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Estimation of electrochrome dyes position in the bilayer through the 2nd harmonic of capacitive current

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Abstract

The depth of location of electrochrome dyes RH-type in a bilayer is evaluated using the magnitudes of intramembrane field $\Delta\varphi$ measured by two methods: from relative change of the rate of transmembrane transport of hydrophobic ions and by means of electrostriction method based on the compensation of the 2nd harmonic of capacitive current, which is generated due to electrostriction phenomenon if sine voltage is applied to the bilayer. The experiments and theoretical analysis are conducted. Comparing the theoretical curves for $\Delta\varphi$ measured by the both methods and the experimental data, the depth of location was estimated as follows: 0.7–1 nm for the dyes RH-421 and RH-160, and 0.9–1.15 nm for the dye RH-237.

Keywords: Bilayer lipid membranes; Intramembrane field; Harmonics of capacitive current; Dyes' position

1. Introduction

Electrochrome dyes are widely used for investigation of the mechanism of work of many membrane ATPases realising transmembrane ion transport. They allow measuring modifications of local electrical fields accompanying conformation transitions of proteins, binding of ions in active sites and their transportation through a membrane [1–12]. A position of a chromofore in a membrane has the important significance for the interpretation of outcomes obtained with these dyes. An evaluation of the position is carried out with the help of various test experiments. For example, the influence is studied on fluorescence of molecules of other substances which position inside a membrane is supposed to be known [4,13,14].

For solving the problem of intramembrane dye location, the electrochemical methods can be applied as well. Their application is based on the fact that the molecules of many dyes, for example, styryl dyes of RH-type, have a significant on magnitude dipole moment, which introduces perturbation to distribution of an electrical field in a membrane [13,15]. In these dyes, the dipole moment is formed by a negatively charged sulphogroup located near membrane–water interface and a positive charge, which is distributed in a chromophore. Therefore, the information about a dye chromophore position in a membrane can be received by studying the distribution of an electrical field inside a membrane generated by the adsorbed dye molecules.

The local electrical fields originating from the adsorption of charged and dipole molecules influence the profile of the potential energy of other ions located in a membrane; therefore, an information about field distribution in the membrane can be obtained by means of measurements of kinetic parameters of the ion passive transport through a membrane. For this purpose, hydrophobic ions are frequently used. Their transposition through the membrane is obeyed by a simple mechanism [16–21]. However, the positively charged groups of styryl dyes of RH-421-type are immersed in a membrane deeper than the position of the adsorption plane of hydrophobic ions [15], and the evaluation of depth of their immersion only by the said method is impossible.

There is another method of estimation of electrical field distribution in a membrane. It is based on the phenomenon of an electrostriction, i.e. the compression of a membrane moiety by an electric field, which outcome is the diminution of its thickness and magnification of the capacitance. This phenomenon is used in a method of compensation of the intramembrane field (CIF), which is successfully applied to

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the measurement of boundary potentials of bilayer lipid membranes over twenty years [22–25]. Its technical realisation can be varied, but the main principle is the same: in short-circuit condition, an asymmetric charge distribution at the membrane interfaces generates an electric field inside a membrane that contracts a membrane moiety and increases its capacitance. In turn, applying a direct potential difference that compensates the generated intramembrane field can reduce this capacitance. The applied potential difference inducing the minimum membrane capacitance corresponds to the complete compensation of the membrane field. Its magnitude is equal to the difference of boundary potentials from the both sides of the membrane that thus can be measured as a direct component of the voltage corresponding to the minimum capacitance. Obviously, this principle is based on the supposition that intramembrane field contracting a membrane is formed by simple summation of the potential differences between the solutions and the potential jumps at the boundaries of a membrane. This, in turn, assumes that the separation of charges causing the formation of the potential jumps happens outside that area of a membrane that is contracted in the electric field. It is considered usually that the compression in an electric field (electrostriction) happens mainly inside a membrane, in the area of hydrocarbonate residuals of the phospholipid [26]. Therefore, CIF method, obviously, should be valid if the charge separation takes place in a diffuse electrical stratum of an electrolyte near the membrane, as it is confirmed by experiment [27]. However, if the separation of charges affects the hydrophobic area of the membrane, in which the electrostriction happens, the method should give the systematic error in the measurement of the difference of boundary potentials. The most obvious example of such separation of the charges is their location in the plane equidistant from both sides of the membrane. In this case, the residual of potentials between the plane of the charge disposition and a water solution is present. However, its measurement by CIF method is impossible because the charge distribution is symmetric, and residual of boundary potentials from two sides of the membrane is equal to zero.

