USE OF THE RADICAL ANION TCNQ$^{\overline{\cdot}}$ AS A SPIN PROBE OF THE POLAR HEADS TRANSITION IN SOME PHOSPHOLIPIDS

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1. Introduction

Biological membranes are constituted of lipids and proteins. Their general molecular organization is now well known: phospholipid bimolecular leaflet in which proteins are more or less embedded [1,2]. Important results have been obtained on the structure of phospholipid aliphatic chains principally on their order–disorder transition by differential scanning calorimetry [3] fluorescent [4] and spin-labelled probes [5].

But the structure and conformational changes of the membrane–water interface constituted mainly of the phospholipid polar head groups is not entirely understood. Recently nmr studies of phosphorus [6] and deuterated choline moiety [7] showed that transition of phospholipid polar heads occurs. In this paper we report preliminary results obtained with a new ESR probe, the tetracyanoquinodimethane (TCNQ) [8]. This molecule is a strong π acid and leads to charge transfer complexes with a variety of Lewis bases; with the quaternary ammonium functions a stable radical anion TCNQ$^{\overline{\cdot}}$ is formed [9].

2. Material and methods

Dimyristoylphosphatidylcholine (DMPC) was purchased from Sigma; TCNQ was from Aldrich and Fluka and purified by sublimation. All reagents were of analytical grade and used without further purification. The best intermediary solvent was the chloroform, because it dissolves both TCNQ and phospholipid. The concentration of TCNQ was 1 mol per cent of phospholipid. The mixture of the three components was lyophilized to remove chloroform and water was then added to obtain liposomes, i.e., multibilayer structures. The ESR spectra were obtained with the X band of VARIAN 4502 equipped with a thermal variation for aqueous cells. The theoretical calculations of spectra were carried out with an IRIS 280 CII computer.

3. Results and discussion

3.1. *Experimental results*

In aqueous solution the mixture phospholipid–TCNQ is green indicating that a radical anion TCNQ$^{\overline{\cdot}}$ is formed [9]. The observed spectrum shows a 9-line hyperfine structure (fig.1a) at high temperatures which progressively disappears when temperature decreases (fig.1b). This result proves that a change in the motion of TCNQ$^{\overline{\cdot}}$ occurs. This effect is certainly related to an increase of the motion of ammonium moiety of the dimyristoylphosphatidylcholine when temperature goes from 6°C up to 30°C. The radical anion interacts by electrostatic forces with $\overset{+}{N}(CH_3)_3$ and forms a stable salt as those known in the solid phase [10]. It is necessary to notice that the salt [10] $(H\text{-}\overset{+}{N}(CH_3)_3)_2\ (TCNQ^-)_2\ TCNQ^\circ$ in dilute aqueous solution exhibits a classical hyperfine spectrum constituted in principle of 45 lines [11]. This comparison indicates indeed that TCNQ$^{\overline{\cdot}}$ interacting with DMPC does not behave as a free radical in solution.

3.2. *Theoretical analysis*

Since we observe nine lines (fig.1a) we deduce that only the hyperfine coupling with nitrogens are visible (four equivalent nitrogens with nuclear spin equal to 1). So we can assume that the pseudo aromatic ring of

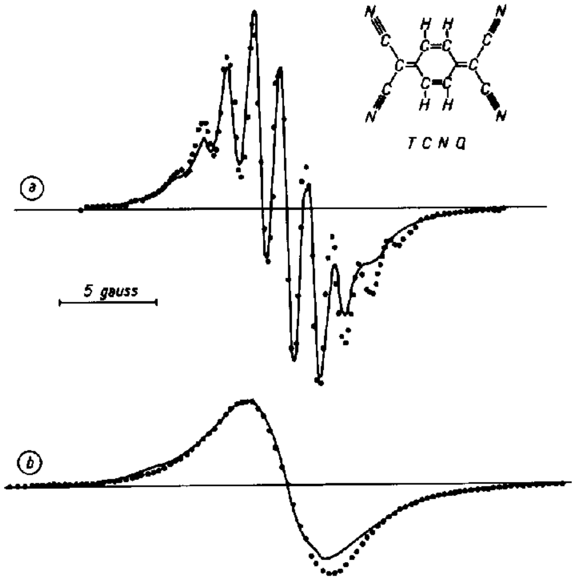

Fig.1. Experimental (full lines) and calculated (black points) EPR spectra of the radical anion $\mathrm{TCNQ}^{\overline{\cdot}}$ on DMPC: At T = 29°C (fig.a) and at T = 10°C (fig.b).

