Yamashita, T., & Takai, Y. (1987) *J. Biol. Chem. 262*, 5536–5539.
Yang, S. F., Freer, S., & Benson, A. A. (1967) *J. Biol. Chem. 242*, 477–484.
Yokohama, H., Tanaka, T., Ito, S., Negishi, M., Hayashi, H., & Hayaishi, O. (1988) *J. Biol. Chem. 263*, 1119–1122.
Yu, C. L., Tsai, M. H., & Stacey, D. W. (1988) *Cell 52*, 63–71.

Molecular Motions and Dynamics of a Diunsaturated Acyl Chain in a Lipid Bilayer: Implications for the Role of Polyunsaturation in Biological Membranes

John E. Baenziger,[‡,§,∥] Harold C. Jarrell,[§] and Ian C. P. Smith*[,§,∥]

Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6, and Department of Biochemistry, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5

Received August 28, 1991; Revised Manuscript Received December 16, 1991

ABSTRACT: The nature and dynamics of the motions of a diunsaturated fatty acyl chain in a lipid bilayer were examined using a comprehensive simulation program for ^2H NMR line shapes developed by Wittebort et al. [Wittebort, R. J., Olejniczak, E. T., & Griffin, R. G. (1987) *J. Chem. Phys. 36*, 5411–5420]. A motional model in which the isolinoleoyl chain (18:2$^{\Delta 6,9}$) adopts two conformations consistent with the low energy structures proposed for 1,4-pentadiene [Applegate, K. R., & Glomset, J. A. (1986) *J. Lipid Res. 27*, 658–680], but undergoes a rapid jump between these states, is sufficient to account for the experimentally observed quadrupolar couplings, the ^2H–^2H and ^1H–^2H dipolar couplings, the longitudinal relaxation times, and the changes in the *average* conformation of the chain that occur with a variation in temperature. The jump motion originates via rotations about the C7–C8 and the C8–C9 carbon bonds and leads to the low order parameters assigned to the C8 methylene segment (0.18) and the C9–C10 double bond (0.11). In contrast, the C6–C7 double bond, which is not involved in the two-site jump, characterized by a relatively large order parameter (0.56). Fatty acyl chains containing three or more double bonds likely cannot undergo the same jump motion and consequently will be highly ordered structures. Correlation times for diffusion of the molecular long axis of the diunsaturated acyl chain about the bilayer normal ($\sim 10^{-10}$ s) and for the local jump motion ($\sim 10^{-10}$ s) were calculated. Relative to their rates of diffusion about their molecular long axes, the rate of the local jump motion in the diunsaturated bilayer is much slower than the rates of the local motions (trans/gauche isomerization) occurring in saturated bilayers. The presence of large amounts of highly unsaturated fatty acyl chains in biological membranes should create a dynamic state that allows considerable intermolecular motion but still maintain a high degree of local order within the hydrophobic region of the bilayer.

ω-3 Polyunsaturated fatty acyl chains perform an important structural role in biological membranes. They are found in large quantities in the membranes of the rod outer segment, postsynaptic neurons, and other excitable cells [see Tinoco et al. (1978)], and the high levels, particularly of docosahexaenoic acid (22:6$^{\Delta 4,7,10,13,16,19}$), are well conserved throughout nature (Crawford et al., 1977). A restriction of the dietary intake of ω-3 polyunsaturated fatty acids has also been correlated with a loss of visual acuity in the rhesus monkey (Neuringer et al., 1984), a decreased electrical response in rat photoreceptor cell membranes (Wheeler et al., 1975), and a poor discrimination-learning response of rats to a Y-maze test (Lamptey & Walker, 1976). Furthermore, the proper photochemical function of rhodopsin is dependent upon the presence of docosahexaenoyl chains (Wiedman et al., 1988).

It is often assumed that the role of polyunsaturated lipids is to lower the gel-to-liquid crystal phase transition temperature and thus to increase and perhaps modulate the "fluidity" of biological membranes. However, recent DSC and NMR[1] studies show that the role of polyunsaturated fatty acyl chains in membrane "fluidity" requires considerable clarification [see Baenziger et al. (1991)]. An alternative hypothesis is that the overlapping 1,4-pentadiene structure imparts unique conformational properties to the polyunsaturated acyl chains (Applegate & Glomset, 1986; Dratz & Deese, 1986). Computer modeling reveals that docosahexaenoyl chains may adopt two low energy conformations in which all six double bond axes are parallel to the bilayer normal, and the consecutive double bond planes form 90° angles with respect to each other (Applegate & Glomset, 1986). It was suggested that these structures pack relatively well in lipid bilayers and may be responsible for the important role of polyunsaturated lipids in biological membranes. Unfortunately, no experimental insight into the structural properties of polyunsaturated fatty acids has yet been obtained.

As a first step to gaining a more detailed understanding of the biological function of polyunsaturated lipids, we synthesized

* To whom correspondence should be addressed.
‡ Present address: Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115, and Department of Anesthesiology, Massachusetts General Hospital, Boston, MA 02114.
§ Institute for Biological Sciences, National Research Council of Canada.
∥ University of Ottawa.

[1] Abbreviations: FID, free induction decay; NMR, nuclear magnetic resonance; iLPPC, 1-isolinoleoyl-2-palmitoyl-*sn*-glycero-3-phosphocholine; PC, *sn*-glycero-3-phosphocholine; PiLPC, 1-palmitoyl-2-isolinoleoyl-*sn*-glycero-3-phosphocholine.

0006-2960/92/0431-3377$03.00/0

a series of specifically deuterated isolinoleic acids (18:2$^{\Delta 6,9}$) and studied the *average* structural and motional properties of the diunsaturated acyl chain in a model lipid bilayer composed of 1-palmitoyl-2-isolinoleoyl-PC (PiLPC). The ^{2}H NMR data revealed that (1) the *average* conformation of the isolinoleoyl chain is "kinked" in a manner that would distort its parallel packing in a lipid bilayer, (2) the chain is highly ordered at the C6–C7 double bond (S_{mol} = 0.55) but disordered at carbons C8 and C9–C10 (S_{mol} = 0.15 and 0.08, respectively; all three values at 40 °C), and (3) the isolinoleoyl chain undergoes a conformational change with a variation in temperature (Baenziger et al., 1991). To account for these observations, we proposed that the chain adopts two conformations, each consistent with the low energy structures suggested for 1,4-pentadiene (Applegate & Glomset, 1986), but undergoes a two-site jump between the two states. The conformation derived from the NMR data therefore reflects the time average of the two structures, as opposed to each individual state, and the two-site jump leads to the low order parameters observed for the C8 methylene segment and the C9–C10 double bond. The change in the average conformation of the chain with temperature could also reflect a change in the relative populations of the states involved in the two-site jump. However, from the *average* conformational data, we could not distinguish between two models differing in both the orientation of the 1,4-pentadiene segment of the chain relative to the bilayer normal and the nature of the two-site jump [Figure 1; see Baenziger et al. (1991)], and no insight into the dynamics of the chain motions was obtained. It is also possible that other types of motions give rise to the experimental data.

In the present study, the temperature dependence of the conformations of the isolinoleoyl chain and the nature and rates of its motions are examined. Spectral simulations indicate that one motional model (the Tilt model in Figure 1b) best accounts for the experimentally observed quadrupolar couplings, the dipolar couplings, and the longitudinal relaxation times. Extrapolation of the data to acyl chains with more than two double bonds provides insight into the physical properties and thus potentially the biological function of polyunsaturated fatty acyl chains.

