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Differences in membrane electrical properties between C3H 10T1/2 mouse embryo fibroblasts and their ionizing radiation and chemically transformed counterparts

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Abstract. Membrane electrical properties of mouse embryo fibroblasts and their ionizing radiation and chemically transformed counterparts were investigated using dielectric relaxation measurements in the radio frequency range. This determination is possible because, in the radio frequency range, suspensions of cells in an electrolyte buffer show a conductivity dispersion due to interfacial polarization. An analysis of the experimental data based on a "single-shell" model showed that conductivity and permittivity of the membranes of both radiation and chemically transformed fibroblasts were lower than in normal cells. In addition, the conductivity of the cytoplasm was higher in both transformed cell types than in the normal mouse fibroblasts. We discuss the significance of these findings in view of the possible structural and functional modifications brought about by the process of neoplastic transformation.

Key words: Membrane conductivity – Radiowave frequencies – Maxwell-Wagner effect – Transformed fibroblasts

Introduction

Cellular transformation induced by either ionizing radiation or by chemical agents has been shown to have a wide variety of effects on cells (Yang and Tobias 1980; Borek 1982). For instance, both ionizing radiation and chemical transformation have been shown to damage the cellular genome (Bowden et al. 1990). Changes at the genetic level are, in turn, also expressed at other cellular sites. The cell membrane is one such very important location where variations at the genetic level may become expressed (Nicolson and Poste 1976; Nicolson 1984). Any changes in the composition, orientation or mobility of membrane components may perturb transmembrane signalling and membrane ionic fluxes, as well as cause alterations in cellular metabolism.

Dielectric relaxation studies in the radiowave frequency range can be a useful tool in examining the electrical characteristics of biological membranes. This technique is based upon the fact that cell membranes separate media of different dielectric properties (for instance, the cytoplasm from the external medium) which also results in charge accumulation at these interfaces. Thus, the cell suspension conductivity which shows a marked frequency dispersion (β-dispersion) between 10^4 and 10^8 Hz, known as the Maxwell-Wagner effect (Pethig and Kell 1987), can be used to determine the characteristics of the membrane by the use of a proper curve-fitting procedure. The electrical properties of the cell membrane (membrane conductivity and membrane permittivity) as well as the conductivity of the cytoplasm can be extracted from the conductivity measurement of the cell suspension.

The possible differences in membrane electrical properties between control and transformed cells, and, for that matter, between radiation and chemically transformed cell types have been the subject of very few investigations. Therefore, the aim of the present study was to determine whether control, ionizing radiation (protons) transformed and chemically (MCA) transformed C3H 10T1/2 mouse embryo fibroblasts have differences in membrane electrical properties. The results show that both types of transformed cells have lower membrane conductivity and membrane permittivity values than controls, but that there appears to be no significant difference between the two transformed cell types themselves. In addition, the conductivity of the cytoplasm is higher in both ionizing radiation and chemically transformed fibroblasts than in controls. Possible mechanisms which can explain the observed differences in dielectric properties between normal and transformed C3H 10T1/2 cells as well as the similarities in membrane characteristics in both radiation and chemically transformed cells are discussed.

Materials and methods

Cell culture and transformation conditions

The C3H mouse embryo fibroblast line 10T1/2 clone 8 developed for transformation assays by Reznikoff et al.

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(1973 a) was used in this study. Control, chemically transformed and ionizing radiation transformed cell lines were cultured in monolayer at 37 °C in a humidified 5% CO_2 atmosphere in Eagle's basal medium prepared in our laboratory with Gibco products and supplemented with 10% fetal calf serum (Flow Laboratories) heat-inactivated for 45 min at 56 °C. The following concentrations of antibiotics were added to the medium: gentamicin, 50 μg/ml; penicillin, 50 U/ml; and streptomycin, 50 μg/ml.

