

Effects of two double bonds on the hydrocarbon interior of a phospholipid bilayer

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Abstract

A phospholipid bilayer was modelled by duplicating a monolayer system of 36 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphatidylcholine (PLPC) molecules (16:0/18:2) plus 1368 water molecules and simulated using molecular dynamics. The analyses revealed distinct characteristics in the membrane structure due to polyunsaturation. The orientational behaviour of the fatty acid chains in the PLPC bilayer was found to be seemingly different from that in monounsaturated or in saturated phospholipid bilayers. The specific attributes responsible for the observed behaviour of the saturated and polyunsaturated chains are discussed and their relative importance assessed.

1. Introduction

Phospholipids are the main components of cell membranes, lipoproteins and other membrane structures in living organisms. There is accumulating evidence that structural and dynamic properties of lipid molecules are important to the overall behaviour and interactions of monolayers and membranes [1–8]. It is also well known that the degree and relative proportion of polyunsaturation varies considerably in membrane systems and the saturation state of the lipid fatty acid chains considerably influences the phase transition temperature and fluidity of bilayers [2,4]. However, the effects of polyunsaturation on the membrane structure and the exact roles of the interactions controlling the phenomena are not adequately known. This is understandable since experimental data from biomembranes (such as order

parameters by ²H NMR) are laborious to obtain and intricate to interpret [4,8].

Recent developments in computer simulation methods and the increase in accessible computational resources have made molecular level simulations possible for biological lipid systems [9–15]. The simulations do not only enable ‘computer experiments’ for experimentally awkward systems or for comparison with measurements, but are most importantly a tool to probe the exact physical mechanisms causing the observed characteristics. However, the majority of published phospholipid simulations have been performed for biologically quite scarce systems consisting of relatively short saturated fatty acid chains. A recent simulation of a 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphatidylcholine (POPC) bilayer [15] has given detailed information about the effects of one double bond on the membrane struc-

ture, but neither experimental nor computational data addressing directly the effects of polyunsaturation on membrane structure appear to be available. In this Letter we report novel structural and dynamical findings from a molecular dynamics (MD) simulation of a polyunsaturated bilayer made up of biologically widespread 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphatidylcholine (PLPC) molecules.

2. Calculations

2.1. Modelling and computational details

A PLPC molecule (Fig. 1) containing a saturated palmitic acid (16:0) and a polyunsaturated linoleic acid (18:2) chain was built up with the two double bonds in *cis* and other chain bonds in *trans* configuration and the energy minimised. The layer construction was started by placing four identical PLPC molecules into a square-like configuration with the

head groups pointing up along the *y* axis. Each PLPC was given a different orientation in the *x*-*z* plane to avoid collective tilting and to ensure the formation of a liquid crystalline phase. A surface area of $8.4 \times 8.4 \text{ \AA}^2$ per PLPC molecule was used based on X-ray scattering experiments for membranes with various lipid compositions [16]. The block of four PLPCs was stabilised by an energy minimisation and a dynamic heating-annealing cycle using periodic boundary conditions (PBC) in the *x*-*z* plane. Such blocks were gradually added together and stabilised in the above manner to construct a monolayer of 36 PLPC molecules in a 6×6 grid with an area of $50.4 \times 50.4 \text{ \AA}^2$. The monolayer was solvated by placing an equilibrated water box of $50.4 \times 22.0 \times 50.4 \text{ \AA}^3$ (density $\approx 1 \text{ kg/dm}^3$) so that it overlapped the head group region. The water molecules overlapping the PLPCs were then removed resulting in a model system consisting of 36 PLPC and 1368 water molecules (in all 8856 atoms).

A rotary-reflection (i.e. a reflection with respect to the plane of the layer and a rotation of 90° around the *y* axis) and PBC in the *x*-*z* plane were applied to model an infinite bilayer. The reflection plane was chosen to result in a phosphorus-to-phosphorus distance of $37 \text{ \AA}$, which was estimated from the experiments [16]. The final energy minimisation was reached by first stabilising the water molecules and then the whole system. A 10 ps dynamical heating period up to a temperature of 310 K was performed and followed by an equilibration period of 150 ps. The actual simulation reported here was carried out for 180 ps. The average simulation temperature was 318 K, which is well above the gel to liquid crystal phase transition temperature of the PLPCs [17].

