LIGAND BINDING ASYMMETRY AND LIPID FLUIDITY CHANGES IN INSIDE-OUT AND RIGHT SIDE-OUT PLASMA MEMBRANE VESICLES

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1. Introduction

A number of studies have suggested that some biological membrane functions might be correlated with the physical state of the phospholipids in these membranes [1–4].

Breaks in the Arrhenius plots of enzymatic or transport activities have indicated a possible relationship between phase transitions in the phospholipids [1–3] and these activities. Recently it has been shown by ^1H NMR and X-ray diffraction that in sarcoplasmic reticulum membranes no order → disorder type of transitions occur in the temperature range 1–40°C, but breaks in the Arrhenius plots for the rates of Ca^{2+} transport and Ca^{2+} activated ATPase were observed between 15°C and 25°C [4]. This observation indicates a possible association between the character of the phospholipid segmental motion and the functional parameters of the membranes.

Binding of Con A on right side-out (RSO) plasma membrane vesicles was shown to affect the activities of 5-'nucleotidase (EC 3.1.3.5) and of $(Na^+ + K^+)$ stimulated Mg^{2+}-ATPase (EC 3.6.1.3), while it had no effect on the inside-out (IO) vesicles of the same plasma membranes. In contrast, a peptidoglycan-like adjuvant was effective in modulating the enzymes, only on the IO vesicles [5].

It is the purpose of this letter to show that binding of these ligands on the reactive membrane face affect the membrane phospholipid fluidity.

2. Materials and methods

2.1. *Cells*

MF_2S cells, growing as ascites in Balb/c mice, were derived from the murine MOPC 173 plasmocytoma [6].

2.2. *Plasma membrane purification*

Described elsewhere [7]. The protein concentration was determined by the Lowry et al. method [8]. Enzymatic assays of the 5'-nucleotidase and $(Na^+ + K^+)$-stimulated Mg^{2+} ATPase were carried out as in [5] (table 1).

2.3. *Isolation of inside-out (IO) and right side-out (RSO) plasma membrane vesicles*

According to Zachowski and Paraf [6].

2.4. *Ligands*

Concanavalin A (Con A) was purchased from Boehringer Werke (Mannheim). A purified peptidoglycan extracted from *M. tuberculosis*, var. *hominis*, strain $H_{37}Ra$ [9] was used. The vesicles were incubated for 20 min at 37°C with either Con A or peptidoglycan, then washed and centrifuged twice for 30 min at 50 000 × *g*, at 4°C. After the last centrifugation, the vesicles were resuspended in Ca^{2+}- and Mg^{2+}-free phosphate buffer saline.

Table 1
Purification of the isolated plasma membranes according to the enzymatic assays

Fraction	5'-Nucleotidase activity		$(Na^+ + K^+)$ stimulated Mg^{2+} ATPase activity		Glucose-6-phosphatase activity	
	Specific	Total	Specific	Total	Specific	Total
Cell lysate	0.9	673	1.2	892	0.4	304
Plasma membranes	10.7	193	15.2	273	0.35	6.3

Specific activities are expressed in μmoles of phosphate liberated × h^{-1} × mg prot.$^{-1}$ Total activities are expressed in μmoles of phosphate liberated × h^{-1}.

2.5. *Fluorescence measurements*

The polarization degree

$$P = \frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}$$

(I_{II}, $I_{\perp}$ are the fluorescence intensities, polarized parallel and perpendicular, respectively to the direction of the excitation beam of 1,6-diphenylhexatriene (Aldrich, Europe) embedded in the membrane vesicles) was measured with an Elscint Microviscosimeter MV-1. The excitation wavelength was 366 nm and the temperature of measurement was 37°C ± 0.5°C. The relationship of P to the viscosity of the matrix in which the probe is dissolved has been extensively discussed [10,11].

The labelling of the vesicles has been described elsewhere [10–12].

3. Results and discussion

The RSO and IO vesicles were characterized by the following criteria: (i) Neuraminidase released 88 ± 9% of total sialic acid from RSO vesicles and 32 ± 5% from IO vesicles. (ii) ^{3}H-labelled Con A bound only on RSO vesicles. (iii) 5'-nucleotidase and $(Na^+ + K^+)$-stimulated Mg^{2+} ATPase activities (hydrolytic sites located at the inner face of the plasma membrane) were about 3-fold higher in IO than in RSO vesicles.

However protein profiles, studied by SDS-polyacrylamide gel electrophoresis, were the same when IO and RSO vesicles were compared. Similarly lipid and fatty acid compositions were the same for both vesicles (data not shown).

The temperature dependence of the fluorescence polarization degree of DPH embedded in the membrane vesicles is shown in fig.1: both vesicles have a common feature: between 4°C and 40°C there is no cooperative phase transition detectable by the fluorescence polarization technique.

