THE ORIGIN OF MULTIPLE QUADRUPOLE COUPLINGS IN THE DEUTERIUM NMR SPECTRA OF THE 2 CHAIN OF 1,2 DIPALMITOYL-*sn*-GLYCERO-3-PHOSPHORYLCHOLINE

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Received 7 February 1981

1. Introduction

Deuterium NMR studies of labeled lipids have provided important details about the conformations and motions of the hydrocarbon chains (reviewed in [1,2]). In most cases, the deuterium NMR spectrum of a lipid dideuterated at a single methylene segment shows a powder pattern characterized by a single quadrupole coupling constant. The derived coupling has commonly been used to calculate a single Saupe order parameter, S_z [3], describing the angular excursions of the methylene segment about an axis normal to the bilayer plane. The deuterium NMR spectra of lipid dideuterated at the C-2 position in the *sn*-2 chain generally contain two quadrupole coupling constants. These are observed in bilayer dispersions of DPPC [1,4] and of several other 1,2-acyl phospholipids [5,6] and also when lipids are incorporated into membranes in bacteria [6–9]. The two splittings could be explained either by the existence of two long-lived conformers, as has been observed in the crystal structure of dimyristoyl phosphatidylcholine [10], or by the non-equivalence of the motions of the *pro-R* and *pro-S* deuterons. Two studies [9,11] have investigated these possibilities through examination of the temperature variation of the splittings. Their conclusions were in contradiction, one favoring two conformations [9] on the basis of spectral simulation, and the other favoring non-equivalence on the basis of signal shape at low temperature [11]. A more direct test of which hypothesis is correct involves measurement of deuterium NMR spectra of DPPC in which only one of the C-2 protons of the *sn*-2 chain is replaced, stereospecifically, by deuterium.

Abbreviations: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine; NMR, nuclear magnetic resonance

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2. Experimental

2.1. *Synthesis of phospholipids*

Lipids were prepared from lysolecithin and palmitic acid using *N,N'*-carbonyldiimidazole [12].

2.2. *Deuterated palmitic acids*

[2,2-$^2H_2$]palmitic acid was prepared by the standard exchange method [13], [2*R*-^{2}H]palmitic acid was prepared by minor modifications of the standard technique for such acids [14], and for [2*R*-^{3}H]palmitic acid [15,16]. In brief, (*S*)-ethyl acetosuccinate, from malic acid [15], and myristic acid were subjected to Kolbe electrolysis [17] of equimolar amounts at 60°C at current densities >0.1 A/cm^2, to yield 2-(*S*)-hydroxyhexadecanoic acid. This was converted to [1,1,2*R*-$^2H_3$]hexadecanol by the action of lithium aluminum deuteride on the intermediate 2-(*S*)-tosylhexadecanoic acid methyl ester, prepared by standard methods. The final product was prepared by two-step oxidation of the alcohol (pyridine–chromium trioxide; CrO_2–sulfuric acid) [18,19].

2.3. *Configurations of products*

The absolute configuration of (–)malic acid has been determined by anamolous X-ray scattering [20], and so (*S*)-ethyl acetosuccinate has a firmly established configuration. Reduction of a tosylate by lithium aluminum deuteride proceeds by S_n2 mechanism

resulting in inversion (reviewed in [21]), at the hexadecanol step. All subsequent steps should proceed with configurational retention. None of the conventional methods of determining optical purity (optical rotation; NMR of the ester of [2*R*-^{2}H]hexadecanol with optically active 3-phenylbutanoic acid; NMR of complexes of [2*R*-^{2}H]palmitic acid with enatiomeric shift reagents) were able accurately to measure the optical purity of the products. Since the deuterium NMR result below is entirely unambiguous for almost complete configurational retention, no further characterization was pursued.

2.4. *Sample preparation*

Dispersions of DPPCs were made up in distilled water, at 50% (w/w). These were sealed into 1 cm lengths of 6 mm o.d. glass tubing, by cementing glass plugs in place with epoxy resin. Samples were incubated above 41°C for ⩾1 h before their spectra were obtained.

2.5. *NMR spectra*

Deuterium NMR spectra were obtained on a 6.8 T spectrometer located at the Francis Bitter National Magnet Laboratory using spin echo technique [22], quadrature detection, an effective bandwidth of 250 kHz, ^{1}H decoupling, and a repetition rate of 1 s. Sample temperature was 51°C.

