Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

In Situ Electrophoresis of Cancer Cell Membrane Components

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Version of record first published: 17 Oct 2011.

To cite this article: Y.-S. Nie , Y.-B. Feng , M.-Q. Tan , N.-M. Chao & D.-P. Zhao (1983): In Situ Electrophoresis of Cancer Cell Membrane Components, Molecular Crystals and Liquid Crystals, 97:1, 317-324

To link to this article: http://dx.doi.org/10.1080/00268948308073160

Mol. Cryst. Liq. Cryst., 1983, vol. 97, pp. 317–324
0026-8941/83/9704–0317/$18.50/0

Printed in the United States of America

In Situ Electrophoresis of Cancer Cell Membrane Components

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(Received January 1, 1983)

Biomembranes show some properties of liquid crystal. The molecules of the membrane can diffuse along the membrane plane. ISE (In Situ Electrophoresis) is one of the new techniques to measure the lateral diffusion of the molecules. By this technique, we studied the membrane fluidity of Ehrlich ascitic cancer cell of a mouse, and obtained the diffusion coefficient $D = 3.2 \times 10^{-10}$ cm^2/sec and the electrophoretic mobility $m = 1.3 \times 10^{-4}$ (μ/sec)/(v/cm) of the Con A receptors of the membrane at 22°C. A larger dispersion of the diffusion coefficient was noticed. We suggest that this is probably one of the characteristics of cancer cells.

I. INTRODUCTION

It is clear that biomembranes show some properties of liquid crystal. The main composition of biomembranes is lipids and proteins. The nonpolar portions of the lipids and the bulk of the nonpolar amino acid residues of the proteins are in the interior of the membrane, while the polar portions of the lipids and the charged amino acid residues of the proteins are both at the surface of the membrane. The lipid molecules are arranged in an orderly way within the membrane, primarily forming a lipid bilayer, and

the protein molecules are embedded in the lipid bilayer. The lipid molecules, as well as some of the proteins, possess a degree of fluidity that allows some lateral movement within the lipid matrix of the membrane.[1]

The membrane fluidity of a cell is closely related to its function. In the last decade, studies have quickly developed in this field, many new physical, chemical and biological techniques have been used. ISE is one of new techniques developed recently to measure the fluidity of biomembranes. It is different from ordinary cell electrophoresis. In ISE experiment, the cells adhere firmly to the bottom of an electrophoresis chamber and are kept in living state while exposed to a suitable steady electric field. Charged membrane component molecules of the cell will then undergo redistribution, migrating to one side of the cell along the membrane plane under the influence of the applied electric field. After removal of the field, the molecules would diffuse back and gradually return to uniform distribution in the membrane, provided the field strength is not too high and the time of cell exposure to the field is not too long. The electrophoretic mobility and the diffusion coefficient of the charged molecules may thus be determined by measuring the migration and back diffusion processes.

The possibility of ISE was first suggested by Jaffe[2] in 1977, and soon demonstrated experimentally by Poo.[3] Many experimental and theoretical studies have been developed by Poo *et al.* with Xenopus myotomal cells.[3–7,9] We think it would be of significance to measure the fluidity of cancer cell membrane by ISE. In fact, the study of the membrane fluidity is one of the most important aspects of cancer cell studies. In this paper, we present our preliminary results on the study of the fluidity of Con A receptors of Ehrlich ascitic cancer cell membrane by ISE.

II. THEORETICAL MODEL

The theory of ISE of membrane component molecules of a spherical cell is based on the model depicted schematically in Figure 1. The hydrophobic segment of the molecule (the charge on it is arbitrarily chosen as negative) is exposed into the aqueous phase around the cell. Normally, the distribution of the molecules is uniform in the membrane plane (Figure 1a). When the cell is placed into a uniform electric field, the field lines around it will be slightly distorted by the cell (Figure 1c) and the charged molecules within the cell membrane will accumulate to one side of the cell along the membrane plane (Figure 1b). In the meanwhile, the density gradient caused by the accumulation results in back diffusion of the molecules. When the flux of the electrophoretic migration equals that of the back diffusion, the

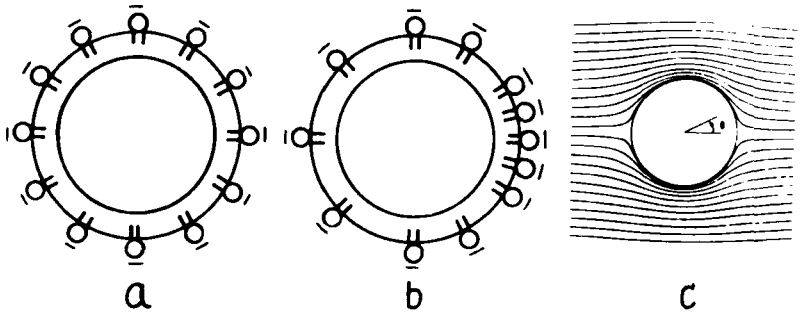


FIGURE 1 Simple model of ISE of spherical cell.