The cell line (F6 10T1/2) transformed by exposure to 31 MeV protons (Bettega et al. 1985) was a kind gift from Prof. Lucia Tallone Lombardi of the University of Milano. Only cells of a single clone selected from a Type III focus using the criteria suggested by Reznikoff et al. (1973 b) were subcultured in the above mentioned conditions and tested for carcinogenesis by standard methods. The cell line (MCA 10T1/2) transformed by the chemical mutagen, 3-methylcholanthrene (MCA) (Reznikoff et al. 1973 b), was generously provided by Dr. Joseph R. Landolph of the University of Southern California.

Conductivity measurements

C3H 10T1/2 cells and their transformed counterparts were grown in BME medium to subconfluence phase, washed twice with cold phosphate-buffered saline (PBS) while still in the culture flasks, trypsinized by standard methods, collected and centrifuged for 10 min at 2 000 g at 4 °C. In order to consider the possible effects of trypsinization on the dielectric properties of the fibroblast membranes, the cells were scraped by rubber policemen and measured. No significant differences in membrane electrical properties with respect to the trypsinized cells were observed. The pellet was washed twice with PBS to remove the trypsin, centrifuged again, the buffer solution removed by aspiration, the sides of the tube were cleared of residual buffer solution and the cell pellet was resuspended in PBS (pH 7.4) so that the volume of the cells (fractional volume ϕ) was about 30–40%, depending on the experiment, of the total volume of the sample.

Cellular volume was determined by using a Coulter Channelyzer. Latex (13 μm diameter) was used as reference standard for the cell size distribution. Only subconfluent phase cells (populations consisted of about 10^4 cells) were analyzed. The mean value was 1 816 μm^3 for control 10T1/2 cells while it was 1 737 μm^3 for both F6 10T1/2 and MCA 10T1/2 cells. The standard deviation was about 3% of the mean value.

Electrical conductivity measurements were carried out in the frequency range from 10 kHz to 100 MHz by means of two Hewlett-Packard impedance analyzers (models 4192A and 4193A) controlled by a Digital Equipment Computer system. The conductivity cell consists of a section of a circular wave-guide excited well beyond its cut-off frequency. The cell constants were determined by calibration measurements with standard liquids of known dielectric constant and conductivity. The experimental apparatus and procedure are described elsewhere (Ballario et al. 1984; Bottomley 1978). The measurements were carried out at a temperature of 20.0 ± 0.1 °C. Errors in conductivity of the cell suspension were estimated to be within 1% throughout the entire frequency range examined.

The integrity of the cell membrane was tested after prolonged incubation in calcium-free saline buffer. Subconfluent cultures were trypsinized and centrifuged in BME medium. Cell pellets were resuspended in PBS at the same concentration as that used during the conductivity measurements and kept at room temperature (20 °C) for various periods of time, t. Trypan blue (0.5%) was added to the cell suspension at the end of t, cells observed under a light microscope and viable cells, i.e. with integral membrane, counted. Dead cells appear blue-stained in this test. For $t < 1$ h, about 75% of the cells were viable and no morphological differences were observed among normal, radiation transformed and MCA transformed fibroblasts.

Dielectric model

The electrical properties of the cell membrane (membrane conductivity σ_s and membrane permittivity ε_s) as well as the conductivity of the cytoplasm σ_p can be extracted from the conductivity of the cell suspension as a function of frequency. The analysis is based on a single-cell model in which the cell is represented as a sphere covered by a shell (the cell membrane) of conductivity $\sigma_s^* = \sigma_s + i\,\omega\,\varepsilon_0\,\varepsilon_s$, a cell interior (cytoplasm) of conductivity $\sigma_p^* = \sigma_p + i\,\omega\,\varepsilon_0\,\varepsilon_p$, randomly distributed in a continuous medium of complex conductivity described by $\sigma_m^* = \sigma_m + i\,\omega\,\varepsilon_0\,\varepsilon_m$. In these expressions, ε_0 is the permittivity of free space, ω is the angular frequency of the applied field and i is the imaginary unit. A fitting procedure similar to that used by Asami et al. (1980) was used. A diameter of 15.1 μm for control cells and 14.9 μm for both transformed cells was used as determined by Coulter analysis. The thickness of the cell membrane was taken as 7.5 nm (Harrison and Lunt 1980).

