On the relationship between C-C and C-D order parameters and its use for studying the conformation of lipid acyl chains in biomembranes

Jean-Paul Douliez, A. Ferrarini, E.J. Dufourc

On the relationship between C-C and C-D order parameters and its use for studying the conformation of lipid acyl chains in biomembranes

J.-P. Douliez[a)]
Laboratoire de Biochimie et Technologie des Protéines, INRA, av. de la Géraudière 44316 Nantes, France

A. Ferrarini
Department of Physical Chemistry, University of Padua, 35131 Padova, Italy

E.-J. Dufourc
Centre de Recherche Paul Pascal, CNRS, av. A. Schweitzer, 33600 Pessac, France

We have used the order tensor method to establish relationships between carbon-carbon and carbon-deuterium order parameters (S^{CC} and S^{CD}, respectively) in saturated and unsaturated lipid acyl chains in biomembranes. We thus confirm the existence of a recursion relation between these order parameters ($-2S_k^{CD} = S_k^{CC} + S_{k+1}^{CC}$) as was previously shown using Wigner matrices formalism [Douliez, Léonard, and Dufourc, Biophys. J. **68**, 1727 (1995)]. This leads to the determination of S_k^{CC} as a function of the position k along the chain, having experimentally measured the corresponding S_k^{CD}. Making use of a mean field approach for computing the energy of chain conformers in the frame of the rotational isomeric model (RIS) we have also calculated S^{CC} and S^{CD} order profile for the *sn*-1 chain of dipalmitoylphosphatidylcholine. A very good agreement is obtained between computational and predicted S_k^{CC} values, reinforcing the validity of the recursion relation. A marked odd-even effect is evidenced on the S^{CC} order profile for positions near the polar head, as was previously experimentally reported for dimyristoylphosphatidylcholine. We propose that this effect reveals an average bent orientation of the beginning of the chain with respect to the normal of the bilayer. Finally, it is shown that our formalism can be applied to some extent to *cis* or *trans* unsaturated lipid. © *1998 American Institute of Physics.* [S0021-9606(98)51530-9]

I. INTRODUCTION

Conformation and dynamics of lipid acyl chains are of strong interest for understanding the functional properties of membranes. For such studies, a great deal of interest has been devoted to computational methods. Information about static and dynamic structure, through the calculation of order parameters or correlation functions may be provided from Monte Carlo,[1–3] master equation,[4] maximum entropy,[5,6] Brownian dynamics[7] and molecular dynamics simulations.[8–11] As an example, order parameters calculated from these methods can be compared to experimental data from NMR (Refs. 12, 13) or Raman scattering[14] whereas correlation functions may be connected to T_1 relaxation experiments.[15,16] The relative ease in deuterating lipid acyl chains and the use of solid state NMR has led to the appearance of C-D order parameter profiles. They have been widely studied theoretically during the last two decades[12,15,17–20] and provide information on acyl chain length or number of *gauche* defects[20,21] as well as on bilayer elastic constants.[22–25] Recent progress in pulse sequence methodology has allowed measurement of sign and intensity of C-H dipolar couplings, which yields similar orientational information.[26–28] Double quantum and separated local field experiments provide valuable determination of C-H and C-C dipolar couplings without requiring isotopic labeling.[19,29–31]

While C-H dipolar coupling and quadrupolar interaction bring the same information, e.g., S_k^{CH} or S_k^{CD}, C-C couplings provide additional details on local segmental motion. We have previously developed a formalism allowing calculation of S_k^{CC} from experimental S_k^{CD}.[20] It links two adjacent C-C order parameters of a methylene unit, k, to the corresponding S_k^{CD} (Fig. 1) and yields the recursion relation:

