Molecular Order in Cis and Trans Unsaturated Phospholipid Bilayers[†]

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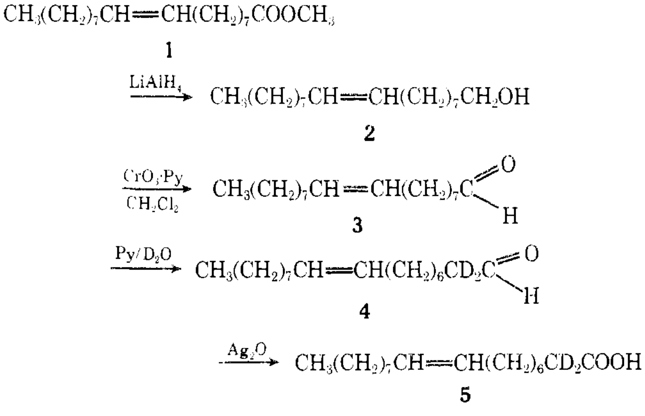
ABSTRACT: The influence of cis and trans double bonds on the hydrocarbon-chain organization of fluid lipid bilayers is investigated by means of deuterium nuclear magnetic resonance. Oleic acid and elaidic acid have been deuterated selectively at six and three different carbon atoms, respectively. The labeled fatty acids are used to prepare 1-palmitoyl-2-oleoyl-, 1-palmitoyl-2-elaidoyl-, and 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine. The residual deuterium quadrupole splittings of the fully hydrated bilayer membranes are measured as a function of temperature. An unusual deuterium order profile is obtained for the cis unsaturated phospholipid membrane. However, taking into account geometric effects, the segmental fluctuations in the sn-1 and sn-2 chain are found to be identical. As a general conclusion, it follows that the segmental fluctuations around the bilayer normal depend only on the distance from the lipid–water interface but not on the specific segment geometry. Conformational differences are observed at the beginning of the two chains in bilayers of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine. The sn-1 chain assumes a straight conformation, while the sn-2 chain is bent at the C-2′ segment. The average orientation of the cis double bond in the membrane can be determined by combining the deuterium NMR results with infrared dichroism data. The C=C bond vector is found to be aligned almost perpendicular to the bilayer surface; i.e., the angle between the bilayer normal and the C=C bond vector is only 7–8°. A similar orientation is likely for the trans double bond.

Biological membranes are characterized by a high percentage of cis unsaturated phospholipids, and therefore the study of the physical–chemical implications of cis unsaturation is of general interest. The deuterium quadrupole splitting of selectively deuterated lipids is a specific and quantitatively useful parameter for the investigation of the phospholipid organization within fluid membranes. The potential of deuterium NMR has now been demonstrated for quite a number of membraneous systems ranging from simple soaplike bilayers (Charvolin et al., 1973; Seelig and Niederberger, 1974; Davis and Jeffrey, 1977) over phospholipid membranes (Seelig and Seelig, 1974; 1975; Stockton et al., 1976; Seelig and Gally, 1976; Stockton and Smith, 1976; Seelig et al., 1977) to true biological membranes (Stockton et al., 1975; Oldfield et al., 1976; Stockton et al., 1977) (for reviews see Mantsch et al., 1977; Seelig, 1977). Considering the specific problem of cis unsaturation, we have previously employed deuterium NMR to study the molecular ordering in bilayers composed of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC[1]) (Seelig and Seelig, 1977). The deuterium labels were attached to the palmitic acyl chain, and the influence of the double bond was probed indirectly by the neighboring chain. We also reported briefly on the motion of the cis double bond itself. In this work we have selectively deuterated various segments of the oleic acyl chain and can now describe in more detail the motional properties of the sn-2 chain of POPC bilayers. As a comparison, we have also synthesized selectively deuterated elaidic acid and the corresponding lipids 1-palmitoyl-2-elaidoyl- (PEPC) and 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine (DEPC). For all bilayer systems, well-resolved deuterium NMR spectra were easily obtained and could be followed as a function of temperature.

