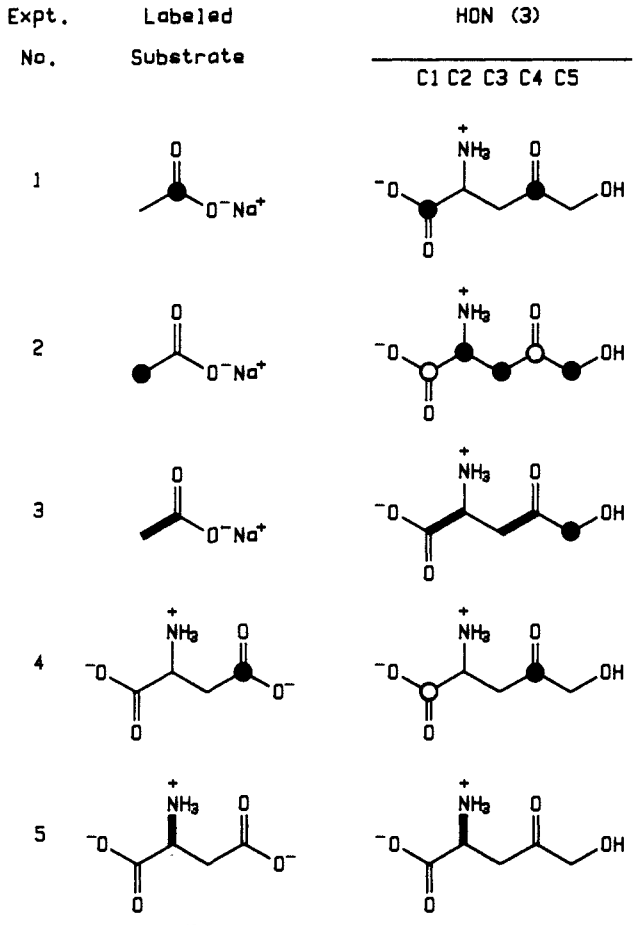


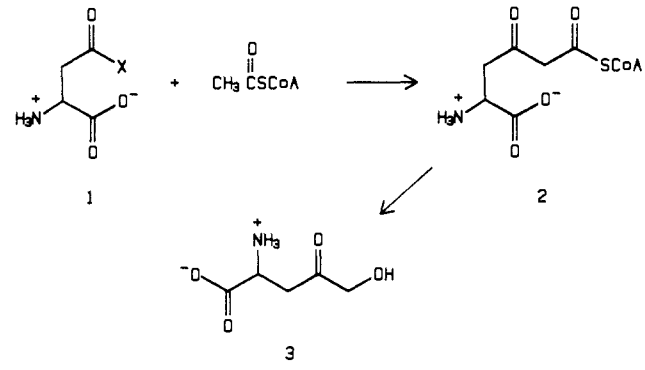
Figure 1. Observed ^{13}C enrichments and couplings in HON derived from labeled substrates. Larger enrichments (2.2-3.7 times natural abundance) are represented by filled circles, smaller enrichments (1.5-1.7 times natural abundance) are represented by open circles, and unlabeled carbon atoms correspond to natural abundance ^{13}C. Coupled nuclei are joined by heavy lines.

by these routes, would have an equal distribution of ^{13}C at C-1 and C-4.

A distinction between aspartate and oxaloacetate or malate was made by feeding DL-[2-^{13}C,^{15}N]aspartate[12] which was synthesized[13] from diethyl [2-^{13}C,^{15}N]phthalimidomalonate[14] and ethyl bromoacetate. The intact incorporation of this C-N unit, and thus the precursor role of aspartate, was demonstrated by the observation of two coupled ^{13}C NMR signals (centered at 49.1 and 48.1 ppm, $^1J_{CN}$ = ca. 6 Hz for each), in the mixture of diastereomeric γ-lactones of 4,5-dihydroxynorvaline, which corresponds to C-2 in each of the diastereomeric γ-lactones and consequently to C-2 of HON.

