MEMBRANE FLUIDITY IN NORMAL AND CYSTIC FIBROSIS FIBROBLASTS

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SUMMARY

We have observed distinct differences in the polarization of fluorescence and temperature dependent emission intensity of the highly fluorescent phospholipid derivative (1-acyl-2-(N-4-nitrobenzo-2-oxa-1,3-diazole)--aminocaproyl phosphatidylcholine (NBD-PC), when incorporated in the plasma membranes of normal and cystic fibrosis fibroblasts. Fluorescence polarization measurements indicate that the fluorochrome has a much higher degree of rotational mobility in cystic fibrosis fibroblasts as compared with normal cells. Temperature dependent transitions in the emission intensity of NBD-PC incorporated in normal fibroblasts are indicated at 17.7 and 21.2^{o} C while the abnormal cell membranes apparently undergo transitions at 8.7 and 13.5^{o} C. These differences might be due to changes in plasma membrane composition and/or organization, in the case of the cystic fibrosis cells.

The thermal motion of various membrane components, sometimes referred to as "membrane fluidity," is now thought by many investigators to play a major role in various cellular regulatory mechanisms (1). The overall mobility of a myriad of receptor sites on the surface of plasma membranes may provide a mechanism for the conduction of specific biochemical signals to the cell interior by the induction of highly ordered protein and/or lipid conformation pertubations. Therefore, the fluid state of surface membrane lipid layer may exert a direct control on signals that are related to the overall flexibility of the membrane and the thermal mobility of receptors embedded in its surface (2). Consequently, a given cell type under controlled conditions should (and does) exhibit specific temperature dependent

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transitions that are characteristic of the cell type under investigation. Any change, therefore, in the physical character of the cell membrane, whether induced experimentally, or resulting from either a genetic alteration and/or disease process, may be reflected directly as a change in the temperature dependent transitions of the membrane, providing the instrumentation utilized in such measurements is of sufficient sensitivity to detect these changes.

In general, an efficient tool for the quantitative evaluation of the degree of mobility of receptor sites (3,4) and of phase transitions characteristics of membrane lipid layers (5,6) has been fluorescence polarization. However, most of the extrinsic fluorescent probes used in such studies are structurally unrelated to naturally occurring membrane constituents. Recently, Monti, et al. reported on the synthesis and properties of a highly fluorescent derivative of phosphatidylcholine (7). Utilizing this compound as a membrane probe, we have observed some striking differences with respect to both membrane transition temperatures and the polarization of fluorescence between normal fibroblasts and fibroblasts from individuals afflicted with cystic fibrosis.

METHODS

GM #142 CF cells were obtained from the Human Genetic Mutant Cell Repository, Camden, New Jersey. Control fibroblasts used in these studies were obtained either from amniotic fluid (N=5) or from skin biopsy of adult males (N=3), and were without any identifiable genetic disorder. Both types of fibroblasts were cultured in McKoys 5A media with 20 per cent fetal calf serum until they had reached confluence. The cells were harvested by scraping the culture flask, collected by centrifugation at 2,000 x g for five minutes, washed twice by resuspension in isotonic saline solution which was 0.05 M in Tris buffer, pH 7.4, and again collected by centrifugation. The cells were then suspended in the washing buffer, containing 0.5 µg/ml of NBD-PC, and centrifuged as before.

The final pellet of each cell type was then suspended in probe free buffered saline and 0.5 ml aliquots were taken and diluted to 3.0 ml for use in subsequent experiements.

The fluorescent phospholipid derivative 1-acyl-2-(N-4-nitrobenzo-2-oxa-1,3-diazole)-aminocaproyl phosphatidylcholine was a gift from Dr. Walter Shaw, Avanti Biochemicals Inc., Birmingham, Alabama. The relative fluorescent intensity of NBD-PC incorporated in control and cystic fibrosis fibroblasts was measured as a function of temperature from $+5^{o}$ to $+40^{o}$ C. Cells were allowed to equilibrate at $+5^{o}$ for 15 minutes before heating at a rate of 0.2^{o} C/minute. Efficient temperature control was achieved by installing a water jacketed cuvette holder in the fluorometer and connecting it to a Forma Scientific Company 2095 circulating water bath. The temperature in the cuvette was monitored continuously with a Yellow Springs Instrument Company 425C telethermometer equipped with a thermistor probe, and the contents in the cuvette were gently stirred with a teflon rod before each determination to insure that the cells remained in suspension. Fluorescence was measured using a Farrand Mark-I spectrofluorometer equipped with a 150 W xenon source, adjustable excitation and emission slits, and a W + W - 1100 recorder. Experiments were done in a 3.0 ml quartz cuvette with a pathlength of 1.0 cm.