surface density distribution at the equilibrium state $C_s(\theta)$ can be expressed by

$$C_s(\theta) = \alpha \exp[-\beta(1 + \cos \theta)] \quad (1)$$

Where $\alpha = 2\beta C_0/[1\text{-}\exp(-2\beta)]$, $\beta = 1.5E_0rm/D$, r is the radius of the cell, C_0 is the initial surface density of the molecules, E_0 is the magnitude of the field applied, D is the diffusion coefficient, m is the electrophoretic mobility, and θ is the polar angle.[5]

An experimentally useful parameter for characterizing the distribution of membrane component molecules is the Asymmetry Index (A), which can be defined as:

$$A(t) = [C(180^0, t) - C(0^0,t)]/[C(180^0,t) + C(0^0,t)] \quad (2)$$

where $C(0^0,t)$ and $C(180^0,t)$ are the surface densities of the molecules facing the anode and cathode of the field at time t, respectively. The field is removed when the equilibrium distribution is attained, then the molecules will undergo a back diffusion. From the equation $\partial C(\theta, t)/\partial t = D\nabla^2 C(\theta, t)$ and initial condition $C(\theta, 0) = C_s(\theta)$, the accurate solution of $C(\theta, t)$ can be obtained. Taking the first-order estimate of the solution, assuming it is within the experimentally accessible accuracy, $A(t)$ can be simplified as

$$A(t) = A_s \exp(-2Dt/r^2) \quad (3)$$

then

$$D = r^2/2\tau_d \quad (4)$$

$$m = (D/3E_0r)\ln[(1 + A_s)/(1 - A_s)] \quad (5)$$

where A_s is the asymmetry index at the equilibrium state, τ_d is the characteristic 1/e decay time of the asymmetry index.[6]

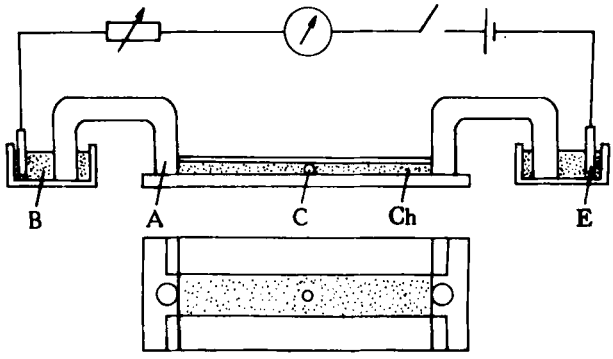


FIGURE 2 Schematic diagram of electrophoresis apparatus (side and top view). B, electrode bath; A, saline bridge; C, cell; Ch, electrophoresis chamber; E, electrode.

III. MATERIALS AND METHODS

The experimental apparatus employed (Figure 2) was similar to Poo's.[5] The Ehrlich ascitic cancer cells were drawn from abdominal cavity of a mouse and washed with physiological saline (pH 7.2), then a suspension of the cell of suitable concentration was prepared. The suspension was filled into a electrophoresis chamber ($50 \times 8.4 \times 0.2$ mm^3) later on. The microscope slide which served as the bottom of the electrophoresis chamber was treated in advance for adhering of the cells to it. The cells were kept alive. An electric field of 12.5 ± 0.5 v/cm was applied through two saline bridges with 2% agarose. The current through the electrophoresis chamber was 2.4 ± 0.2 mA. The electrodes were made of graphite. Both field application and the post-field back diffusion processes of the molecules were at 22°C. The cell membrane component was labeled with 20 μg/ml FITC-Con A (Vector Laboratory Inc. USA) at 2°C for detecting the migration and back diffusion of the membrane component molecules. Con A (Concanavalin A), a sort of lectins, is able to combine specifically with some glycoproteins of the cell membrane.[8] The flourescence intensity of the membrane surface is proportional to the density of the Con A receptors, therefore the migration and back diffusion of the Con A receptors may be determined by measuring the changes of the distribution of fluorescence intensity of the membrane surface. The fluorescence intensity was measured by a Type SMP-05 microspectrophotometer (OPTON, W. Germany). The effective diameter of the measuring aperture is about 4 μm. The fluorescence intensity of the ring stain on the spherical cell was measured at the poles of the cell facing the anode ($\theta = 0°$) and the cathode ($\theta = 180°$)

of the field, respectively. The background fluorescence intensity was measured at the adjacent regions outside the cell. From these data we obtained the asymmetry index of each cell $A = (I_{180°} - I_{0°})/(I_{180°} + I_{0°})$, where $I_{0°}$ and $I_{180°}$ refer to the net fluorescence intensity, that is, the background intensity was subtracted from the ring stain intensity measurements. The cells measured were randomly selected. We rejected the cells whose size or shape was too abnormal and the cells which were quite close to each other. The diameter of the cells measured was about 16 μm. In this report, the average value of 40 cells measured was presented as a single point on the curves.