Materials and Methods

Sample Preparation. The biosynthesis of [4,4-$^2H_2$]-, [5,5-$^2H_2$]-, [6-^{2}H]-, [7-^{2}H]-, [8,8-$^2H_2$]-, [9,10-$^2H_2$]-, [14,14-$^2H_2$]-*cis,cis*-octadeca-6,9-dienoic (isolinoleic) acid and the preparation of aqueous dispersions of the deuterated 1-palmitoyl-2-isolinoleoylphosphatidylcholines (PiLPC) have been described previously (Baenziger et al., 1987, 1990). PiLPC deuterated at either carbon 8 or carbons 9 and 10 of the isolinoleoyl chain (18:2$^{\Delta 6,9}$) was oriented as multibilayers between glass plates. In each case, the lipid was dissolved in 0.1–0.2 mL of $CHCl_3$ and spotted on approximately 40 microscope cover slips (~8 × 15 mm) with a glass pasteur pipette. After drying, the plates were stacked in a 10-mm NMR tube, placed under vacuum for at least 2 h to remove all traces of $CHCl_3$, and hydrated overnight at 37 °C in an atmosphere saturated with deuterium-depleted H_2O. After addition of a drop of deuterium-depleted H_2O, each tube was sealed with epoxy. All manipulations were carried out under an argon atmosphere.

NMR Spectroscopy. ^{2}H spectra were acquired using a modified quadrupolar echo pulse sequence with full phase cycling of the radiofrequency pulses (Rance et al., 1980) at either 46.06 MHz on a Bruker MSL-300 spectrometer or 30.7 MHz on a home-built spectrometer. The 90° pulse lengths were generally 4.6 μs (10-mm solenoid coil) or 2.3 μs (5-mm coil) in length, and the echo pulse spacing was 60 μs. Recycle times were always >5 × T_1. Proton-decoupled ^{2}H spectra (at 46 MHz) were acquired with WALTZ-16 decoupling (Shaka et al., 1983) during acquisition of the free induction decay (FID). The decoupling was performed with ^{1}H 90° pulses of 60 μs at a decoupler power of 6 W. The time of decoupling (the acquisition time) was never allowed to exceed 2% of the recycle time in order to avoid sample heating. ^{2}H longitudinal relaxation times (T_1) were recorded using the standard inversion–recovery sequence coupled with a quadrupolar echo pulse sequence (Dufourc et al., 1983).

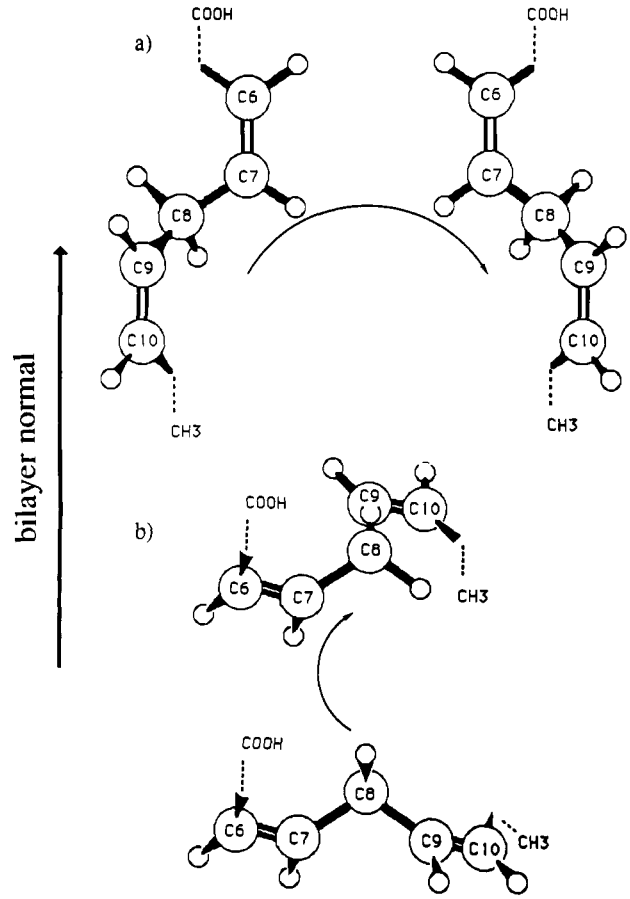

FIGURE 1: Two possible models for the motions of the isolinoleoyl chain. (a) The Angle-Iron model. The chain adopts two essentially identical conformations in which both double bond directions are parallel to the bilayer normal and the two double bond planes are at 90° with respect to each other, but interconvert, at a fast rate (>10^5 s^{-1}) between the two conformations. The two conformations are the same as those proposed for docosahexaenoyl acyl chains by Applegate and Glomset (1986). (b) The Tilt model. The 1,4-pentadiene segment of the chain adopts the same two conformations, but in each case the whole segment is tilted away from the bilayer normal. The chain still interconverts between the two states, but in this case the C6–C7 double bond is not involved in the two-site jump whereas, in the former model, the double bond participates in the two-site jump.

For the oriented samples, the angle between the bilayer normal and the magnetic field direction was determined with a small protractor, and the angular settings were found to be accurate to within ±3°. Phosphorus spectra were recorded with the sample at both the 0° and 90° orientations of the bilayer normal relative to the magnetic field direction to examine the sample alignment. At each orientation, both samples gave spectra dominated by a single resonance, indicating a predominantly uniaxial alignment of the lipid.

Spectral simulations were performed on a Sun 4-260 computer using a modified line shape simulation program (Wittebort et al., 1987) based on the general formalism of Torchia and Szabo (1982). The geometries of the two double

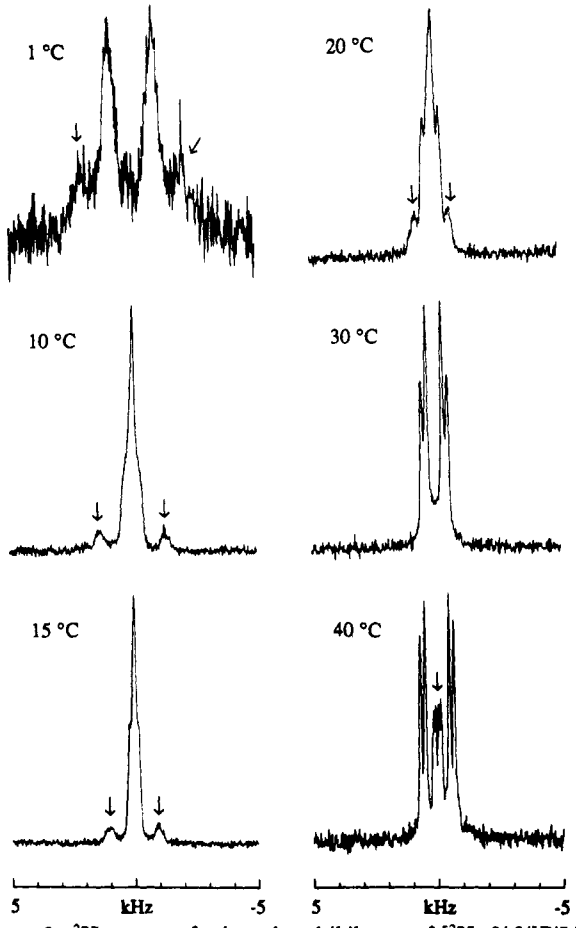

FIGURE 2: ^2H spectra of oriented multibilayers of [$^2H_2$-8′,8′]PiLPC ($\theta' = 90°$) acquired as a function of temperature with a spectral width of 50 kHz, 2K data points zero-filled to 4K, and 15 000 scans. The arrows indicate peaks arising from [$^2H_2$-8′,8′]iLPPC (see text).

