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Microviscosity of the cell membrane

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SUMMARY

The microviscosity in the membrane interior of hemoglobin-free erythrocyte membranes, lymphocytes and submitochondrial particles was derived from an adequate comparison of the degree of fluorescence polarization of perylene when dissolved in the tested membranes and in a reference oil of known viscosity. The membranes tested showed a highly viscous liquid interior with microviscosities in the range of 100–200 cP.

Trypsin digestion of hemoglobin-free erythrocyte membranes had almost no effect on the microviscosity while, on the other hand, phospholipase C digestion made the membranes much more fluid. The derived fusion activation energies for the different membranes are reported.

In a recent publication Shinitzky *et al.*[1] showed that the viscosity in the detergent micelle interior (referred to as microviscosity) could be derived from an adequate comparison of the degree of fluorescence depolarization of perylene or 2-methylanthracene when dissolved in the tested micelles and in a reference oil of known viscosity. The results showed the micelle interior to be highly liquid, the determined microviscosities at 27 °C were all in the range of 17–50 cP. The same procedure has now been extended to measure the microviscosity in the interior of hemoglobin-free erythrocyte membranes, intact lymphocytes and submitochondrial particles.

The procedure used was essentially that previously published[1]; perylene (K and K Laboratories) was dissolved in acetone and evaporated with stirring onto clean glass beads

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(160–200 mesh). A given amount of perylene-coated glass was incubated with the cell suspension at 37 °C for periods of up to 60 min. The glass was eliminated by low speed centrifugation and the cell suspension was sedimented at the appropriate high speed. Fluorescence was measured in a polarization apparatus built according to the design of Weber and Bablouzian[2]. The excitation wavelength (411 nm) was selected from a 500-W Xenon lamp by a Bausch and Lomb 500 grating monochromator and a Bausch and Lomb interference filter. The emission was measured at 470 nm by the use of a Jarrell–Ash monochromator and a Corning 273 sharp-cut glass filter. Under the conditions measured, the scattering of the membrane suspensions was in all cases less than 2% of the fluorescence signal. Life-time measurements and corrections were as previously reported[1]. We assumed that the life-time of the excited state of perylene in the membranes was that measured in rabbit erythrocyte membranes by A.C. Dianoux and G. Weber (personal communication) (τ = 6.5 ns).

Human hemoglobin-free erythrocyte membranes were prepared as described[3], human peripheral lymphocytes by the method of Mendelsohn *et al.*[4], and bovine heart submitochondrial particles by the procedure of Fessender and Racker[5].

The membranes were found to readily take up the perylene when incubated with the coated glass beads at 37 °C. Fig. 1 shows the time course of the incorporation of perylene into human hemoglobin-free erythrocyte membranes. The measured polarization of the fluorescence emission was found to be independent of the amount of perylene incorporated into the membranes. This indicates that within this range of concentration (approx. 10^{-7} M),

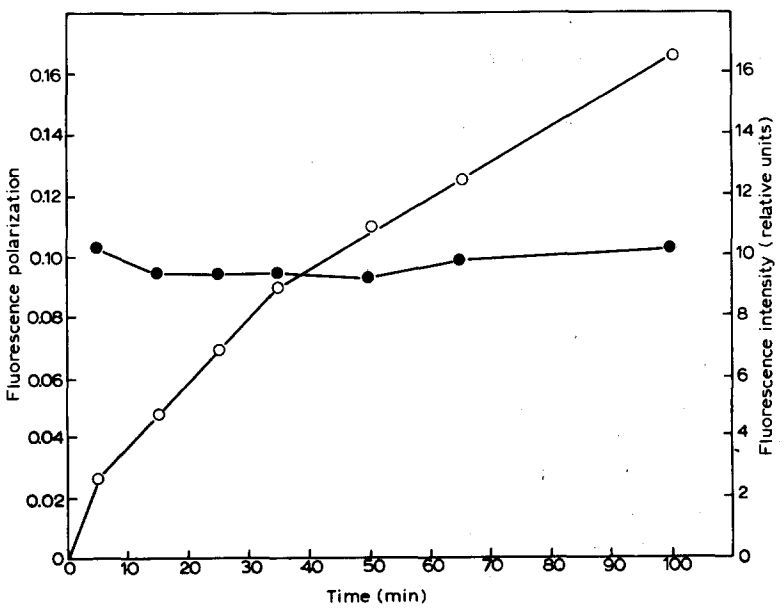

Fig.1. Degree of incorporation of perylene into hemoglobin-free erythrocyte membranes (o——o) and the polarization of its fluorescence emission (•——•).

