Heterogeneous Lipid Distribution in Rod Outer Segment Membranes: a Spin Label Study

An important effort in biophysical research is at present being devoted to gaining knowledge of the molecular organization of biological membranes. A variety of physical techniques such as differential scanning calorimetry[1–3], X-ray diffraction[4], fluorescence[5,6] and nuclear magnetic resonance spectroscopy[7,8], have been applied to study lipid phase transitions.

From recent evidence, a new concept concerning structural properties of biomembranes has been developed. The sustaining idea tends to consider the membrane as a two dimensional fluid in which liquid and semicrystalline phases are in equilibrium within a specific range of temperature[1,9,10]. For elaborating this model, information was derived from spin label studies[9–12]. Paramagnetic probes are very useful for detecting structural and dynamic modifications occuring within a lipid layer. Phase transitions were detected with such techniques in mycoplasma membranes[13] as well as in mitochondrial membranes of sweet potato roots and of rat liver[14]. More recently, a spin label study of *E. coli* membrane vesicles revealed the presence of two phase transitions[15].

The purpose of the present communication is to report our results on the influence of temperature on spin probe mobilities incorporated in retinal rod outer segments (ROS). Several transitions were detected, suggesting the presence of heterogeneous lipid distribution in ROS membranes.

Materials and methods. ROS membranes from cattle eyes were prepared as described previously by de Grip et al.[16]. All manipulations were carried out in dim red light. Within 2 h after death of the animals, 30 retinas were dissected and gently homogenized in 12 ml ice cold *Tris*-HCl buffer (0.16 *M*, pH 7.1). The homogenate was filtered through 120 mesh screen and the residue washed with *Tris*-HCl buffer. The combined filtrates (30 ml) were mixed with aqueous sucrose (10.5 ml of 66% sucrose w/w) to a final concentration of 0.42 *M*. This suspension and a 45 ml volume of 40% (w/w) sucrose solution were used to prepare continuous gradients (0.42–0.18 *M*). After 1 h of centrifugation in a swing-out rotor (27,000 × *g*, 10 °C), the upper layer containing ROS was collected. This fraction was then incubated with 11-*cis* retinaldehyde[17] (25 n*M* per retina) in order to regenerate rhodopsin. The retinaldehyde in excess was then eliminated by enzymatic reduction with NADPH (0.1 mg per retina). After the enrichment procedure, a second density gradient was prepared with 40% sucrose (1 h, 27,000 × *g*, 10 °C) yielding a single layer. The ROS were washed once in *Tris*-HCl buffer and twice in distilled water. After washing, the material was lyophilized. The yield was 1.5 to 2 mg per eye. 1 mg of the lyophilized material dissolved in 1 ml of Triton-X-100 detergent (2% w/w) gives a difference in absorbance before and after illumination at least equal to 0.25 O.D. at 500 nm (1 cm light path).

For the present investigation, 3 spin labels were used: 2,2,6,6 tetramethylpiperidine-1-oxyl (Tempo) (structure I) and two N-oxyl-4,4′ dimethyloxazolidine derivatives of stearic acid (structure II).

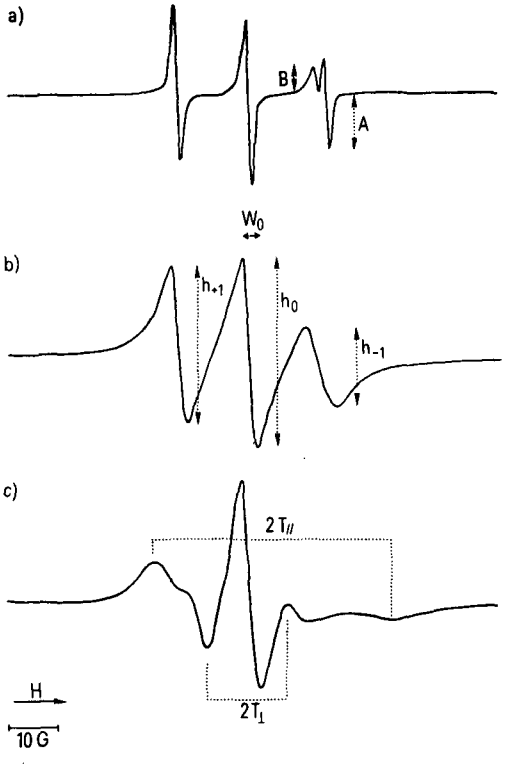
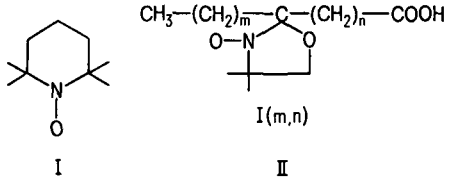