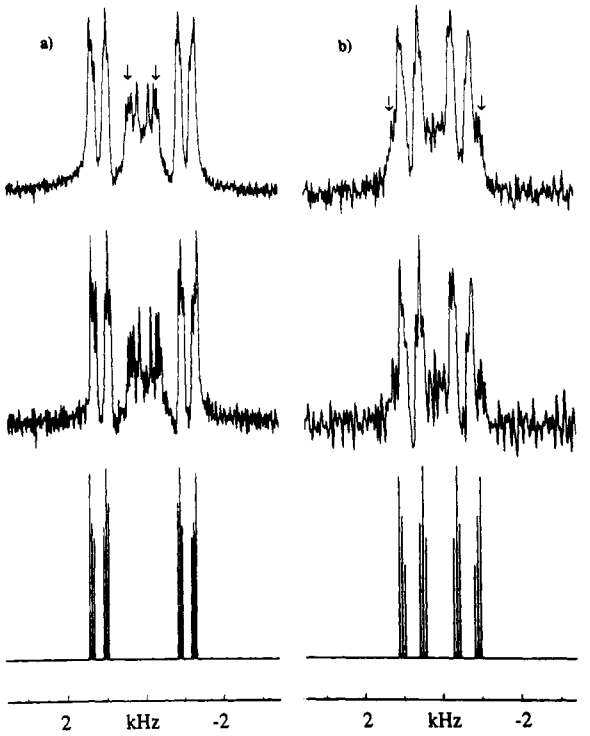

FIGURE 3: ^2H spectra of oriented multibilayers of [$^2H_2$-8′,8′]PiLPC acquired at (a) 40 °C and (b) 25 °C with the bilayer normal aligned parallel to the magnetic field direction (top). Spectra were acquired at a spectral width of 50 kHz and 4K data points. The spectra were also zero-filled to 8K data points (middle) and treated with a Gaussian resolution enhancement function (GB = 0.4 and LB = −50 Hz, using the standard Bruker software). Spectra were simulated (bottom) using the PANIC software on the Bruker MSL-300 spectrometer with a digital resolution of 3 Hz per point and a negligible line width. Input parameters were (a) $\nu_Q(90°)$ = 1312 and 958 Hz, D_{DD} = 30 Hz, and (b) $\nu_Q(90°)$ = 928 and 440 Hz, D_{DD} = 40 Hz. The arrows indicate peaks arising from [$^2H_2$-8′,8′]iLPPC.

bonds were assigned from the crystal structure of linoleic acid (18:2$^{\Delta 9,12}$) as described previously (Baenziger et al., 1991).

Results

Oriented Sample Spectra. A deuterated lipid undergoing axially symmetric motion in an *oriented* lipid bilayer gives rise to a ^2H NMR spectrum consisting of a doublet. The separation between the two peaks of the doublet, the quadrupolar splitting ($\Delta\nu_Q$), is defined as (Dufourc et al., 1983)

$$\Delta\nu_Q = \frac{3}{2}\frac{e^2qQ}{h}\left(\frac{\overline{3\cos^2\alpha - 1}}{2}\right)\left(\frac{3\cos^2\beta - 1}{2}\right)\left(\frac{3\cos^2\theta' - 1}{2}\right) \quad (1)$$

where e^2qQ/h is the quadrupolar coupling constant (170 or 175.3 kHz for deuterium in saturated or unsaturated C–^2H bonds, respectively); the term involving the angle α, S_{mol}, is a quantitative measure of the amplitudes of fluctuation of the C–^2H bond about its average orientation (the line denotes a time average); β describes the average angle between the C–^2H bond vector and the director of motional averaging (usually the bilayer normal); and θ' is the angle between the director and the magnetic field direction, H_0. The quadrupolar splitting is therefore sensitive to both the average structural and the motional properties of a deuterated acyl chain segment.

In a previous ^2H NMR study (Baenziger et al., 1991), we examined aqueous dispersions of 1-palmitoyl-2-isolinoleoyl-PC (PiLPC) deuterium-labeled at 10 individual positions along the isolinoleoyl chain (18:2$^{\Delta 6,9}$). From the quadrupolar splitting data and the dipolar coupling measured between the two C8 geminal deuterons (Baenziger et al., 1988), the average conformation of the 1,4-pentadiene segment of the isolinoleoyl chain was defined at 40 °C. However, the quadrupolar splittings measured for several of the deuterated acyl chain segments actually increase with increasing temperature, indicating that the average orientation of the carbon segments (the angle β), and thus the conformation of the acyl chain as a whole, changes with temperature.

Proton-decoupled ^2H NMR spectra of *oriented bilayers* ($\theta' = 90°$) of [8′,8′-$^2H_2$]PiLPC are presented in Figure 2. Each spectrum consists of two doublets arising from the two C–^2H bonds of the C8 methylene segment and two additional doublets of much lesser intensity (see also Figure 3) assigned to deuterated 1-isolinoleoyl-2-palmitoyl-PC (iLPPC; Baenziger et al., 1991). The quadrupolar splittings are essentially identical to those obtained previously from spectra of aqueous dispersions of [8′,8′-$^2H_2$]PiLPC and, for both samples, initially decrease in magnitude to zero but subsequently increase in size with a further increase in temperature. The unusual temperature dependences of the splittings suggest, in agreement with our previous observations, that the average orientation of the C8 methylene segment with respect to the director of motional averaging (the bilayer normal, see below) changes with temperature. The collapse of the splittings to zero at 15 °C also requires that each C–^2H bond vector (i.e., the angle β, eq 1) be oriented, on the average, at the magic angle of 54.7° with respect to the director.

Proton-decoupled spectra of [8′,8′-$^2H_2$]PiLPC were acquired at 25 °C as a function of the angle between the bilayer normal

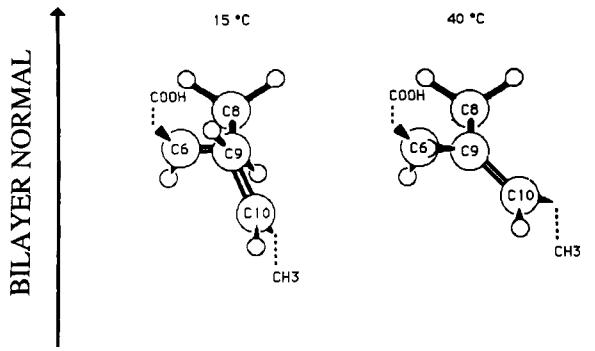

FIGURE 4: Defined average orientations of the 1,4-pentadiene segment of the isolinoleoyl chain of PiLPC at 15 and 40 °C. For details, see Appendix and Baenziger et al. (1991).

and the magnetic field direction, H_0 (not shown). The splittings follow a $(3\cos^2\theta' - 1)$ dependence and collapse to zero at an orientation of $\theta' = 54.7°$ implying a zero average tilt of the hydrocarbon chains relative to the bilayer normal. In addition, spectra acquired with the normal to the glass plates (the bilayer normal) oriented parallel to the magnetic field direction contain additional fine structure which, for each ^2H resonance, is clearly resolved into a triplet by treating the acquired FIDs with a Gaussian deconvolution function before Fourier transformation (see Figure 3 for details). The fine structure arises from dipolar coupling between the two geminal C8 deuterons and provides the additional information necessary to define, without assumptions, both the average orientation and the order parameter tensor for the C8 methylene unit (Appendix) at both 25 and 40 °C. In each case, the defined orientation is such that the bilayer normal essentially bisects the ^2H–C–^2H tetrahedral angle (see Figure 4). Consequently, both C–^2H bonds are oriented very close to the magic angle, as was suggested for the methylene segment at 15 °C. In addition, the molecular order parameters, S_{mol} (or S_{33}), defined for the C8 unit at 25 and 40 °C are 0.18 and 0.15, respectively, indicating considerable motion of the carbon segment. The unusual temperature dependences of the splittings are a consequence of their sensitivity to slight changes in the orientation of the methylene unit (<0.4° change for each C–^2H bond between 15 and 40 °C), when the average angle between each methylene C–^2H bond vector and the director axis (angle β, eq 1) is very close to 54.7°.