The pattern of incorporation of ^{13}C into HON is consistent with the condensation of acetyl coenzyme A or malonyl coenzyme A with a β-activated aspartate (**1**) to form a 6-carbon intermediate (e.g., **2**) that is converted to HON (**3**) by hydrolysis and either oxidative decarboxylation or separate decarboxylation and hydroxylation steps (Scheme I). A similar condensation between acetyl coenzyme A and a γ-activated glutamate[5] or glutamic semialdehyde[15] has been proposed as the first step in the biosynthesis of the carbapenem antibiotics. Whether the analogy

Scheme I. Biosynthetic Formation of HON (3) from Acetyl Coenzyme A and a β-Activated Aspartate (X = Activating Group)



extends to the enzymes that catalyze these two condensations is under investigation.

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Direct Measurement of Deuterium-Deuterium Dipolar Coupling and Analysis of the Ordering of a Specifically Deuteriated Diunsaturated Lipid

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Although polyunsaturated lipids are of considerable biological interest,[1] relatively little is known of their physico-chemical properties in membranes. In order to achieve a better understanding of their biological function we have initiated ^2H NMR studies to elucidate the average structural properties and associated molecular dynamics of liposomal polyunsaturated phospholipids.[2] As part of these studies we examined model bilayers composed of 1-palmitoyl-2-isolinoleoyl phosphatidylcholine (PiLPC) specifically deuteriated at the 8 position of the isolinoleoyl (18:2$^{\Delta 6,9}$; *cis,cis*-octadeca-6,9-dienoyl) chain (inset of Figure 1). We report here that acquiring spectra with proton decoupling facilitates the line shape analysis of the spectra and has lead to the first direct observation of geminal ^2H-^2H dipolar coupling and calculation of the complete ordering tensor for the methylene segment.

The ^2H NMR spectra of aqueous dispersions of [8'-$^2H_2$]PiLPC are relatively narrow and featureless (Figure 1a) suggesting that either the average orientation of both methylene C-^2H bonds is close to the "magic angle" (54.7°) or their molecular motion is axially asymmetric. Spectra of the sample oriented between glass plates (Figure 1c) did not resolve the uncertainty; however, acquiring spectra of both the aligned and dispersed samples, with proton decoupling (Figures 1b and 1d), established that the two deuterons are magnetically inequivalent and that their molecular

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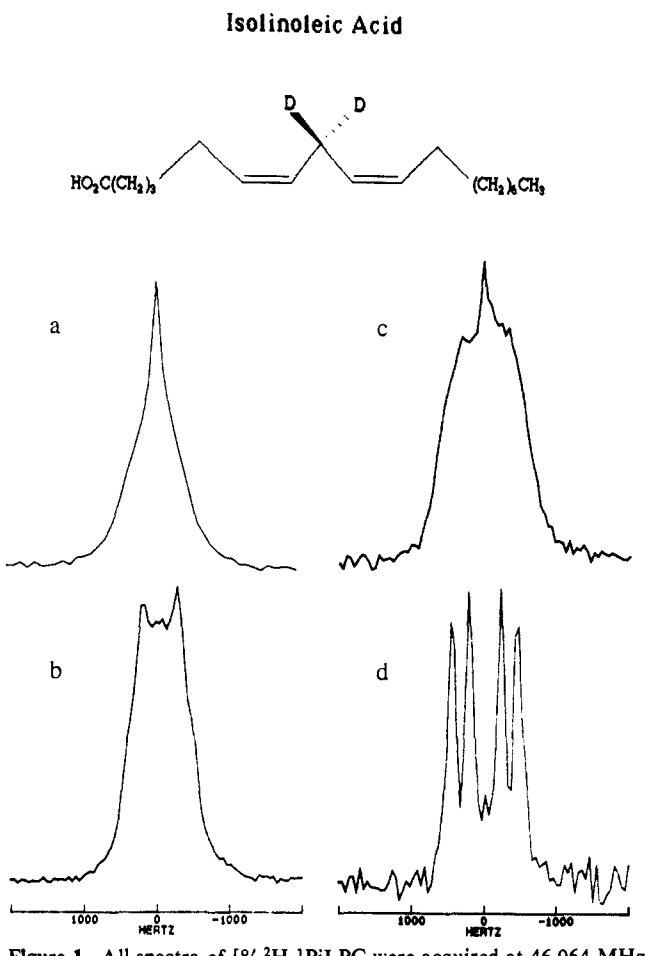

