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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

A Model of Electro-Osmosis in a Leaky Tight Junction of Epithelial Cells[1]

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Coupled solute-solvent transport across epithelial cell layers is an important physiological phenomenon [1–5]. The problem of the possibility of isotonic water transport over epithelial layers, which divided the solutions with the same concentration, was discussed in [4]. The mechanism of this phenomenon is still unclear [1, 2]. Electro-osmotic coupling (EO) in the channels between epithelial cells can explain the possibility of existence of isotonic transport. The theory of EO in the lateral intracellular space has been developed in [5]. However, the possibility of the existence of EO in the tight junctions (TJs) between epithelial cells was not considered in [5]. The authors of [6] demonstrated that the claudin macromolecules create the cation—anion selectivity in TJs. The role of TJ in generation of EO has been shown experimentally in [1]. Theoretical possibility of EO in TJs was reported in [7]. The authors of [1, 6, 7] substantiate the necessity of analysis of the role of TJ macromolecules in EO. Important effect of macromolecules adsorbed on the membrane on electrokinetic processes has been analyzed in [8–10]. The TJ strands where claudin macromolecules are located create the main hydraulic resistance for water flow. Thus, the key moment in the problem of epithelial transport is the explanation of the mechanism by which water overcomes this restriction.

The role of tight junction's surface and volume charges. The ion fluxes influence water with the force $EF[C_{Na}(x) - C_{Cl}(x)]$. This force depends on the TJ membrane surface charge σ_m and TJ macromolecule volume charge $\rho(f)$. Axe x is chosen perpendicular to TJ membrane surfaces, and the distance between these membranes h = 4 nm (Fig. 1). The electric field E, which arises from the existing transepithelial potential, is parallel to TJ membranes, and F is the Faraday constant. Let us express the concentrations in the point "x" in TJ by the Boltzmann formula as $C_{Na}(x) = cn_1e^{-\Phi(x)}$, $C_{Cl}(x) = cn_2e^{\Phi(x)}$, where c = 0.15 M is the concentration of NaCl in the free solution, $\Phi(x) = F\varphi(x)/RT$, R is the gas constant, T is absolute temperature, and $\varphi(x)$ is the electrostatic potential in TJ. The coefficients n_1 and n_2 are the distribution coefficients for Na^+ and Cl^- between free solution and TJ; they can be calculated according to the Born formula. Water velocity $V(x)$ is parallel to the TJ membranes and equals to zero on membranes surfaces. The length of Brinkman's boundary layer λ_B characterizes friction between water and macromolecules in TJ. Let us introduce the dimensionless velocity $V(x) = v(x)/v_m$, where $v_m = -\varepsilon\varepsilon_0E\zeta_m/\eta$ is the velocity according to the Helmholtz–Smoluchowsi formula; it corresponds to membrane zeta-potential ζ_m = –15 mV. Here, η is the viscosity of water, ε = 81 is the dielectric constant for water, and ε_0 is the permittivity of vacuum. Let us apply the Brinkman equation [7, 8], which describes water flow across polymeric media, for water velocity $V(x)$ in TJs:

$$(d^2V/dx^2) - (1/\lambda_B)^2V(x) = f(x); \quad V(-h/2) = V(h/2) = 0; \tag{1}$$

$$f(x) = -(RT/F)\{n_2\exp[\Phi(x)] - n_1\exp[-\Phi(x)]\}/(2\zeta_m\lambda_D^2). \tag{2}$$

The Debye length $\lambda_D = [(\varepsilon\varepsilon_0RT)/(2cF^2)]^{1/2}$ = 0.8 nm at the concentration c = 0.15 M. The solution of Eq. (1) depends on $\varphi(x)$ and can be represented as Eqs. (3) and (4):

$$G(x) = \int_0^x f(y)sh[(x-y)/\lambda_B]dy, \tag{3}$$

$$V(x) = \lambda_B\{G(x) - G(h/2)[ch(x/\lambda_B)/ch(h/(2\lambda_B))]\}. \tag{4}$$

It is supposed that the fixed centers in TJs are negatively charged, and their concentration is constant and equals $c(f)$; dimensionless concentration $C(f) = c(f)/c$. The