Such situations, when the plane of the ion adsorption is immersed into the membrane, were marked in the literature; for example, it was proposed in the model describing the distribution of the potential generated by the hydrophobic ions [18]. The similar model of immersing of the charge into the nonpolar area of the membrane was used as well for investigation of adsorption of the hydrophobic ions, which thanking to a hydrophobic tail, are capable to be built in a membrane, but can not freely penetrate through it [28]. CIF method gave correct outcomes from the investigation of the amphiphilic ion adsorption that was shown by a comparison of these results with the outcomes obtained from other methods [21,29]. However, for want of study of an adsorption of dipole molecules of styryl dyes which molecules have length compared to the length of phospholipid molecules, CIF method gave the underestimated values of the boundary potential as compared with the other method based on the measurement of kinetic parameters of the transport of hydrophobic ions [30]. Such distinction was marked earlier for want of study of an adsorption of other chemicals and was usually explained by the penetration of these molecules through the membrane affecting the potential jump on the opposite membrane interface. This process results in a diminution of magnitude of a difference of boundary potentials [31]. However, in the case of styryl dyes, this explanation is unlikely, as it was shown that these dyes do not practically penetrate through the membrane [32]. Therefore, the other explanation of a divergence of outcomes obtained by CIF method and conductivity measurements should be proposed. It should take into consideration that dipole dye molecules penetrate into the membrane area where an electrostriction takes place.

In the present work, the method is proposed to determine the chromophore position in a membrane. It is based on the comparison of the electric field measured by two methods: (1) from the dependence of penetration of the charged hydrophobic ions through the membrane modified by a dye; (2) from the method of compensation of the intramembrane field [25].

2. Experimental

The dependencies of $\Delta\varphi$ on dye concentration were investigated. For details, see Ref. [15]. The intramembrane field $\Delta\varphi_Q$ was calculated from relative change of the rate of transmembrane transport of tetraphenilborate (TFB^-) ions. The RH-dyes were added to both sides of the bilayer. The values of $\Delta\varphi_C$ estimated by the method of compensation of intramembrane field were measured in a standard way [25]. RH-dyes were added from one side of the membrane.

3. Results and discussion

An intramembrane field measured by means of second harmonic compensation method (solid circles) and by TPB transport (triangles) depends linearly on concentrations of three RH-dyes (Fig. 1). For all the dyes, the ratio of the slopes K of these lines is less than unity, $K<1$. To compute the depth of the chromophore location, the model is proposed. It is based on the properties of electrostriction in an inhomogeneous membrane [33,34]. The latter is a three-layer solid, its dielectric properties and compliance varying across its thickness (x-coordinate). Its outer layers with thickness Δ are hydrophilic with dielectric permittivity $\varepsilon(x)$ changing from the value $\varepsilon_w \approx 2$ typical for water to $\varepsilon_m \approx 2$ typical for a membrane hydrophobic region [35] as a linear function. Bilayer compliance $M(x)$ is zero in two outer layers. In the inner one, it changes from a low value M_0 at its borders to a maximum value $\sim M_1$ in the centre. Using the method [33,34], the intramembrane field $\Delta\varphi_C$ measured by