Materials and Methods

Synthesis of Selectively Deuterated Oleic Acid and Elaidic Acid. Five *cis*-9-octadecenoic acids selectively deuterated at carbon atoms 2, 8, 9–10, 11, and 12 have been synthesized. Among these, methyl *cis*-9-octadecenoate-8,8-d_2, -11,11-d_2, and -12,12-d_2 have been prepared via a stereoselective Wittig reaction which will be described in detail in a separate publication (N. Waespe-Šarčevič and J. Seelig, manuscript in preparation). *cis*-9-Octadecenoic acid-2,2-d_2 has been synthesized according to the following scheme



Methyl *cis*-9-octadecenoate (**1**) was reduced with lithium aluminum hydride to the respective alcohol **2**, which upon oxidation with the chromium trioxide–pyridine complex in methylene chloride according to Ratcliffe and Roderhorst (1970) gave *cis*-9-octadecenal (**3**). *cis*-9-Octadecenal-2,2-d_2 (**4**) was prepared by multiple exchanges with pyridine–heavy water mixtures (Tucker et al., 1971). The acid **5** was obtained by oxidizing the aldehyde with silver oxide (Mayer et al., 1976). *cis*-9-Octadecenoic acid-9,10-d_2 was prepared from stearolic acid (Adkins and Burks, 1955) by reduction with deuterium using Lindlar catalyst (Lindlar and Dubius, 1973).

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[1] Abbreviations used are: NMR, nuclear magnetic resonance; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PEPC, 1-palmitoyl-2-elaidoyl-*sn*-glycero-3-phosphocholine; DEPC, 1,2-dielaidoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine.

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TABLE I: Residual Deuterium Quadrupole Splittings, $\Delta\nu_Q$, in kHz for Unsaturated Phospholipid Bilayers (Powder-Type Spectra).[a]

oleic acid chain C atom	temp, °C		
	0	27	46
	POPC[b]		
2	18.75	16.4	15.6
	9.4	10.8	10.6
8	19.1	15.6	13.8
9	15.8	13.4	12.0
10	3.6	2.5	2.3
11	9.2	6.2	5.0
12		13.0	10.8
elaidic acid C atom	temp, °C		
	28	46	57
	PEPC[c]		
9[d]	27.2	20.5	18.4
10[d]	27.2	20.5	18.4
11	25.2	19.3	17.8
both elaidic acid chains deuterated at C atom	temp °C		
	16	26	57
	DEPC[e]		
9[f]	24.9	21.6	17.0
10[f]	24.9	21.6	17.0

[a] The assignment of the two quadrupole splittings of the cis double bond has been made on the basis of the analysis given in the Appendix. The gel to liquid-crystal transition temperatures T_c were determined by the loss of the deuterium NMR signal. For POPC and DEPC the results agree with those obtained by differential scanning calorimetry (van Dijck et al., 1976). For PEPC, no such data are available. [b] $T_c = -5$ °C. [c] $T_c = 26$ °C. [d] One doublet only. [e] $T_c = 10$ °C. [f] One doublet only.

trans-9-Octadecenoic acid-9,10-d_2 and -11,11-d_2 were synthesized by light-induced isomerization of the corresponding *cis*-9-octadecenoic acid using the procedure of Gerlach et al. (1976). The acids were characterized by their IR, MS, and NMR spectra and, in the case of the elaidic acids, by their melting point. The products were shown to be better than 95% pure with respect to cis or trans unsaturation by gas chromatography of the epoxide methyl esters.

Synthesis of Phospholipids. Deuterated POPC and PEPC were synthesized by reacylation of 1-palmitoyl-2-lyso-*sn*-glycero-3-phosphocholine with deuterated oleic or elaidic acid anhydride (Cubero Robles and van den Berg, 1969; Seelig and Seelig, 1977). DEPC was prepared by reacylation of *sn*-glycero-3-phosphocholine with elaidic acid anhydride. The purified lipids were characterized by thin-layer chromatography, ^{1}H and ^{2}H NMR, and their optical rotation ($[\alpha]^{20}_{546}$ +6.9–7.8° for all lipids, $c = 10$ in $CHCl_3/CH_3OH$, 9:1). A further control was provided by the gel to liquid-crystal transition temperature of the lipid–water mixtures as determined by the loss of the deuterium signal below the phase-transition temperature. The transition points were generally found to be within 2 °C of those established by differential-scanning calorimetry (cf. Table I).