Figure 1. All spectra of [8′-$^2H_2$]PiLPC were acquired at 46.064 MHz with a modified quadrupolar echo pulse sequence[11] except "a" which was acquired at 30.7 MHz. Spectra in "a" and "b" correspond to aqueous dispersions of PiLPC at 30 °C, while those in "c" and "d" were recorded from oriented multibilayers with the bilayer normal at an angle of 90° with respect to the magnetic field direction, at 25 °C. The spectra in "b" and "d" were acquired while proton decoupling with the WALTZ pulse sequence.[12] Details will be published elsewhere. The inset is a schematic diagram of the deuteriated methylene segment of isolinoleic acid.

motion has effective axial symmetry. In addition, at most temperatures two less intense doublets are also resolved (Figure 2). We believe that the less intense peaks arise from 1-isolinoleoyl-2-palmitoyl PC formed, by acyl chain migration, during the synthetic procedure.[2]

Spectra acquired at 40 °C with the sample oriented such that the bilayer normal is parallel to the magnetic field direction (0° orientation) show not only four doublets but also, each major peak is split into three peaks of unequal intensity, separated by 60 Hz (Figure 2a). The fine structure of each quadrupolar doublet is symmetric about the Larmor frequency and arises from dipole–dipole interactions between the two geminal C-8 deuterons. We simulated spectra of an oriented sample containing two deuterons with homonuclear dipolar coupling, and, as shown in Figures 2b and 2c, the simulations for both the 0° and 90° orientations correspond very closely to their experimental counterparts. Simulations performed with different combinations of signs of the quadrupolar splittings and dipolar coupling (not shown) also clearly indicate that all three have the same sign.

Order parameters for the two carbon–deuterium bonds and for the deuteron–deuteron internuclear vector were calculated to be S_{CD} (1) = 7.51 × 10^{-3}, S_{CD} (2) = 1.02 × 10^{-2}, and S_{DD}[3] = −6.18 × 10^{-2} (or the reverse sign for all three values), and following Seelig[4] and Seelig and Waespe-Šarčevič[5] the complete ordering