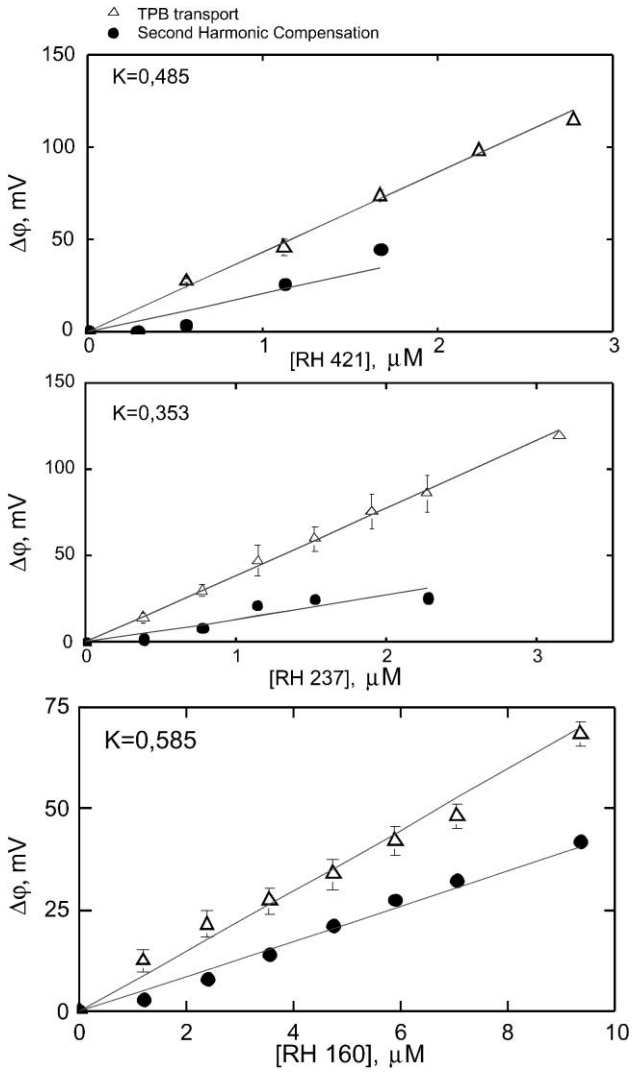

Fig. 1. Intramembrane field estimated for three RH-dyes by means of second harmonic compensation method (solid circles) and TPB transport (triangles) for various dye concentration. The values of K are the ratio of the slopes of the corresponding lines for the each dye.

the method of minimisation of specific membrane capacitance C (and proportional to the amplitude of the 2nd harmonic) is as follows:

$$\Delta\varphi_C(d) = \sigma\frac{C_{h-d}A_{h-d} - C_dA_d}{C_dC_{h-d}(A_{h-d}+A_d)}, \quad (1)$$

where σ stands for a surface density disposed at a depth $\Delta \le d \le h-\Delta$, ε_0 is the permittivity of free space; $D = \varepsilon_m\Delta\ln(\varepsilon_w/\varepsilon_m)/(\varepsilon_w-\varepsilon_m) \approx 0.095\Delta$ stands for the contribution of the layer with varying dielectric permittivity into C; $1/C_d = (1/\varepsilon_0)\left[\int_0^{\Delta}(1/\varepsilon(x))\mathrm{d}x + (1/\varepsilon_m)\int_{\Delta}^{d}\mathrm{d}x\right] = (1/\varepsilon_0\varepsilon_m)[d-\Delta+D]$, $1/C_{h-d} = \int_d^h(1/\varepsilon_0\varepsilon(x))\mathrm{d}x = (1/\varepsilon_0\varepsilon_m)[h-d-\Delta+D]$, $A_d = (1/\varepsilon_0^2\varepsilon_m^2)\int_{\Delta}^{d}M(x)dx$, $A_{h-d} = (1/\varepsilon_0^2\varepsilon_m^2)\int_d^{h-\Delta}M(x)dx$. The intramembrane field $\Delta\varphi_Q$ estimated from the ion transport [15] is:

$$\Delta\varphi_Q(d) = \sigma/C_d, \quad (2)$$

where $d \le h/2$. From Eqs. (1) and (2), the ratio $K = \Delta\varphi_C/\Delta\varphi_Q$ of the intramembrane field estimated by two methods is as follows:

$$K(d) = \frac{(d+D-\Delta)\int_d^{h-\Delta}M(x)\mathrm{d}x - (h-d+D-\Delta)\int_{\Delta}^{d}M(x)\mathrm{d}x}{(d+D-\Delta)\int_{\Delta}^{h-\Delta}M(x)\mathrm{d}x}. \quad (3)$$