$$-2S_k^{CD} = S_k^{CC} + S_{k+1}^{CC}. \qquad (1)$$

This equation was initially derived by performing coordinate transformations from the C-C or C-D bonds of a lipid chain to the bilayer normal, **n**. For ease of calculation, Wigner matrices were used and a complete decomposition of motions occurring at the NMR time scale was performed. As a consequence, it was not clearly demonstrated whether this recursion relation depends or not on the dynamics occurring in the membrane. In the same way, the terminal bond order parameter, S_n^{CC}, was determined by a simple coordinate transformation from the C_n-D bond of the methyl terminus to the C_{n-1}-C_n bond (which is also the rotation axis of the methyl group). Subsequently, the recursion relation was used to calculate all the C-C bond order parameters along the chain having started from the methyl-end. Let us note that Eq. (1) stands only for the case of saturated chains and to our knowledge, no similar relation has been yet established in the case of a double bond. It should be mentioned that, as a consequence of Eq. (1), S_k^{CD} and S_k^{CC} bring the same structural and dynamical information. However, it is more conve-
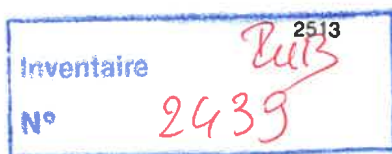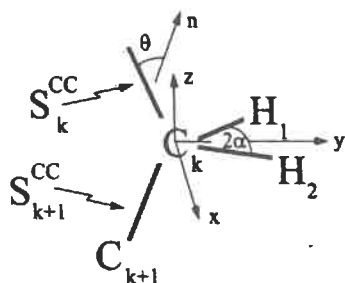
FIG. 1. Schematic representation of a methylene group in a lipid acyl chain with the axes systems needed for the description of order parameters. n is the normal to the bilayer and the x,y,z frame is defined as in van der Ploeg and Berendsen (Ref. 9). S_k^{CC} and S_{k+1}^{CC} is respectively the order parameter of the C_{k-1}-C_k and C_k-C_{k+1} bond. 2α is the angle between the CH_1 and CH_2 vectors.

nient to use the S^{CC} formalism as a basis to calculate physical parameters such as acyl chain length or number of gauche defects. Moreover, the S^{CC} order profile may reveal an odd-even effect that cannot be observed for S^{CD} measured from perdeuterated chains. The amplitude of such an effect has been found to increase with temperature for sn-2 chains of several lipids whereas it remains constant for sn-1 chains.[20,21] The validity of the formalism has been tested experimentally from ^{13}C- and ^{2}H-NMR experiments. Palmitic acid deuterated on the whole chain and labeled with ^{13}C on the C_1 and C_2 positions were embedded in a liquid crystal. S_k^{CD} were then measured allowing calculation of S_k^{CC} (as described above) and especially S_2^{CC}, the order parameter of the C_1-C_2 bond. This former order parameter was also experimentally determined with one and two-dimensional ^{13}C-NMR by measuring the dipolar coupling between C_1 and C_2. Both values of S_2^{CC} determined separately as a function of temperature were found in very good agreement.[21]

In order to generalize the validity of our formalism, we use in this paper the order tensor concept for calculating S_k^{CC} and S_k^{CD} order parameters. This approach has a strong advantage since it does not require any decomposition of motions occurring in the system. It also leads to the recursion relation, Eq. (1), demonstrating that the S^{CC} formalism is dynamically independent and rather depends on the molecular geometry. By using a thermodynamical model to compute the configurational energy of the sn-1 chain of DPPC, we calculate S_k^{CC} and S_k^{CD}. S^{CC} order parameter profiles exhibit an odd-even effect especially for positions near the polar head. It is proposed that such an effect is a consequence of the average bent conformation of the beginning of the chain. In a parallel way, we have extended our formalism to double bonds as in the oleoyl POPC chain. In such a case, it is not possible *a priori* to obtain a recursion relation. However, whatever the *trans* or *cis* configuration, it is shown how the S^{CC} formalism can help for the attribution of the *pro*-R and *pro*-S C-D order parameters of the double bond.