3. Results

Deuterium NMR spectra of dispersions of 2-[2,2-$^2H_2$]DPPC (upper) and 2-[2*R*-^{2}H]DPPC are shown in fig.1. The deuterium quadrupole couplings in the spectra as measured by peak-to-peak separation, are 17.8 and 11.9 (upper), and 17.9 kHz. In the lower spectrum, the line shape closely resembles that of a random dispersion [1]. Additional minor features are a central peak arising from deuterium in water, a pair of small peaks corresponding to the smaller coupling in the upper spectrum, and a pair of shoulders just outside the main peaks having a separation equal to the observed coupling for deuterons at the C-2 position of the *sn*-1 chain.

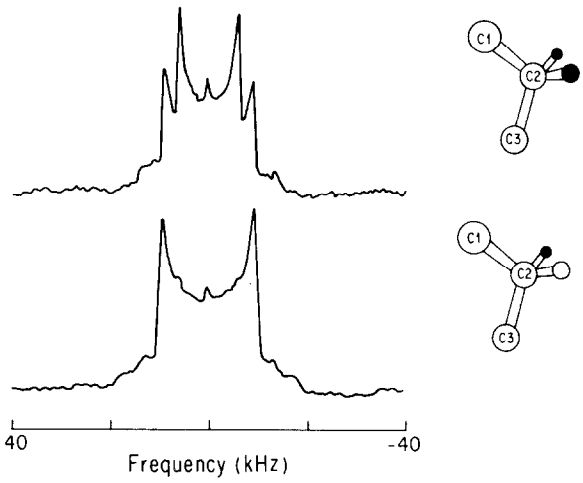

Fig.1. Deuterium NMR spectra of aqueous dispersions of 2[2-$^2H_2$]dipalmitoyl-*sn*-glycero-3-phosphorylcholine (upper, 1000 transients) and 2[*R*-^{2}H]dipalmitoyl-*sn*-glycero-3-phosphorylcholine (lower; 7000 transients). Black circles in the cartoons of molecular fragments represent deuterium atoms.

4. Discussion

These experiments unambiguously prove that the two couplings in the spectrum of 2-[2-$^2H_2$]DPPC arise from 2 non-equivalent deuterons/molecule, rather than from 2 classes of relatively long-lived conformations each having, separately, equivalent deuterons. This result shows that a single Saupe order parameter cannot represent the motion of the *sn*-2 C-2 segment. For most other methylene segments in saturated acyl chains of phospholipids, it is generally accepted [1] that the equivalence of the two methylene hydrogen positions arises from rapid rotation which is about the axis normal to the HCH plane, and which is coplanar with the carbon atom. The simplest explanation of the result for the *sn*-2 C-2 segment is then that this condition is violated. Either the motions of the two positions in this segment are averaged about quite different angles relative to the chain normal, or the angular excursions of each position differ. If it is assumed that the major contribution to segmental motion of this fragment is from kink propagation [1,23,24], i.e., concerted two-segment *gauche–trans* isomerisation up and down the acyl chain, then the different couplings presumably arise mainly from different average orientations.

The approximate positions can be deduced from crystallographic studies [10,25] performed at lower temperatures, the *pro-R* position to C-2 is normal to the extended chain axix, and *pro-S* to C-2 is at ~34°. These values would give rise to predicted Saupe order parameters about the extended chain axis of ~±1/2 [1,3]. Since the observed quadrupole couplings are

much smaller than would be expected if this order parameter were the only non-zero order parameter, it is obvious that significant molecular motions in other directions are taking place. This is not inconsistent with kink propagation being the major mechanism, since the last C2–C3 bond of the *sn*-2 chain is differently orientated with respect to the extended chain axis, compared to lower carbon–carbon bonds.

In conclusion, the multiple deuterium quadrupole couplings of the C-2 segment of the *sn*-2 chain in DPPC arise from magnetic non-equivalence, not from multiple conformations and it is reasonable to assume that this result is applicable to similar observations in biological membranes, for which contrary conclusions have been drawn [9]. This kinked start of the *sn*-2 chain of phosphilipids may be a general conformational feature.

Acknowledgements

Supported in part by NIH AM-20357. We are grateful to Dr T.-H. Huang and Dr R. G. Griffin for use of the deuterium NMR spectrometer at the National NMR Facility for Biomolecular Research, MIT, supported by NIH RR-00795, and to professor W. C. Agosta for discussion.

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