Lipid peroxidation and fluidity of plasma membranes from rat liver and Morris hepatoma 3924A

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Plasma membranes isolated from the fast-growing, maximal-deviation, Morris hepatoma 3924A exhibit remarkable changes in lipid composition, lipid peroxidation and to some extent in the physical state with respect to rat liver plasmalemmas. A correlation appears to exist between the lower phospholipid: protein ratio, higher cholesterol: phospholipid ratio, lower rate of lipid peroxidation and decrease in fluidity in tumor plasma membranes.

Hepatoma plasma membrane *Lipid peroxidation* *Lipid composition* *Membrane fluidity* *Lipid order* *Fluorescent probe*

1. INTRODUCTION

Interest in the physical and chemical properties of cellular membranes in cancer research has been steadily growing in the last few years [1,2]. Among other techniques, fluorescence anisotropy measurements have been widely applied. In particular steady-state experiments of fluorescence polarization of DPH show dramatic changes of this parameter in membranes isolated from normal and malignant cells. Solid tumor membranes show an increase in fluorescence polarization with respect to the normal ones [3–7]. In contrast in leukemic cell membranes a decrease in polarization has always been found on going from normal to lymphoma cells [8–10]. In both cases it has been suggested [11–13] that fluorescence polarization measurements can be of diagnostic and/or prognostic value.

Recent studies on microsomal membranes, isolated from tumors with different growth rates, have shown that the degree of unsaturation of phospholipids [14], their peroxidizability [15] and the cholesterol content [16] modify the static and dynamic properties of the membrane in a manner which is related to the growth rate of the tumor [16]. While the major functions of microsomes in the cell are related to metabolism, plasma membranes are fundamental in cell–cell interactions. For this reason it appears important for a more general insight into the role of cellular membranes in neoplastic growth to extend these studies to the latter membranes.

We here report data on the structural organization of lipid in the plasma membrane from hepatoma 3924A, compared to that from normal hepatocytes.

Order parameters and rotational diffusion coefficients for the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene (DPH) have been determined using the approach in [16–18] combining steady-state and fluorescence decay measurements. These physical parameters, connected to the order of lipids and fluidity in the membrane, respectively, are also discussed in terms of lipid peroxidation and composition.

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2. MATERIALS AND METHODS

Tumor preparation and isolation of plasma membranes from rat liver (ACI/T strain) and hepatoma 3924A were carried out as in [19,20]. Proteins were estimated as in [21] using bovine serum albumin as standard and phospholipids as in [22]. Cholesterol was determined using the Boehringer monotest method, as modified in [23].

Lipid peroxidation was measured at 25°C as malondialdehyde formation by the thiobarbituric acid assay [24] and as lipid hydroperoxide formation [25]. Plasma membranes, suspended in 0.15 M KCl, 50 mM Tris–HCl (pH 7.5) saturated with oxygen, were incubated in the presence of 1 mM ADP, 0.05 mM $FeCl_3$ and 0.33 mM xanthine. The reaction was started by 50 μg/ml xanthine oxidase (Sigma, type I).

Polarization measurements were carried out using a Perkin Elmer MPF 44A spectrofluorimeter equipped with a DCSU2 unit and two Polaroid film polarizers. Fluorescence lifetimes were obtained using a home-assembled single-photon apparatus with an Edinburgh Instrument nanosecond flashlamp, filled with nitrogen, a Jasco emission monochromator and an EMI 98160B photomultiplier. The data were collected on a Silena multichannel analyzer; single-photon counting electronic devices were purchased from ORTEC.

DPH was obtained from Aldrich-Europe (Beerse, Belgium) and purified by sublimation. Acetone and all organic solvents were of fluorimetric grade. All other chemicals were of reagent grade quality.

Samples for both steady-state and fluorescence lifetime determinations were prepared by diluting a suitable amount of membrane suspension (300 μg as protein) in 2 ml of 20 mM Tris–HCl buffer (pH 7.4) and adding DPH, dissolved in acetone, to a final concentration of 5×10^{-7} M.

The DPH : phospholipid ratio in the experiments was maintained between 1 : 150 and 1 : 300. No depolarization due to light scattering was observed by diluting the membrane suspension. In all experiments the samples were thermostatted using a Braun Frigomix-Thermomix unit (±0.1°C).

Steady-state polarization anisotropy was calculated as in [16]. Fluorescence decay curves were deconvoluted using a non-linear least-square fit–fast Fourier transform method developed in [26]. The fluorescence intensities were assumed to decay via a biexponential, i.e.

$$F(t) = C_1 \exp(t/\tau_1) + C_2 \exp(t/\tau_2) \quad (1)$$

Fits were also performed using a single exponential decay. However, when comparing the fitted and experimental decays, poor χ^2 and residue distribution were found.

