# MICROVISCOSITY IN THE SURFACE MEMBRANE LIPID LAYER OF INTACT NORMAL LYMPHOCYTES AND LEUKEMIC CELLS

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## 1. Introduction

Fluorescent hydrocarbon probes that can be embeded in membrane lipid layers [1–4], can be used to elucidate dynamic structural changes in cell surface membranes in relation to changes in the cellular regulatory mechanisms for cell growth. One of the most efficient such probes is 1,6-diphenyl 1,3, 5-hexatriene (DPH) which can be easily introduced into the hydrocarbon region of membrane lipid layers [5]. By analyzing the fluorescence polarization properties of the embeded DPH, the fluidity of membrane lipid layers can be quantitatively determined [5,6].

In the following we report results on the differences in fluidity of the surface membrane of normal lymphocytes and malignant transformed lymphoma cells obtained from experimental animals and humans. The experiments, which were carried out with intact viable cells labeled with DPH, show that malignant transformation of normal lymphocytes is associated with an increase in fluidity of the surface membrane lipid layers. It is suggested that the higher fluidity in the transformed cells is associated with a lower level of membrane cholesterol.

## 2. Materials and methods

Normal rats or mice lymph-node lymphocytes were collected by teasing the tissue apart in a solution of 0.15 M KCl and allowing the pieces to sediment [7,8]. The cells were then washed three times with 0.15 M KCl. The lymphoma cells were from an ascites form of a Maloney virus-induced lymphoma grown in A strain mice [9]. Cells ($10^5$) were inoculated intra-peritoneally into adult mice, and the cells were collected and washed with 0.15 M KCl 10 days after inoculatation [10]. Normal lymphocytes and chronic lymphatic leukemic cells from eight human patients and eight human donors were obtained from peripheral blood. Cells were isolated from freshly drawn human blood by Ficoll–Hypaque gradient centrifugation [11] and washed three times with 0.15 M KCl.

Labeling of cells with DPH was performed as follows: 0.1 ml of $2 \times 10^{-3}$ M DPH in tetrahydrofuran was injected into vigorously stirred 100 ml 0.15 M KCl. Stirring was continued for 15 min at 25°C, and a clear dispersion of $2 \times 10^{-6}$ M DPH, which is practically void of fluorescence, was obtained. One volume of cell suspension ($5 \times 10^6 - 2.5 \times 10^7$ cells/ml) was mixed with one volume of the DPH dispersion. Upon penetration of DPH into the surface membrane the fluorescence signal increases steeply and levels off after about 60 min of incubation at 25°C. The labeled cells were then twice washed, resuspended in 0.15 M KCl, and immediately used for fluorescence measurements. Suspensions of unlabeled cells were used as reference samples.

Fluorescence polarizarion and intensity were measured with an instrument which was previously described [12]. A 366 nm band generated from a 500 W mercury arc, which was passed through a Glan–Thompson polarizer, was used for excitation. The emitted light was detected in two independent cross

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Table 1

Degree of fluorescence polarization *, $P$, and the apparent microviscosity, $\bar{\eta}$, obtained with normal lymphocytes and lymphoma cells labeled with DPH at different temperatures.

| Cells | 4°C | | 25°C | | 37°C | |
|---|---|---|---|---|---|---|
| | $P$ | $\bar{\eta}$ (poise) | $P$ | $\bar{\eta}$ (poise) | $P$ | $\bar{\eta}$ (poise) |
| Normal lymphocytes (rats or mice) ($5 \times 10^6$ cells/ml) | 0.335–0.340 | 6.7–7.0 | 0.270–0.275 | 2.5–2.7 | 0.224–0.228 | 1.5–1.6 |
| Lymphoma cells (mice) ($2 \times 10^6$ cells/ml) | 0.278–0.286 | 3.7–4.1 | 0.206–0.219 | 1.3–1.6 | 0.156–0.167 | 0.8–1.0 |
| Normal lymphocytes (humans) ($5 \times 10^6$ cells/ml) | 0.340–0.347 | 7.1–7.7 | 0.291–0.296 | 3.0–3.2 | 0.252–0.257 | 1.9–2.0 |
| Chronic lymphatic leukemic cells (humans) ($5 \times 10^6$ cells/ml) | 0.308–0.322 | 5.2–6.0 | 0.254–0.275 | 2.2–2.7 | 0.215–0.228 | 1.4–1.6 |

* $\lambda_{ex}$ = 366 nm.

polarized channels equipped with Glan–Thompson polarizers after passing through a 2 M sodium nitrite solution used as a cut off filter. The emission intensities polarized parallel ($I_{\parallel}$) and perpendicular ($I_{\perp}$) to the direction of polarization of the excitation beam, were obtained by a simultaneous measurement of $I_{\parallel}/I_{\perp}$ and $I_{\perp}$. These intensities relate to the degree of fluorescence polarization, $P$, and to the total fluorescence intensity, $F$, by the following equations:

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}; \quad F = I_{\parallel} + 2I_{\perp} \qquad (1)$$

With the aid of $P$ and $F$ the microviscosity, $\bar{\eta}$, was determined [1,2].

## 3. Results and discussion

Fluorescence polarization measurements with cell suspensions are liable to serious experimental errors [13,14]. The two main sources of errors originate from depolarization by scattering of the fluorescence signals, and from penetration of scattered excitation or stray light into the emission channels. However, with highly efficient fluorescence probes such as DPH these errors can be easily quantitized or even eliminated. Upon sequential dilutions of the DPH labeled cell suspensions with 0.15 M KCl a constant value of $P$ is reached at concentrations of $1 \times 10^6 - 5 \times 10^6$ cells/ml. At these cell concentrations scattering depolarization of the fluorescence light is negligible, and the contribution of scattered light to the fluorescence signal, as checked with the reference unlabeled cell samples, was found to be less than 2% in all systems analyzed. According to these findings errors of only about 1% were estimated for the determined $P$ values, and no corrections were therefore made.

The degree of fluorescence polarization, $P$, and the fluorescence intensity, $F$, of the DPH labeled cells were measured at 4°C, 25°C and 37°C. For each type of cells, at least 10 samples taken from different individuals, were analyzed. The microviscosity, $\bar{\eta}$, of the hydrocarbon region for each cell sample was determined with the aid of the corresponding $P$ and $F$ according to the method which was previously outlined [1,2]. Table 1 presents the range of $P$ and $\bar{\eta}$ values obtained with all cell samples tested. Identical values of $P$ and $\bar{\eta}$ were obtained in the presence of $10^{-2}$ M $NaN_3$ which excluded the possibility of DPH pinocytosis. It was therefore assumed that the determined values of $P$ and $\bar{\eta}$ relate only to the lipid layer of the cell surgace membrane.

As shown in table 1 the ranges of the determined $P$ and $\bar{\eta}$ are rather narrow which strongly suggests that the surface membrane of each type of the analyzed cells has a specific lipid composition. The malignant transformed cells tested possess distinctively lower $\bar{\eta}$ values than those of the normal lymphocytes of the same species. The ration between the $\bar{\eta}$ values in the normal and respected transformed cells is approximately the same at 4°C, 25°C and 37°C. Such a characteristic is typical of lipid bilayers containing

different amounts of cholesterol [2,4]. Since in animal cells cholesterol is present in the surface membrane only [15], this suggests that the increase in fluidity of the surface membrane lipid layer upon malignant transformation of normal lymphocytes mainly originates from decrease in cholesterol level. It can be therefore suggested that the relative amount of cholesterol present in the cell surface membrane [15] may play a major role in the control mechanisms for cell replication and malignancy.

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