems and cubic phases in particular. Here a brief summary of our recent studies of the translational diffusion and the structure of some phases will be reported.

Experimental. The diffusion coefficients were measured at 61 MHz on a Bruker 322s spin-echo NMR spectrometer according to the method of Stejskal and Tanner.[1] By introducing pulsed magnetic field gradients the spin-echo height, observed at 2τ in a Carr-Purcell pulse sequence, can be made sensitive to the diffusional motion of the molecules or ions containing the magnetic nuclei (Fig. 1a). The translational diffusion will attenuate the echo amplitude E according to the equation:

$$\ln(E_g/E_o) = -(\gamma\delta g)^2 D(\Delta - \delta/3)$$

where g is the magnitude, δ the width and Δ the time spacing between the gradient pulses (*cf.* Fig. 1a). The measurements were performed as described previously.[2,3]

For isotropic systems like micellar solutions and cubic liquid crystals the NMR signals are narrow and the determination of the diffusion coefficient is straight forward. For lamellar liquid crystals, however, very broad signals due to dipolar couplings are usually observed and the standard techniques have to be modified. This problem can be solved by aligning the lamellar sample and orienting the normal of the lamellae at an angle of 54.7° (the magic angle) relative to the external magnetic field, B_o (Fig. 1b and c).

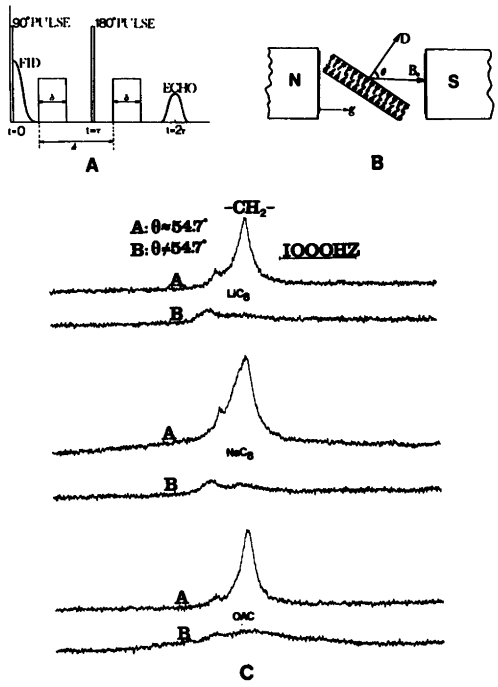

Fig. 1. a. Illustration of the radio frequency and the magnetic field pulse sequences. Δ is the spacing between the field gradients and δ is the width of them; b. Schematic picture of the orientation of the aligned lamellar phase in the magnetic field, B_0 and the field gradient, g; c. Proton NMR spectra of macroscopically aligned lamellar samples of lithium (LiC_8), sodium (NaC_8) octanoate and of octylammonium hydrochloride (OAC).

Applications. A. The effect of cholesterol on lecithin diffusion. The lecithin lateral diffusion coefficient in lecithin-cholesterol bilayers has been measured [4] for varying cholesterol contents. As shown in Fig. 2 cholesterol does not appreciably affect the lecithin diffusion for the three different lecithins studied. It is well known that cholesterol increases the molecular ordering in the bilayer but this is not accompanied by a decrease in the lipid diffusion. Previously, the results obtained with various methods were usually discussed in terms of cholesterol as a moderator of "fluidity" or "microviscosity". This explanation is obviously not correct (for a detailed discussion see Refs. 4–6).

B. The phase structure of the cubic phase in the system monoolein-water. Structural information can be obtained from an investigation of the ratio be-

* Communication at the Joint Meeting of the Swedish Biochemical and Swedish Biophysical Societies in Uppsala, 28–29th November, 1980.

0302-4369/81/010061-02$02.50

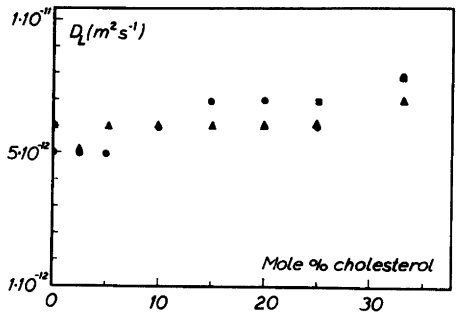

Fig. 2. The lipid lateral diffusion coefficient, D_L as a function of the cholesterol content in the bilayer. (▲) egg yolk lecithin, (■) palmitoyl oleoyl lecithin and (●) dioleoyl lecithin.