II. EQUATIONS AND METHODS

A. Order tensor for saturated chains

Order parameters are of strong interest in mean field, Maier-Saupe, theories.[33-36] They are defined as an averaged

ordinary Legendre polynomial of second order. In the most general case, this definition can be generalized by introducing the order parameter tensor, S, having the following components:[37,38]

$$S_{ij} = \langle 3 \cos \theta_i \cos \theta_j - \delta_{ij}\rangle/2, \qquad (2)$$

where θ_i denotes the angle between the bilayer normal and the molecular coordinates axes: $i,j = x,y,z$, δ_{ij} the Kronecker symbol and the angular brackets a time and space average. For methylene groups of saturated acyl chains in membranes this molecular frame is usually defined as follows:[9] x is parallel to the D-D (or H-H) vector, y bisects the D-C_k-D (or the H-C_k-H) angle and z is the normal on the plane spanned by the two C_k-D (C_k-H) bond vectors (Fig. 1). This yields the well-known relation for S^{CH} or S^{CD}.[39]

$$S_k^{CD} = S_k^{CH} = (2S_{xx}^k + S_{yy}^k)/3. \qquad (3)$$

For a better understanding on how to get the recursion relation, Eq. (1), it is now necessary to give more details on the way that leads to determine Eq. (3). Let us then define the C_k-D_1 and C_k-D_2 vectors as x and y components,

$$C_kH_1 = C_kD_1 = \cos(\alpha)y - \sin(\alpha)x,$$

$$C_kH_2 = C_kD_2 = \cos(\alpha)y + \sin(\alpha)x, \qquad (4)$$

where $2\alpha = 109.5°$ is the D-C_k-D angle, with $\cos \alpha = 1/\sqrt{3}$ and $\sin \alpha = \sqrt{2/3}$. Let n be the bilayer normal and β_a ($a = 1$ or 2) the angle between the C_k-H_a bond and n (Fig. 1),

$$\cos \beta_1 = n \cdot C_kH_1 = n \cdot C_kD_1 = \frac{1}{\sqrt{3}} \cos \theta_y - \frac{\sqrt{2}}{\sqrt{3}} \cos \theta_x,$$

$$\qquad (5)$$

$$\cos \beta_2 = n \cdot C_kH_2 = n \cdot C_kD_2 = \frac{1}{\sqrt{3}} \cos \theta_y + \frac{\sqrt{2}}{\sqrt{3}} \cos \theta_x,$$

where θ_i ($i = x,y,z$) is the angle between the axis i and the normal to the bilayer. It is then easy to calculate the order parameter of the C_kD_1 and C_kD_2 bonds using $S_k^{CD_a} = \langle 3 \cos^2(\beta_a) - 1\rangle/2$ which yields, with the help of Eqs. (2) and (5) to

$$S_k^{CD_1} = \frac{2}{3} S_{xx}^k + \frac{1}{3} S_{yy}^k - \frac{2\sqrt{2}}{2} S_{xy}^k,$$

$$\qquad (6)$$

$$S_k^{CD_2} = \frac{2}{3} S_{xx}^k + \frac{1}{3} S_{yy}^k + \frac{2\sqrt{2}}{3} S_{xy}^k.$$

Let us note that in the case where deuterons are equivalent, it follows from Eq. (6) that S_{xy}^k is zero. Then, summation of both lines in Eq. (6) yields

$$S_k^{CD_1} + S_k^{CD_2} = 2S_k^{CD} = 2(\tfrac{2}{3}S_{xx}^k + \tfrac{1}{3}S_{yy}^k) \qquad (7)$$

which is similar to Eq. (3).

We now take the advantage that methylene units bring the tetrahedral geometry which means that the C_{k-1}-C_k-C_{k+1} angle is also 2α (Fig. 1). It is then easy to rewrite Eqs. (4) to (6) for S_k^{CC} and S_{k+1}^{CC} by replacing C_k-D_1 and C_k-D_2 by C_{k-1}-C_k and C_k-C_{k+1}, respectively (Fig. 1)