IV. RESULTS AND DISCUSSION

Figure 3 shows the changes of the asymmetry index during the electrophoretic migration and the back diffusion processes. Before exposure to the electric field, the average value of A was equal to zero. The uniform ring stain of the cells was seen through the fluoromicroscope (Figure 4a). While exposed to an applied electric field, the Con A receptors were gradually accumulated to one side of the cells and the value of A was quickly increasing. At the end of 15 min of exposure to the electric field, the A value of the cells reached 0.52 and approached to a steady state, showing

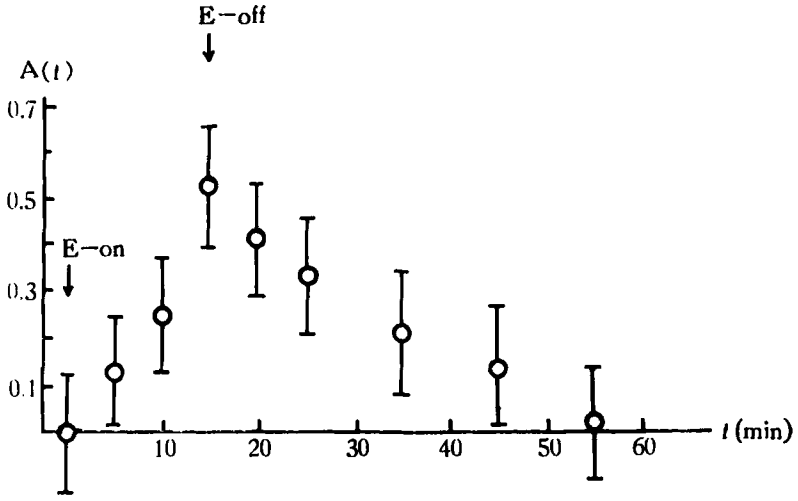
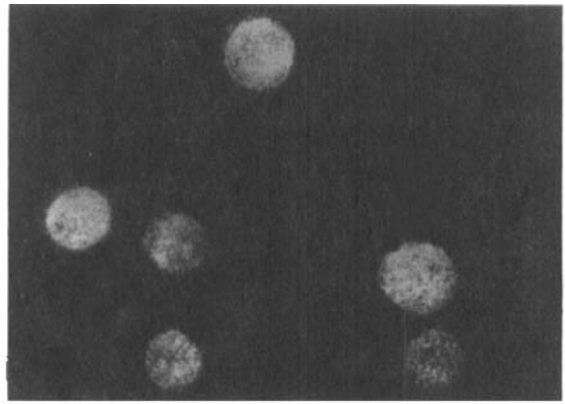
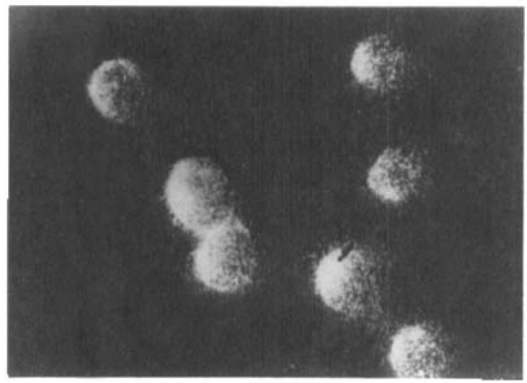


FIGURE 3 Changes of A during electrophoretic migration and back diffusion processes. E_0 = 12 v/cm, T = 22°C.



(*a*)



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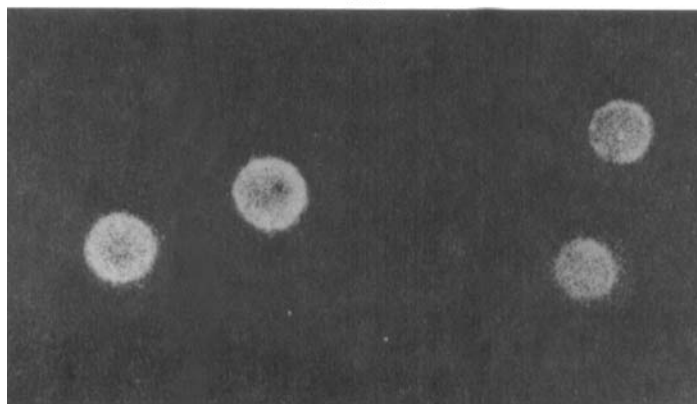

(*c*)

FIGURE 4 The cells seen through the fluoromicroscope.