The average orientation and the associated molecular order parameters, S_{mol}, for the C6–C7 and the C9–C10 double bond were also defined at 15, 25, and 40 °C using the quadrupolar splitting data obtained previously from dispersions of PiLPC and the average orientation of the C8 methylene segment defined above [see Appendix and Baenziger et al. (1991)]. As shown in Figure 4, the C6–C7 double bond orientations are essentially identical at all three temperatures, and the motions of the double bond are described by a large order parameter indicating restricted motion, $S_{mol} = 0.60$, 0.56, and 0.55 at 15, 25, and 40 °C, respectively (Appendix). In contrast, between 15 and 40 °C the average orientation of the C9–C10 double bond changes by a rotation about the C8–C9 single bond of 35°, and the double bond undergoes relatively large amplitudes of motion described by order parameters varying from 0.15 to 0.08. As with [8′,8′-$^2H_2$]PiLPC, the quadrupolar splittings are very sensitive to the orientation of the C9–C10 double bond because the angle between each C–^2H bond vector and the magnetic field direction is close to the magic angle of 54.7° (or 180°–54.7°).

It is also interesting to note that there is little difference in the line widths of the ^2H peaks in spectra of [9′,10′-$^2H_2$]PiLPC

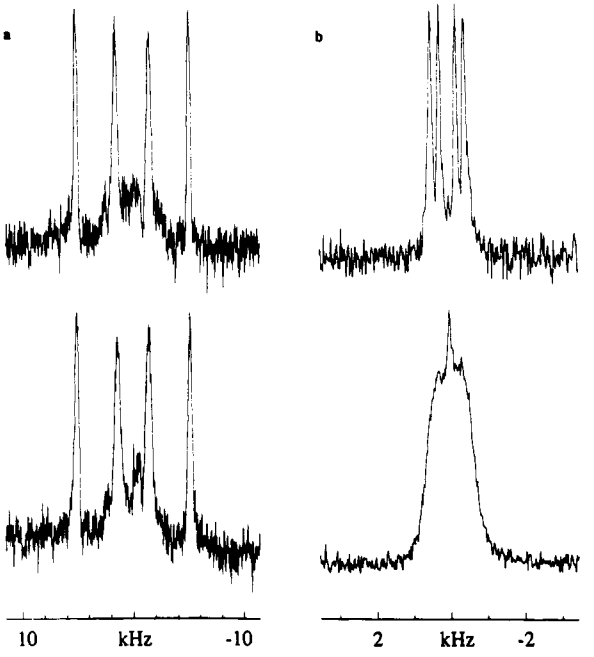

FIGURE 5: Proton-coupled (bottom) and decoupled (top) ^2H spectra of (a) [$^2H_2$-9′,10′]PiLPC oriented multibilayers (left) and (b) [$^2H_2$-8′,8′]PiLPC oriented multibilayers acquired at 25 °C.

oriented multibilayers acquired with (Figure 5a, bottom) and without (Figure 5a, top) proton decoupling. This suggests that the methylene carbons on either side of the C9–C10 double bond undergo motions which are of sufficiently large amplitude to average the ^1H–^2H heteronuclear dipolar couplings between the C9 and C10 deuterons and adjacent protons to small values (complete averaging of the ^1H–^2H couplings also occurs in spectra of specifically deuterated saturated phospholipid acyl chains; Seelig & Seelig, 1974; Kang et al., 1979). In contrast, residual ^1H–^2H dipolar couplings contribute roughly 650-Hz line broadening to the spectra of [8′,8′-$^2H_2$]PiLPC (at 25 °C and $\theta' = 90°$; Figure 5b, bottom) suggesting, in agreement with the order parameter analysis and the Tilt model (Figure 1b), that the amplitudes of motion of the C6–C7 double bond and the C5 methylene segment are relatively restricted.

Spectral Simulation of the ^2H Line Shapes and the Quadrupolar Splittings. The order parameter analysis presented in the previous section gives insight into the average orientation relative to the bilayer normal and the amplitudes of motion of the various carbon segments but no insight into either the nature of, or the specific conformations involved with, the molecular motions. For an unambiguous interpretation of the results, and thus insight into the molecular motions of the isolinoleoyl chain, we have examined a variety of motional models and determined, by spectral simulation (Wittebort et al., 1987), whether any of the models could account for the experimentally observed ^2H line shapes and quadrupolar splittings. Initially, we focused on simple models in which the isolinoleoyl chain was restricted to a rotational motion about is molecular long axis, but the 1,4-pentadiene segment of the acyl chain was allowed to adopt several different conformations, including each of those illustrated in Figure 1. The rotational motion was modeled by a three-site jump of 120° about the bilayer normal between equally populated states. It was also assumed that the phospholipid undergoes limited whole-body fluctuations perpendicular to the plane of the membrane and that this motion is characterized by an order parameter of $S_{zz} = 0.6$. Consequently, each of the splittings measured from the simulated spectra were multiplied by a factor of 0.6. An order parameter, S_{zz},

describing similar motions in membranes composed of DMPC has been assigned values ranging from 0.60 to 0.46 between 25 and 45 °C [see Figure 6 of Moser et al. (1989)]. *No attempt was made to model the temperature dependence of* S_{zz} (see below).

The simple rotational diffusion models predict spectra with line shapes indicative of axially symmetric motions (not shown) when the rotational rate is faster than 10^5 s^{-1} (i.e., in the rapid motional regime). However, in each case, the quadrupolar splittings predicted for the C6, C7, C8, C9, and C10 carbons differ in magnitude from the experimental values, and the ratios of the splittings observed for the C6 and C7 and for the C9 and C10 deuterons, which are very sensitive to the average orientation of each double bond (Baenziger et al., 1991), deviate substantially from the experimental ratios. Therefore, as was suggested by the order parameter analysis presented above, the carbon atoms in the vicinity of the two double bonds must undergo local motion, possibly as described by either the Angle-Iron or the Tilt model illustrated in Figure 1.

We investigated whether either the Angle-Iron or the Tilt models could account for the experimentally observed quadrupolar splittings. Each model was simulated using a six-site jump which accounts for both rotation of the lipid about its molecular long axis (as described above) and the two-site jumps illustrated in Figure 1, panels a and b, respectively. The temperature dependences of the splittings were approximated by changing the relative populations of the two states, and the derived quadrupolar splittings were multiplied by a factor of 0.6 to account for the limited whole-body fluctuations of the lipid perpendicular to the plane of the membrane (S_{zz}; see above). As with the simple rotational models, spectra with line shapes characteristic of axially symmetric motions are observed when the diffusion rate occurs at a frequency greater than 10^5 s^{-1} (spectra not shown). In the fast motional limit for the diffusive motion, the two-site jump described by each model had no effect on the ^2H line shapes but for some of the labeled positions led to additional averaging of the residual quadrupolar splittings to smaller values.