The simulation was performed applying the CHARMM software [18]. Lipid force field parameters were used [19], except for the partial charges of the linoleic acid double bonds, which were estimated by an *ab initio* local density functional calculation [20] (charges partitioned using the method of Hirshfeld [21]) and adapted to the charge distribution of the rest of the molecule. For water molecules the TIP3P parameters [22] were used. All atoms were independently taken into account in the MD simulation, which was performed in a microcanonical ensemble. The lengths of the bonds involving hydrogen atoms were fixed using the SHAKE algorithm to

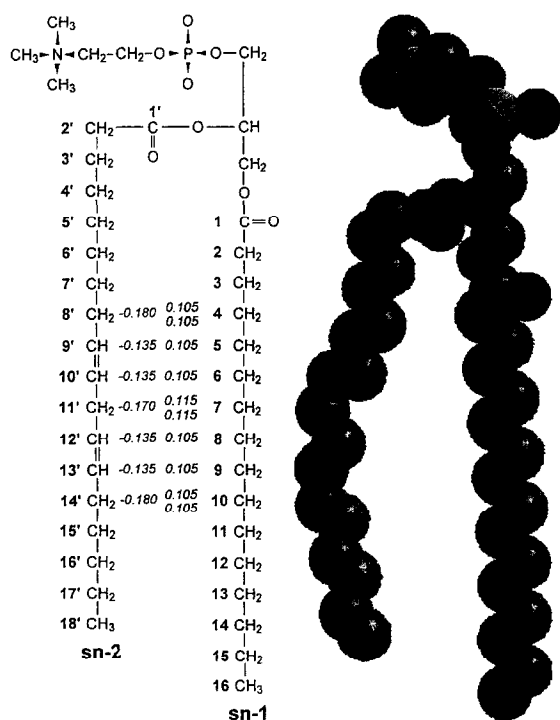


Fig. 1. The structure of an individual PLPC molecule together with the numbering of the carbon atoms. The partial charges (in units of *e*) for the double bond region are also shown.

allow the use of a 1 fs time step [23]. Long-range interactions were truncated using a cut-off radius of 13 Å with a shifting function for the electrostatic and a switching function for the van der Waals interactions [24]. The nonbonded interactions were updated every 25th step (0.025 ps) during the heating and the first 80 ps of equilibration after which an update was performed every 10th step. Energies, coordinates and velocities were saved every 25th step. The simulation was carried out on a SGI Power Challenge/10 + 6 computer achieving a simulation time of 1 ps with approximately 9 CPU h.

2.2. Simulation analysis

The local orientation of a chain segment associated with the carbon atom C_j is defined by the orientation of the H– C_j –H plane. The order parameter profiles were calculated by

$$S_j^{\text{mol}} = \frac{1}{2} \langle 3 \cos^2 \theta_j - 1 \rangle, \quad (1)$$

in which j is the number of the carbon atom C_j and θ is either the angle between the normal of the H– C_j –H plane and the bilayer normal or, for the unsaturated carbons, the angle between the vector from C_{j-1} to C_{j+1} and the bilayer normal. The standard error of mean (SEM) of the time averages was calculated to estimate alterations in the S_j^{mol} values. When isotropic rotations around the y axis are assumed, order parameters S_{CD} from ^2H NMR experiments can be compared with the calculated values according to the relation $-2S_{\text{CD}} = S_j^{\text{mol}}$ [25].

The average gauche fractions were computed considering torsion angles between 20° and 100° , and -100° and -20° as gauche bonds. The carbon atom distributions along the bilayer normal were obtained by calculating the number of individual carbons in 178 horizontal slices (height 0.25 Å). The rms fluctuations of the individual atoms were also calculated.

3. Results and discussion

3.1. General aspects

The calculated order parameter profiles of the saturated palmitic acid (16:0) and polyunsaturated linoleic acid (18:2) chains of the PLPC molecules

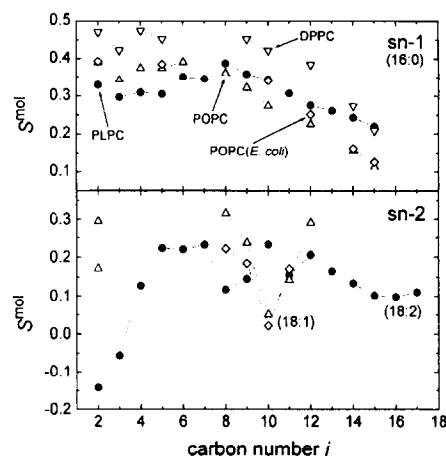


Fig. 2. The calculated order parameter profiles of the palmitic acid (sn-1) and the linoleic acid (sn-2) chains of the PLPCs (●). The SEMs of the time averages range from 0.03 to 0.07. The ^2H NMR results ($|S^{\text{mol}}|$) for the sn-1 chains of DPPCs (16:0/16:0) at 315 K [25] (▽) and of POPCs (16:0/18:1) at 315 K [25,26] (△), and of POPCs in *E. coli* at 313 K [27] (◇) are also given for comparison.