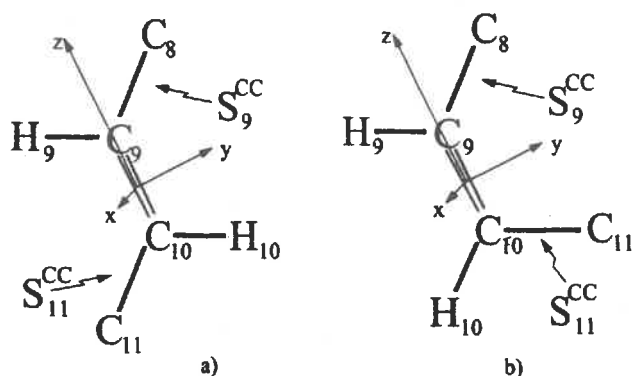$$C_{k-1}C_k = \cos(\alpha)y - \sin(\alpha)z, \qquad (8a)$$

FIG. 2. Schematic representation of a *trans* (a) and *cis* (b) double bond standing at positions 9 and 10. The x,y,z frame is slightly modified compared to Fig. 1 and is defined as in Seelig and Waespe Sarcevic (Ref. 40). Order parameters are defined as in Fig. 1, i.e., S_k^{CC} stands for the C_{k-1}-C_k bond.

$$\mathbf{C}_k\mathbf{C}_{k+1} = -\cos(\alpha)\mathbf{y} - \sin(\alpha)\mathbf{z},$$

$$S_k^{CC} = \frac{2}{3} S_{zz} + \frac{1}{3} S_{yy} - \frac{2\sqrt{2}}{3} S_{yz},$$

$$S_{k+1}^{CC} = \frac{2}{3} S_{zz} + \frac{1}{3} S_{yy} + \frac{2\sqrt{2}}{3} S_{yz}. \qquad (8b)$$

Equation (8b) expresses S^{CC} as a function of the xx,yy order parameters and of the off-diagonal element S_{yz}. As mentioned in the case of equivalent deuterons, let us note that $S_k^{CC} = S_{k+1}^{CC}$ implies S_{yz} is zero. Then, summation of both lines in Eq. (8b) gives

$$S_k^{CC} + S_{k+1}^{CC} = 2(\tfrac{2}{3} S_{zz} + \tfrac{1}{3} S_{yy}). \qquad (9)$$

Because the trace of the order tensor is zero, the combination of Eqs. (3) and (9) leads to the recursion relationship between S^{CC} and S^{CD}, Eq. (1). It must be mentioned that the above equations are derived without requiring any decomposition of motions occurring in the system. This demonstrates that the recursion relation does not depend on the motions occurring in the system, as long as motional averaging happens, but rather on the tetrahedral geometry of the methylene group.

At this point of the development, it may be interesting to extend such calculation to cases where double bonds are present in lipid chains.

B. Order tensor for unsaturated chains

We envisage here the case of the oleoyl chain of POPC where the double bond stands at position 9–10 (Fig. 2). The molecular frame has then to be slightly modified.[40] z is along the C_9-C_{10} bond; x is perpendicular to the plane defined by the C_9-D (or C_{10}-D) bond and z; and y is perpendicular to x and z axes. Let us note that following this definition, $S_{10}^{CC} = S_{zz}$. Writing similar equations as in (4) and (5), one obtains

$$S_9^{CD} = \frac{1}{4} S_{zz} + \frac{3}{4} S_{yy} - \frac{\sqrt{3}}{2} S_{yz}, \qquad (10)$$

$$S_{10}^{CD} = \frac{1}{4} S_{zz} + \frac{3}{4} S_{yy} + \frac{\sqrt{3}}{2} S_{yz}.$$

It must be mentioned that, in the present case, we have not been able to derive a recursion relation similar to Eq. (1). However, one can take advantage that the double bond is rigid, that is to say no rotation around the C_9-C_{10} can occur, and note the following: (i) C_9-D is parallel to the C_{10}-C_{11} bond and (ii) C_{10}-D is parallel to the C_8-C_9 bond. Then, one should get for the *cis* double bond in position 9–10,

$$S_9^{CD} = S_{11}^{CC} \quad \text{and} \quad S_{10}^{CD} = S_9^{CC}. \qquad (11)$$