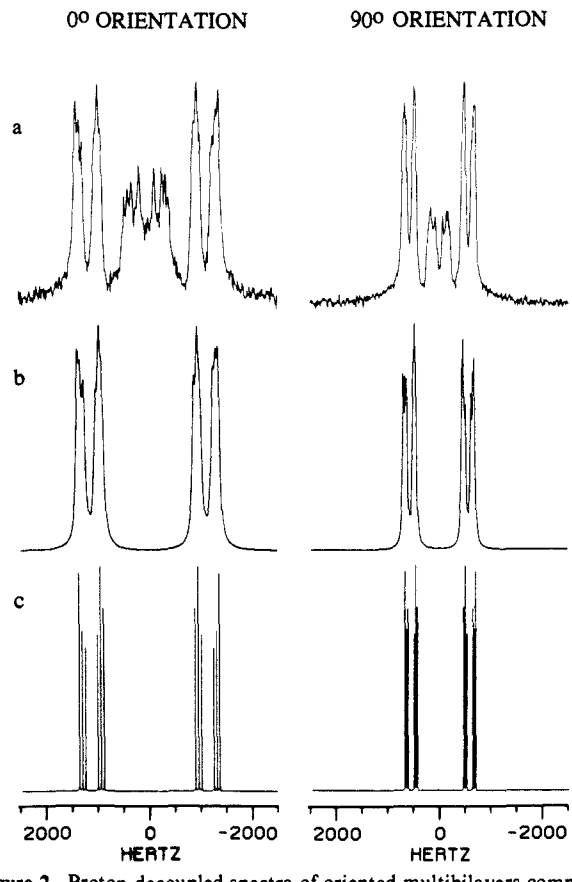

Figure 2. Proton-decoupled spectra of oriented multibilayers composed of [8′-$^2H_2$]PiLPC oriented with the bilayer normal at angles of 0° and 90° with respect to the magnetic field direction are shown in "a". Both were recorded at 40 °C with a sweep width of 50 kHz and were zero-filled from 2048 to 4096 "real" data points and left shifted to the top of the echo before Fourier transformation of the trailing edge of the FID. No other data manipulations were performed. The spectra in "b" were simulated by using the PANIC routine of the Bruker software with the following parameters for the 0° orientation: dipolar coupling = 30 Hz; quadrupolar coupling = 1312 and 958 Hz (these correspond to what is normally referred to as the "quadrupolar splittings" of the powder, i.e., the 90° orientation); Lorentzian line width = 70 Hz;[13] and the same digital resolution as the experimental spectra, 12 Hz/Pt. For the 90° orientation all input parameters (except for the digital resolution) were halved. The spectra in "c" were simulated by using zero line width and a digital resolution of 3 Hz/Pt.

tensor for the methylene unit was defined. The tensor was then diagonalized and the result (**S***) indicates that the ordering is not axially symmetric ($S^*_{xx} \neq S^*_{yy}$)

$$\mathbf{S}^* = \begin{pmatrix} -0.089 & & \\ & -0.062 & \\ & & 0.150 \end{pmatrix}$$

The methylene segment appears to undergo motions of relatively large amplitude (i.e., all three order parameters are smaller than those for saturated systems labeled at the same position), and its average orientation is such that the director (bilayer normal) lies in the ^2H–C–^2H plane tilted at an angle of ±0.4° from the bisector of the ^2H–C–^2H geminal angle.[6]

The C8 methylene unit links two rigid double bonds (see Figure 1), and, therefore, we expected its ordering to reflect a restricted geometry and to yield order parameters greater than those measured for saturated systems. We also expected the average orientation to be such that both double bonds could adopt orientations parallel to the bilayer normal, as has been suggested by others.[7,8] This is clearly not the case. However, the methylene

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unit can adopt two conformations that allow both double bonds to assume the proposed orientation. If interconversion between these two conformations is rapid, the spectra (and thus the calculated orientation) would reflect the average of both. Furthermore, jumping between the two could also reduce the effective order parameters to values such as those found for our system. Our results may, therefore, reflect the existence of two interconverting, relatively ordered states. We plan to use specific motional models to investigate this hypothesis and thus gain a better understanding of the average structure and ordering of lipids containing more than one double bond.

The analysis presented in this paper is unique in that no assumptions were required to define the ordering of the system. Direct observation of the deuteron–deuteron dipolar coupling provided the additional parameter necessary to define completely both the ordering tensor and the average orientation of the methylene segment. Although deuterium homonuclear dipolar couplings have been measured in other cases,[3,9,10] this is the first direct observation in spectra recorded from a deuteriated acyl chain in a phospholipid bilayer. The spectra and subsequent analysis illustrate the salutary effect of proton decoupling and demonstrate that more detailed information regarding the ordering of lipid systems can be obtained if an approach similar to ours is used.

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(13) The line width was chosen so that the simulation for the 0° orientation would resemble the experimental spectrum. The value used is within ±10% of the line width calculated from the T_2 of the sample.

Ordered DNA–Polypeptide Complexes of Extreme Chirality: Effects of Polypeptide Handedness on DNA Long-Range Asymmetry

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Under appropriate conditions of ionic strength and in the presence of various polymers or dehydrating agents, DNA molecules undergo a remarkable cooperative compaction process.[1] The resulting structures, which attracted much interest due to their potential relevance to compact states of DNA invariably found in vivo, are characterized by nonconservative circular dichroism spectra whose magnitudes are significantly larger than those of dispersed DNA molecules.[2] Both the shape and the size of the CD signals indicate that the condensation process is accompanied by the formation of ordered tertiary structures in which well-defined, long-range couplings between chromophores are induced. Of particular interest are the phenomena observed when the DNA is condensed by dehydrating agents at high ionic strength conditions.[3] In 35% (v/v) ethanol and 0.8 M NaCl the DNA molecules undergo a cooperative transition into compact species that exhibit a nonconservative CD spectrum with a large positive signal centered around 265 nm. The magnitude of the signal is diminished as the salt concentrations are increased; a sign reversal and large negative bands are finally obtained (Figure 1a).[3] It occurred to us that reactions between the ordered structures thus obtained and species of a given, long-range, secondary conformation should be strongly affected by the asymmetry of the DNA molecules. This assumption is borne out: the interaction between condensed forms of nucleic acids and polypeptides result in hitherto unknown types of complexes of extremely large asymmetry which is determined by the DNA long-range order and the handedness of the polypeptide.