Preparation of the Bilayer Phases. The lipid in chloroform solution was filled in an ampule. The chloroform was evaporated first under nitrogen and then under high vacuum. Water (51.5 wt %) was added to the phospholipid (48.5 wt %), and the ampule was sealed under argon. A homogeneous phase was obtained by shaking the mixture at temperatures above the phase transition.

Deuterium Magnetic Resonance Measurements. The deuterium NMR measurements were made at 55.2 and 13.8 MHz with a Bruker WH-360-FT and HX-90-FT spectrometer, respectively, both equipped with a calibrated variable-temperature unit.

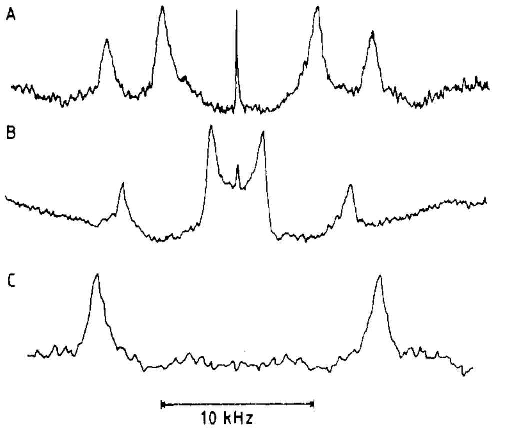

FIGURE 1: Deuterium magnetic resonance spectra of unsonicated phospholipid multilayers (48.5 wt % phospholipid; 51.5 wt % H_2O). (A) 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine deuterated at the C-2 atom of the oleic acyl chain (2′-d_2). Deuterium NMR frequency 55.2 MHz; 2800 transients; measuring temperature 10 °C. (B) 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine deuterated at the cis double bond (*cis*-9′-10′-d_2). Deuterium NMR frequency 55.2 MHz; 4700 transients; 5 °C. (C) 1-Palmitoyl-2-elaidoyl-*sn*-glycero-3-phosphocholine deuterated at the trans double bond (*trans*-9′-10′-d_2). Deuterium NMR frequency 13.81 MHz; 10 000 transients; 57 °C.

Results

Almost all CD_2 segments investigated in this study give rise to just one quadrupole splitting. The only exception is the oleic acid C-2′ segment in POPC, which has a deuterium NMR spectrum consisting of two doublets (Figure 1A). Both splittings are distinctly smaller than that of the palmitic acid C-2′ segment. Two quadrupole splittings are also observed for the deuterated cis double bond in POPC (Figure 1B), suggesting motional inequivalence of the C-9 and C-10 deuterons. However, only one quadrupole splitting is obtained for the deuterated trans double bond in either PEPC (Figure 1C) or DEPC (spectrum not shown) bilayers.

The residual quadrupole splittings, $\Delta\nu_Q$, of the segments investigated are summarized in Table I. These splittings are related to the order parameter, S_{CD}, of the deuterium bond vector according to

$$\Delta\nu_Q = (3/4)(e^2qQ/h)S_{CD}$$

The static deuteron quadrupole coupling constant (e^2qQ/h) is 170 kHz for aliphatic C–D bonds (Burnett and Muller, 1971) and 175.3 kHz for olefinic C–D bonds (Kowalewski et al., 1976). In both cases the asymmetry parameter is very close to zero. In this work the most extensive measurements have been performed with POPC bilayers. Figure 2 shows a comparison of the deuterium order parameters, S_{CD}, of the *sn*-1-palmitic acyl chain and the *sn*-2-oleic acyl chain at 27 °C. The cis double bond appears as a pronounced discontinuity in the curve drawn through the order parameters.

A similar comparison can be made between a cis and trans unsaturated lipid. This is illustrated in Figure 3 for bilayers composed of either POPC or PEPC where the deuterium labels are contained in the oleic acid and the elaidic acid chain, respectively. from Figures 2 and 3 it is obvious that the behavior of the *sn*-2-elaidic acyl chain resembles more that of a fully saturated chain than that of a cis unsaturated chain.