Results

Figure 1 shows electrical conductivity as a function of frequency of suspensions of control CH3 10T1/2 (curve a), MCA 10T1/2 (curve b) and F6 10T1/2 (curve c) fibroblasts. The conductivity of the buffer solution is also shown (curve d). A marked electrode polarization effect exists at low frequencies (up to $3 \cdot 10^4$ Hz) due to the high values of the d. c. ionic conductivity of these samples (data not shown). This phenomenon is related to the existence of an electrical double layer at the electrode-buffer interface that behaves as a capacitor dependent mainly on the frequency of the applied field, buffer conductivity and electrode surface area. This capacitance-like behavior is responsible for the lowering of the measured conductivity values at low frequencies.

Curve d shows that the conductivity of the buffer solution is constant from 10^5 to 10^8 Hz. This may be expected since there is no interfacial polarization in this salt solution. In contrast, curves a, b and c show the marked conductivity dispersion typical of interfacial polarization

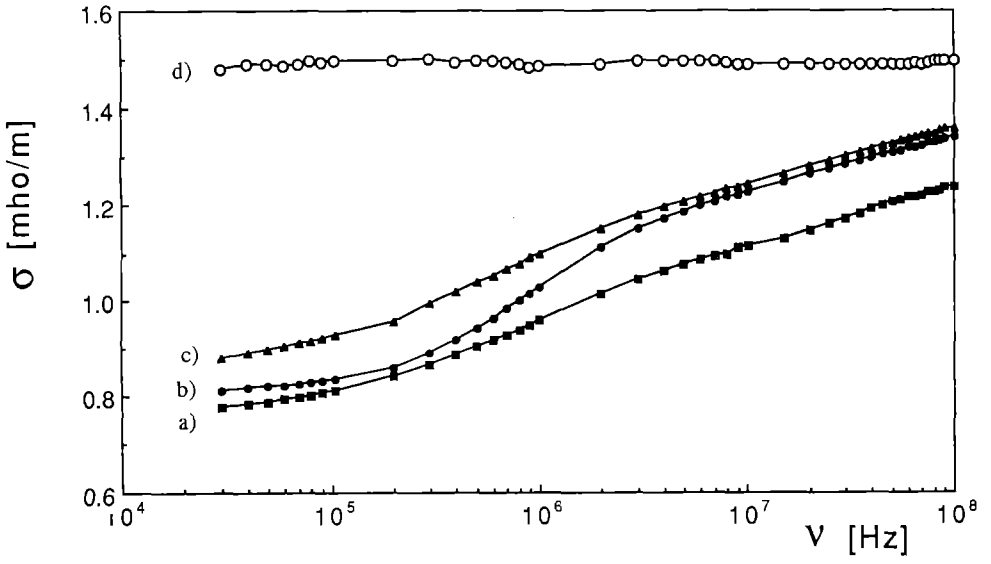

Fig. 1. Conductivity of suspension of mouse embryo fibroblasts at 20 °C as a function of frequency: *a)* control C3H 10T1/2 cells with a fractional volume (ϕ) of 0.34, *b)* MCA 10T1/2 (chemically transformed) fibroblasts with a fractional volume of 0.32, *c)* F6 10T1/2 (radiation transformed) cells with a fractional volume of 0.33, and *d)* phosphate-buffered saline (pH 7.4). The solid lines are drawn for visual purposes only. The frequency dependence of conductivity (at frequencies below 10^4 Hz) due to electrode polarization is not shown

