suggests that it was more susceptible than heterochromatin to mechanical degradation. If this reasonably evident hypothesis is confirmed, the technique we have developed will provide the research worker with fractions of weakly fragmented domains of euchromatin and complete circular domains of heterochromatin. The importance of the study of these chromatin fractions for biology and medicine is undisputed, especially in the light of the idea [10] that different kinds of physiological and pathological processes are based on differential gene expression controlled by endogenous and exogenous factors.

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COMPARATIVE FLUORESCENCE STUDY OF CELL MEMBRANES AND RECONSTITUTED LIPOSOMES

V. S. Posokhov, A. Yu. Sungurov, O. A. Rozenberg, K. P. Khanson, and E. F. Davidenkova

UDC 612.112.94:612.397.8.014.2]:576.314

KEY WORDS: fluorescence; liposomes; cell membranes; thymocytes; Ehrlich's ascites carcinoma

The essential role of the plasma membranes of cells in various functional and pathological cell processes, and also in the development of responses to external injurious influences is the factor which determines the importance of the development of new and promising approaches to their study. One such approach is the use of proteoliposomes, reconstituted from solubilized cell membranes as an experimental model, and their use to modify the cell surface.

The aim of this investigation was to compare the properties of membranes from thymus lymphocytes and Ehrlich's ascites carcinoma (EAC) cells and those of liposomes reconstituted from them. The liposomes were characterized by the use of fluorescence methods, yielding quantitative information on the state of the test object while inflicting minimal damage on it during the experiments [4]. The intrinsic ultraviolet fluorescence (UVF) of the cells characterizes the quantity and state of the cell membrane proteins. The nonpolar fluorescent probe pyrene, localized in the hydrophobic part of the lipid bilayer, can be used to assess the state of the membrane lipids [2].

EXPERIMENTAL METHOD

Thymus lymphocytes were isolated from noninbred albino rats weighing 120-130 g by the method in [3]. EAC cells, transplantable into noninbred albino mice, were isolated on the 7th day after inoculation. The characteristics of fluorescence of the cells and proteoliposomes in Krebs–Ringer solution (pH 7.3) were measured on an MP-650-40 fluorescence spectrophotometer (Hitachi, Japan). Characteristics of UVF were measured within the bounds of the linear region of dependence of UVF on concentration: with concentrations of thymocytes and EAC cells of $4 \cdot 10^6$ and $0.7 \cdot 10^6$/ml, respectively, and of proteoliposomes equivalent to 0.1 μmoles of phospholipids in 1 ml. Fluorescence of membrane-bound pyrene was measured in the presence of pyrene in concentrations of 3 μmoles/ml, thymocytes $20 \cdot 10^6$/ml, EAC cells $3 \cdot 10^6$/

Central Roentgeno-radiologic Research Institute, Ministry of Health of the USSR, Leningrad.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 11, pp. 559-561, November, 1987. Original article submitted August 8, 1986.

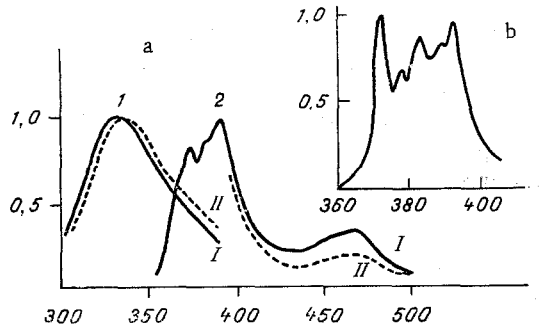

Fig. 1. Fluorescence spectra: a) UVF (1) and fluorescence spectra of pyrene (2) for thymocytes (I) and of proteoliposomes from thymocyte membranes (II) with slit widths of monochromators of 5 nm; b) fluorescence spectrum of pyrene, imbedded in thymocyte membranes, with slit width of monochromators of 1 nm. Abscissa, wavelength (in nm); ordinate, intensity of fluorescence (in relative units).

ml, and proteoliposomes 0.4 μmole phospholipids/ml, which guaranteed complete imbedding of the pyrene into the membranes. The wavelength of excitation for cells and proteoliposomes was 290 nm, the wavelength of fluorescence of pyrene was 335 nm, and the intensity of UVF of cells and proteoliposomes was recorded at 335 nm and of the monomer band of pyrene at 392 nm. Polarization of fluorescence was determined at the same wavelengths with the aid of polarization plates. The degree of polarization P was calculated by the equation:

$$P = \frac{I_{\parallel} + I_{\perp}}{I_{\parallel} + I_{\perp}},$$

where $I_{\parallel}$ and $I_{\perp}$ denote the intensity of fluorescence when the plates are arranged parallel and perpendicular to one another respectively, with correction for different characteristics of the polarization plates and for light scattering of the sample. To prepare the proteoliposomes, thymocytes were isolated in a medium of 135 mM NaCl, 4 mM KCl, 5 mM HEPES, 5 mM $NaHCO_3$, 4 mM $MgCl_2$ (pH 7.4), and EAC cells were washed twice with this same medium. Plasma membranes were obtained by solubilization of the cells by a modified method [5] at 4°C by gentle shaking for 10 min in buffer containing 0.025% Triton X-100, 0.1 mM phenylmethylsulfonyl fluoride, 140 mM NaCl, with pH 7.4, adjusted with 20 mM Tris-acetate buffer to pH 8.4. The supernatant containing the solubilized membrane components was obtained by sedimentation of the nuclei and cell organelles twice at 15,000g for 20 min, followed by freezing at −25°C. The proteoliposomes were formed by treating the frozen supernatant with ultrasound in the tubular magnetostrictor of a UZDN-1 ultrasonic disintegrator at 22 kHz and 4°C for 15 sec. Detergent was removed by sedimentation of the proteoliposomes at 100,000g for 1 h, and the residue was resuspended in solution containing 140 mM NaCl, 3 mM $MgCl_2$, pH 7.4, and then again treated with ultrasound for 60 sec at 4°C. To obtain proteoliposome containing a mixture of membrane components of EAC cells and thymocytes the supernatants were pooled in the ratio of 1:1 for phospholipid concentration. The phospholipid concentration was determined by the method in [7].