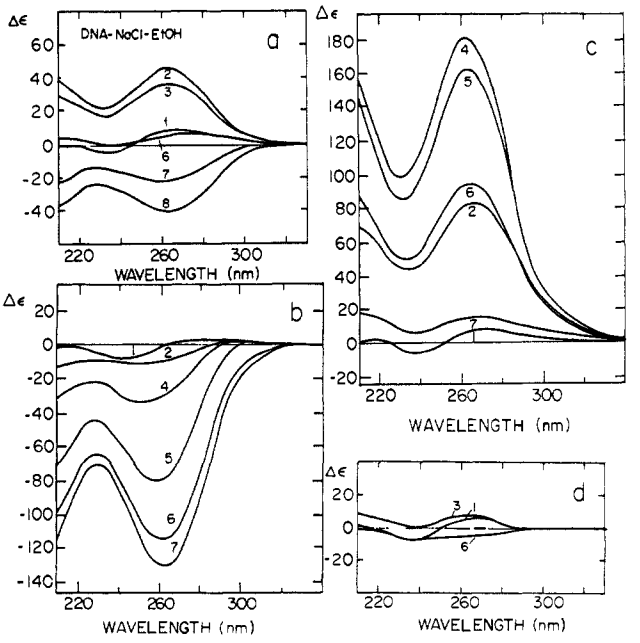

Figure 1. Circular dichroism spectra of condensed DNA molecules in H_2O–ETOH (35% v/v) solution containing the following NaCl concentrations (in M): (1) 0; (2) 0.8; (3) 1.0; (4) 1.2; (5) 1.4; (6) 1.6; (7) 1.8; (8) 2.0. a, DNA; b, DNA + poly-L-lysine; c, DNA + poly-D-lysine; d, DNA + poly-DL-lysine (DNA concentration, $5 \cdot 10^{-5}$ M in bp, polypeptide concentration $5 \cdot 10^{-5}$ M in amino acid residues).

When DNA condensation is induced in the presence of poly-L-lysine, the CD absorptions are substantially altered; instead of the large positive band obtained at 0.8 M NaCl, a negative signal is observed. Increasing NaCl concentrations are accompanied by a gradual increase of the absolute magnitude of the CD bands. Significantly, *negative ellipticities are observed at all salt concentrations* (Figure 1b), in clear contrast with the system in which the polypeptide is absent. The CD signals which characterize the DNA-poly-D-lysine complexes are, on the other hand, *invariably positive* with maximal intensity exhibited at 1.4 M NaCl (Figure 1c). It should be noted that the highest intensity of the CD bands in both cases where polypeptides are present is about four times larger than the maximal signal magnitude revealed by the compact species devoid of polylysines. In contrast, inclusion of a random polymer composed of L- and D-lysines in a 1/1 ratio in the condensation mixtures results in very small CD bands (Figure 1d), indicating complete disruption of any long-range order.[4]

In order to obtain the phenomena illustrated in Figure 1, strict length requirements have to be met by both nucleic acids and polypeptides: CD anomalies are detected only when the DNA

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(4) The described CD spectra are basically different from those reported for species obtained by a high to low salt dialysis of DNA–polylysine mixtures in the absence of dehydrating agents. In these cases, the condensation process is *induced by the polypeptides*, probably by excluded volume effects similar to those revealed by other polymers that affect condensation. In these cases, identical CD spectra for both poly-L- and poly-D-lysines are observed. See: (a) Shapiro, J. T.; Leng, M.; Felsenfeld, G. *Biochemistry* **1969**, *8*, 3219–3232. (b) Carroll, D. *Biochemistry* **1972**, *11*, 421–426.

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