tween the diffusion coefficient in the lamellar phase and the apparent diffusion coefficient of the liquid crystalline phase of unknown structure.[2,3] The measured diffusion coefficient of a cubic phase will depend on how many directions that are effectively available for the lipid diffusion within the aggregates building up the phase structure. Thus conclusions about the geometry of the aggregates can be drawn from the apparent diffusion coefficients if the lipid diffusion within an aggregate is known. The latter is assumed to be equal to the lateral diffusion coefficient in the bilayer where it can be *directly* measured.[2] It is assumed that the shape of the aggregate does not influence the diffusion coefficient in the aggregate to any appreciable extent.

The monoglyceride monoolein forms with water a lamellar and a cubic phase. It was found that the local lipid diffusion, assuming two-dimensional diffusion in the cubic phase, was the same for the two phases. A combination of X-ray diffraction and NMR diffusion studies leads to the conclusion that the cubic phase was composed of bilayer units.[3]

C. The structure of the cubic and the hexagonal phase in the system lecithin-sodium cholate-water. By using the same method as above it was found that for both the hexagonal and the cubic phases the lipid diffusion was of the same order of magnitude as in the lamellar phase.[7,8] From these findings together with other NMR data it was concluded that both phases consisted of continuous rod-like aggregates.

D. Phase structures of lipids from Acholeplasma laidlawii *membranes.* The lipid composition in membranes of *A. laidlawii* is extensively regulated as a response to changes in temperature and incorporation of fatty acids and cholesterol.

Due to their different molecular geometries MGDG (wedge shape) forms a reversed hexagonal phase and DGDG (rod shape) forms a lamellar phase.[9] Thus, the phase structure of mixtures of the glucolipids strongly depends on the ratio MGDG/DGDG. A mixture of MGDG and DGDG having only oleoyl chains forms a *cubic* phase.[10] Information about the aggregate structure in this phase was obtained by comparing diffusion coefficients for lamellar and cubic phases as described above. These findings lead to a structure compatible with the structure proposed by Luzzati and Spegt.[11] The lamellar structure is the only one compatible with a functional biological membrane and none of the non-lamellar phases have been observed with lipid compositions occurring *in vivo*. The results obtained from these lipid mixtures are highly relevant to the *regulation* of membrane lipid composition observed in *A. laidlawii* grown under different conditions. It is inferred that the molecular geometry of the lipids is of vital importance for the membrane stability.

Acknowledgements. The collaboration with K. Fontell, L. B.-Å. Johansson, A. Khan, O. Söderman and H. Wennerström, Physical Chemistry 2, Lund, G. Arvidson, Medical and Physiological Chemistry, Uppsala and A. Christiansson, L. Rilfors and Å. Wieslander, Microbiology, Lund is gratefully acknowledged.

1. Stejskal, E. O. and Tanner, J. E. *J. Chem. Phys. 42* (1965) 288.
2. Lindblom, G. and Wennerström, H. *Biophys. Chem. 6* (1977) 167.
3. Lindblom, G., Larsson, K., Johansson, L. B.-Å., Fontell, K. and Forsén, S. *J. Am. Chem. Soc. 101* (1979) 5465.
4. Lindblom, G., Johansson, L.B.-Å. and Arvidson, G. *Biochemistry. In press.*
5. Wieslander, Å., Christiansson, A., Rilfors, L. and Lindblom, G. *Biochemistry 19* (1980) 3650.
6. Khan, A., Rilfors, L., Wieslander, Å. and Lindblom, G. *Eur. J. Biochem. In press.*
7. Arvidson, G., Fontell, K., Johansson, L.B.-Å., Lindblom, G., Ulmius, J. and Wennerström, H. *Ber. Bunsenges. Phys. Chem. 82* (1978) 977.
8. Ulmius, J., Lindblom, G., Wennerström, H., Johansson, L.B.-Å., Söderman, O., Arvidson, G. and Fontell, K. *To be published.*
9. Wieslander, Å., Ulmius, J., Lindblom, G. and Fontell, K. *Biochim. Biophys. Acta 512* (1978) 241.
10. Wieslander, Å., Rilfors, L., Johansson, L.B.-Å. and Lindblom, G. *Biochemistry. In press.*
11. Luzzati, V. and Spegt, P. A. *Nature (London) 215* (1967) 701.

Received November 20, 1980.