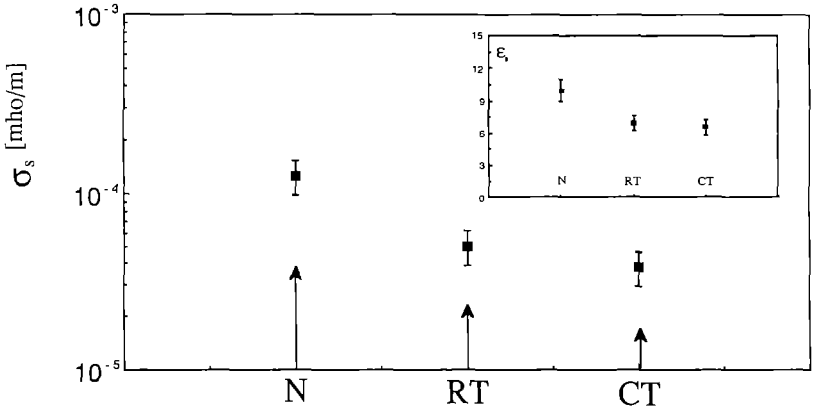

Fig. 2. Membrane conductivity (σ_s) and membrane permittivity (ε_s) (*insert*) values of control (N), radiation transformed (RT) and chemically transformed (CT) mouse embryo fibroblasts. Each point represents the mean and the maximum uncertainty of three separate experiments conducted for each cell type

in heterogeneous systems. The conductivity increment (from low to high frequencies) was less pronounced in controls (curve a) than in transformed fibroblasts (curves b and c) when normalized with respect to fractional cell volume. Although these differences appear quite small in the figure, they indicated to us possible variations in membrane surface properties between these cells or other more subtle variations in membrane structure and/or function. These observations prompted further examination of the differences noted by elaboration of the data with the "single-shell" model which is able to yield more quantitative variations.

Figure 2 gives mean values of membrane conductivity σ_s of control, radiation transformed and chemically transformed mouse embryo fibroblasts after the data in Fig. 1 had been analyzed by the fitting procedure based upon the "single-shell" model. As can be seen, elaboration did indeed yield differences in membrane electrical properties. CH3 10T1/2 cells have a higher conductivity value ($(1.25 \pm 0.35) \cdot 10^{-4}\ \Omega^{-1}\ m^{-1}$) than both F6 10T1/2 ($(5.0 \pm 0.9) \cdot 10^{-5}\ \Omega^{-1}\ m^{-1}$) and MCA 10T1/2 ($(3.8 \pm 0.9) \cdot 10^{-5}\ \Omega^{-1}\ m^{-1}$) fibroblasts. A similar behavior in membrane permittivity ε_s also occurs in these mouse fibroblasts (insert). Again, control cells have a higher permittivity value (10 ± 2) than both radiation transformed (6.9 ± 0.9) and chemically transformed (6.6 ± 0.9) mouse embryo fibroblasts.

Finally, in Fig. 3 are shown values of the conductivity of the cytoplasm σ_p of CH3 10T1/2 cells and their transformed counterparts. As can be seen, control cells have a lower conductivity ($0.70 \pm 0.07\ \Omega^{-1}\ m^{-1}$) than both types of transformed cells ($0.97 \pm 0.09\ \Omega^{-1}\ m^{-1}$ for ionizing radiation transformed cells and $0.94 \pm 0.09\ \Omega^{-1}\ m^{-1}$ for chemically transformed fibroblasts, respectively). Thus, there is an increase in cytoplasmatic conductivity in transformed cells while both membrane conductivity and membrane permittivity are lower (Fig. 2).

Discussion

The results presented in this report indicate that neoplastic transformation induces structural changes in both the plasma membrane and cytoplasm of mouse embryo fibroblasts. Evidence for the membrane variations comes from the observed decrease in both membrane conductivity (σ_s) and membrane permittivity (ε_s) in radiation as well as chemically transformed cells. Membrane conductivity is a measure of the overall ionic transport across the