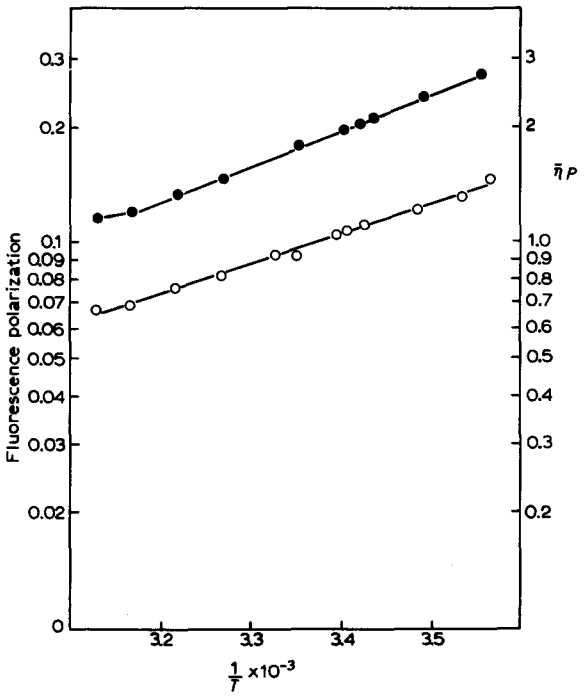

Fig.2. Temperature dependence of the fluorescence polarization, P (o—o) and derived microviscosities $\bar{\eta}$ (●—●) for perylene incorporated into hemoglobin-free erythrocyte membranes.

the perylene is present in equivalent sites and there is no apparent depolarization due to energy transfer among neighbouring molecules[6].

Fig. 2 shows the temperature dependence of the fluorescence polarization and of the derived microviscosity ($\bar{\eta}$) for perylene incorporated into human hemoglobin-free erythrocyte membranes. With the possible exception of the highest temperature tested, all the data fit a simple exponential relationship, which, in the case of the variation of $\bar{\eta}$ with temperature, can be described by the equation[1]

$$\bar{\eta} = Ae^{\Delta E/RT}$$

where ΔE is the so-called fusion activation energy. Table I shows the derived values of $\bar{\eta}$ and ΔE for perylene incorporated into different membranes and under various conditions. The hemoglobin-free erythrocyte membranes show the highest viscosity of the membranes tested. Increasing the external ionic concentration from hypotonicity to isotonicity decreases the apparent microviscosity although it does not alter the ΔE values. Submitochondrial particles and preliminary results with cholinergic synaptosomes ($\bar{\eta} = 85$) indicate that in these membranes the perylene is located in a more fluid environment. The ΔE values observed are all similar to those found previously[1] for perylene incorporated into cetyltrimethylammonium bromide micelles containing cholesterol (ΔE 5.2), but much lower

than those recorded for perylene in detergent micelles in the absence of additives (ΔE 9.6), or for those of the reference oil. Since submitochondrial particles are devoid of cholesterol an explanation for the ΔE values is, as yet, not apparent. The viscosity values recorded are of the same magnitude as those calculated by Frye and Edidin[7] based on the diffusion of antigenic determinants in the membrane plane and of Cone[8] from rhodopsin rotational movements.

TABLE I

DEGREE OF FLUORESCENCE POLARIZATION (P), DERIVED MICROVISCOSITIES AND FUSION ACTIVATION ENERGIES FOR PERYLENE INCORPORATED INTO DIFFERENT MEMBRANES

At 37 °C the values of $\bar{\eta}$ were derived from the equation

$$r_0/r = 1 + \frac{k\,T\,\tau}{\eta\,v\,(r)}$$

where r is the observed molecular anisotropy, r_0 is the observed anisotropy for an immobilized molecule; T is the absolute temperature, η is the viscosity of the medium, $v(r)$ is the variable effective volume of the rotating fluorescent sphere, τ is the average life-time of the excited state and k is the Boltzmann constant.