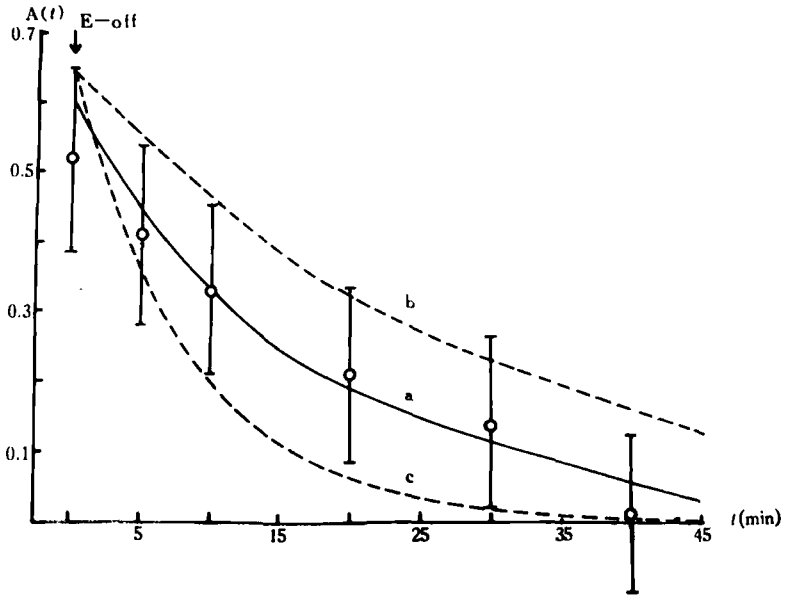

FIGURE 5 Theoretical curves fitted to the experimental data: a: $D = 3.2 \times 10^{-10}$ cm^2/sec, b: $D = 1.9 \times 10^{-10}$ cm^2/sec, c: $D = 6.5 \times 10^{-10}$ cm^2/sec, $E_0 = 12$ v/cm, T = 22°C.

that an equilibrium distribution was almost attained. At this time, the asymmetric distribution of fluorescence on the cell membrane was seen clearly (Figure 4b). Just after this time the field was removed, the value of A gradually decreased, and at 40 min after the removal of the electric field the average value of A was about 0.01. This suggested that the Con A receptors had already nearly recovered to a state of uniform distribution similar to the state before exposure to the field (Figure 4c).

Based on the above theoretical analysis, the diffusion coefficient D can be determined by the Eqs. 3 or 4. Figure 5 shows the theoretical curves fitted in with the experimental data. According to these curves, we obtained $D = 3.2 \times 10^{-10}$ cm^2/sec with a range from 1.9 to 6.5×10^{-10} cm^2/sec. We also obtained $m = 1.3 \times 10^{-4}$ (μm/sec)/(v/cm) by Eq. 5.

FPR (Fluorescence-Photobleaching Recovery) technique can also be used for measuring the lateral diffusion of cell membrane component.[5] For FPR technique, with the exception of some specific samples (*e.g.* the disc membrane of ROS), the membrane components have to be labeled in advance; therefore, one cannot directly obtain the fluidity of the membrane component itself, but that of the component-fluorescein complexes. For ISE technique, membrane component is post-field labeled, the results obtained reflect directly the fluidity of the component itself, so the diffusion coefficient with FPR is, generally, less than that with ISE.[9] In FPR experi-

ment, the microarea of the cell membrane would be possibly damaged by the powerful light, which would influence the normal physiological state of the cell. In ISE experiment, the applied electric field E_0 will produce a field E_i within the cell. The calculation shows that the intracellular field E_i is less than $10^{-4}\ E_0$, in other words, E_i is less than 1 mv/cm (taking $E_0 = 10$ v/cm). Such a small field is unlikely to produce significant intracellular effects,[6] but the effect of an external electric field on membrane potential is possibly involved in altering the topography and mobility of membrane components.[10]

The behavior of a cancer cell is different from a normal cell. The fluidity of their membranes may be different from each other, too. In Figures 3 and 4, it can be seen that the error bars of the experimental data are larger, about twice that of normal cells under similar experimental conditions.[5] That may be due to the irregularity of the cancer cells. Since the cells are in an active proliferating state, their membrane fluidity is different from each other at different physiological states. It may be true that a larger dispersion of the diffusion coefficient of membrane components is one of characteristics of cancer cell.

Acknowledgments

The authors are grateful to Prof. M.-M. Poo for his valuable comments and supports, to Prof. S. M. Shen and Prof. J. Liu for their helpful advice, to Dr. L.-S. Cheng and Dr. Q.-E Chen for their kind help.

Dr. R.-G. He and Dr. K.-S. Hu took part in the earlier experiment.

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