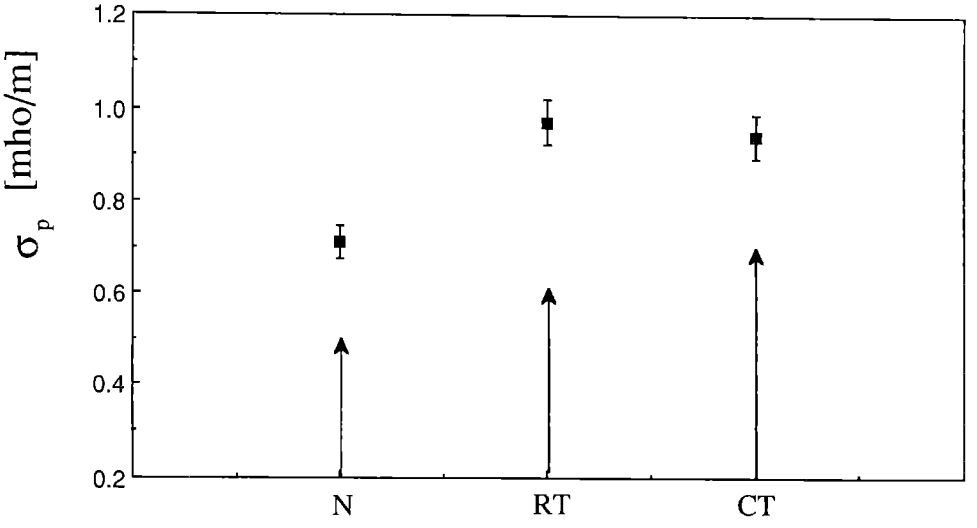

Fig. 3. Conductivity of the cell interior (σ_p) of control (N), radiation transformed (RT) and chemically transformed (CT) mouse embryo fibroblasts. Each point again represents the mean and the maximum uncertainty of three separate experiments conducted for each cell type

cell membrane through ion channels while membrane permittivity takes into account the distribution of charges and/or polar groups inside the lipid double layer and the contribution due to ion distribution at the aqueous-hydrophilic interface. Therefore, conductivity may be viewed as a measure of the dynamic processes dependent upon net ionic fluxes whereas permittivity, although indirectly dependent upon these same dynamic processes, is indicative of both the static and external field-induced charges present in the membrane. Since both of these parameters vary, it is apparent that both dynamic as well as static ionic properties of the fibroblasts are influenced by the transformation process.

The lower membrane conductivity observed in transformed fibroblasts seems to indicate that a decrease in ionic flux across the fibroblast membrane, perhaps by the closing of specific membrane channels and/or pumps, has taken place. It has been demonstrated that in transformed cells the Na^+/K^+-ATPase is altered (Borek and Guernsey 1981). Alteration in this pump may therefore explain, at least in part, the variation in membrane conductivity observed here. In fact, it has been previously demonstrated using the same dielectric relaxation method described in this work that variations in membrane conductivity in chick embryo myoblasts may be due to variations in ion channels and/or pumps, particularly in an alteration in K^+ channels and, consequently, the Na^+/K^+ equilibrium (Santini et al. 1988). Perhaps, a similar mechanism is in force in transformed fibroblasts. In addition, it has also been shown that transformed cells require a lower calcium concentration than normal cells in order to survive (Swierenga et al. 1978; Borek 1980). This change in the necessary calcium concentration may also be related to changes in the Na^+/K^+ equilibrium since a number of calcium-activated potassium channels which respond to the calcium ion concentration are present in cells (Hille 1984). Further study of this problem is necessary in order to establish if this hypothesis is correct.