Fig. 1. Electron spin resonance spectra at 25 °C of rod outer segment membranes. a) suspended in Tempo solution (10^{-3} *M* in *Tris*-HCl, pH 7.1); b) labelled with I(1.14) and c) labelled with I(12.3): Label concentration 2:100 w/w. Membranes suspended in *Tris*-HCl, pH 7.1. Modulation amplitude: 0.5 Gauss. Microwave power: 4 mW.



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Labels I(1.14) and I(12.3) were purchased from Synvar (Palo Alto). Tempo was synthetized as described by ROZANTZEV[18].

Usually 5 mg of lyophilized material were resuspended in 35 µl of Tempo solution (10^{-3} *M* in *Tris*-HCl buffer, pH 7.1). Fatty acid spin labels were incorporated in ROS membranes as follows: the labels were first dissolved in ethanol (1 mg/ml). 100 µl of the solution were evaporated to dryness under N_2. Lyophilized material (5 mg) was then added and the suspension made up with 35 µl of *Tris*-HCl buffer. The suspension was carefully homogenized. For electron spin resonance (ESR) measurements, the labelled membranes were introduced in Pasteur pipettes. Spectra were recorded on a Varian E-3 spectrometer equipped with a variable temperature control unit. Temperature could be controlled within a 0.25 °C range and was measured with a pyrometric resistor (Brion-Paris).

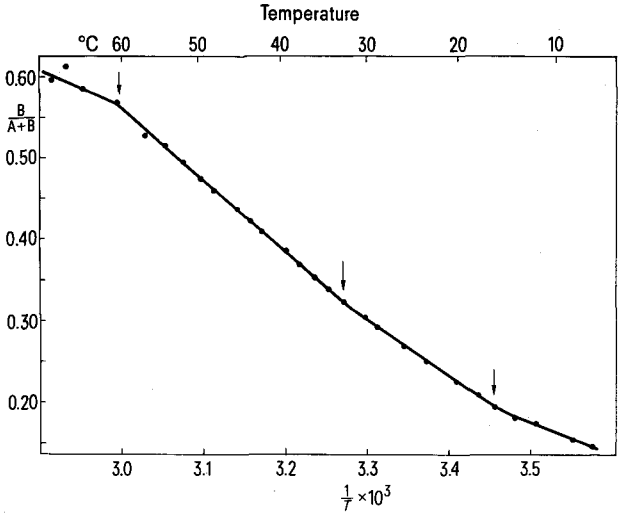

Fig. 2. Tempo spectral parameter B/A + B versus $1/T$ for rod outer segment membranes. 5 mg of lyophilized material in 35 µl of Tempo solution 10^{-3} *M* in *Tris*-HCl.