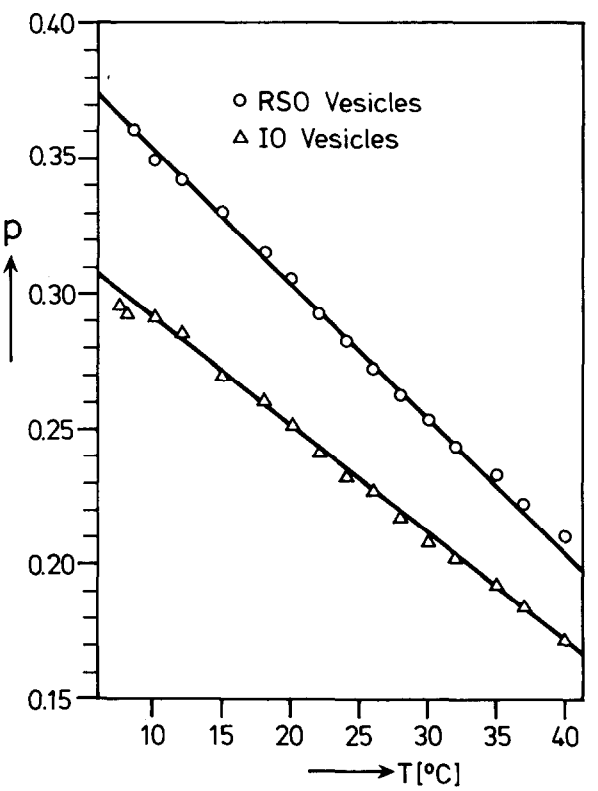

Fig.1. Temperature dependence of the fluorescence polarization degree of DPH embedded in the IO and RSO plasma membrane vesicles.

Table 2
The fluorescence polarization degree of DPH in RSO and IO vesicles in the presence and absence of ligands

Ligand	Ligand concentration ($\mu g.\ ml^{-1}$)	Polarization degree of DPH, $P = \frac{I_{II} - I_{\perp}}{I_{II} + I_{\perp}}$, at 37°C	
		RSO vesicles	IO vesicles
None		0.232 ± 0.005	0.190 ± 0.005
Con A	2	0.232 ± 0.005	0.190 ± 0.005
	2000	0.256 ± 0.005	0.195 ± 0.005
Peptidoglycan	2	0.236 ± 0.005	0.205 ± 0.005
	30	0.234 ± 0.005	0.210 ± 0.005

The absolute values of the polarizations in the IO vesicles are lower than in the RSO vesicles, and the decrease of the polarization with increasing temperature is slightly faster in the RSO than in the IO vesicles.

Addition of the ligands alters the fluidity of the membrane as measured by fluorescence polarization in both vesicle types. The results are listed in table 2.

While Con A, at rather high concentration, significantly increases the polarization degree of DPH embedded in the RSO vesicles, it does not affect the fluidity of the IO vesicles even at lectin concentrations of 1000 $\mu g.ml^{-1}$. In contrast, the peptidoglycan affects only the IO vesicles fluidity, while the RSO vesicles fluidity remained completely unchanged.

These results are in agreement with the observation by Zachowski et al. [5], that Con A modulates the activity of 5'-nucleotidase and of ($Na^+ + K^+$) stimulated Mg^{2+}-ATPase only on RSO vesicles, but not on IO vesicles, while the peptidoglycan modulates the activity of the two enzymes solely on the IO vesicles.

The fluorescence polarization degree of DPH was shown to reflect quite faithfully phase transitions in lipid bilayers [13,14]. Phase diagrams could be traced, in agreement with other methods, even if a pretransition cannot be seen with this label. An important feature is that it shows little difference in the partitioning between the two phases in phospholipid bilayers.

The absence of phase transitions in our systems – transitions of the type order → disorder, which are well evidenced by DPH – agrees also with other data concerning the physical state of phospholipids in cholesterol containing membranes [12–15].

The incertitudes concerning the orientation of the DPH molecules within the membrane, as well as the exact meaning of rotational relaxation of the probe in the membranes induced us to use the polarization degree values instead of microviscosity.

While the lipid, fatty acid and protein compositions were the same, the temperature dependence of P shows for IO vesicles a smoother slope than it does with RSO. Moreover, IO vesicles show a lower polarization degree than do RSO vesicles. The reasons for these differences are presently unclear.

The binding of face-specific ligands alters the polarization degree of DPH embedded in the RSO and IO vesicles. Interactions of Con A with RSO vesicles and of peptidoglycan with IO vesicles enhance significantly the values of P (table 1). However, this enhancement becomes significant only with concentrations 100- to 1000-fold higher than those required to modulate the plasma membrane enzyme activities [5]. Thus there appears to be no correlation between changes in membrane fluidity and modulation of enzyme activities by ligands.

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