The points discussed thus far are sufficient to explain the changes in membrane conductivity, which are influenced by active ionic transport. However, the variations in membrane permittivity, which depend upon field-induced motions of membrane components, need further discussion. First, it must be noted that the membrane permittivity values found in both controls (10 ± 2) and transformed fibroblasts (6.9 ± 0.9 and 6.6 ± 0.9) appear to have a value higher than that reported in the literature. For instance, for black lipid membranes (BLM) the value of total membrane permittivity is between 2.2 and 2.4 (Pethig and Kell 1987). However, it must also be noted that the use of this β-dispersion method to evaluate membrane permittivity may lead to an overestimation of this value (Pethig and Kell 1987). Thus, the higher values observed may be the result of the method used. Nonetheless, the absolute values of membrane permittivity (and for that matter for membrane conductivity and the conductivity of the cytoplasm as well) are not of extreme importance in this study. In fact, it was the purpose of this paper to see if changes in these parameters between control and transformed fibroblasts exist. Since large differences in these parameters appear to exist, the results presented here seem to confirm the literature that large variations in membrane structure and function are induced by neoplastic transformation. It has been shown that in transformed cells neoantigens form (Embleton and Heidelberger 1975) and glycoproteins decrease, disappear, or no longer are completely glycosylated (Gahnberg and Hakomori 1973). Thus, transformed cells have a completely different membrane protein distribution than normal cells. Consequently, the charge distribution changes in these cells.

In addition, changes in charge distribution and induced dipoles may also be due to membrane lipids which are, together with proteins, one of the main constituents of membranes and are, in fact, the major contributors of membrane permittivity. Transformed cells have a reduction in higher gangliosides (Brady et al. 1969; Borek et al. 1977) perhaps due to an incomplete glycosylation of sialoglycolipids (gangliosides), a major group of membrane glycolipids (Gahnberg and Hakomori 1973). Thus, it may be theorized that in the fibroblasts studied here similar protein/lipid changes occur which can, at least in part, help to explain the lower membrane permittivity value observed in the transformed cells.

Membrane conductivity and membrane permittivity are strongly interrelated and their effects cannot be easily separated. For instance, the NA^+/K^+-ATPase or similar pumps may not only affect conductivity, but may also influence membrane permittivity since any changes in structure of these pumps may also cause variations in fixed membrane charges. In addition, phospholipid type and/or charge can interfere with active transport processes directly. In fact, the type of phospholipid environment in which ion channels are embedded can vary not only permittivity, but also the transport properties of the channels (Apell et al. 1979; Moczydlowski et al. 1985; Benz and Janko 1976). Conductivity is much higher in negatively charged membranes than in neutral ones (Apell et al. 1979; Moczydlowski et al. 1985). Thus, phospholipid class is important in protein channel conductance.

Membrane conductivity and membrane permittivity in both radiation and chemically transformed cell types differed in the same manner from normal cells. From this, it may be hypothesized that the type of transforming agent is not important once neoplastic transformation has occurred. Recent studies involving mouse skin cells suggest that the target gene(s) for oncogenic activation are different for chemical carcinogens and ionizing radiation (Bowden et al. 1990). Thus, if this is the case, it may be theorized that the effects observed here are a consequence of the oncogenic process once initiated and not the transforming agent. This view is also supported by a previous study which demonstrated, using electron paramagnetic resonance (EPR) spectroscopy, that membrane order in both radiation and chemically transformed mouse embryo fibroblasts was higher at 20 °C than normal fibroblasts (A. Lanzone, personal communication). In that work, another membrane parameter (membrane order) differed in the same manner in both types of transformed cells indicating again that the neoplastic state and not the transforming agent is important. In addition, a rigidification of the transformed membrane may also lend support to the hypothesis discussed earlier of a closing of ion channels in the transformed cells since a more rigid membrane may be speculated to be more compact and less permeable to ions. Whether or not this is the case, it is nonetheless evident that neoplastic transformation induces important structural and functional membrane changes.

The conductivity of the cytoplasm of transformed fibroblasts also showed a variation from normal cells. In this case, however, conductivity increased in transformed cells indicating a possible concomitant increase in ionic flux in the cellular cytoplasm. Although the reasons for this increase still remain unclear, it may be speculated that the much higher metabolic activity known to occur in neoplastic cells (Reznikoff et al. 1973 b) may also be responsible for the increase in conductivity in transformed cells. On the other hand, it may also be that the higher ion and water concentrations in the cytoplasm of these cells may be the cause of the higher metabolic activity found in neoplastic cells and not just an effect. In either case, further study is required in order to evaluate these possibilities.

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