The values of the quadrupolar splittings predicted by the Angle-Iron model (Figure 6b) for the C8 deuterons are similar to those observed experimentally for [8′,8′-$^2H_2$]PiLPC (Figure 6a). The model gives rise to distinct, although essentially identical, splittings for each of the two C8 deuterons and a change in the relative populations alters slightly the predicted values. Note that the maximum splitting for a deuterated methylene carbon undergoing diffusion about the molecular long axis in a lipid bilayer and $\theta' = 90°$ is 128 kHz (eq 1). Note also that both C–^2H bond vectors in each of the two conformations (Figure 1a) are close to the magic angle and their splittings are very sensitive to slight changes in the orientation of the C–^2H bond vectors. Consequently, a slight modification of the two methylene segment orientations also leads to spectra with splittings that decrease to an isotropic value and then increase in magnitude as the relative populations of the two states are varied (data not shown).

In contrast, exchange between the two conformations does not lead to additional averaging of the residual quadrupolar splittings predicted for the C6, C7, C9, or the C10 deuterons (Figure 6b) as the angle β (eq 1) for each of the four C–^2H bond vectors is identical in either state. The predicted values are substantially larger than those observed experimentally, and the ratio of the C6 and C7 deuteron splittings, which is sensitive to the average orientation of the C6–C7 double bond (Baenziger et al., 1991), is not compatible with the experimentally observed data. In addition, a change in the relative

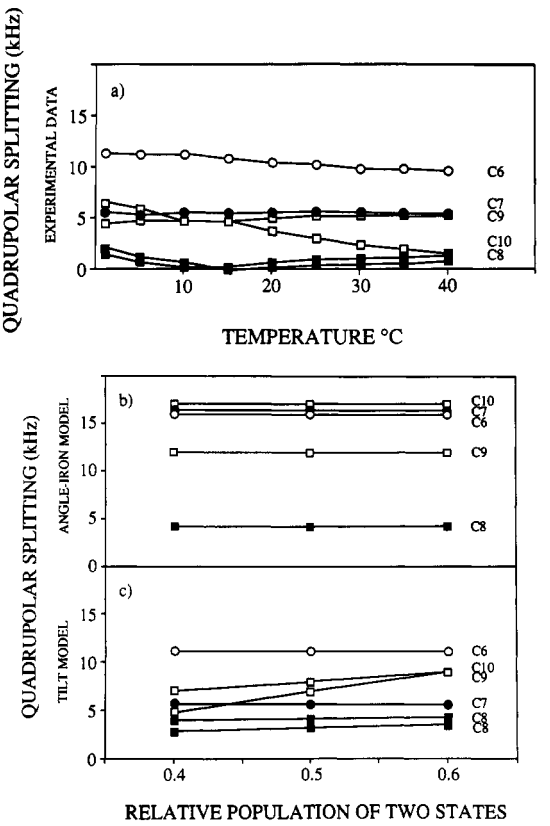

FIGURE 6: Comparison of the (a) experimental quadrupolar splittings measured for carbons C6 through C10 between 0 and 40 °C and those measured from spectra simulated using the (b) Angle-Iron and (c) Tilt models with varying relative populations of the two states involved in the two-site jump.

populations of the two states does not mimic the temperature dependences of the splittings observed for either the C9 or the C10 deuterons. The simulations provide strong evidence that the isolinoleoyl chain is *not* oriented with its two double bond axes parallel to the bilayer normal, as was suggested for the double bonds of docosahexaenoyl acyl chains (22:6$^{\Delta 4,7,10,13,16,19}$; Applegate & Glomset, 1986), and does *not* undergo the motions described by the Angle-Iron model in Figure 1a.

The Tilt model also predicts a distinct splitting for each of the two C8 deuterons. The values of the two splittings are close to the experimental values and the values change with a variation in the relative populations of the two interconverting states. With a slight modification of the two C8 segment orientations, the temperature dependences of the splittings observed experimentally can be mimicked with a change in the relative populations of the two interconverting conformations (data not shown). In contrast to the previous (Angle-Iron) model, the splittings predicted for the C6, C7, C9, and C10 deuterons match closely the experimental values (Figure 6a and 7c). Furthermore, a change in the relative populations of the two interconverting conformations alters the values of the splittings predicted for the C9 and C10 deuterons in a manner that resembles the changes in the experimental values with temperature. The simulations therefore provide strong support for the Tilt model and suggest that the deduced changes in the *average* orientation of the C8 methylene segment and the C9–C10 double bond with temperature (Figure 4) arise from a change in the relative populations of the two conformations shown in Figure 1b.

Although the splittings predicted by the Tilt model fit the experimental data resonably well, an *exact* fit is not achieved. However, this is not expected given the uncertainty in the

Table I: Experimental and Simulated Longitudinal T_1 Relaxation Data

labeled position	experimental T_1 values (ms) 15 °C	30 °C	T_1 values (ms) predicted by the Tilt Model to match the experimental data at 15 °C (left) and 30 °C (right) τ_R[a] = 1.6 × 10^{-10} s; τ_J = 1.0 × 10^{-9} s	τ_R = 1.1 × 10^{-10} s; τ_J = 2.1 × 10^{-10} s
C6	11.4 ± 0.2	16.0 ± 1.0	11.4	16.0
C7	8.5 ± 0.1	14.8 ± 0.3	11.3	15.9
C8a[b]	15.6 ± 0.4	27.3 ± 0.2	15.6	27.3
C8b			16.0	29.3
C9	12.1 ± 0.2	21.6 ± 0.5	13.2	22.0
C10	12.1 ± 0.2	18.0 ± 0.9	13.3	21.5

[a] τ_R, correlation time for rotation about the long molecular axis; τ_J, correlation time for the two-site jump motion described by the Tilt model (Figure 1b). Note that the experimental values are powder averages over all possible angles θ' between 0 and 90° whereas the predicted values are the weighted average of the T_1's calculated for the θ' = 90° and 35° orientations. θ' refers to the angle between the bilayer normal and the magnetic field direction. [b] The two splittings for each of the two C8 deuterons are superimposed in the proton-coupled spectra of dispersions of [$^2H_2$-8′,8′]-PiLPC. Only one T_1 was measured for the two C8 deuterons.

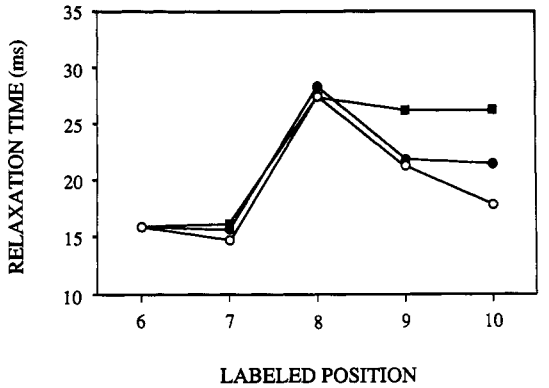

FIGURE 7: Comparison of the experimental T_1 values at 30 °C for carbons C6 through C10 and the best fit of the values predicted using the Angle-Iron and Tilt models (see text). Only one T_1 was measured experimentally for the two C8 deuterons. The Angle-Iron model predicts identical values for each C8 deuteron. The Tilt model predicts a separate value for each C8 deuteron (see Table I), the average of which is shown here. ○, experimental T_1 values; ●, T_1 values predicted by the Tilt model; ■, T_1 values predicted by the Angle-Iron model (for details, see text).

simulations. In particular, the relative populations of the two states involved in the proposed two-site jump are not known and could differ from the relative populations used to calculate the quadrupolar splittings presented in Figure 6c. The important point is that the predicted splittings have a *similar* magnitude to the experimental values and that a change in the relative populations of the two states alters the splittings in a manner that explains the unusual temperature dependences of the observed splittings. In contrast, the data predicted by the Angle-Iron model and the simple diffusion models do not mimic the experimental data under any conditions. It can be concluded that the double bond axes are not aligned parallel to the bilayer normal as suggested by the Angle-Iron model and therefore must be tilted away from the bilayer normal, perhaps as suggested by the Tilt model.