Membrane	*P × 100*	*$\bar{\eta}$ (cP)*	*ΔE (kcal/mole)*
Hemoglobin-free erythrocyte membranes in 10 mM Tris–HCl buffer (pH 7.4)	8.4	132	3.9
Hemoglobin-free erythrocyte membranes in 0.155 M NaCl (pH 7.4)	8.0	120	3.9
Submitochondrial particles	7.5	95	4.9
Lymphocytes	7.9	109	4.2
Reference oil	5.9	65	11.0

Table II describes the effect of trypsin and of phospholipase C digestion of hemoglobin-free erythrocyte membranes on the polarization, $\bar{\eta}$ and ΔE values derived from the perylene fluorescence. Treatment with trypsin has almost no effect while that with phospholipase C markedly decreases the viscosity while leaving the ΔE values unchanged. These latter observations are in agreement with the recent findings of Simpkins *et al.*[9], who observed an enhancement in the mobility of an incorporated spin-labelled fatty acid following phospholipase C treatment of axonal membranes.

It is not possible, based on the present results, to establish unequivocally the actual localization of the perylene in the membrane. However, it would seem that it is probably located in the vicinity of the terminal portions of the phospholipid chains. Thus, when incorporated into dipalmitoyllecithin liposomes, a phase transition is detected at about

TABLE II

DEGREE OF FLUORESCENCE POLARIZATION, DERIVED MICROVISCOSITIES AND ACTIVATION ENERGIES FOR PERYLENE INCORPORATED INTO HEMOGLOBIN-FREE ERYTHROCYTE MEMBRANES WITH DIFFERENT TREATMENTS

At 37 °C the values of $\bar{\eta}$ were derived as in Table I, assuming the same value of τ for the different treated membranes.

Treatment	$P \times 100$	$\bar{\eta}$ *(cP)*	ΔE *(kcal/mole)*
None	8.6	138	4
Trypsin digestion	8.4	125	4.3
Phospholipase C digestion	5.7	45	4

40 °C which closely relates with the melting of the hydrocarbon chains. In addition, the fact that digestion with trypsin, which removes some 50% of the membrane protein, does not alter the determined fluorescence polarization while treatment with phospholipase C markedly reduces the apparent microviscosity, strongly suggests that the apolar perylene is located in the hydrocarbon region of the membrane lipids. Furthermore, perylene bound to serum albumin shows a polarization of the fluorescence emission (0.30) much higher than any measured in the membrane systems. Hubbell and McConnell[10] reported an increasingly restricted mobility of a spin-labelled fatty acid incorporated into nerve, as the location of the label was moved from the terminal position of the fatty acid towards the polar headgroup. These results are in agreement with the findings that 8-anilinonaphthalene-1-sulfonate fluorescence polarization shows much higher values than those observed in the present study for perylene fluorescence.

It should be noticed that all the experiments were carried out with fresh cell preparations. Hemoglobin-free erythrocyte membranes kept 2–3 days at 4 °C showed higher polarization and microviscosity values.

The absence of detectable phase transitions in the various membranes indicates that the complex mixture of lipids present in them precludes the presence of a distinct melting behaviour. In general, phase transitions in intact membranes have only been observed in those organisms where growth conditions or genetic defects allow the predominant incorporation of a single fatty acid species. Moreover, Pinto da Silva[11] did not observe a transition in the movement of the proteins of erythrocyte membranes from −16 to 35 °C.

The ease with which the results were obtained and the sensitivity of the measurements indicate that the present method represents a valuable tool for the study of transitory and steady state membrane properties.

The implications of the liquid nature of the membrane components have been recently discussed. These include carrier movements implied in transport systems, modification in the topology of surface elements, plastic responses to the interaction

with effectors, such as the activation of adenyl cyclase, and even the possibility of the hereditary perpetuation of a membrane state without genic changes[12–14].

A.C. Dianoux, M. Shinitzky and G. Weber (personal communication) have independently obtained results which are in qualitative agreement to those presented herein.

Dr Richard Spencer made this investigation possible by building our polarization of the fluorescence apparatus.

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