are presented in Fig. 2. The values for the sn-2 carbons are regularly smaller than the corresponding values for the sn-1 carbons and vary from -0.14 to 0.23 and from 0.22 to 0.39 in the case of the sn-2 and sn-1 chains, respectively. A general comparison with ^2H NMR based order parameters of DPPC (16:0/16:0), POPC (16:0/18:1) and POPC in *Escherichia coli* [26–28] is also presented in Fig. 2. A detailed discussion of the points of similarity and dissimilarity between the obtained PLPC order parameters and the different experiments and the theoretical order parameters for POPC [15] is given in Sections 3.2 and 3.3. DePaked ^2H NMR based smoothed order profiles [8,29], even though capable of describing the general orientational behaviour for the end of the chains, are intrinsically unable to describe the variations of the order at the beginning of the chains and cannot thus be used as a reference material here.

Due to the lack of experimental and theoretical data for direct comparison with the present results, the quality and reliability of the PLPC simulation were also assessed using alternative measures. The properties of the PC head group region were examined and compared with both experimental and theoretical data available for other PC membranes. For

instance, the radial distribution of water oxygens and hydrogens around the head group atoms in this simulation shows a similar behaviour as in a large-scale simulation of a DMPC bilayer (14:0/14:0) [10,13]. Furthermore, corresponding to the experimental findings [30], the average head group orientation of the PLPCs is nearly parallel to the membrane surface. These and other results of the PLPC simulation will be discussed in detail elsewhere, but the good agreement of the general characteristics with both experiments and recent simulations of other phospholipid systems gives a sound basis for the novel structural information given here.

According to Eq. (1) the order parameter can vary between 1 and $-1/2$ corresponding to fully, ordered chains parallel and perpendicular to the bilayer normal, respectively. Values in between are affected by different contributions such as preferred local orientation, distribution of local geometries (long-scale dynamics) and short-scale dynamic fluctuations. A preferred local orientation means that a particular carbon segment orients similarly in all PLPC molecules. The decrease of the order parameter from 1 to $-1/2$ can thus be interpreted as a change in the preferred local orientation angle from 0 to 90° (the zero value of the order parameter corresponds to an angle of 54.7°). However, variations in local geometries lead to distributions of local orientations allowing to interpret the decrease of the order parameter from 1 to 0 also as a disappearance of the parallel order of the chains towards complete disorder. Accordingly, a further decrease of the order parameter from 0 to $-1/2$ can be interpreted as an appearance of perpendicular chain order towards a fully ordered situation. On the other hand, the short-scale dynamic fluctuations tend to decrease the order. Thus, the order parameters always contain a mixture of different contributions and must be assessed with care.

3.2. Palmitic acid chain

The order parameter profile of the saturated sn-1 chains is characterised by a small dip in the beginning for carbon C_3 , an increasing trend from C_3 to C_8 , and an evenly decreasing end part (Fig. 2). It is interesting to note that despite the general success of recent POPC [15] and DMPC [14] bilayer simula-

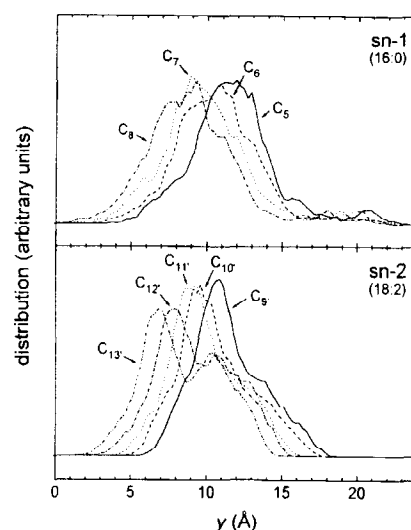


Fig. 3. The calculated carbon atom distributions along the PLPC bilayer normal for the sn-1 carbons C_5 – C_8 and for the sn-2 double bond region carbons C_9 – C_{13} ($y = 0$ in the centre of the bilayer).