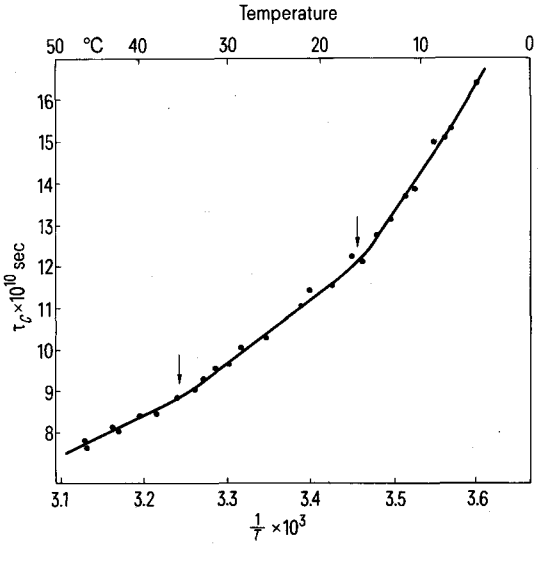

Fig. 3. Correlation time for spin label I (1.14) incorporated in rod outer segment membranes versus $1/T$. 5 mg of lyophilized material in 35 µl of *Tris*-HCl buffer. Label concentration 2:100 w/w.

Results and discussion. Figure 1 shows representative ESR spectra of ROS membranes labelled with the 3 different labels. The first spectrum (a) was obtained with Tempo. It has been shown[19] that such molecule partitions between aqueous solution and the hydrophobic region of the membrane. A semi-quantitative analysis of Tempo partition between the two phases can be obtained by measuring the solubility parameter B/A + B[20]. The second spectrum (b) characterizes ROS labelled with I(1.14). As the nitroxide group is located close to the apolar end of the fatty acid molecule, the label motion is virtually isotropic and consequently a correlation time can be measured by using the well known formula[21]:

$$\tau_c = 6.82 \times 10^{-10} \Delta W_o \left[\sqrt{\frac{h_o}{h_{-1}}} - \sqrt{\frac{h_o}{h_{+1}}}\right]$$

where ΔW_o is the width of the central peak in gauss while h_{+1}, h_o and h_{-1} are respectively the heights of the low, middle and high field lines. The third spectrum (c) describes the anisotropic motion of label I(12.3) around the longitudinal molecular axis. An order parameter S has been introduced to measure the amplitude of the chain flexibility[22,23].

$$S = \frac{T_{//} - T_{\perp}}{T_{zz} - T_{xx}}$$

The separation between the two outer and the two inner hyperfine extrema of the spectra corresponds respectively to $2T_{//}$ and $2T_{\perp}$. On the other hand, $2T_{zz}$ (61.6 gauss) and $2T_{xx}$ (11.6 gauss) represent the maximum and minimum hyperfine splittings and can be derived from crystal analysis[22].

Figure 2 shows an Arrhenius plot of B/A + B over a large temperature range (5–70 °C). Three small breaks are apparent at 17 °C ± 2 °C, 33 °C ± 2 °C and 60 °C ± 2 °C respectively. The change in slope observed at high temperature occurs simultaneously with thermal rhodopsin denaturation[24].

The two breaks observed with Tempo below the temperature of rhodopsin denaturation were reinvestigated using fatty acid spin labels. Figure 3 relates to probe I(1.14) and gives a plot of the correlation time τc versus $1/T$. Two changes in slope are clearly visible at 16 °C ± 2 °C and 34 °C ± 2 °C. For label I(12.3), S was plotted as a function of $1/T$ (Figure 4). Although the breaks are less pronounced in this latter case, they seem to occur about at the same temperature as with the two other labels.

The set of results presented above establish unambiguously the presence of three specific thermotropic modifications in ROS membranes at 16 °C ± 2 °C, 34 °C ± 2 °C and 60 °C ± 2 °C. At the present time, it is difficult to describe the origin of these modifications at a molecular level. The transition occuring at 34 °C may reveal a modification in the lipid-protein interaction. We observed that the chemical reduction of Tempo in

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ROS membranes involves mainly the membrane proteins[25] and that the activation energy relating to this reaction undergoes a drastic change around 30 °C[26].

Whatever the molecular origin of the various transitions may be, they indicate unquestionably the presence of an heterogeneous structure in ROS. If a liquid semi-crystalline equilibrium exists in ROS membranes, it could be modified under light influence since the membrane fluidity increases after illumination[27, 28].

