THEORETICAL STUDY OF SPIN-LABELLED ALIPHATIC CHAINS IN BILAYERS

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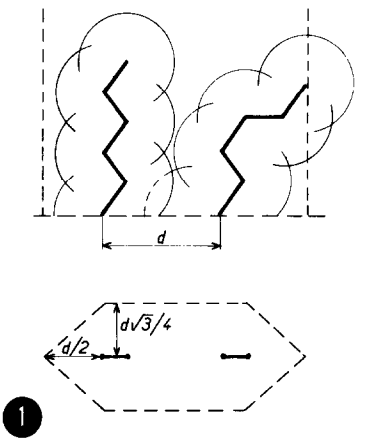
SUMMARY

Some authors have studied the spin-labelled order parameter in phospholipidic bilayers. It is now possible to calculate this parameter through rotation isomer theory and molecular interactions (WIRI model). Its variation along the chain is described. A good agreement is shown with experiments. Therefore an important theoretical parameter is the distance d between closed chains. Theoretical d values for agreement are a little too large.

We recently proposed a theoretical model to study some structural properties of aliphatic chains in mono and bilayers (1-4). WIRI model implies rotational isomerism around carbon bonds and chain interactions (dispersion forces). Two chains are enclosed in an hexagonal box. First of all, calculations were applied to hexan chains. More recently large chain melting entropy was calculated (4) (n = 14 to 22 carbon atoms). We now present a new application for large chains : the calculation of spin label parameter S_3. For many years spin label techniques have proved a very powerful tool to study aliphatic chain fluidity in bilayers and biological membranes (5). A nitroxide radical is bound to a fatty acid or phospholipid carbon atom. Microscopic fluidity change of the labelled carbon atom can be followed by putting consecutively the radical on carbon atoms along the chain. A theoretical order parameter S_3 can be measured with this technique. It is defined as : (6)

$$S_3 = \frac{1}{2}\,(3 < \cos^2 \theta_3 > -1).$$

θ_3 is the angle between the axis normal to the layer and the $p\Pi$ orbital of the nitrogen atom of the radical.



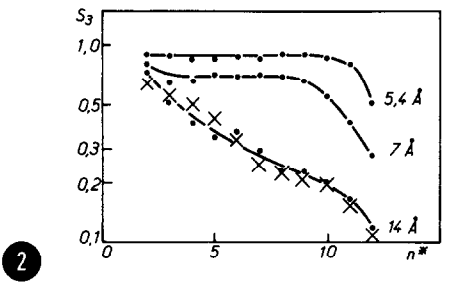


Fig. 1 - The two aliphatic chains in the hexagonal box (WIRI model).

Fig. 2 - Order parameter as a function of number n^* of the carbon atom (semilog plot) : (•) theoretical data for various d values ; (X) experimental data for decanol-sodium decanoate mixture and stearic acid as spin label (see figure 8).

Order parameter calculation

It is now possible to calculate the S_3 parameter with methods described earlier for short (3) and large chains (4). It must be remembered that only interacting chains are enclosed in an hexagonal box (see figure 1).

Figure 2 gives a semilog plot of the order parameter S_3 versus n^* the carbon atoms number between paramagnetic N-oxylaxazolidine ring and polar head. Each curve corresponds to a specific d value for the distance between the two chains in the box. Calculations were carried out at room temperature (T = 25°C) and with an energy difference between gauche and trans isomers $\Delta U^\circ_\circ$ = 600 cal. $mole^{-1}$. Lennard-Jones potentials we have used have been previously described (see table I in reference 3).

Figure 2 shows a general S_3 decrease along the chain (maximum value is obtained near polar head). The log S_3 variation is a linear one for large d values (when dispersion forces are going to cancel). On the contrary, linear variations is not observed when distance d is small. In both cases, carbon atoms have little freedom near the polar head. For very small distances,

the S_3 parameter increases and tends towards 1 which is the value for the rigid chain as in the crystal. On the contrary S_3 values can be very small at the chain end. Therefore we can discern two carbon atom states along the chain for intermediate d values : i) a rigid state (for small n^* values). The chain between the polar head and the nitroxide radical is extended, almost in the trans conformation. This state is close to that of crystalline chains ii) a fluid state (at the chain end). Each carbon atom is delocalized between trans conformation and numerous gauche conformations. Disorder is almost the same as in liquid alkanes. Therefore we can see along the chain, at the molecular level, a melting-like transformation from "crystalline" carbon sequences to "liquid" ones (indeed chains form a "liquid crystal" in the second state).

Comparison between theoretical and experimental results is not very easy. Spin label and bilayer molecules are very often different (5-7). The S_3 values and the shape of the curve depend on the nature of the spin label(6). In the lecithin-cholesterol bilayer log S_3 shows a linear variation versus n^* with a fatty acid as a spin label (as for theoretical variation with large d value). On the contrary the experimental curve is not linear with a phospholipid as spin label in the same lecithin-cholesterol bilayer. In the last case, the shape of the experimental curve is analog to the theoretical one for small d values.

We have tried to compare our calculations with the results of Seeling and al. (8). In a particular experiment, these authors studied bilayers made of a mixture of sodium decanoate and decanol. As the bilayer molecules, the stearic acid label has only one chain and is saturated. Unfortunately, the spin label molecule is longer than the chains of the bilayer (n = 16 and 10 carbon atoms respectively).

The data from Seelig and al. are included in figure 2. A good agreement is observed with d = 14Å. But this theoretical value is very high. In the crystalline state, the distance between the chains is only

4.8 Å. In the liquid crystal state, this distance must be higher than in the crystal but no as much as 14 Å. It is also important that this experiment gives stearic acid label S_3 values and not decanol-decanoate parameter. The local fluidity is probably higher near the spin label carbon atom than in the bulk bilayer for two reasons : i) the steric hindrance of the nitroxide group, ii) the spin label chain length larger than neighbour molecules. This experimental perturbation can give us an apparent d value higher than the actual one.

However, the theoretical value of 14 Å is probably too high. The explanation of this enhancement is clear. Until now our calculations were made with two packed chains in an hexagonal box (see figure 1). The actual chain end can escape from this theoretical box. Therefore they have a little more fluidity than in our two chains model. For an experimental S_3 value, agreement with theory is obtained with a d_{th} value higher than the actual value d_a. The positive difference, $d_{th} - d_a$, allows us to correct the fluidity loss due to the hexagonal box (the fluidity increases as d).

New calculations are now carried out in our laboratory, without the box but with 10 interacting chains. Preliminary results are in agreement with the former ones, but with lower d values.

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