This can be generalized in the case of a *cis* or *trans* double bond at positions C_p-C_{p+1},

cis $\quad S_p^{CD} = S_{p+2}^{CC} \quad$ and $\quad S_{p+1}^{CD} = S_p^{CC}, \qquad (12)$

trans $\quad S_p^{CD} = S_{p+1}^{CD} \quad$ and $\quad S_{p+2}^{CC} = S_p^{CC}, \qquad (13)$

where the notation for order parameters is similar to that indicated in Fig. 1. As will be discussed later, these properties may be of great interest for the assignment of labeled positions and allow calculation of S^{CC} order parameters for all carbon positions when going towards the polar head from the methyl terminal.

C. The thermodynamical model

In order to check the validity of Eq. (1) and analyze the S^{CC} profile, we calculated S^{CC} and S^{CD} by making use of a thermodynamical model. In what follows, we will briefly remind the general procedure employed to determine the energy of the chain. For more details, one may consult the following references.[34,41,4] Under the assumption of fixed bond lengths ($l_{C-C} = 1.53$ Å) and bond angles (C_{k-1}-C_k-$C_{k+1} = 112°$ and H-C-H $= 109°$),[42,43] the internal dynamics of the chain is described in the frame of the rotational isomeric state (RIS) approximation. The rotation around the C-C bonds is assumed to be subject to a *trans-gauche* torsional potential.[42,44,45] A stable configuration of the chain is then identified by a sequence of *trans* and *gauche*±. The energy of such a configuration is given in terms of intra- and intermolecular interactions. The intramolecular contribution arises from the isomerizations (rotation around the C-C bonds) and is assumed to be 0 for the *trans* state and V_g for *gauche*±. For a given configuration, the intermolecular interaction is described by a mean field potential,[34,35] $V = -\epsilon\sum_{k=1}^{15} S_{zz}^k$, where ϵ gives the strength of the contribution. Let us note that steric criteria as detailed as in the original work[4] yield 37227 allowed configurations for the sn-1 chain of DPPC. S^{CC} and S^{CD} order parameters may then be determined as

$$S_k = \sum_J P_J S_{k,J}, \qquad (14)$$

where P_J and $S_{k,J}$ are respectively the fractional population (according to the Boltzman equation) and the order parameter of the position k for the Jth configuration.[41,46]

III. RESULTS AND DISCUSSION

The S^{CC} order parameter formalism is of great interest for our understanding of conformational properties of acyl chains in lipid membranes. It affords calculation of conformer probabilities, acyl chain length and in combination with neutron scattering experiments, provides information about protrusion of lipids in the membrane.[32] The basis of this formalism is a recursion relationship between S^{CC} and S^{CD} which has been derived by making use of Wigner matrices.[20] This requires a complete decomposition of the different acyl chain motions and a set of Euler angles allowing coordinate transformations from the initial tensor axis system to the laboratory frame of reference. We thus accounted for the isomerization around C-C bonds, lipid rotation around its long molecular axis and wobbling (oscillation around the bilayer normal).[15,17] However, it was not clear whether the recursion relation depends or not on the dynamics occurring in the system. When using the concept of order tensor equations are derived without requiring any decomposition of motions and the average of order parameters is performed over time and space. Please note also that the introduction of the bilayer normal in the calculation could have been replaced by the magnetic field vector. Using this approach, we have thus shown that the recursion relation between S^{CD} and S^{CC} still stands and then does not depend on the type of motions of the acyl chain. It is rather a consequence of the tetrahedral geometry of the methylene group. Even if we have considered that both dihedral angles H-C_k-H and C_{k+1}-C_k-C_{k-1} are equal it will be shown in the following, from a calculation performed with the thermodynamical model where this condition is not rigorously satisfied, that the recursion relation still stands.

