Membrane dynamics in human leukemia and lymphoma cells

pH dependency of diphenylhexatriene fluorescence polarization

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Received 31 May 1983

Membrane dynamics of human leukemia and lymphoma cell lines were analyzed by investigating the effect of pH on fluorescence polarization (*P*) of the lipophilic probe diphenylhexatriene (DPH). The degree of *P* varied as a function of pH, depending on the cell lines. These variations were not detected in phospholipid vesicles. In addition, they were prevented by treatments with glutaraldehyde, sodium azide or phenylmethylsulfanyl fluoride, a specific protease inhibitor. Therefore, these *P* value changes might be influenced by protein modification.

Membrane dynamics *Leukemic cell* *Proliferation* *DPH fluorescence polarization* *pH effect* *Protein modification*

1. INTRODUCTION

Dynamic interactions between proteins and lipids of the plasma membrane are considered to be implicated in certain cellular functions which are altered in cancer cells. Numerous works, based on fluorescence polarization of the hydrophobic probe DPH [1], or on other methods, showed membrane dynamic changes associated with malignancy, cell growth and differentiation [2–9]. Such changes were frequently attributed to primary modifications in the composition and fluidity of the membrane lipids [2–4,6,9].

However, the influence of proteins on the physical state of lipids has been demonstrated [10–12]. In addition, it was shown that a conformational modification of lactalbumin, induced by acidification, resulted in a shift of the fluorescence polarization (*P*) of DPH-labeled phospholipid vesicles [13,14]. Variations of pH are known to affect not only protein conformation, but also enzyme activity [15] and hormone–membrane receptor binding [16].

We reported that *P*-value alterations of human leukemia and lymphoma cell membranes relate to cell proliferation and immaturity [17]. To analyze the factors involved in these changes, we have investigated the effect of pH on the degree of *P* in various cell lines in growing state.

2. MATERIALS AND METHODS

2.1. *Cells*

Six human cell lines routinely tested for phenotypic markers were used: the non-B–non-T cell line REH, initiated in our laboratory from a common acute lymphocytic leukemia [18], the pre-B cell line Nalm-1, established from a chronic myeloid leukemia in blastic crisis [19], and 4 well-characterized B-cell lines established from

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Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

Burkitt's lymphomas: Bjab, Daudi, Raji and Namalva [19]. All cells were subcultured every 2 or 3 days in RPMI 1640 medium containing 10% foetal calf serum and kept at 37°C in a CO_2 incubator. Experiments were performed 24 h after seeding at 5×10^5 cells/ml.

2.2. *Fluorescence labeling and measurements*

Hydrophobic regions of cell membranes were labeled for 30 min at 37°C with 2 μM 1,6-diphenyl-1,3,5-hexatriene (DPH) in phosphate buffers of different pH, as in [20]. Labeling was also carried out with DPH-phosphate buffer solutions containing either 2.5% glutaraldehyde, or 10 mM sodium azide, or 0.2% phenylmethylsulfanyl fluoride (PMSF).

Steady-state polarization (*P*) measurements were done at 37°C with a MV-1 Elscint instrument, as in [20]. For the excitation of DPH, a plane polarized 365 nm band generated from a 200 W mercury arc was employed. The emitted fluorescence was detected in two independent cross-polarized channels equipped with cut-off filters for wavelengths below 390 nm. Light scattering did not cause depolarization as monitored by serial dilutions of samples. The ratio of the intensity of the light transmitted through the suspension, on that through the buffer, remained nearly constant during the experiments, indicating that the different *P* values were not due to extracellular changes. The *P* values of duplicate samples did not differ from $\geqslant 0.005$.

Fluorescence spectra were performed with a spectrofluorimeter made for detection of low emission of luminescence [21]. Excitation spectra were recorded with an emission wavelength of 450 nm, and emission spectra were recorded with an excitation wavelength of 340 nm.