tions, they did not reproduce the experimentally observed dip for carbon C_3 . Comparison of the PLPC order parameters for carbons C_2 – C_5 with the experimental values obtained at approximately the same temperature shows a clear decrease along the increase in the number of double bonds, i.e. in the chain DPPC(16:0/16:0)–POPC(16:0/18:1)–PLPC(16:0/18:2). This probably reflects differences in the gel to liquid crystal phase transition temperatures of the bilayers: PLPCs have the lowest transition temperature, and accordingly, the lowest order parameters. For other parts of the chain the comparison is hampered due to missing experiments but for carbons C_8 – C_{15} the trend seems to be that the order parameters for DPPC are the highest and those for the POPC the lowest.

The y -distributions of sn-1 carbons C_5 – C_8 and sn-2 carbons C_9 – C_{13} of the PLPCs are illustrated in Fig. 3. The double bond region is seen to have an almost exactly overlapping distribution with carbons C_5 – C_8 , which should reflect close interaction of these parts in the interior of the membrane. In fact, the double bond distribution overlaps partly with all the sn-1 carbon distributions indicating that the whole sn-1 chain may be affected by the presence of the double bonds. The distributions also indicate that a

marked proportion of the double bond regions lies closely parallel to the membrane surface. Compared to the situation with one or no double bonds in the sn-2 chain, this is an obviously different situation for the sn-1 carbons C_5 – C_8 which are situated at the same depth in the membrane. This is most likely one reason for the clear increase from C_5 to C_8 in the order parameter profile. This is also supported by the fraction of gauche bonds in the sn-1 chains of the PLPCs and DPPCs (Fig. 4). The experimentally estimated percentage of gauche bonds for the sn-1 carbons C_6 in a DPPC bilayer [31] is twice that obtained from the PLPC simulation although the fraction of gauche bonds from the DPPC experiments and from the PLPC simulation for the carbons C_4 and C_{10} are almost identical.

As discussed by Seelig and Seelig [26], the incorporation of one rigid *cis* double bond between the sn-2 carbons C_9 and C_{10} probably reduces the number of available rotational isomeric states in the sn-1 chain and causes a stiffening and accordingly slight increase in the sn-1 order parameters. The incorporation of an additional *cis* double bond between the carbons C_{12} and C_{13} would thus probably extend the region of stiffening towards the end of the sn-1 chain and also limit the possible orientations of the end part. This matches well with the observed increasing trend in the order parameter profile for the carbons C_3 – C_8 as well as with the higher order parameters for the PLPC carbons C_8 – C_{15} than for those of the POPC.

The evenly decreasing order parameter profile for the carbons C_8 – C_{15} is in accordance with the steep-

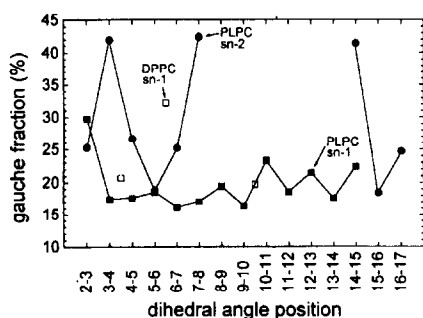


Fig. 4. The calculated fraction of gauche bonds for the carbon segments in the sn-1 (■) and sn-2 (●) chains of the PLPCs. The experimental results for the sn-1 palmitic acid chains of DPPCs at 312 K [29] (□) are shown for comparison.

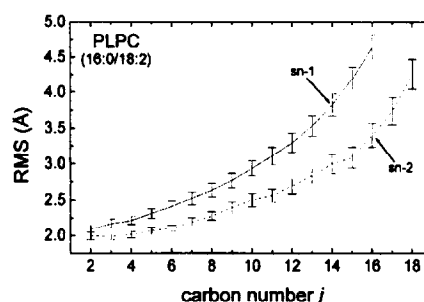


Fig. 5. The calculated atomic RMS fluctuations of the carbon segments in the sn-1 (solid line) and sn-2 (dashed line) chains of the PLPCs. The bars represent the SEMs.

ening increase of the dynamic fluctuations illustrated in Fig. 5. This also suggests that within the end part of the sn-1 chain the sn-2 double bonds hardly affect the rotational freedom of the torsion angles. Interestingly, the values of the order parameters for the sn-1 carbons C_{10} and C_{12} obtained from the POPCs at the *E. coli* membranes are closer to the simulated values of the PLPCs than those of pure POPCs (Fig. 2). This most likely reflects the existence of polyunsaturated chains in the real cell membranes and their effect on the saturated sn-1 chains of the investigated POPCs.