3. RESULTS

Table 1 shows the lipid content of plasma membranes isolated from rat liver and Morris hepatoma 3924A. It can be observed that in the tumor the phospholipid content is decreased about 2-fold while cholesterol is only slightly increased.

Lipid peroxidation, induced by xanthine oxidase (to generate superoxide radicals), is strikingly depressed in tumor with respect to liver plasma membranes (fig.1). Indeed, malondialdehyde production during 40 min incubation was only about 5% of the control and lipid hydroperoxides were undetectable in tumor membranes with the method employed. Fig.2 shows the results of the steady-state fluorescence anisotropy of DPH as a function of temperature for the tumor and control membranes. The steady-state fluorescence anisotropy for a rod-like molecule with transition moments parallel to the long axis, expressed according to the theory in [16,18] by the equation

$$r_s = r_0 \sum_{Fkq}\sum\sum (b^{q0})_k / (1 + (a_{q0})_k D_{per} \tau_F)$$

$$(q = 0, \pm 1, \pm 2; k = 1, 2, 3 \ldots; F = 1, 2) \quad (2)$$

can be used to derive the order parameter $\langle P_2 \rangle$ and D_{per}, that is the component of the probe rotational

Table 1

Lipid content of rat liver and hepatoma plasma membranes

	Rat liver	Hepatoma 3924A
Phospholipid : protein (w/w)	0.46	0.28
Cholesterol : protein (w/w)	0.10	0.12
Cholesterol : phospholipid (w/w)	0.22	0.43

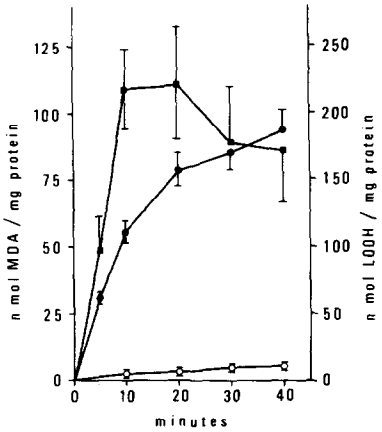

Fig.1. Lipid peroxidation induced by superoxide radicals of plasma membranes isolated from rat liver (■,●) and Morris hepatoma 3924A (○). Membranes at 0.2 mg protein/ml were suspended in the medium described in section 2 and incubated at 25°C in a Dubnoff metabolic shaker under oxygen tension. (■) Lipid hydroperoxide (LOOH), (●,○) malondialdehyde (MDA). Values are means ±SE of 4–6 experiments.

diffusion tensor perpendicular to the long axis. The latter parameter tells us how easy it is to reorient the long axis and is therefore a measure of 'fluidity'. The order parameter $\langle P_2 \rangle$ has been derived from a slight generalization of the theory to allow for double exponential fluorescence decay as in eq. 1. The coefficients $(b^{q0})_k$, $(a_{q0})_k$ are obtained from the solution of the diffusion equation [17,18]. They are functions of the order parameter $\langle P_2 \rangle$ as discussed in [17,18]. The input parameters

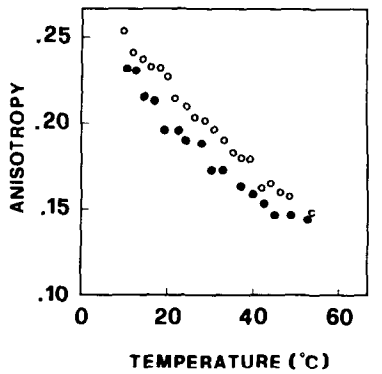

Fig.2. Dependence of steady-state fluorescence anisotropy on temperature for DPH in plasma membranes from hepatocytes (●) and hepatoma 3924A (○) (λ_{ex} = 360 nm, λ_{em} = 440 nm).

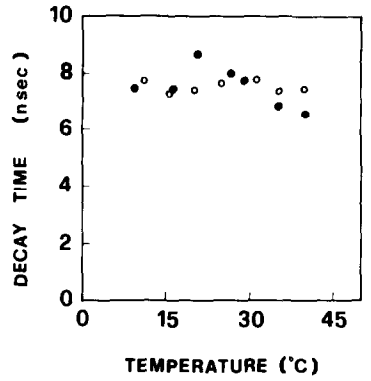

Fig.3. Average fluorescence decay times *vs* temperature plot of DPH in plasma membranes of control (●) and hepatoma 3924A (○) (λ_{ex} = 360 nm, λ_{em} = 440 nm).

needed to predict r_s at a certain temperature are τ_F, D_{per} and $\langle P_2 \rangle$.