Fluorescence polarization spectra, including grating correction factors, were obtained by the method of Chen and Bowman (8), using quartz polacoat excitation and emission filters. Since the turbidity of cell suspensions might introduce errors in fluorescence polarization measurements, the contribution of scattered light to the fluorescence signals was measured with a reference system consisting of an identical unlabeled cell suspension. At the protein concentrations used in these experiments, the contribution of scattered light to the measured fluorescence intensities was $\leq$ 3 per cent for all cell suspensions.

RESULTS AND DISCUSSION

Figure 1 indicates the temperature dependence of NBD-PC emission

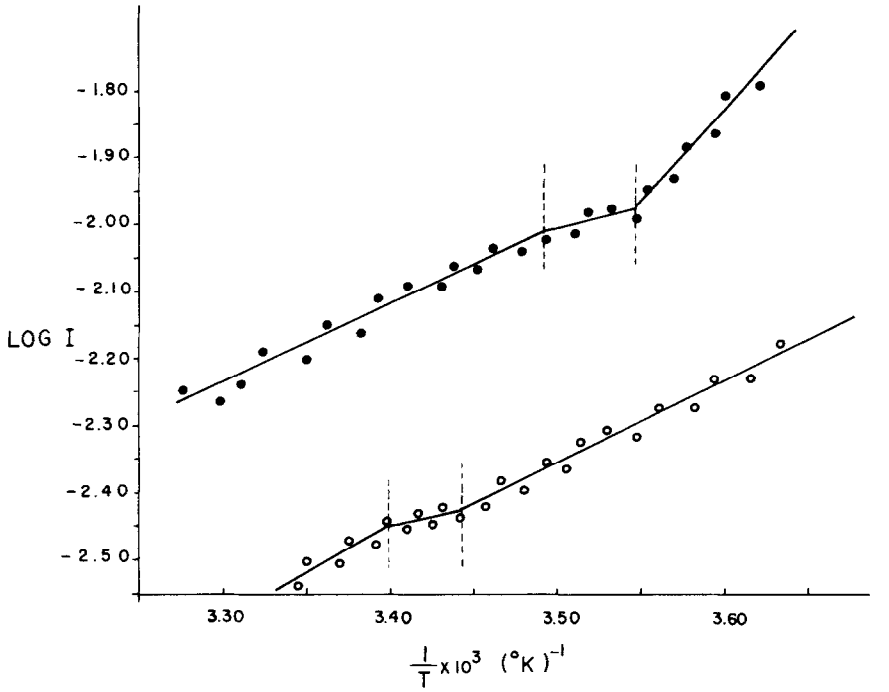

Figure 1: Plot of the fluorescence intensity of the NBD-PC phospholipid probe incorporated into the plasma membranes of control (closed circles) and cystic fibrosis (open circles) fibroblasts as a function of the reciprocal of the absolute temperature. The exciting wavelength was 465 nm and emission was observed at 525 nm. Excitation and emission slits were 10 and 5 nm respectively. Experimental conditions are the same as those described in the text. The cell concentration in each case was approximately 5 x 10^4 cells/ml in a 3 ml cuvette. The phase transition diagrams of the cystic fibrosis fibroblasts indicate both a different slope and widely different phase transition points from the normal cells. Phase transitions occur in these cells at 13.5^0 and 8.7^0 C respectively. Normal fibroblasts under identical conditions show phase transitions at 21.2 and 17.7 C respectively. All values are the average of triplicate determinations.

intensity when the fluorochrome in incorporated in the membrane of both cell types. This figure shows a distinct difference between normal fibroblasts and cystic fibroblasts with respect to their thermal transition characteristics. The normal cells show discontinuities at 21.2^0 C and 17.7^0 C while the abnormal cells apparently undergo transitions at 13.5^0 C and 8.7^0 C. As previously stated, such differences could be a reflection of the changes in the overall composition of their respective cytoplasmic membranes, or