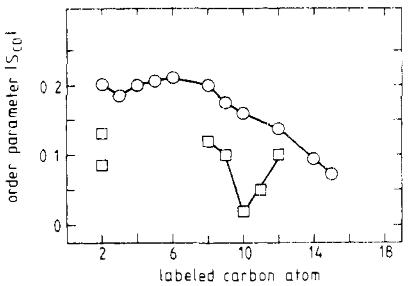

FIGURE 2: *Bilayers of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine.* Variation of the deuterium order parameter, S_{CD}, with the segment position: (○) *sn*-1 palmitic acyl chain; (□) *sn*-2-oleic acyl chain. Measuring temperature 27 °C.

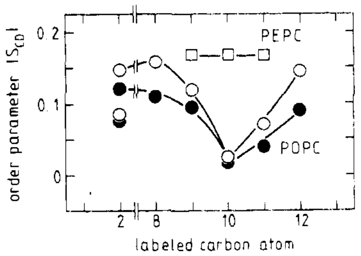

FIGURE 3: Comparison of cis and trans unsaturated lipid bilayers. The deuterium label is attached to the unsaturated chain: ○ (●), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine at 40 °C (0 °C). □, 1-palmitoyl-2-elaidoyl-*sn*-glycero-3-phosphocholine at 40 °C.

Discussion

Straight sn-1 Chain and Bent sn-2 Chain. Figure 2 shows obvious differences between the sn-1 and sn-2 chain at the C-2′ position. This effect is not caused by the incorporation of the cis double bond into the sn-2 chain, since a very similar spectral pattern has been observed for bilayers composed of fully saturated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (Seelig and Seelig, 1975). Addition of cholesterol to DPPC enhances the differences between the two chains (Haberkorn et al., 1977). The spectral differences of the C-2′ segments are also independent of the nature of the polar head group. We have investigated bilayers composed of phosphatidylethanolamine and phosphatidylserine and have obtained very similar results as for the phosphatidylcholines (J. L. Browning and J. Seelig, unpublished work). We therefore conclude that the inequivalence of the sn-1 and sn-2 chains at the C-2′ position is a rather general feature of phospholipid organization within membranes.

The differences in the quadrupole splittings can be understood by assuming different orientations at the beginning of the sn-1 and sn-2 chains with respect to the plane of the membrane (Seelig and Seelig, 1975). The sn-1 chain is perpendicular to the membrane surface at all segments, while the sn-2 chain begins parallel to the membrane surface and is bent perpendicular to it after the C-2′ segment. This structure is in agreement with the crystal structure of *rac*-dilauroylglycerophosphoethanolamine (Hitchcock et al., 1974) and is further supported by neutron-diffraction studies of DPPC/H_2O bilayers (Büldt et al., 1978). The origin of the two quadrupole splittings of the sn-2 chain is not yet clear. The two signals could be due to the magnetic inequivalence of the two deuterons or to two different conformations of the lipid molecule. A decision between the two alternatives is not possible on the basis of the available data.

Motion of the Cis and Trans Unsaturated Chains. For the molecular interpretation of deuterium order parameters, it is essential to note that they depend on (i) the statistical fluctuations within the bilayer and (ii) the orientation of the deuterated segment with respect to the bilayer normal (cf. Seelig, 1977). It cannot be expected a priori that segments of different geometry such as the methylene CD_2 group and the cis double bond will have the same average orientation in the lipid bilayer. Depending on the orientation, different quadrupole splittings can be obtained even if the two segments are subjected to identical molecular fluctuations around the bilayer normal. Thus, the main problem in the analysis of deuterium order parameters is to differentiate between purely geometric effects and statistical fluctuations.

An unambiguous interpretation can be given for the cis double bond. The deuterium NMR spectrum of POPC bilayers deuterated at the 9′,10′ carbon atoms of the oleoyl chain consists of two doublets (Figure 1B). In principle, it is conceivable that the two deuterons are motionally equivalent and that the two signals arise from two different phospholipid conformations. However, the CD_2 segments preceding and following the cis double bond exhibit just one quadrupole splitting and thus give no indication of two conformational states. We therefore attribute the two signals to the magnetic inequivalence of the C-9′ and the C-10′ deuteron. The relative areas of the two doublets are apparently not equal. This is due to the insufficient sensitivity and radiofrequency power of the NMR spectrometer used.