It should also be noted that the limited whole body fluctuations of PiLPC were assigned a value of S_{zz} = 0.6 for all the simulations, whereas, for DMPC, S_{zz} has been assigned values from 0.60 to 0.46 between 25 and 45 °C (Moser et al., 1989). A similar variation in S_{zz} would lead to a better fit between the splittings predicted by the Tilt model and the experimentally observed quadupolar splittings. Furthermore, the Tilt model assumes that the only motion of the C6–C7 double bond is due to diffusion of the whole lipid about its molecular long axis. This assumption was made in order to simplify the calculations and is based on the order parameter analysis, which indicates that the amplitudes of motion of the C6–C7 double bond are relatively restricted and are characterized by an order parameter $S_{mol} \approx 0.6$. Averaging the order parameter tensor to this value could result predominantly from limited whole-body fluctuations, i.e., S_{ZZ}. However, the double bond likely undergoes some local motion of small amplitude that does contribute to the two-site jump. The Tilt model is intended to represent a reasonable approximation of the structure and motions of the isolinoleoyl chain, and the similarity between the predicted and experimental data provides strong support to this interpretation.

The Tilt model also predicts a dipolar coupling between the two C8 methylene deuterons of D_{DD} = 34 Hz (S_{zz} = 0.6), for equal populations of the two states. This value is intermediate between the values of D_{DD} = 40 and 30 Hz observed experimentally at 25 and 40 °C, respectively (Figure 3), and changing the relative populations of the two states alters the magnitude of the coupling. Therefore, the Tilt model predicts all the *average* spectral parameters observed experimentally and is sufficient to describe the molecular motions of the isolinoleoyl chain.

Spectral Simulation of the Longitudinal Relaxation (T_1) Times. Further support for the Tilt model and insight into the dynamics of the acyl chain motions were obtained from the T_1 relaxation times measured from *aqueous dispersions* of PiLPC deuterated at several locations along the isolinoleoyl chain. Experimental T_1 values at both 15 and 30 °C, and T_1 values predicted using the Tilt model, are listed in Table I. Note that the simulated values represent the weighted average of the T_1 values predicted for the 90° and 35° orientations of the director (bilayer normal) relative to the magnetic field direction, whereas each experimental value is a *powder* average of the T_1 from each orientation (0–90°), possibly due to rapid lateral diffusion of the lipids around the circumferences of the lipid vesicles. T_1 anisotropy was not observed in any of the spectra acquired from the deuterated PiLPCs. In addition, the simulations assume that the states involved in the two-site jump are equally populated.

For the Tilt model, the correlation time for rotation about the molecular long axis was calculated by matching the T_1 predicted for the C6 deuteron, given a specific rotational rate, to the experimental value (in the Tilt model, the C6 deuteron undergoes essentially no local jump motion, only rotation about the long molecular axis). The correlation time for the jump motion was defined by superimposing the two-site jump on top of the rotational motion and then matching the predicted T_1 for the C8 deuteron to one of the two values observed experimentally. The T_1 values for the second C8 deuteron and the C7, C9, and C10 deuterons were then calculated using these two correlation times defined for the local jump and

rotational diffusion motions. A T_1 profile predicted by the Tilt model is presented in Figure 7 and is very close to the T_1 profile observed experimentally 30 °C. In particular, the model reproduces the unusual T_1 maximum that is observed for the two C8 deuterons. The relaxation data therefore provide strong support to the Tilt model.

The corresponding relaxation profile was also calculated using the motions described by the Angle-Iron model. Initially, *the local motion of the C6–C7 double bond was restricted to rotation of the whole lipid about its molecular long axis*, and the two-site jump was assumed to arise solely from motions about the C7–C8 and C8–C9 bonds (as is the case for the Tilt model). With these motions, the model predicts a relaxation profile with a T_1 maximum at the two C8 deuterons, but the maximum is not as pronounced as in the experimental data (Figure 7). *Furthermore, if the C6–C7 double bond is allowed to undergo motion that contributes to the conformational exchange, an essentially flat T_1 profile is observed.*

In agreement with the order parameter analysis and the proton-coupled and -decoupled spectra of [$^2H_2$-8′,8′]- and [$^2H_2$-9′,10′]PiLPC (Figure 5), the relaxation profile is indicative of a two-site jump motion which originates mainly from motions of the C8 and C9–C10 carbon segments. The local motions of the C6–C7 double bond must be relatively restricted, and the double bond must therefore be tilted away from the bilayer normal as defined by the order parameter analysis (Figure 4) and subsequently described by the Tilt model (Figure 1b). The structure and motions described by Tilt model account for not only the average structural (Figure 4) but also the dynamical data observed of the isolinoleoyl chain.

At 15 and 30 °C, the correlation times calculated using the Tilt model for rotation about the molecular long axis are 2.0×10^{-10} and 1.4×10^{-10} s, respectively, and for the local jump motion 3.9×10^{-10} and 1.1×10^{-10} s, respectively. In saturated bilayers of 1,2-dimyristoyl-PC between 25 and 55 °C, correlation times calculated for rotation about the molecular long axis range from $\sim 10^{-8}$ to $\sim 10^{-9}$ s and for the local trans–gauche isomerizations remain relatively constant at 10^{-11}–10^{-12} s, depending on the labeled position (Moser et al., 1989). A comparison of the absolute magnitude of the correlation times for the diunsaturated and saturated bilayers is not warranted given that for PiLPC they were calculated using a molecular model that does not allow for limited whole-body fluctuations of the lipid in a plane perpendicular to the bilayer normal. However, it is interesting to note that, relative to the rates of rotation of the lipids about their long axes (τ_R), the rate of the putative two-site jump (τ_J) for PiLPC is much slower than the rate of trans–gauche isomerization in the saturated bilayers (see Discussion).

Discussion

Implications of the Tilt Model. The order parameter analysis and molecular modeling strongly support the Tilt model shown in Figure 1b. In this model, the 1,4-pentadiene segment of the isolinoleoyl chain adopts two conformations but undergoes a two-site jump between the two states. In each of the conformations, the two double bond axes are parallel but are tilted away from the bilayer normal, and the two double bond planes form 90° angles with each other. The two-site jump arises mainly from motions about the C7–C8 and C8–C9 single bonds.