3.3. Linoleic acid chain

The order parameter profile of the polyunsaturated sn-2 chains is characterised by a steep increase from the value of -0.14 for the carbon $C_{2'}$ to the value of 0.22 for the carbon $C_{5'}$, a short plateau at $C_{5'}$ – $C_{7'}$, dips at $C_{8'}$ – $C_{9'}$ and at $C_{11'}$, and decreasing values at the end (Fig. 2). Since ^2H NMR reveals only $|S_{\text{CD}}|$ there is no experimental verification for the negative order parameters of $C_{2'}$ and $C_{3'}$ revealed by the PLPC simulation. However, the calculated distributions of the angles between the vector from the first to the third carbon and the bilayer normal peak relatively sharply at 29° for the sn-1 and at 70° for the sn-2 chain explain the negative order parameters in the beginning of the unsaturated chain (see also Fig. 1). On the basis of this conformational model [27,32] it is also likely that the order parameters for the first sn-2 carbons in the DPPC and POPC bilayers behave in a qualitatively similar manner as in the PLPC bilayer. In fact, the average of the two

^2H NMR values (reflecting the motional non-equivalence of the $\text{C}_{2'}$ deuterons [33]) shown in Fig. 2 for the order parameter of the sn-2 carbon $\text{C}_{2'}$ of a POPC bilayer is close to the corresponding absolute value obtained for the PLPCs.

The sn-2 chains bend downwards after carbon $\text{C}_{2'}$, which causes the change of the order parameter from negative to positive (corresponding to a change of the angle θ from over 60° to below 50°). This bending is also reflected by the large 42% gauche fraction in the bond $\text{C}_3\text{--C}_4$ (Fig. 4). The plateau region in the order parameter profile at $\text{C}_5\text{--C}_7$ is in accordance with the relatively small gauche fraction between the bonds $\text{C}_5\text{--C}_6$ and $\text{C}_6\text{--C}_7$: trans bonds keep the distribution of local structures relatively uniform in this region. The order parameter for carbon C_8 is only half that for carbon C_7 , which reflects more variability in the local orientations as implied by the high 42% gauche fraction for the bond $\text{C}_7\text{--C}_8$.

Comparison of the order parameters for the sn-2 linoleic acid carbons of the PLPCs with the available experimental values for the sn-2 oleic acid carbons of the POPCs reveals remarkable differences (Fig. 2). In the order parameter profile of the oleic acid chain the dip is at C_{10} , but for the linoleic acid chain the dips are at $\text{C}_8\text{--C}_9$ and at C_{11} . The dip in the oleic acid profile is also much more pronounced than the dips in the linoleic acid profile. According to Gally et al. [28] and Heller et al. [15] the observed dip in the oleic acid order parameter profile is more likely a geometric effect than due to increased fluctuations. The increase in the RMS fluctuations of the sn-2 carbons of the PLPCs (Fig. 5) is not revealed in the double bond region of the order parameter profile (Fig. 2), which points out the importance of geometric effects for the linoleic acid chains as well. These effects are probably even enhanced in the case of two double bonds, a high proportion of which lies at the same depth in the membrane (Fig. 3). At the end of the linoleic acid chain the effect of increased RMS fluctuations becomes noticeable although a kind of end plateau at $\text{C}_{15}\text{--C}_{17}$ seems to occur as a result of strong local orientations in relation to the large gauche fraction in the bond $\text{C}_{14}\text{--C}_{15}$.

The results discussed above give evidence that the geometrical constraints of the sn-2 double bond regions induce structural changes especially into the

parts of the saturated sn-1 chains which are at the same depth in the layer. This suggests that (poly)unsaturation does not only induce changes to the co-operative properties of membranes, but can also cause direct local effects in the vicinity of the double bonds.

4. Conclusions

The reported MD simulation of a bilayer consisting of biologically abundant polyunsaturated PLPC molecules reveals a considerable difference in the structure of the hydrocarbon interior of the membrane compared to other PC systems containing only saturated or saturated and monounsaturated chains. These novel results indicate that marked changes in the membrane structure can arise from slight changes in the unsaturation state, which elucidates the crucial role of the double bonds in membrane functions. Hence it will be of great interest to investigate and compare structural properties of membranes with different degrees of polyunsaturation.