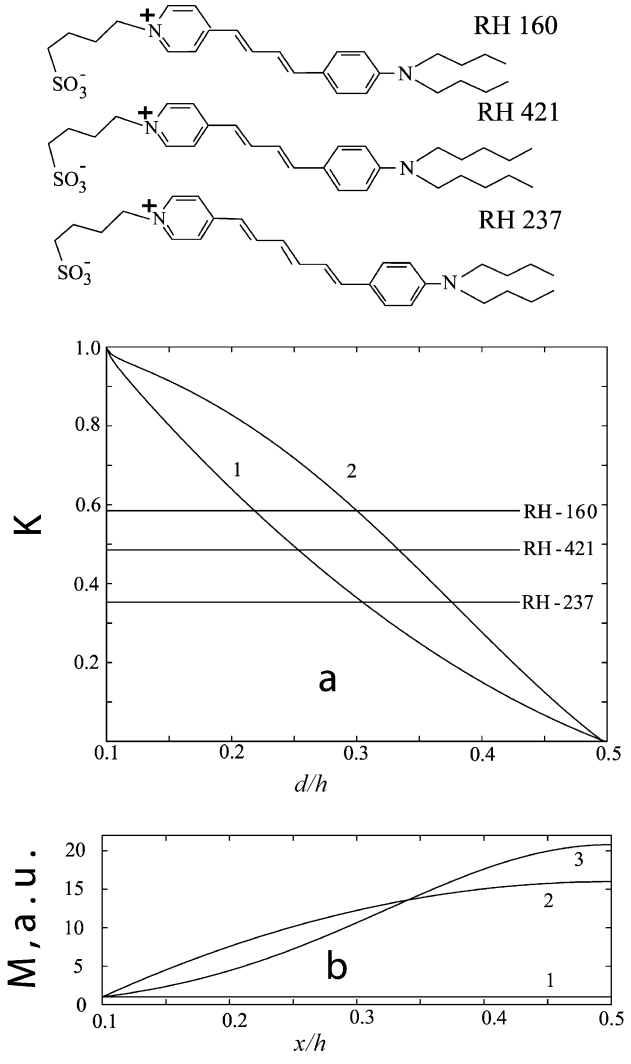

Fig. 2. Estimation of depth of chromophore immersion for various RH-dyes (upper inset) in a bilayer. (a) Curves 1 and 2 show the theoretical dependence of the ratio $K(d) = \Delta\varphi_C/\Delta\varphi_Q$ of the intramembrane field estimated by two methods on relative depth d/h of charge location in the membrane (d is the depth, h is the membrane thickness) for (b): a parabolic (curve 1) and a Gaussian (curve 2) dependencies of the compliance on the normalised coordinate x/h (see (b)). Horizontal lines in (a) represent the values of K measured for the dyes. The abscissas of their cross-point with curves 1 and 2 determine the values of depth of immersing of the positively charged moieties of the dyes molecules. (b) The dependence of membrane compliance M on the normalised coordinate x/h. 1—minimum compliance $M(x) = M_0$ of the bilayer. 2,3—$M(x)$ for parabolic and Gaussian dependencies, correspondingly, described by Eq. (4).

The computing was fulfilled for two dependencies of bilayer compliance on x-coordinate (Fig. 2b):

$$\left.\begin{aligned} M_P(x) &= M_0 + 4(M_1 - M_0)(x - \Delta)(h - \Delta - x)/h^2 \\ M_G(x) &= M_0 + 1.2732(M_1 - M_0)\left[\exp(-(x - h/2)^2/2s^2h^2) - \exp(-1/8s^2)\right]/\left[1 - \exp(-1/8s^2)\right] \end{aligned}\right\}, \quad (4)$$

where $M_P(x)$ is a parabolic curve (2) and $M_G(x)$ is a Gaussian curve (3); they differ substantially near the bilayer interfaces. The coefficient 1.2732 equalises the mean values of bilayer compliance given by both equations for the parameter $s = 0.25$. The corresponding dependencies $K(d)$ are presented in Fig. 2a for the both distributions (1 and 2, respectively) for $M_1/M_0 \gg 1$. The horizontal lines in Fig. 2a represent the values of K determined experimentally (Fig. 1). The points of their interception with curves 1 and 2 determine the depths of immersion of the chromophores into the bilayer. They increase in a row: RH-160, RH-421, RH-237 correlating with their molecular structure. The first two dyes have the same distance l from SO_3^- group to the second nitrogen atom, but the second one has a longer hydrophobic tail, which anchors the molecule deeper into bilayer. The value of l is higher for RH-237, so it is buried deeper into the bilayer. The absolute depth of location of dye positive moiety in the membrane depends on real membrane thickness. For the values of $h = 3$ nm (or $h = 4$ nm), the depth of immersion of the dye moiety can be estimated as follows: 0.7–1 (0.93–1.14) nm for the dyes RH-421 and RH-160, and 0.9–1.15 (1.24–1.52) nm for the dye RH-237.

4. Conclusion

In the row of dyes, the relative intramembrane position of their charged moieties can be estimated by means of conjoint usage of two bioelectrochemical methods, the results being in good agreement with the structures of the dyes. The exact quantitative determination of the position of the charged group in the bilayer needs precise data about membrane thickness as well as the dependence of the bilayer compliance on coordinate. The detailed comparison with literature data needs special study and is out of the scope of this paper. The main difficulty of such investigation is the fact that all electrochemical and optic methods measure some effective relative parameters. Perhaps NMR studies of the dyes are capable of getting the corresponding information. However, such data are not yet present.

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