The observation of the two different quadrupole splittings for the C-9′ and C-10′ deuteron rules out the possibility that the C═C bond vector is *exactly* parallel to the bilayer normal. If this were the case, both deuterons would make the same angle with the rotation axis and would produce the same quadrupole splitting. The two deuterium order parameters alone are not sufficient to determine the average orientation of the cis double bond. The cis double bond is characterized by C_{2v} symmetry, and three independent order parameters are necessary to completely specify its average position (cf. Saupe, 1964). Recently, Fringeli has measured the infrared dichroism of the HC═CH stretching frequency in bilayers of egg-yolk lecithin and deduced an order parameter of $S_{33} = 0.36 \pm 0.03$ for the C═C bond direction (Fringeli, 1977). Since egg-yolk lecithin contains about 70 wt % POPC (Tattrie et al., 1968), we have assumed that this order parameter can also be used for pure POPC bilayers. By combining the deuterium NMR data with the infrared dichroism measurements, the complete order matrix, referred to a molecular coordinate system containing the C═C bond and the C_2 symmetry axis of the cis fragment, can be determined. The order matrix is then diagonalized by referring it to a new coordinate system which is rotated by 7–8° with respect to the previous one and has the bilayer normal as the *z* axis (cf. the Appendix). To be more precise, four calculations must be carried out, since both deuterium order parameters may be positive or negative and the assignment of the two quadrupole splittings to the C-9′ and the C-10′ deuteron is not obvious. In all four cases the deviation of the C═C bond vector from the bilayer normal has been found to be limited at most to ±9°. However, only the assignments and the orientation shown in Figure 4 lead to an axially symmetric order matrix as required by the uniaxial properties of the fluid bilayer. Details of this calculation are given in the Appendix. The principal element of the axially symmetric order matrix is $S_{mol} = 0.37 \pm 0.04$ at 27 °C. S_{mol} is free of geometric effects and truly represents the molecular fluctuations of the double bond around the bilayer normal. This result may be compared with the molecular ordering in the neighboring palmitic acyl chain for which the molecular order parameter has been estimated to be $S_{mol} = 2|S_{CD}|$ (Seelig and Seelig, 1977). From Figure 2 one calculates that for the first

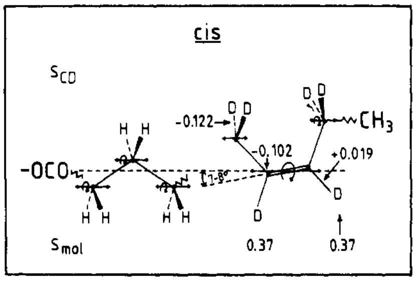

FIGURE 4: Comparison between deuterium order parameters, S_{CD}, and segmental order parameters, S_{mol}, for the cis double bond. The direction of the normal to the plane of the bilayer is indicated by the dashed line. Numbers above this line refer to the experimentally measured S_{CD} and numbers below this line to S_{mol}. Segment axes are denoted by double-headed arrows. S_{mol} describes the fluctuations of the segment axes. From the analysis of the deuterium order parameters and the infrared dichroism, it follows that the C=C vector is tilted by 7-8° with respect to the bilayer normal. Measuring temperature 27 °C.

nine segments $S_{mol} \approx 0.35$–0.4, which is in good agreement with the above analysis.

The simple relation $S_{mol} = 2|S_{CD}|$ cannot be applied to the CD_2 segments in the oleic acyl chain. Owing to the tilting of the C=C bond vector relative to the bilayer normal, CD_2 segments next to the double bond must also be tilted. This is illustrated for the C-8′ and C-11′ segment in Figure 4 where bond angles of 120° and 112° have been used for sp^2 and sp^3 hybridization, respectively. If one *assumes* segmental rotation axes as indicated by the arrows in Figure 4, the molecular order parameters of these axes are $S_{mol} = 0.36$ for the C-8′ segment and $S_{mol} = 0.89$ for the C-11′ segment. The first value agrees well with the order parameters established for the cis double bond and the palmitic acyl chain supporting this particular choice of rotation axis for the C-8′ segment. For the C-11′ segment, the angle between the C–D bond vector and the bilayer normal is close to the magic angle and S_{mol} is the ratio of two small numbers. A change in the rotation axis by only a few degrees will reduce S_{mol} to 0.4. The indicated rotation axis is therefore at least a reasonable approximation. An unambiguous determination of the rotation axes of the CD_2 segments would be possible if a second-order parameter could be determined, for example, by measuring the D–D dipolar coupling (cf. Diehl and Niederberger, 1974; Seelig, 1977).

