Dielectric Properties of Epithelial Monolayer Cultured on Planar Permeable Support

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Mardin-Darby canine kidney (MDCK) cells were cultured on membrane filters. The cells formed monolayers, which were subjected to admittance measurements over a frequency range 0.1 kHz to 10 MHz. A single dielectric dispersion was found around 1 kHz, being analyzed based on a simple equivalent circuit model composed of the cell monolayer capacitance and conductance and a series conductance for the aqueous medium. Mean value of the monolayer capacitance was 1.8 μF cm⁻². The monolayer conductance was 1 × 10⁻¹ mS cm⁻², which was sensitive to Ca²⁺ in the bathing solution of the cell monolayer. The removal of Ca²⁺ from the basal solution increased the monolayer conductance by about ten times, the change being reversible by the addition of Ca²⁺ into the basal solution. On the other hand, Ca²⁺ in the apical solution had no effect on the monolayer conductance. This suggests that the opening and closing of the intercellular junctions are regulated by Ca²⁺ levels of the basal solution.

KEY WORDS: Epithelial monolayer/ Intercellular junction/ Ca²⁺/ Monolayer capacitance/ Monolayer conductance

INTRODUCTION

Cultured epithelial cell layers offer a good model system for studying the mechanism of epithelial transport. MDCK cells derived from dog kidney form monolayers on permeable planar supports. The cell-monolayer showed a transport function similar to natural epithelia of kidney¹,²). In the present study, we carried out dielectric analysis of the MDCK-cell monolayer to characterize its structural and electrical properties related to the transport function.

MATERIALS AND METHODS

Mardin-Darby canine kidney (MDCK) cells were cultured on membrane filters (Millipore HAWP 01300) in Dulbecco’s modified Eagle medium (DMEM) supplemented with 100 mg/l kanamycin and 5% fetal calf serum. The cells formed a confluent monolayer on the membrane filter after 1 day culture. The monolayer was mounted between two lucite chambers having a platinized Pt electrode attached to a brass block as a water jacket (see Fig. 1) in order to measure admittance across the monolayer. Admittance measurements were carried out with an HP 4192A Impedance Analyzer over a frequency range 10 Hz to 10 MHz.

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RESULTS AND DISCUSSION

Dielectric dispersion of cell monolayers

Figure 2 shows frequency dependence of the capacitance ($C_t$) and conductance.

Fig. 2. Dielectric dispersion of MDCK-cell monolayer measured in DMEM at 37°C
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$(G_\ell)$ of a MDCK-cell monolayer including bathing solutions (fetal-free DMEM with 10 mM Hepes-NaOH, pH 7.4). A single dielectric dispersion was found around 1 kHz and the Cole-Cole plots traced a semicircle with a slightly depressed center (Fig. 3).

Fig. 3. Cole-Cole plots of the data in Fig. 2.

Since the cell monolayer-bathing solution system is represented by a simple equivalent circuit model including the monolayer capacitance $C_e$ and conductance $G_e$ and a series conductance $G_a$ for the bathing solutions (Fig. 4), the capacitance $C_i$ and conductance $G_i$ of the system are given by

$$C_i = \frac{C_e}{1 + (\omega r)^2}, \quad (1)$$

Fig. 4. An equivalent electrical circuit for a MDCK-cell monolayer including bathing solutions. $C_e$, cell monolayer capacitance; $G_e$, cell monolayer conductance; $G_a$, series conductance for the bathing solutions.
where $\omega=2\pi f$, $f$ is frequency, and the dielectric parameters, $C_t$, $G_t$, $G_h$ and $\tau$ are as follows:

$$C_t = \frac{G_e^2}{(G_e+G_a)^2},$$

$$G_t = \frac{G_e G_a}{G_e+G_a},$$

$$G_h = G_a,$$

$$\tau = \frac{1}{2\pi f_0} = \frac{C_e}{G_e+G_a}.$$

Rearranging eqs. (3) and (4), the monolayer capacitance $C_e$ and conductance $G_e$ are given by

$$C_e = C_t \frac{(G_t+G_a)^2}{G_a^2} = C_t \frac{(G_t+G_h)^2}{G_h^2},$$

$$G_e = \frac{G_t G_a}{G_a-G_t} = \frac{G_t G_h}{G_h-G_t}.$$

The monolayer capacitance was calculated from eq.(7) to be 1.8 $\mu$Fcm$^{-2}$, being in good agreement with that obtained by Cereijido et al.\textsuperscript{3} from dc transient measurements. The monolayer conductance was found to be 1-10 mScm$^{-2}$.

**Effect of Ca$^{2+}$ on monolayer conductance**

In general, cell monolayer conductance depends on a paracellular shunt conductance corresponding to intercellular junctions, which are modulated by Ca$^{2+}$ in bathing solutions. The MDCK-cell monolayer has two surfaces: the one is the basal surface attached to the membrane filter and the other is the apical surface. Since the two surfaces differ in structure, Ca$^{2+}$ in the apical and basal solutions are expected to have different effects on the intercellular junctions.

By replacing the apical solution (fetal-free DMEM) with Ca$^{2+}$-free saline containing 2.5 mM EGTA, the monolayer conductance $G_e$ remained constant (Fig. 5). On the other hand, under the reverse condition (i.e., the apical solution contains Ca$^{2+}$, the conductance decreased significantly. The effect of Ca$^{2+}$ on the conductance is shown in Fig. 5.

![Fig. 5. Effect of Ca$^{2+}$ on monolayer conductance. The bathing solutions were changed at the points indicated by arrows.](222)
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Ca²⁺ and the basal one is Ca²⁺-free), $G_e$ increased up to about 10 times the control value within 20 min. The value of $G_e$ returned to the control level by replacing the basal solution with a Ca²⁺ containing medium. This suggests that the opening and closing of the intercellular junctions are regulated by Ca²⁺ levels of the basal solution.

REFERENCES