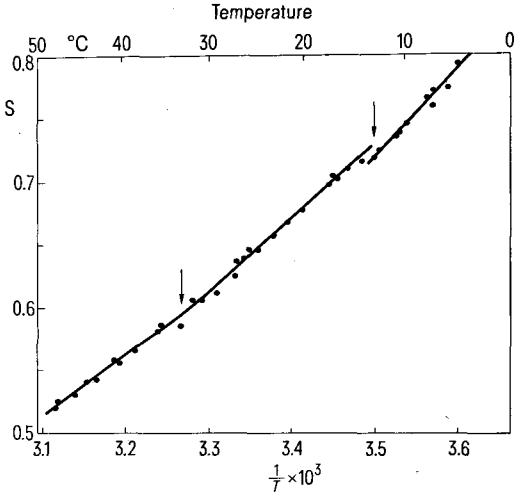

Fig. 4. Order parameter for spin label I(12,3) incorporated in rod outer segment membranes versus $1/T$. 5 mg of lyophilized material in 35 µl of *Tris*-HCl buffer. Label concentration 2:100 w/w.

Résumé. La technique du marquage par spin est utilisée pour étudier l'inhomogénéité de la distribution des lipides dans les membranes des segments externes des bâtonnets. Cette étude révèle la présence de trois transitions respectivement à 16°, 34° et 60 °C. La transition à 34 °C traduit vraissemblablement une modification de l'interaction entre les lipides et la rhodopsine.

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Effect of Water Temperature on the Predatory Efficiency of *Gambusia affinis*

Predation of mosquito larvae by the fish *Gambusia affinis* has so far been studied with reference to the influence of several biotic factors concerning either the prey or the predator[1, 2]. The occurrence, distribution and success of aquatic predators may also be largely dependent on the physico-chemical conditions operative in natural waters[3]. The physico-chemical complex of freshwater bodies where mosquitoes breed are known to fluctuate from time to time[4]. Maglio and Rosen[5] observed that the feeding behaviour of *G. affinis* was a direct response to the water temperature. A deeper knowledge on the effect of water temperature influencing the predatory efficiency of *G. affinis* would give an insight into the regulatory control mechanism of several mosquito populations by this predator.

Materials and methods. A B.O.D. incubator, with arrangements for illumination, was used to maintain constant temperature of 20, 25 or 30 °C. Illumination was controlled for 10 h per day during the experimental period. *Gambusia affinis* collected from field were grouped into male, non-gestating female and gestating female, depending on their sex and physiological state. 5 individuals of similar body size were taken from each group by random choice for each experiment. The fish were placed in aquaria containing 1 l aerated freshwater. The fish were acclimated in these aquaria to the respective temperature for 3 days prior to the start of the experiment. The experimental food organisms, consisting of 25 live 4th instar larvae of *Culex fatigans*, were introduced into each of the aquaria without disturbing the test fish. The prey was exposed to predation for 10 h a day. Since the experiments were repeated on 3 successive days, the performance of 5 individuals yielded a total of 15 observati ons in each series.

Results. On an average, a non-gestating female predated 12 larvae in 10 h at 20 °C and its predatory efficiency increased to a maximum of 23 larvae with increase in temperature to 30 °C (Table). Gestating females also increased their predatory efficiency from 16 larvae at 20 °C to 24 larvae at 30 °C. The differences observed in the predatory efficiency at the 3 selected temperatures for these fish were statistically significant. Male *Gambusia affinis* predated only 10.5 larvae at 20 °C, while at 30 °C the fish predated 15 larvae. The increase observed in predatory efficiency of male at 25 °C was not significant. Thus, the magnitude of reduction in predatory efficiency depends upon sex and physiological status of the fish. Nongestating and gestating females exhibited increase in predatory efficiency with 5 °C rise in temperature, while male displayed similar increase only when there was 10 °C rise in temperature. Similar dependence of predatory efficiency on sex and physiological state of this fish was also observed when the space provided per fish was changed[6].

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