The two C–D bond vectors of the deuterated trans double bond are parallel to each other and make the same angle with the bilayer normal. Only one quadrupole splitting per chain will therefore be observed under all circumstances (Figure 1C). From the available experimental data, it is not possible to evaluate the orientation of the trans double bond with respect to the bilayer normal. However, the simplest assumption is to draw the trans chain in the fully extended configuration perpendicular to the bilayer surface. The tilt angle between the trans C=C bond vector and the bilayer normal is then 25°, and S_{mol} is calculated to be 0.30 for the C-9′, C-10′, and the C-11′ segment. This is distinctly smaller than is usually anticipated at this position in the bilayer. However, a reduction in the tilt angle from 25° to 10° will increase the calculated S_{mol} from 0.30 to 0.45. A tilt angle of 10° and a corresponding S_{mol} of ~0.45 would also be in good agreement with the data established for the cis double bond. It is interesting to note that for DEPC the sn-1 and sn-2 chains give rise to the same quadrupole splitting at the 9′–10′ positions.

Conclusions

Almost all *saturated* bilayer systems investigated so far exhibit a similar variation of the segmental order parameter, S_{mol}, with the segment position. After a plateau region with $S_{mol} \approx 0.4$–0.6, comprising the first 8 to 10 segments, S_{mol} decreases gradually to almost zero at the methyl terminal of the chain. For the unsaturated POPC bilayer, the same shape of the order profile has been observed for the sn-1 palmitic acyl chain. However, the whole curve was found to be shifted toward lower S_{mol} values when compared with a DPPC order profile at the same temperature (Seelig and Seelig, 1977). The present analysis shows that the cis unsaturated sn-2 chain of POPC behaves similarly to the saturated sn-1 chain. The dramatic differences in the deuterium order parameters, S_{CD}, of the two chains in Figure 2 can be explained by the unique orientation of the cis double bond in the membrane. After correcting for this geometric factor, the molecular order parameters are identical within error limits in both chains.

The molecular origin of hydrocarbon chain disorder is generally thought to be rapid transitions between trans and gauche states of carbon–carbon single bonds. The cis double bond cannot execute such rotational jumps due to its specific chemical structure. Still, it is subjected to the same molecular fluctuations as the neighboring methylene segments. The main conclusion of the present study is that the segmental fluctuations in POPC bilayers are not dependent on the segment geometry but are determined by the distance of the segment from the lipid–water boundary. This suggests that to a good approximation the hydrocarbon interior of a POPC bilayer may be divided into strata running parallel to the bilayer surface. Segments which are located in the same stratum are then characterized by the same segmental order parameter S_{mol}. This certainly simplifies the statistical-mechanical analysis of the POPC bilayer.

As a second conclusion, it follows that the POPC molecules in the bilayer exhibit distinct conformational preferences. One prominent feature is the bend in the sn-2 chain after the C-2′ segment, another the alignment of the C=C bond vector almost parallel to the bilayer normal. Both properties seem not to be unique to the POPC bilayer. A bend in the sn-2 fatty acyl chain has been observed for a variety of chemically different phospholipids in the crystalline state, in the gel state, and in the liquid-crystalline state (cf. above). The parallel orientation of the trans double bond has been investigated less extensively, but is supported by the results obtained for PEPC membranes. Thus, only to a first approximation can the hydrocarbon chains in a lipid bilayer be described as disordered and liquidlike (cf. Tardieu et al., 1977). The detailed and quantitative results provided by deuterium NMR reveal a more structured bilayer interior than is generally anticipated by a simple comparison with a paraffinic liquid.

Acknowledgment

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Appendix

Orientation of the Cis Double Bond. We attach a right-handed cartesian coordinate system (x, y, z) to the double bond as shown in Figure 5.