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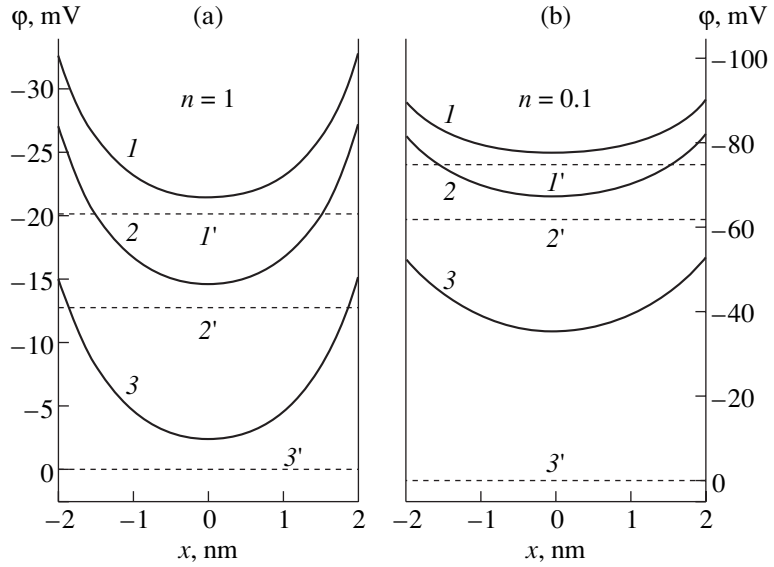

Fig. 1. Distribution of electrostatic potential in tight junctions between epithelial cells. Notations: (*1*), $C(f) = 1.65$; (*2*), $C(f) = 1$; (*3*), $C(f) = 0$. For solid lines, $\zeta_m = -15$ mV; for dotted lines, $\zeta_m = 0$. The ion distribution coefficients between TJs and free solution: (a) $n_1 = n_2 = n = 1$; (b) $n_1 = n_2 = n = 0.1$.

distribution of dimensionless potential $\Phi(x) = \Phi(x)/RT$ across TJ is described by Poisson equation (5) with boundary condition on TJ membranes (6):

$$d^2\Phi/dx^2 = [1/(2\lambda_D^2)]\{n_2\exp[\Phi(x)] - n_1\exp[-\Phi(x)] + C(f)\}; \quad (5)$$

$$[d\Phi/dx]|_{x = \pm h/2} = \pm(2/\lambda_D)sh[F\zeta_m/(2RT)]. \quad (6)$$

The zeta potential of the membrane is connected with its own surface charge σ_m by the Gouy-Chapmen formula [10]. Its value is no more than 15 mV at $c = 0.15$ M for biological membranes [5]. So, it was assumed that $\zeta_m = -15$ mV. The fourth-order numerical scheme of Runge–Kutta was applied for the solution of Eqs. (5) and (6). Substituting this solution for $\Phi(x)$ to formula (2) and numerically integrating in expression (3), we determined water velocity in TJs. The effective ζ-potential in TJs was calculated by integration of this velocity:

$$\zeta = -(Q\eta)/(\varepsilon\varepsilon_0 E) = (\zeta_m/h)\int_{-h/2}^{h/2} V(x)dx. \quad (7)$$

The Brinkman length is connected with the permeability $K(v_p)$ of TJs as $\lambda_B = [K(p)]^{1/2}$, and according to the Kozeni–Karman model, at small v_p values, $K(p) \sim 1/v_p^2$, where v_p is the partial volume occupied by macromolecules in TJs. As the concentration $C(f)$ is proportional to v_p, the following equation should hold:

$$\lambda_B = \gamma/C(f). \quad (8)$$

Electro-kinetic potential in tight junction. The ratio of water flow to electrical current for EO in the rabbit cornea endothelium, according to [1], should be $Q/I = 66$ μL/(A s). If we substitute this value to the Helmholtz–Smoluchowski formula, we will obtain:

$$\zeta = -\{(Q\eta\kappa)/(\varepsilon\varepsilon_0 I)\} = -114 \text{ mV}, \quad (9)$$