3. RESULTS AND DISCUSSION

Changes of fluorescence polarization of DPH-labeled human leukemia and lymphoma cell membranes were associated with cell proliferation [16]. We therefore studied the effect of pH on the degree of *P* from cells in the growing state. We found variations of *P* value as a function of pH, depending on the cell lines. Over pH 4.5–9.0, the degree of *P* rose either continuously in Daudi cells, or according to a saturation-like curve in Bjab cells, whereas it diminished continuously with Namalva cells. The degree of *P* was also found to increase by acidification and alkalinization from a minimum value observed at neutral pH, in the non-B–non-T REH cells (fig.1), in the pre-B Nalm-1 cells and in the Raji B-lymphoblasts (fig.2,3).

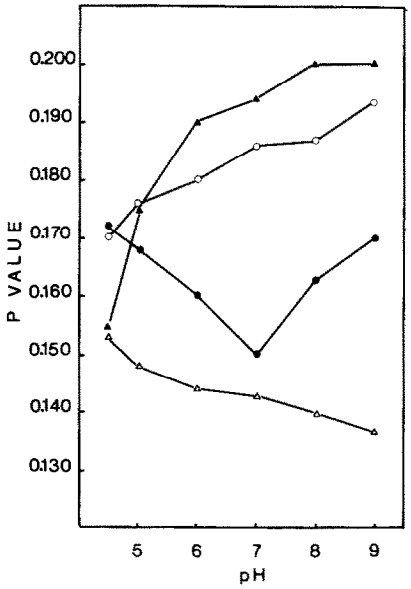

Fig.1. Effect of pH on fluorescence polarization (*P*) values at 37°C in different human lymphoma-leukemia cell lines: Bjab (▲), Daudi (○), REH (●) and Namalva (△). DPH labeling and *P* measurements were carried out as in section 2.

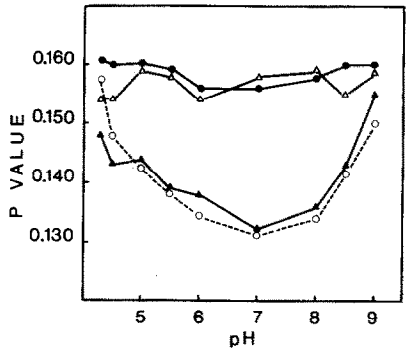

Fig.2. Effect of glutaraldehyde and sodium azide treatments on the pH-dependent *P* values in Raji cells: untreated control (○); glutaraldehyde (●); sodium azide (△); sodium azide removed by washing (▲). DPH labeling and *P* measurements were done as in section 2.

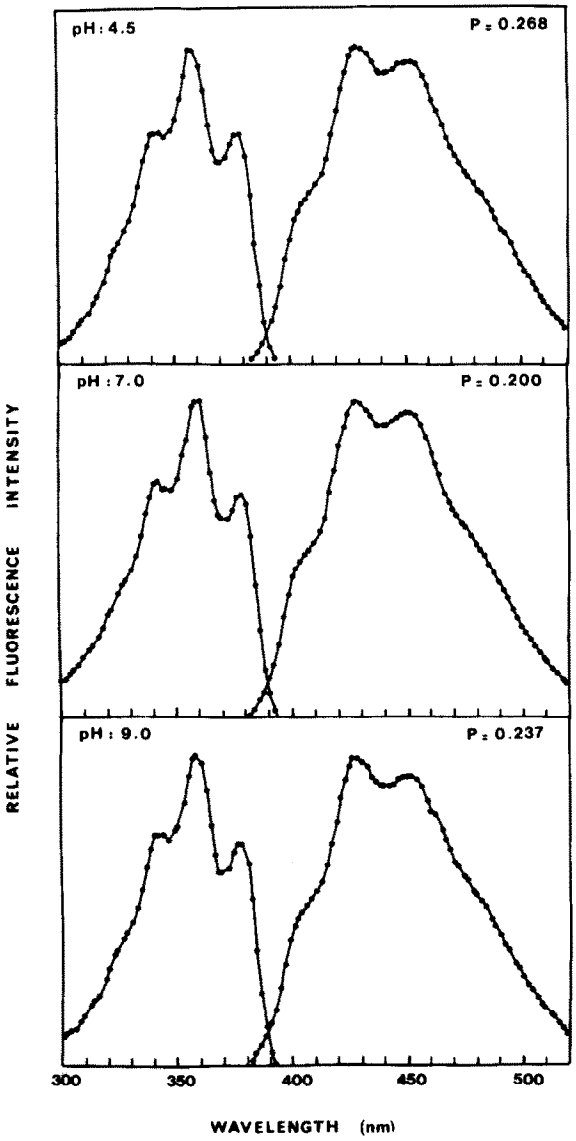

Fig.3. Normalized excitation and emission spectra of DPH incorporated into Nalm-1 cell membranes at different pH-values, as in section 2. Measurements were performed at 25°C.