The two deuterons are differentiated as D_a and D_b. In the coordinate system of the C–D bond $(x', y', z'; z' \mathrel{\hat{=}} \text{C–D bond vector})$, the electric-field gradient tensor is axially symmetric ($\eta = 0$, $e^2qQ/h = 175.3$ kHz; Kowalesky et al., 1976) and has

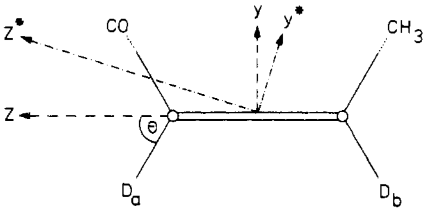

FIGURE 5: Coordinate systems of the cis double bond.

the form

$$\mathbf{V} = \begin{pmatrix} -\frac{1}{2}eq & 0 & 0 \\ 0 & -\frac{1}{2}eq & 0 \\ 0 & 0 & eq \end{pmatrix} \qquad (1)$$

Referred to the double-bond coordinate system, the electric-field gradient tensors $\mathbf{V}^{(r)}$ (r = a, b) can be written as

$$\mathbf{V}^{(r)} = eq \begin{pmatrix} -\frac{1}{2} & 0 & 0 \\ 0 & 1 - \frac{3}{2}\cos^2\theta & \pm\frac{3}{2}\sin\theta\cos\theta \\ 0 & \pm\frac{3}{2}\sin\theta\cos\theta & \frac{3}{2}\cos^2\theta - \frac{1}{2} \end{pmatrix} \qquad (2)$$

where the + (−) sign of the off-diagonal elements refers to D_a (D_b). The double bond is rotating around the director axis n of the bilayer. Let us denote with γ_i (i = 1,2,3) the direction cosines between the director axis (the normal to the bilayer surface) and the x, y, z coordinate axis of the double bond. The component of the average field gradient tensor parallel to n is given by:

$$\mathbf{V}^{(r)}_{\parallel} = \left[-\frac{1}{2}\overline{\gamma_1^2} + \left(1 - \frac{3}{2}\cos^2\theta\right)\overline{\gamma_2^2} + \left(\frac{3}{2}\cos^2\theta - \frac{1}{2}\right)\overline{\gamma_3^2} \pm 2\overline{\gamma_2\gamma_3} \times \frac{3}{2}\sin\theta\cos\theta\right] eq \qquad (3)$$

where the bars denote time averages. Introducing order parameters according to

$$S_{ij} = \left(\frac{3}{2}\overline{\gamma_i\gamma_j} - \frac{1}{2}\delta_{ij}\right) \qquad (4)$$

leads to the following expression for $\mathbf{V}^{(r)}_{\parallel}$

$$\mathbf{V}^{(r)}_{\parallel} = eq[S_{22}\sin^2\theta + S_{33}\cos^2\theta \pm 2S_{23}\sin\theta\cos\theta] \qquad (5)$$

If we express $\mathbf{V}^{(r)}_{\parallel}$ in terms of the experimentally available order parameters, $S^{(r)}{}_{CD}$, of the C-D bond vectors we obtain

$$\mathbf{V}^{(r)}_{\parallel} = eqS^{(r)}{}_{CD} \qquad (6)$$

The experimental results, $S^{(r)}{}_{CD}$, are thus related to the order parameters, S_{ij}, of the double bond according to

$$S^{(r)}{}_{CD} = S_{22}\sin^2\theta + S_{33}\cos^2\theta \pm 2S_{23}\sin\theta\cos\theta \qquad (7)$$

$$S^{(a)}{}_{CD} + S^{(b)}{}_{CD} = 2(S_{22}\sin^2\theta + S_{33}\cos^2\theta) \qquad (8)$$

$|S^{(a)}{}_{CD}|$ and $|S^{(b)}{}_{CD}|$ are evaluated from the deuterium NMR spectra; S_{33} has recently been determined from the infrared linear dichroism of the HC=CH stretching frequency (Fringeli, 1977). The results are summarized in Table II. The error in the S_{CD} values is about 2-3%, while that in S_{33} amounts to about 10%.