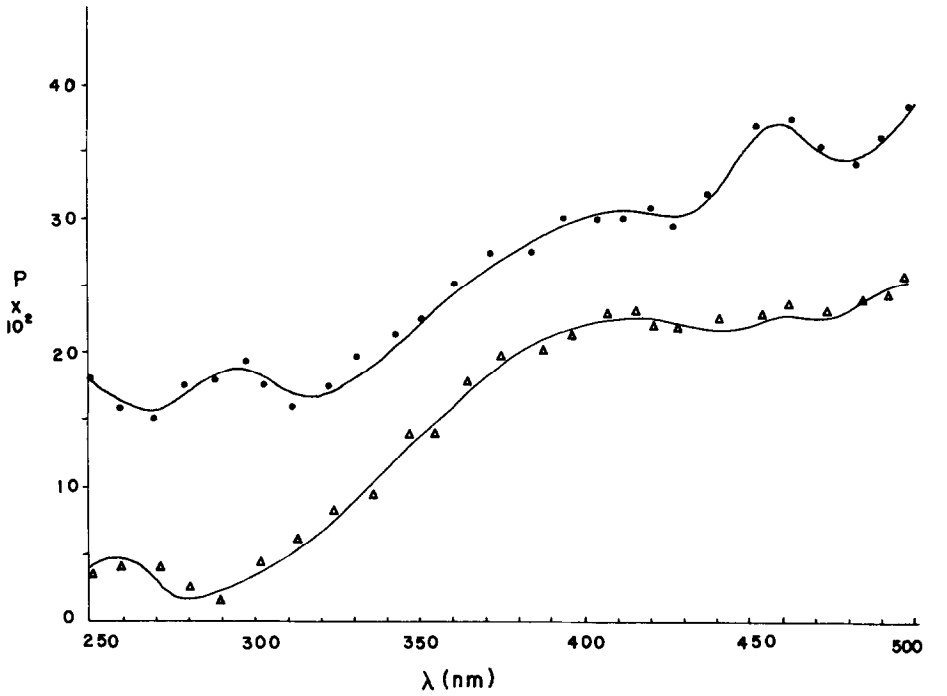

Figure 2: Polarization of fluorescence of the NBD-Cl phospholipid probe in the membrane of both normal and abnormal cell types measured as a function of the excitation wavelength. Emission was observed at 525 nm and excitation and emission slits were 10 and 5 nm respectively. The normal fibroblasts (closed circles) show a consistently higher degree of polarization than do the cystic fibrosis cells (triangles). Each data point represents the average of eight separate determinations in the case of normal fibroblasts and three separate determinations in the case of the cystic fibrosis cell.

alterations in the organization of the membrane's lipid layer (9). However, at this time, we do not know what specific difference(s) between normal and CF fibroblast membranes might account for our observation.

Figure 2 shows the differences in the polarization of fluorescence between these two cell types. These data show that not only are the polarization spectra different between the two cell types but that the polarization of the normal cells is higher than the CF cells. This may be explained by more restricted rotational mobility of the fluorescent probe in the normal fibroblasts. The abnormal cells, however, indicate a much lower degree of polarization. These data appear to be in agreement

with the transition temperature data, since higher transition temperatures usually indicate a higher membrane viscosity than do lower transition temperatures. Consequently, one might expect to find the polarization of fluorescence of the phospholipid probe in these membranes to change in the same direction as does the transition temperature. That is, the higher the transition temperature, the higher the degree of polarization.

The data presented in this report appear to clearly indicate physical-chemical differences between normal fibroblasts and fibroblasts obtained from patients with cystic fibrosis. Other investigators (10-14), using spin-label techniques, have reported finding lower surface mobility of membrane proteins in cells from patients with muscular dystrophy, and more recently, Huntingdon's disease (14). Our own data would suggest that cellular membranes from persons with cystic fibrosis contain regions of greater fluidity than membranes in normal cells.

Cystic fibrosis is a recessively inherited disease and no known chromosomal markers are present which would allow either an in utero diagnosis to be made, or the carriers themselves to be identified. Although our work in this area is in the early stage of development, we believe that our findings suggest that it may eventually be possible to diagnose the disease on a prenatal basis by obtaining cells from amniotic fluid, and using sufficiently sensitive instrumentation, identify the carriers of the disease by utilizing the phospholipid fluorescent probe--fibroblast membrane technique.

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