where $\kappa = 1.79$ (Ω m)$^{-1}$ is the electroconductivity of NaCl solution at $c = 0.15$ M and $t = 36$°C. In [1], this value of ζ-potential was not calculated, as the authors at that time had not the theory that can explain such a big zeta-potential value. The electro-osmosis in TJs has a non-Helmholtzian character, as the charged centers are located not only on the membranes surfaces but also in the TJ volume. Therefore, formula (9) cannot be applied for TJs in this form. The ratio between the concentrations of Na^+ and Cl^- in TJs is determined as $\exp(-2F\varphi(x)/RT)$ according to the Boltzmann formula, and the potential distribution is demonstrated on Fig. 1. The minimum of C_{Na}/C_{Cl} is 3.2 for curve (*2*) in Fig. 1a. As can be seen in Fig. 1b (curve *3*), if $n = 0.1$, then Na^+ cations predominate in TJs even in the absence of fixed charges in the TJ volume, because Cl^- ions are completely displaced by the negative membrane charge. The effect of small values n on the potential distribution in TJs explains how TJs can function as a cation-anion selective filter even in the case of a nonregular distribution of fixed charges inside TJs. In the developed model of TJs, the concentration of Na^+ is sufficiently higher than that of Cl^-, so the TJ is a filter which detains the anions. We have to take into account this circumstance in Eq. (9) by replacing the value of κ in free solution for

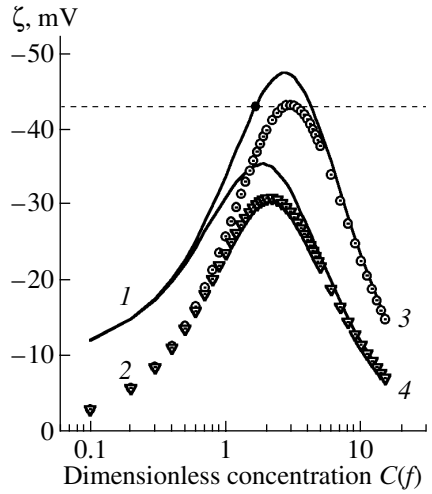

Fig. 2. Effective electro-kinetic potential for epithelial TJ as a function of dimensionless concentration of negatively charged centers, calculated by formulas (7) and (8). Dotted line indicates the electrokinetic potential calculated by formula (10) from the data of [1]. Curves (*1*) (solid lines) correspond to membranes with $\zeta_m = -15$ mV; curves (*2*) (circles and triangles), to $\zeta_m = 0$. For curves (*3*), $\gamma = 3.8$ nm from formula (8); curves (*4*) are plotted at $\gamma = 2.8$ nm.

its value in TJs: $(TJ) = \kappa t_{Na}$, where $t_{Na} = 0.38$ is the transference number for Na^+.

$$\zeta = -\{[Q\eta\kappa t_{Na}]/(\varepsilon\varepsilon_0 I)\} = -43 \text{ mV}. \quad (10)$$

The concentration of fixed centers is $C(f) = c(f)/c = 1.65$ for the intersection of line *1* in Fig. 2 and the dotted line corresponding to $\zeta = -43$ mV, and $\lambda_B = 2.3$ nm, as calculated by formula (8). The next formula has been calculated in the case of uncharged TJ membranes ($\zeta_m = 0$):

$$\zeta = -(\lambda_B/\lambda_D)^2(RT/2F)C(f) \times [(2\lambda_B/h)th(h/(2\lambda_B)) - 1]. \quad (11)$$

The qualitative character of the dependence of ζ on $C(f)$, calculated by analytical formula (11) is the same as that calculated by formula (7). The difference was observed only at small concentration $C(f)$ (Fig. 2). The extreme character of the dependence ζ on $C(f)$ can be explained by the fact that an increase in fixed charges in TJs requires an increase in the volume in the TJs occupied by polymers. For this reason, according to formula (8), the Brinkman length will decrease, and the effect of EO amplification, which stems from the fact that fixed charges exist in TJs, will be reduced as a result of friction between water flow and TJ polymers.

The main conclusion of this study is that macromolecules located in tight junctions of epithelial and endothelial cell layers create cation-anion selectivity and simultaneously generate the electro-kinetic potential whose value depends on the concentration of fixed charges of macromolecules in TJs.

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