TABLE II

parameter	result	remarks
S_{33}	0.36 ± 0.03	Fringeli, 1977; IR dichroism, rm temp, egg lecithin, hydration not specified
$S^{(a)}{}_{CD}$	±0.101	^{2}H NMR; 27 °C; 48.5 wt % lipid–51.5 wt % H_2O; only the absolute value of the order parameters can be determined; the assignment of the two quadrupole splittings to a and b is arbitrary
$S^{(b)}{}_{CD}$	±0.019	

TABLE III

$S^{(a)}{}_{CD}$	$S^{(b)}{}_{CD}$	η^*
−0.101	+0.019	0
−0.101	−0.019	0.11
+0.101	+0.019	−0.75
+0.101	−0.019	−0.59

Using eq 8 and 7 it is then possible to calculate the order parameters S_{22} and S_{23}. S_{11} is obtained from the relation

$$\sum_{i=1}^{3} S_{ii} = 0 \qquad (9)$$

Since the signs of the deuterium order parameters are unknown, four solutions to the problem are possible. As an example, we give the result for $S^{(a)}{}_{CD} = -0.101$; $S^{(b)}{}_{CD} = +0.019$. The order matrix (S_{ij}) reads

$$S = \begin{pmatrix} -0.185_3 & 0 & 0 \\ 0 & -0.174_7 & +0.069 \\ 0 & +0.069 & 0.36 \end{pmatrix}$$

This matrix is almost diagonal. Complete diagonalization is achieved by referring it to a coordinate system (x, y^*, z^*) which is rotated around the x axis by −7.2° with respect to the original (x, y, z system) (cf. Figure 5). The diagonal matrix has the elements

$$S^* = \begin{pmatrix} -0.185 & 0 & 0 \\ 0 & -0.183 & 0 \\ 0 & 0 & 0.368 \end{pmatrix}$$

Performing the same calculation for the remaining three combinations of $S^{(a)}{}_{CD}$ and $S^{(b)}{}_{CD}$ also leads to matrices which can be diagonalized by rotations around the x axis with small rotation angles, $-9° < \alpha < +9°$. Thus, it can be concluded that the true rotation axis of the DC=CD segment is close to the direction of the C=C bond vector. The difference between the solution given above and the remaining three rests in the asymmetry of the diagonal elements. Defining an asymmetry parameter η^* as

$$\eta^* = \left|\frac{S^*_{11} - S^*_{22}}{S^*_{33}}\right|$$

this is found to be in the range of $0 \leq |\eta^*| \leq 0.75$ (Table III).

Thus, the assignment $S^{(a)}{}_{CD} = -0.101$ and $S^{(b)}{}_{CD} = +0.019$ seems to be the most likely result, since it leads to an axially symmetric order tensor which is in agreement with the uniaxial properties of the lipid membrane.

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Dissociation Kinetics of Tetra-*S*-carbamidomethylated Plasmin-Modified Human Somatotropin[†]

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ABSTRACT: The dissociation of tetra-*S*-carbamidomethylated, plasmin-modified human somatotropin at 25 ± 2 °C has been found to be a slow, exergonic equilibrium reaction; an equilibrium constant of 1.77 mol/L and a standard free energy of dissociation of −340 cal/mol have been calculated. The major products of the dissociation have been shown to consist of a monomeric form of the 51-residue carboxyl-terminal fragment and a trimeric form of the 134-residue amino-terminal fragment. The carboxyl-terminal fragment appears to be a random coil, while the amino-terminal fragment retains a considerable degree of secondary and tertiary structures.

It has been reported that human somatotropin (HGH)[1] can be subjected to limited digestion with human plasmin resulting in the loss of a hexapeptide comprising residues 135 to 140 (see Figure 1) without loss in biological or immunological activities (Clarke et al., 1974; Li and Gráf, 1974; Mills et al., 1973; Yadley and Chrambach, 1973). In the absence of denaturants, reduction and carbamidomethylation of the two disulfide bonds in the plasmin-modified hormone (PL-HGH) remove the remaining covalent link (the disulfide bond linking half-cystine-53 to half-cystine-165) between the amino terminal 134-residue fragment and the 51-residue carboxyl-terminal fragment (Li and Gráf, 1974). Preliminary studies (Li and Bewley, 1976) showed that the tetra-*S*-carbamidomethyl-PL-HGH does not immediately dissociate but remains intact

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[1] Abbreviations used: HGH, human somatotropin (growth hormone); PL-HGH, the product produced by limited proteolysis of HGH with human plasmin; DTT, dithiothreitol; CD, circular dichroism; UV, ultraviolet; V_e/V_0, the ratio of elution to void volume on exclusion chromatography.

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