Let us now give a general comment concerning the determination of S^{CD} from molecular dynamics simulation. This is a powerful tool allowing theoretical calculation of physical properties of lipids embedded in a membrane. However, the time used when performing a molecular dynamics simulation (ps to ns time scale) could be too short for acyl chains to explore all their possible configurations (isomerization). As a consequence, due to insufficient time and space averaging, both deuterons on a methylene could be nonequivalent. It must be mentioned that usually in the literature,[10] Eq. (3) (i.e., S^{CD} vs S_{xx} and S_{yy}) is employed to calculate order parameters. We would like to stress that such an equation is only valid under the assumption that the order parameters on a methylene unit are equal. Care should be taken and the general definition of order parameters, Eq. (2), should preferentially be used, since the angle between the C-D bond and the bilayer normal can always be determined from the calculation. Note that it must also be possible to calculate the off-diagonal term S_{xy}, which could help as a criterion of convergence for the simulation since S^{CD} are often experimentally equivalent.

We shall now present the results concerning the calculation of S^{CD} and S^{CC} from the thermodynamical model. As mentioned above, the allowed configurations of the DPPC sn-1 chain are computationally generated. A given configuration is a sequence of *gauche* and *trans* from carbon C_2 to the methyl terminal where the energy is calculated as de-

TABLE I. S_k^{CD} and S_k^{CC} order parameters as a function of carbon position, k, for the sn-1 chain of DPPC at 50 °C.

k	$S_k^{CD\,a}$	$S_k^{CD\,b}$	$2S_k^{CD\,c}$	$S_k^{CC\,d}+S_{k+1}^{CC}$
2	−0.282	n.d.[e]	0.564	n.d.[e]
3	−0.266	0.214	0.532	0.533
4	−0.289	0.319	0.578	0.579
5	−0.284	0.260	0.568	0.568
6	−0.286	0.308	0.572	0.571
7	−0.274	0.263	0.548	0.547
8	−0.269	0.284	0.538	0.537
9	−0.256	0.253	0.512	0.513
10	−0.249	0.260	0.498	0.498
11	−0.236	0.238	0.472	0.471
12	−0.226	0.233	0.452	0.452
13	−0.207	0.219	0.414	0.415
14	−0.190	0.196	0.380	0.380
15	−0.143	0.184	0.286	0.287
16	n.d.[e]	0.103	n.d.[e]	n.d.[e]

[a]From Ref. 4.
[b]From the thermodynamical model (vide supra).
[c]Calculated from column a.
[d]Calculated from column b. The quantity $S_k^{CC}+S_{k+1}^{CC}$ is reported at line k for ease of comparison with $-2S_k^{CD}$.
[e]Not determined.

scribed above with the following parameters: Vg = 500 cal/mol and ϵ = 0.3 kT.[4,35–42] The order parameters have been calculated according to Eq. (14) and reported in Table I (columns a and b). These values should be scaled by a molecular order parameter (S_{mol}) accounting for the wobbling of the chain[17] to be compared to experimental data.[15,47] However, multiplication by S_{mol} on both sides of Eq. (1) does not modify the recursion relation and the test can be performed at this level. From columns a and b we calculated the quantities $-2S_k^{CD}$ and $S_k^{CC}+S_{k+1}^{CC}$ (Table I, columns c and d). For a given position k, data presented in these columns are identical within only an error of 0.001. This is not surprising since the recursion relationship is a consequence of bond geometry. However, it is very interesting to note that Eq. (1) was established using a geometrical model[20] based on a pure tetrahedral geometry, i.e., both C_{k-1}-C_k-C_{k+1} and H-C-H angles were taken to be 109.5°, whereas calculation from the thermodynamical model was performed with an angle of 112° for C_{k-1}-C_k-C_{k+1}. Since the agreement between both methods is very good, this shows that the recursion relation does not depend too much (within a few degrees) on the dihedral condition.