The same experiments carried out with phosphatidylcholine and phosphatidylcholine–cholesterol vesicles showed that low and high P values, respectively, did not vary with the pH (table 1), indicating that the latter has no effect on the P values of membrane lipids. These results plus the fact that pH variations are able to affect the conformation and activity of proteins [13,15,16], suggested that the P value changes described above could be due to modifications of membrane proteins. This assumption was supported by the observation that the P value changes related to pH were prevented by treatments with glutaraldehyde, with sodium azide, known to block the active site of proteins, or with PMSF, a specific protease inhibitor. The resulting P values were shifted to either their maximum or minimum levels, according to the treatment and to the cell line (fig.2, table 2). We therefore conclude that the pH-dependent P value changes are characteristic for each cell line and that they can be associated with protein modifications.

Previous studies on lipoprotein systems have demonstrated the modulating effect of 'integral' membrane proteins on the physical state of phospholipids, including the DPH fluorescence polarization [10–12]. Moreover, a study on interactions of lactalbumin with DPH labeled phospholipid vesicles showed that a conformational change of the protein, induced by acidification, results in a shift of the P value [13,14]. Thus, the pH-dependent P value changes observed in human leukemic cell membranes could be achieved through such modifications in the protein–lipid interactions.

The effects of sodium azide and PMSF suggest that the P value changes could, on the other hand, be directly due to local alterations of the proteins: It has been suggested that interactions of the DPH probe with hydrophobic folds of proteins might be involved in the degree of P of cell membranes [22]. Fluorescence energy transfer studies indicated that hydrophobic interactions between tryptophan residues and DPH molecules are possible in serum lipoproteins [23], but not in oligopeptide-bound phosphatidylserine (T. Le Doan, personal communication). If such interactions were involved in the P value changes described here, it would be expected that the local environment of the DPH be affected by pH variations. However, no shift in the excitation and emission spectra of DPH embedded in cell membranes were detected by varying the pH (fig.3). Furthermore, the structure and position of maxima and minima were quite identical than those of DPH dissolved in the tetrahydrofuran solvent of reference. Therefore, further experiments are required in order to elucidate the question whether our results can be explained by local

Table 1

Effect of pH on the P values at 37°C of lipid vesicles

Vesicle	pH					
	4.5	5.0	6.0	7.0	8.0	9.0
Phosphatidylcholine	0.098	0.096	0.090	0.102	0.100	0.098
–cholesterol	0.320	0.316	0.323	0.318	0.320	0.320

Phosphatidylcholine vesicles (8 mg/ml egg lecithin) were sonicated, mixed or not with cholesterol (4 mg/ml), and labeled with DPH in phosphate buffer of different pH values

Table 2

Effects of different treatments on pH-dependent P values of Namalva cells

Treatment	pH		
	4.5	7.0	9.0
Control	0.153	0.143	0.137
Glutaraldehyde	0.136	0.133	0.131
Sodium azide	0.161	0.159	0.154
PMSF	0.140	0.137	0.139

DPH labeling and P measurements were done as in section 2

alterations of proteins or by modifications in the protein–lipid interactions.

Nevertheless, these data favor the view that alterations of membrane dynamics in human leukemia and lymphoma cells, which were currently attributed to primary lipid changes, may also be influenced by protein modifications. With those reported on the pH dependency of enzyme activity and hormone-receptor binding [15,16], these data also support the idea that membrane dynamic alterations could be associated with changes in functional activities.

ACKNOWLEDGEMENTS

We are grateful to Dr F. Gaboriau and Dr Z. Mishal for their amicable and generous collaboration, and to Martine Billard for her very helpful assistance. This work was supported by grants from CNAMTS (541) and INSERM (82-79114 and 822034).

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