EXPERIMENTAL RESULTS

Spectra of intrinsic UVF and fluorescence of pyrene for thymocytes and proteoliposomes reconstituted from the plasma membranes of these cells are given in Fig. 1a. The spectra recorded for EAC cells and proteoliposomes reconstituted from their membranes were similar in shape. To characterize the position of the UVF spectrum, depending on polarity and rigidity of the environment of the tryptophanyl groups of the protein, a parameter A was used: the ratio between the intensities of fluorescence on the slopes of the UVF spectrum at 320 and 360 nm [1]. The fluorescence spectrum of pyrene incorporated into the membrane had excimer and monomer bands, the ratio of the peak intensities of which (I_{470}/I_{392}) depended on the coefficient of forward diffusion of pyrene in the lipid bilayer. This coefficient decreases with an increase in microviscosity.

Two peaks are distinguishable in the spectrum of the monomer band of pyrene fluorescence, and if the width of the slits of the two monochromators of the spectrophotometer was reduced, five peaks of the fine or vibration structure were distinguishable in the monomer band (Fig. 1b). The size of the first of these depended essentially on the polarity of the environment of the probe, increasing as the latter increased [6]. To characterize the polarity of the environment of pyrene localized in the lipid bilayer the ratio between peaks of fluorescence at 374 and 383 nm was used.

The results of investigation of the fluorescence characteristics of EAC cells and thymocytes and also of proteoliposomes reconstituted from the solubilized plasma membranes of

TABLE 1. Parameters of Intrinsic Fluorescence and Fluorescence of Pyrene for Cells and Proteoliposomes Reconstituted from Their Plasma Membranes (M ± m, n = 6)

Test object	Parameters of fluorescence					
	intensity of intrinsic UVF	ratio of intensities of UVF spectrum at 320 and 360 nm	polarization of UVF	ratio of intensities of fluorescence of peaks 1 and 3 of pyrene spectrum	ratio of fluorescence activities of excimer and monomer peaks of pyrene	polarization of pyrene fluorescence
Thymocytes	0,118±0,01	1,19±0,02	0,128±0,003	1,14±0,03	0,33±0,04	0,015±0,03
Proteoliposomes						
from thymocyte membranes	660±40	1,02±0,01	0,141±0,005	1,17±0,02	0,17±0,09	0,032±0,01
from mixture of membrane components of thymocytes and EAC cells	725±40	1,03±0,02	0,137±0,04	1,14±0,02	0,14±0,02	0,037±0,01
from membranes of EAC cells	850±50	1,01±0,01	0,138±0,02	1,1±0,01	0,16±0,02	0,03±0,05
EAC cells	0,144±0,007	1,13±0.01	0,122±0,08	1,07±0.02	0,25±0,07	0,023±0,01

these cells are given in Table 1. The intensity of UVF, determined primarily by the quantity of fluorescent membrane proteins, was normalized for the volume and concentration of the cells and for proteoliposomes, for phospholipid concentration. As will be clear from Table 1, corresponding to the higher value of the normalized UVF in EAC cells than in thymocytes, higher values of fluorescence also were found in proteoliposomes prepared from membranes of EAC cells than those prepared from thymocyte membranes. This last observation also was indirectly confirmed by the higher protein content in proteoliposomes from EAC cell membranes, when calculated relative to phospholipids. The intensity of UVF of proteoliposomes from a mixture of membrane components of thymocytes and EAC cells and the value of the parameter A for all three types of proteoliposomes were less than the corresponding values for cells. Comparison of these data with the polarization of UVF, which is higher for proteoliposomes than for the original cells, leads to the conclusion that the microenvironment of the tryptophanyl groups of the protein of the proteoliposomes is more polarized and rigid than that of membrane proteins of the cells studied.

Polarization of pyrene fluorescence in proteoliposomes is higher than in cells, evidence of the greater microviscosity of the lipid bilayer of the liposomes. This was confirmed by the lower values of the ratio between excimer and monomer peaks of fluorescence of the probe, which also depends on the microviscosities of the environment of the fluorochrome. The ratio between the intensities of the peaks of fine structure of the monomer band of pyrene fluorescence reveals the greater polarity of its microenvironment in membranes of proteoliposomes than in those of the corresponding cells. It may perhaps be due to the presence of bound water in the lipid bilayer.

The analysis thus showed greater polarization and rigidity of the microenvironment of the membrane proteins and the greater microviscosity of the lipid bilayer in membranes of proteoliposomes than in membranes of the original cells. Species specificity of proteoliposomal membranes is manifested as a higher content of membrane proteins and lower polarization of the lipid bilayer in liposomes made from EAC membranes than in liposomes reconstituted from thymocyte membranes. This fact indicates that reconstituted proteoliposomes may be usable as models with which to study the structural and functional properties of cell plasma membranes.

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