Both structures defined by the Tilt model are consistent with the low energy conformations suggested for the individual 1,4-pentadiene segments of docosahexaenoyl acyl chains (Applegate & Glomset, 1986), but in contrast to these models the double bond axes are tilted away from the bilayer normal. This difference in the orientation of the two double bonds may arise because the docosahexaenoyl acyl chain structures are suggested for lipids with a small headgroup, such as phosphatidylethanolamine, whereas the double bonds of PiLPC tilt with respect to the bilayer normal in order to accommodate the larger choline headgroup. It is also interesting to note that the orientations of the double bond axes in the two proposed structures of the isolinoleoyl chain are similar to the orientations of the double bond axes in the crystal structure of linoleic acid (18:2$^{\Delta 9,12}$; Ernst et al., 1979) if the saturated segments of the chain are aligned parallel to the bilayer normal. In the linoleic acid crystal structure, the methylene carbons on either side of the double bonds adopt a compact all-trans conformation that would pack reasonably well in a lipid bilayer. In contrast, if the double bond axes are oriented parallel to the bilayer normal, as proposed by the Angle-Iron model, the saturated segments of the fatty acid would have to undergo severe distortions from the energetically favorable all-trans conformation in order to pack within the bilayer. It therefore appears that the two double bond axes of isolinoleic acid are tilted away from the bilayer normal to allow a more compact packing arrangement of the entire isolinoleoyl chain. The presence of six double bonds, as opposed to two, will clearly have a dramatic effect on the conformation of the docosahexaenoyl chains within a lipid bilayer. We therefore suggest that our data are consistent with and lend support to the unique structures proposed for docosahexaenoyl acyl chains by Applegate and Glomset (1986).

The two-site jump motion described for the Tilt model arises mainly from rotations about the C7–C8 and the C8–C9 single bonds and leads to the low molecular order parameters observed experimentally for the C8 methylene segment and the C9–C10 double bond. In contrast, the C6–C7 double bond is characterized by a relatively large order parameter. It is unlikely that an acyl chain containing three or more double bonds could execute a similar jump motion as this motion would project the chain parallel to the bilayer surface. Highly unsaturated fatty acyl chains should *not* undergo local motions of significant amplitude and will be *highly ordered structures*. Furthermore, such highly ordered, compact structures, which pack well in lipid bilayers (Applegate & Glomset, 1986), might be important in forming a tightly sealed membrane that is not permeable to mono- and divalent cations. A tightly sealed membrane is clearly an important requirement for membranes involved in the transmission of electrical signals.

The molecular modeling also suggests that the dynamic properties of the diunsaturated acyl chain differ from the same properties of saturated acyl chains in lipid bilayers. Relative to their respective rates for rotation about their molecular long axes, the rates of the local motions in the diunsaturated bilayer (the two-site jump) are much slower than in saturated bilayers (trans–gauche isomerizations).

An important effect of polyunsaturated lipids could be to maintain membranes in a sufficiently dynamic state to allow lateral diffusion of the lipids and proteins within the plane of the bilayer by lowering the transition temperature of the membrane. Note, however, that bilayers composed of choline phospholipids with a monounsaturated acyl chain in the *sn*-2 position have a lower gel-to-liquid crystal transition temperature than do those composed of the corresponding lipid with an *sn*-2 position hexaunsaturated acyl chain [Dratz & Deese, 1986; see also Coolbear et al. (1983)]. Such monounsaturated lipids could create the same dynamic environment necessary to allow rapid translational diffusion within the plane of a

Table II: Average Orientations of the C6–C7 and C9–C10 Double Bonds

double bond	temp (°C)	rotation angle θ	S_{mol}
C6–C7	15	±120°	0.60
	25	±120°	0.56
	40	±120°	0.55
C9–C10	15	±25°	0.15
	25	±39°	0.11
	40	±59°	0.08

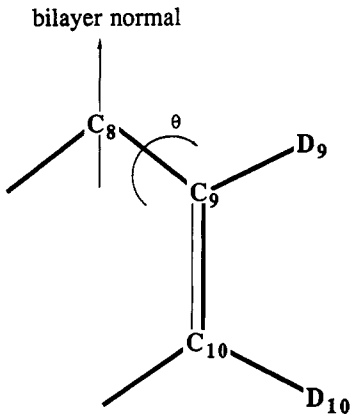

FIGURE 8: Starting orientation of the C9–C10 double bond (and the C6–C7 double bond, not shown) for the calculations described in the Appendix. The average orientations of the double bond, as shown in Figure 4, arise from rotations about the C8–C9 (C7–C8) single bond by the angles θ defined in Table II. The bilayer normal bisects the H–C8–H tetrahedral angle.

membrane, but the acyl chains are relatively flexible (Seelig & Browning, 1978) and at physiological temperatures would undergo rapid local motions of relatively large amplitude (bilayers of POPC are roughly 45 °C above their transition temperature at 37 °C; Keough et al., 1979). Consequently, they might not pack sufficiently well to form a tightly sealed lipid bilayer. In contrast, our data suggest that bilayers composed of a large number of polyunsaturated acyl chains should exist in a dynamic state, but the presence of *polyunsaturation* should maintain a high degree of local order with relatively restricted motions within the hydrophobic region of the bilayer.

Conclusions. Our data indicate that the structural and motional properties of a diunsaturated acyl chain in a lipid bilayer differ dramatically from the same properties of saturated and monounsaturated fatty acyl chains and suggest that highly unsaturated chains will be relatively ordered structures. An important role of polyunsaturated lipids in excitable membranes may be to create a dynamic lipid environment that allows rapid lateral diffusion within the plane of the membrane but still maintains a high degree of local order within the hydrophobic region of the lipid bilayer. In excitable cells, these properties might be important in forming a bilayer with a relatively tight seal that is less permeable to small cations.

ACKNOWLEDGMENTS

J.E.B. is supported by a Natural Sciences and Engineering Research Council (NSERC) of Canada fellowship and by a Graduate Scholarship from the province of Ontario.

APPENDIX

Orientation of the C8 Methylene Segment. As described previously (Baenziger et al., 1991), the complete order parameter matrix for the C8 methylene segment can be defined from the quadrupolar splittings and the homonuclear dipolar couplings of the two C8 deuterons. Diagonalization of this matrix defines the average orientation of the C8 methylene segment. At 40 °C, the order parameter matrix was diagonalized by rotations about the Euler angles $\alpha' = \pm 90.0°$ and $\beta' = \pm 0.40°$ [see Baenziger et al. (1990) for details] to give

$$S^* = \begin{bmatrix} -0.089 & 0 & 0 \\ 0 & -0.062 & 0 \\ 0 & 0 & 0.150 \end{bmatrix}$$

At 25 °C, the order matrix was diagonalized by rotations $\beta' = \pm 0.39°$ and $\alpha' = \pm 90.0°$ to give

$$S^* = \begin{bmatrix} -0.096 & 0 & 0 \\ 0 & -0.082 & 0 \\ 0 & 0 & 0.178 \end{bmatrix}$$

Orientation of the C6–C7 and the C9–C10 Double Bonds. Given the defined orientations of the C8 methylene segment at 15 (see text), 25, and 40 °C, and the quadrupolar splitting data obtained previously from ^2H spectra of aqueous dispersions of PiLPC, the average orientation of both the C6–C7 and the C9–C10 double bonds at all three temperatures were defined. The details of the calculations are described in Baenziger et al. (1991), and the results are presented in Table II. The two double bond orientations at 15 and 40 °C are shown in Figure 4 and arise from the starting orientations shown in Figure 8, by rotations of the C6–C7 and the C9–C10 double bond segments about the C7–C8 or the C8–C9 single bonds, respectively, by the angles θ.

Registry No. PiLPC, 111210-16-3.

REFERENCES

Applegate, K. R., & Glomset, J. A. (1986) *J. Lipid Res. 27*, 658–680.

Baenziger, J. E., Smith, I. C. P., & Hill, R. J. (1987) *Biochemistry 26*, 8405–8410.