We shall now comment the S^{CD} and S^{CC} profiles. Data from Table I have been plotted in Fig. 3 as a function of carbon position, k. S^{CD} exhibit a classical behavior, e.g., a plateau region followed by a decrease when going towards the methyl end.[47] S^{CC} experiences a marked odd-even effect, especially at the beginning of the chain, with $S_{odd}^{CC}<S_{even}^{CC}$. This effect has been previously observed in the case of several lipids.[20,32] It may be interesting to mention here that if one plots separately odd and even CC order parameters (not shown), one gets two smoothed independent curves, which join at around position 11. Odd order parameters start to increase from position 3 to 7 and then slowly decrease. The behavior for S_{even} is markedly different since it continuously
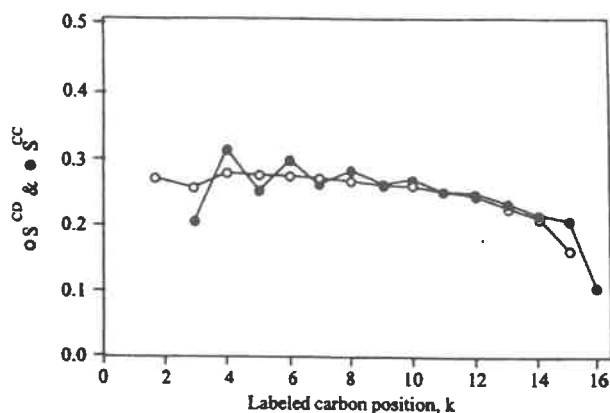
FIG. 3. S_k^{CD} (O) and S_k^{CC} (●) order parameters as a function of the carbon position, k, for the sn-1 chain of DPPC at 50 °C.

decreases from position 4 to the end. Let us make here a parallelism with the work of Lafleur.[18] These authors described the S^{CD} order profile with a smoothed function, assuming a monotonic decrease of the order along the chain. In the same way, one could use two different empirical functions to approximate odd and even S^{CC} profiles. However, while monotonic decrease appears to be justified for S_{even}, the S_{odd} profile should rather be described with a bell curve.

One can try to give an explanation to the odd-even effect by considering a chain in the *all trans* configuration with its long axis slightly tilted with respect to the rotational axis Z_D [Figs. 4(a) and 4(b)]. In such a case, all S^{CD} are equal since the angle between the C-D bond and Z_D remain the same with position k. On the contrary, S^{CC} oscillates between two values. Indeed, the angle between the C_k-C_{k+1} bond is similar to that of the C_{k+2}-C_{k+3} bond (with respect to Z_D), whereas it differs from that of the C_{k+1}-C_{k+2} bond. Let us remind here that in our computational calculations, the odd-even effect occurs eventhough no tilt of the acyl chain is accounted for. However, such a tilt of the chain can appear when a *gauche* defect stands on a methylene group [Fig. 4(c)]. Following the isomerization, the long axis of the rest of the chain is tilted with respect to Z_D. This then would
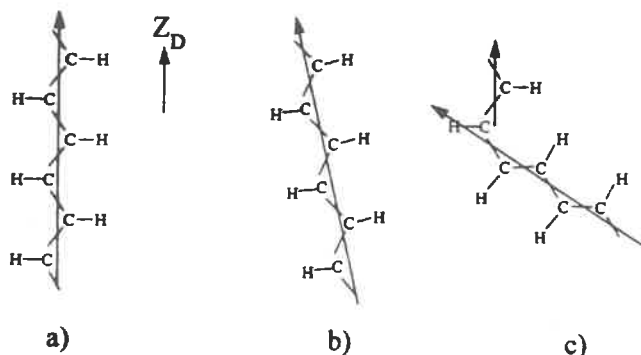

FIG. 4. Schematic representation of an acyl chain needed for the description of the S^{CC} odd-even effect. Z_D is the diffusion axis of the molecule, while the other arrows indicate the long axis of an acyl chain portion. (a) In the all trans case with the long axis parallel to Z_D. (b) In the all trans case where the long axis is slightly tilted with respect to Z_D. (c) With an isomerization occurring in the middle of the chain: the long axis of the beginning of the chain is parallel to Z_D, while the rest is tilted.

lead to odd-even S^{CC} for this part of the chain. In other words, the occurrence of the odd-even effect gives evidence that, in average, the chain is tilted with respect to Z_D. In our computational calculations this effect occurs at the beginning of the chain and slowly decreases on going to position 11. This could mean that the average orientation of the chain is bent, the curvature decreasing as one reaches position 11. Towards the chain end, the S^{CC} profile follows that of S^{CD} accounting for the increase in conformational freedom. The average axis orientation of this part of the chain would be parallel to Z_D.