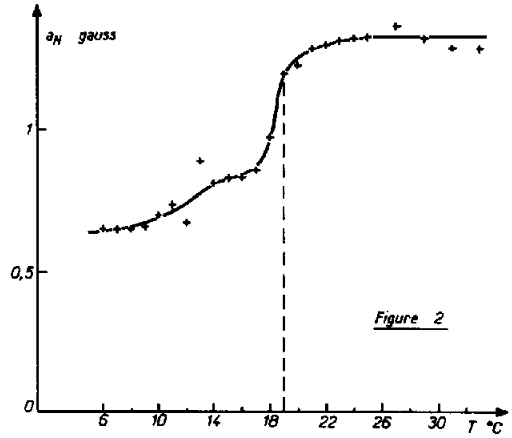

Fig.2. Thermal variation of the calculated hyperfine splitting constant a_N.

$\mathrm{TCNQ}^{\overline{\cdot}}$ is rigidly bound to the three methyl of the $\overset{+}{\mathrm{N}}$-$\mathrm{CH_3}$ group. Consequently we are not able to detect the hyperfine structure due to the four protons because the linewidth is too broad due to the absence of motional narrowing.

In order to carry out the spectra simulation we used: (a) the static spin Hamiltonian in which the mean values of the g factor and the hyperfine coupling constant with nitrogen a_N are involved [12] and (b) the lines have a Lorentzian shape characterized by the linewidth ΔH between inflexion points.

We compare the experimental and calculated spectra by selecting the best values for the two parameters a_N and ΔH. A least square calculation on the ordinates gives a good fit (see fig.1).

Every experimental spectrum has been fitted by applying this technique and the thermal variation of the two parameters has been deduced (fig.2 and 3): a significant change of their behaviour is found around 19°C. We attribute this phenomenon to an order-disorder transition on the polar heads of DMPC in agreement with RMN results [6,7].

We can analyse each parameter: (a) The linewidth variation around 19°C indicates that the spin–spin relaxation time T_2 ($T_2 \simeq 10^{-8}$ s) is modified in a similar way as the aliphatic chains labelled by nitroxyde radicals [12]. (b) The hyperfine constant coupling a_N changes from 0.8 to 1.3 guass while going through the transition temperature; for an homogeneous solution Fischer and McDowell [11] gave a_N = 1.02 guass, value which is just in a rough agreement. Following theoretical calculations from Jonkmann and Kommandeur [13], who have extended McConnell's empirical relation between a_N and the spin density at the nucleus, we obtained a_N = 1.227 gauss. This value is close to ours at high temperature. The π electron spin density is supposed to be ρ_N = 0.03 for such calculation [14]. Now we must

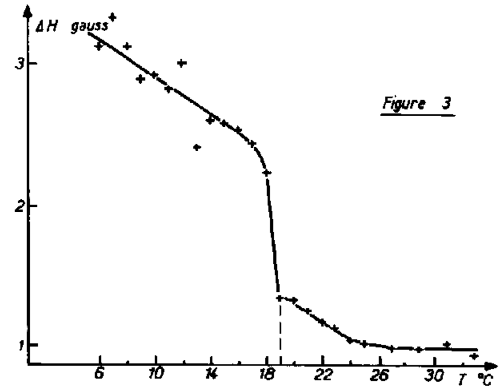

Fig.3. Thermal variation of the linewidth ΔH for a supposed Lorentzian line.

assume that, at the phase transition, there is a variation of this value related to a change of the positive charge distribution of the neighbours. Evidently a quantitative analysis has to be carried out to explain how the spin density of $TCNQ^-$ can be modified through its variation of the charge density and from the surrounding water.

3.3. *Conclusion*

In this paper we have shown that it is possible to obtain a radical anion $TCNQ^{\overline{\cdot}}$ interacting with the quaternary ammonium moiety of DMPC. During the preparation the electron can come from the ions present in the aqueous solution but this must be proved in further experiments.

The found transition temperature is a little lower than that of aliphatic chains (T_c = 23°C) and has to be compared with the pretransitional effect [3]. So this radical plays the role of a spin label for the polar head at least for phospholipids bearing a positively charged ammonium group. Experiments are underway to demonstrate if this interesting specificity really exist.

Acknowledgements

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