We have first measured τ_F at a series of T. The suggestion in [27] of assuming an effective average decay time $\tau_F = C_1\tau_1 + C_2\tau_2$ was first followed. The results for the control and tumor values of τ are given in fig.3. In the final analysis the full form of eq. 2 was used with τ_1 and τ_2, whose values are not tabulated here in full for reasons of space. In any case τ_1 and τ_2 values needed at the polarization measurement temperatures were obtained by fit and interpolation. Having reduced by experimental means the unknowns in eq. 2, we have further assumed an Arrhenius-type dependence for the variation of D_{per} with temperature,

$$D_{per}(T) = (D_{per})_{T_1} \exp\left[-E_R\left(\frac{1}{T} - \frac{1}{T_1}\right)\right] \qquad (3)$$

where T_1 is the highest experimental temperature for a certain series and E_R a rotational activation energy. We assumed moreover a cubic dependence of $\langle P_2 \rangle$ on T, i.e.

$$\langle P_2 \rangle_T = \langle P_2 \rangle_{T_1} + a(T_1 - T) + b(T_1 - T)^2 + c(T_1 - T)^3 \qquad (4)$$

The remaining parameters for a given set of r_s *vs* T data have been determined using a non-linear least-squares fitting procedure. Our results for the parameters D_{per} along with $\langle P_2 \rangle$ E_R and a, b and c values, as from eqs 3 and 4, are listed in table 2.

Table 2

Best-fit values for the temperature variation of $\langle P_2 \rangle$ and D_{per} as from eqs 3,4

Sample	T_1(°C)	$\langle P_2 \rangle_{T_1}$	$a \times 10^2$ (°C^{-1})	$b \times 10^4$ (°C^{-2})	$c \times 10^6$ (°C^{-3})	$(D_{per})_{T_1}$ (ns^{-1})	E_R (kcal/mol)
Rat liver	51.5	0.508	0.334	−0.218	1.308	0.138	4.15
Hepatoma 3924A	52.6	0.502	0.170	1.035	−0.885	0.095	5.08

In fig.4 the order parameter $\langle P_2 \rangle$ for the tumor and control is shown. $\langle P_2 \rangle$ decreases with temperature but is always similar for the tumor membranes compared to the control. Table 2 shows that the D_{per} values are higher for the normal than for the tumor membranes.

4. DISCUSSION

We have shown in comparative experiments with microsomal membranes isolated from rat liver and hepatomas that the tumor membranes catalyze relatively low rates of lipid peroxidation [15]. Such resistance to peroxidation has been correlated with the lipid composition rather than the content in antioxidants like vitamin E [14,28]. We report here that plasma membranes from hepatoma 3924A also exhibit a much lower susceptibility to peroxidative agents with respect to rat liver plasmalemmas. Although antioxidants have not yet been determined, the observation that in hepatoma plasma membranes the phospholipids are decreased to 60% of the control indicates that substrate availability is the major limiting factor in their lipid peroxidation.

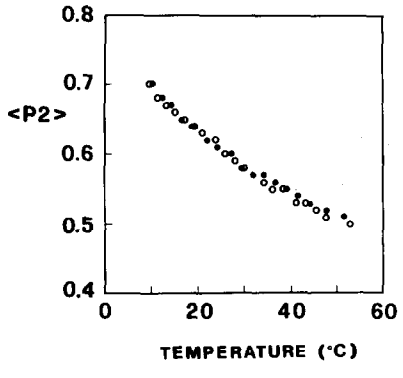

Fig.4. Dependence of order parameters $\langle P_2 \rangle$ on temperature for DPH in plasma membranes from hepatocytes (●) and hepatoma 3924A (○).

As is well known the parameter $\langle P_2 \rangle$ affords a measure of the structural order of the membrane. D_{per}, on the other hand, tells us how easy it is to reorient, after excitation, the long axis of the rod-like molecule DPH within the lipid matrix. The results of fig.4 show no increase in ordering on going from the control to the fast-growing tumor plasma membranes. This effect is perhaps surprising at first in view of the differences in chemical composition of the two membranes, namely the decrease of the phospholipid: protein ratio and the remarkable increase of the cholesterol: phospholipid ratio in the tumor. It is however noteworthy that the order parameter is quite high for control plasma membranes: in fact it ranges from 0.5 to 0.7, while values between 0.05 and 0.3 have been found for control microsomal membranes [16]. On the other hand, the dynamic parameters D_{per} and E_R indicate a decreased fluidity in the tumor plasma membranes. Comparing these data, both for chemical composition and for the physical parameters, with those found for microsomal membranes [16], a pattern seems to exist whereby the tumor membranes generally are structurally changed towards a higher rigidity. The observation that the liver microsomal membranes are richer in phospholipids than the plasma membranes and that their cholesterol: phospholipid ratio is lower can explain their lower order parameters [16]. Conversely, their availability to peroxidation is higher when compared to that of plasma membranes [28]. This further supports the hypothesis [16,29] that the rate-limiting step of lipid peroxidation is substrate availability. In conclusion the experiments reported here strengthen the model according to which the process of neoplastic transformation leads to a dramatic change in the lipid structural organization within the membrane.

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