It must be reminded here that this odd-even effect has already been observed on sn-2 DL- DM- and DPPC,[20] palmitic acid[21] and sn-1 DMPC chains.[32] While the amplitude (Δ) of this odd-even effect was roughly constant as a function of temperature for palmitic acid and sn-1 chains, sn-2 chains experience a marked increase of Δ with temperature having started to roughly zero near the phase transition. This could mean that sn-1 chains are tilted, in average, independently of the temperature. At contrary and close to the phase transition, the average orientation of the beginning (from C_3) of sn-2 chains would be parallel to the long molecular axis. This could be due to the fact that the carbonyl region of this chain is already bent.[48-53] For higher temperature, the amplitude of the odd-even effect increases, in other words, the average orientation start to be bent what could be a consequence of the increase of lipid area.[54]

In order to reinforce the above remarks, it would be necessary to determine the orientational distribution function of the acyl chain. This could be done experimentally from IR-ATR experiments by determining the distribution function of the transition moment.[55,56] This could also be studied from computational methods, with the method as described above, or from molecular dynamic simulations, where it is possible to calculate the average orientation of the chain and then predict whether it is tilted or not. These calculations are under progress in our laboratory.

We were not able to determine any recursion relationship between S^{CD} and S^{CC} in the case of unsaturated chains. When the double bond stands in position C_9-C_{10}, it is not possible, *a priori*, to calculate S_{10}^{CC} even if one knows S_{11}^{CC} and S_{10}^{CD} (Fig. 2). However, we can use the advantage that the double bond is rigid for continuing to calculate S^{CC} order parameters along the whole chain. Let us first envisage the case of a *cis* double bond in position 9, 10 [Fig. 2(b)]. Let us suppose that it is possible, as we have shown above in the case of saturated chains, to calculate S^{CC} from the methyl terminus to S_{11}^{CC}, from a perdeuterated chain for instance. As a consequence of the double bond (no rotation occurring around the C_9-C_{10} bond) C_{10}-C_{11} and C_9-H bonds on one hand and C_9-C_8 and C_{10}-H bonds on the other hand remain parallel and then Eq. (11) applies. It is then possible continuing calculation of S^{CC} from S_9^{CC} to S_2^{CC}. In the case of the *trans* conformation, a similar approach can be carried out from the experimental knowledge of all S^{CD} order parameters and especially S_9^{CD} and S_{10}^{CD}. The latter order parameters can be predicted to be equal because the C_9-H and C_{10}-H bonds are parallel [Fig. 2(a)]. Then for the same reasons [Eq. (13)] it comes: $S_{11}^{CC} = S_9^{CC}$. If one is able to cal-

culate S_{11}^{CC} using Eq. (1) one can also determine S_9^{CC} and then all other S^{CC} order parameters on going towards the C_2 position. One can therefore conclude that whatever the *cis* or *trans* conformation of the double bond, it is still possible to calculate S^{CC} for all positions, but that of the double bond, when going from the methyl-end towards the polar head using a perdeuterated sample and Eqs. (1), (12) and (13).

IV. CONCLUSION

We have demonstrated from a thermodynamical point of view that there exists a recursion relationship linking S^{CC} to S^{CD} order parameters which offers a valuable basis to calculate physical parameters of saturated acyl chains embedded in biomembranes. This reinforces the geometrical model we already successfully applied to experimental data.[20,21,32] However, it was unsuccessful, *a priori*, to develop an analog relation for unsaturated chains. Nonetheless, S^{CC} can still be calculated along the whole unsaturated chain except for one carbon of the double bond. We have also proposed an interpretation of the odd-even effect occurring on the S^{CC} order profile in terms of average tilt of the chain.

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