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References

- [1] A.G. Lee, N.J.M. Birdsall, J.C. Metcalfe, P.A. Toon and G.B. Warren, *Biochemistry* 13 (1974) 3699.
- [2] P.R. Cullis and M.J. Hope, in: *Biochemistry of lipids and membranes*, eds. D.E. Vance and J.E. Vance (Benjamin/Cummings, Menlo Park, 1985).
- [3] J.A. Ibdah, S. Lund-Katz and M.C. Phillips, *Biochemistry* 28 (1989) 1126.

- [4] M. Bloom, E. Evans and O.G. Mouritsen, *Quart. Rev. Biophys.* 24 (1991) 293.
- [5] J.S. Owen, S. Rafique, E. Osman and A.K. Burroughs, *Drug. Invest.* 4 (1992) 22.
- [6] S. Jain, M. Thomas, G.P. Kumar and M. Laloraya, *Biochem. Biophys. Res. Commun.* 195 (1993) 574.
- [7] H. Kadowaki, G.M. Patton and S.J. Robins, *J. Lipid Res.* 34 (1993) 180.
- [8] L.L. Holte, S.A. Peter, T.M. Sinnwell and K. Gawrisch, *Biophys. J.* 68 (1995) 2396.
- [9] J.J. Wendoloski, S.J. Kimatian, C.E. Schutt and F.R. Salemme, *Science* 243 (1989) 636.
- [10] H.E. Alper, D. Bassolino-Klimas and T.R. Stouch, *J. Chem. Phys.* 99 (1993) 5547.
- [11] R.M. Venable, Y. Zhang, B.J. Hardy and R.W. Pastor, *Science* 262 (1993) 223.
- [12] P. Ahlström and H.J.C. Berendsen, *J. Phys. Chem.* 97 (1993) 13691.
- [13] K.V. Damodaran and K.M. Merz Jr., *Biophys. J.* 66 (1994) 1076.
- [14] A.J. Robinson, W.G. Richards, P.J. Thomas and M.M. Hann, *Biophys. J.* 67 (1994) 2345.
- [15] H. Heller, M. Schaefer and K. Schulten, *J. Phys. Chem.* 97 (1993) 8343.
- [16] B.A. Lewis and D.M. Engelman, *J. Mol. Biol.* 166 (1983) 211.
- [17] C.D. Niebylski and N. Salem Jr., *Biophys. J.* 67 (1994) 2387.
- [18] CHARMm Version 22.2 (Molecular Simulations Inc., Waltham, 1992).
- [19] M. Schlenkrich, A.D. MacKerell Jr. and M. Karplus, manuscript in preparation.
- [20] DMol Version 2.3 (Biosym Technologies, San Diego, 1993).
- [21] F.L. Hirshfeld, *Theoret. Chim. Acta* 44 (1977) 129.
- [22] W.L. Jorgensen, J. Chandrasekhar and J.D. Madura, *J. Chem. Phys.* 79 (1983) 926.
- [23] W.F. van Gunsteren and H.J.C. Berendsen, *Mol. Phys.* 34 (1977) 1311.
- [24] B.R. Brooks, R.E. Bruccoleri, B.D. Olafson, D.J. States, S. Swaminathan and M. Karplus, *J. Comput. Chem.* 4 (1983) 187.
- [25] J. Seelig and W. Niederberger, *J. Am. Chem. Soc.* 96 (1974) 2069.
- [26] A. Seelig and J. Seelig, *Biochemistry* 16 (1977) 47.
- [27] J. Seelig and N. Waespe-Šarčević, *Biochemistry* 17 (1978) 3310.
- [28] H.U. Gally, G. Pluschke, P. Overath and J. Seelig, *Biochemistry* 18 (1979) 5605.
- [29] M. Lafleur, B. Fine, E. Stermin, P.R. Cullis and M. Bloom, *Biophys. J.* 56 (1989) 1037.
- [30] J. Seelig and A. Seelig, *Quart. Rev. Biophys.* 13 (1980) 19.
- [31] R. Mendelsohn, M.A. Davies, J.W. Brauner, H.F. Schuster and R.A. Dluhy, *Biochemistry* 28 (1989) 8934.
- [32] A. Seelig and J. Seelig, *Biochim. Biophys. Acta* 406 (1975) 1.
- [33] A.K. Engel and D. Cowburn, *FEBS Letters* 126 (1981) 169.