Baenziger, J. E., Smith, I. C. P., Hill, R. J., & Jarrell, H. C. (1988) *J. Am. Chem. Soc. 110*, 8224–8231.

Baenziger, J. E., Smith, I. C. P., & Hill, R. J. (1990) *Chem. Phys. Lipids 54*, 17–23.

Baenziger, J. E., Jarrell, H. C., Hill, R. J., & Smith, I. C. P. (1991) *Biochemistry 30*, 894–903.

Browning, J. L. (1981) in *Liposomes: From Physical Structure to Therapeutic Applications*, pp 189–242, Elsevier/North-Holland Biomedical Press, Amsterdam.

Coolbear, K. P., Berde, C. B., & Keough, K. M. W. (1983) *Biochemistry 22*, 1466–1473.

Crawford, M. A., Hassam, A. G., Williams, G., & Whitehorse, W. (1977) *Adv. Exp. Med. Biol. 83*, 135–143.

Dratz, E. A., & Deese, A. J. (1986) in *Health Effects of Polyunsaturated Fatty Acids in Seafoods* (Simopoulos, A. P., Kifer, R. R., & Martin, R. E., Eds.) pp 319–351, Academic, New York.

Dufourc, E. J., Smith, I. C. P., & Jarrell, H. C. (1983) *Chem. Phys. Lipids 33*, 153–177.

Ernst, J., Sheldrick, W. S., & Fuhrhop, J.-H. (1979) *Z. Naturforsch. 34*, 706–711.

Kang, S. Y., Gutowsky, H. S., Hsung, J. C., Jacobs, R., King, T. E., Rice, D., & Oldfield, E. (1979) *Biochemistry 18*, 3257–3267.

Kar, L., Nevy-Igner, E., & Freed, J. (1985) *Biophys. J. 48*, 569–595.

Keough, K. M. W., & Davis, P. J. (1979) *Biochemistry 18*, 1453–1459.

Lamptey, M. S., & Walker, B. L. (1976) *J. Nutr. 106*, 86–93.

Meier, P., Ohmes, E., & Kothe, G. (1986) *J. Chem. Phys. 85*, 3598–3614.

Moser, M., Marsh, D., Meier, P., Wassmer, K.-H., & Kothe, G. (1989) *Biophys. J. 55*, 111-123.
Neuringer, M., Connor, W. E., Van Petton, D., & Barstad, L. (1984) *J. Clin. Invest. 73*, 272-276.
Rance, M., Jeffrey, K. R., Tulloch, A. P., Butler, K. W., & Smith, I. C. P. (1980) *Biochim. Biophys. Acta 600*, 245-262.
Seelig, A., & Seelig, J. (1974) *Biochemistry 13*, 4839-4845.
Seelig, J., & Browning, J. L. (1978) *FEBS Lett. 92*, 41-44.
Shaka, A. J., Keeler, J., Frenkiel, T., & Freeman, R. (1983) *J. Magn. Reson. 52*, 335-341.
Tinoco, J., Babcock, R., Hincenbergs, I., Medwadowski, B., Miljanich, P., & Williams, M. A. (1978) *Lipids 14*, 166-173.
Torchia, D. A., & Szabo, A. (1982) *J. Magn. Reson. 49*, 107-121.
Wheeler, T. G., Benolken, R. M., & Anderson, R. E. (1975) *Science 188*, 1312-1313.
Wiedmann, T. S., Pates, R. D., Besch, J. M., Salmon, A., & Brown, M. F. (1988) *Biochemistry 27*, 6469-6474.
Wittebort, R. J., Olejniczak, E. T., & Griffin, R. G. (1987) *J. Chem. Phys. 36*, 5411-5420.

Reactions of Benzylamines with Methylamine Dehydrogenase. Evidence for a Carbanionic Reaction Intermediate and Reaction Mechanism Similar to Eukaryotic Quinoproteins†

Victor L. Davidson,* Limei Hsu Jones, and M. Elizabeth Graichen
Department of Biochemistry, The University of Mississippi Medical Center, 2500 North State Street, Jackson, Mississippi 39216-4505

Received August 14, 1991; Revised Manuscript Received December 19, 1991

ABSTRACT: It had been previously reported that aromatic amines were not substrates for the bacterial quinoprotein methylamine dehydrogenase. In this study, benzylamine-dependent activity was also not observed in the steady-state assay of this enzyme with the artificial electron acceptor phenazine ethosulfate (PES). Benzylamines did, however, stoichiometrically reduce the protein-bound tryptophan tryptophylquinone (TTQ) prosthetic group and acted as reversible competitive inhibitors of methylamine oxidation when the enzyme was assayed with PES. When methylamine dehydrogenase activity was monitored using a steady-state assay which employed its physiological electron acceptor amicyanin instead of PES, very low but detectable benzylamine-dependent activity was observed. The reactions of a series of para-substituted benzylamines with methylamine dehydrogenase were examined. A Hammett plot of the log of K_i values for the competitive inhibition by these amines against σ_p exhibited a negative slope. Rapid kinetic measurements allowed the determination of values of k_3 and K_s for the reduction of TTQ by each of these amines. A Hammett plot of log k_3 versus σ_p exhibited a positive slope, which suggests that the oxidation of these amines by methylamine dehydrogenase proceeds through a carbanionic reaction intermediate. A negative slope was observed for the correlation between log K_s and σ_p. Plots of log k_3 and log K_s against substituent constants which reflected either resonance or field/inductive parameters for each para substituent indicated that the magnitude of k_3 was primarily influenced by field/inductive effects while K_s was primarily influenced by resonance effects. No correlation was observed between either k_3 or K_s and the relative hydrophobicity of the para-substituted benzylamines or steric parameters. The K_i values which were obtained from steady-state kinetic experiments correlated strongly with the K_s values which were obtained from rapid kinetic experiments. On the basis of these results, a mechanism is proposed for the reactions of benzylamines with this enzyme. These data are also discussed in light of results of similar studies of the reactions of para-substituted benzylamines with two eukaryotic quinoproteins, lysyl oxidase and plasma amine oxidase.

Methylamine dehydrogenase from *Paracoccus denitrificans* is a soluble enzyme which catalyzes the oxidation of methylamine to formaldehyde plus ammonia. It possesses an $\alpha_2\beta_2$ structure and subunit molecular weights of 46 700 and 15 500 (Husain & Davidson, 1987). Each small subunit contains a covalently bound quinone prosthetic group, which is involved both in catalysis and in the subsequent electron transfer to a type I copper protein, amicyanin (Husain & Davidson, 1985), which is its physiological reoxidant. This enzyme is a member of a newly characterized family of bacterial and eukaryotic oxidoreductases which are referred to as quinoproteins [reviewed by Duine and Jongejan (1989)]. These enzymes had all been thought to possess pyrroloquinolinequinone (PQQ)[1] (Salisbury et al., 1979) as a prosthetic group. It now seems that while most bacterial quinoproteins, such as methanol and glucose dehydrogenases, do possess tightly, but noncovalently-associated, PQQ, certain eukaryotic amine oxidases possess other novel covalently-bound quinone species at their active sites. For bovine plasma amine

† This work was supported by National Institutes of Health Grant GM-41574.
* Corresponding author.

[1] Abbreviations: PQQ, pyrroloquinolinequinone; TTQ, tryptophan tryptophylquinone; PES, phenazine ethosulfate; DCIP, 2,6-dichlorophenolindophenol; R, resonance parameter; F, field/inductive parameter; P, octanol-water partition coefficient; E_s, steric parameter.

0006-2960/92/0431-3385$03.00/0