Redistribution of Positively Charged Probes in Membrane Suspension under the Action of Transmembrane Potential

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Abstract. The distribution of amphiphilic and hydrophobic positively charged probes in suspensions of closed membrane vesicles was analysed by the methods of equilibrium thermodynamics. Two versions of the probe location in the membranes are discussed: near the boundary between the polar and hydrocarbon regions, and in the centre of the hydrocarbon region of a lipid bilayer. It is shown that, in the first case, the action of the inside-negative transmembrane potential results in an increased average probe concentration in the lipid phase and in a significant redistribution of the probe in the membrane interior. An inside-positive transmembrane potential causes a decrease in the average probe concentration in membranes, the probe being redistributed in the membrane bilayer. If the probe is sited in the centre of the hydrocarbon layer, the transmembrane potential evokes only an increase or decrease in the probe concentration in membranes at a negative or positive sign of the potential inside the vesicles.

Key words: Partition coefficient — Lecithin liposomes — Potential-sensitive probe — Transmembrane potential — Equilibrium distribution — Chemical potential — Probe redistribution

Introduction

Redistribution of charged amphiphilic probes in membrane suspensions under the action of transmembrane potential results in a local increase in their concentration in some regions of this two-phase system and thus in a fluorescence quenching of the dye. In our previous papers we described equilibrium distribution of a carbocyanine probe, diS-C_3-(5), in a membrane suspension without potential, and concentration dependences of the fluorescence intensity of this probe in aqueous and membrane phases (Ivkova et al. 1982; Ivkova et al. 1983a; Ivkova et al. 1983b).

The present work deals with the analysis of the equilibrium probe redistribution in a suspension of closed unilamellar vesicles in the presence of transmembrane potential. This approach can be used for molecules that 1) carry a charge, 2)

are capable of crossing membranes, and 3) have sufficiently small dimensions so that, in the thermodynamic sense, the membranes can be regarded as a phase with the probe molecules dissolved in them.

Analysis of the permeability (Finkelstein 1976) and thermodynamic parameters of phase transition (Ivkov and Berestovsky 1981) in phospholipid bilayers shows that phospholipid membranes can be regarded as a phase if the dimensions of the molecules dissolved do not exceed those of several methylene groups. In addition, intermolecular interactions between the substance dissolved and the phospholipids are mainly determined by dispersion forces rather than by hydrogen bonds. Both these requirements are apparently met in the carbocyanine probes.

In our studies of membrane potentials, as a rule, small concentrations of the probe are used and the probe solutions in aqueous and lipid phase can thus be considered as nearly ideal.

In order to simplify the calculations, concentration values can then be used instead the activity values. The partition coefficients expressed in terms of mole fractions* (K) and molar concentrations (K_p) are in a simple linear relationship:

$$K_p \simeq K \cdot \frac{\bar{V}_a}{\bar{V}_l}, \tag{1}$$

where $\bar{V}_a$ and $\bar{V}_l$ are the partial molar volumes of water and lipid equal to 18 cm^3 and 775 cm^3 for water and egg lecithin, respectively. Thus, for this system, $K_p \simeq 0.023K$.

In an ideal two-phase system, the partition constant for a dissolved substance is determined by the difference in the chemical potentials, $\Delta\mu$ (Prigogine and Defay 1966):

$$RT \ln K = -\Delta\mu \tag{2}$$

where R is the universal gas constant and T is the temperature (degrees Kelvin). Equation (2) is in fact the Bolzmann distribution for solutions. If the molecules are charged, the electric field elicits changes in the chemical potential, and thus in the molecule distribution.

According to the experimental conditions, the salt concentrations in the external and internal vesicular aqueous volumes are identical (concentration changes due to the transmembrane potential can be neglected). Thus, the surface potential of the membrane itself can be ignored, since it is allowed for in K, when the membrane is considered as symmetrical.

So far, quantitative estimations have been made only for the redistribution of diS-C_3-(5) between the external and the internal aqueous volumes of vesicles

* We use the term "partition constant" for this parameter.

because, in this case, the concentrations are governed directly by the Nernst law. In addition, it has been suggested by several authors that the probe would be redistributed between the aqueous and the membrane phase (Waggoner et al. 1977) or in the membrane interior (Krasne 1980; Ivkova et al. 1983a). However no calculations for these hypothetical processes are available.

In our experiments we have attempted to describe quantitatively the redistribution of a positively charged probe in a suspension of closed unilamellar vesicles, under the action of both the inside-negative and inside-positive transmembrane potentials, using the methods of equilibrium thermodynamics. Unfortunately, no information concerning the exact localization of the probe in the membrane interior, and the potential profile across the membrane is available. Due to this, approximate quantitative estimations can be only based on general physical considerations which take into account the properties of both the probe and the lipid membrane.

Results and Discussion

Location of probes in membranes

In the two-phase system under consideration, the transmembrane electric field applied results in changes in the chemical potential of the charged particles. As a result, the difference of the electrochemical potentials ($\Delta\mu$) of the probe molecules in these phases is

$$\Delta\mu = \Delta\mu_0 + zF\Delta\varphi \tag{3}$$

where $\Delta\mu_0$ is the chemical potential difference, z is the charge of the particle, F is the Faraday constant and $\Delta\varphi$ is the interphase electric potential. The location of the probe in the membranes can be estimated from the solubility and from the range of the absorption and fluorescence spectra of the probe in various solvents. For example, diS-C_3-(5) is practically insoluble in decane or benzene; it is slightly soluble in water and readily soluble in alcohols.

As discussed in previous reports, the dye is soluble in membranes at concentrations greatly exceeding that in aqueous media. In addition, both the absorption and fluorescence spectra of the probe diS-C_3-(5) in membranes are similar to those in alcohols, especially in octanol (Sims et al. 1974). These data can be regarded as an indirect evidence for this probe being located near the boundary between the polar and the hydrocarbon region of the bilayer, i. e. in the region, where glycerol residues and carboxyl groups of hydrocarbon chains are located (Ivkova et al. 1983a).

The conductivity of the lipid membrane is about 10^{-3} ohm^{-1}.cm^{-2} for the

polar layer and about 10^{-7} ohm^{-1} cm^{-2} for the hydrocarbon layer (Coster and Smith 1974). Thus, the entire transmembrane potential essentially falls on the central region between the zones of location of glycerol residues. Due to two short hydrocarbon chains of the probe, each of them about 0.5 nm in length, the probe must be slightly immersed in the hydrophobic layer. For example, the thickness of the hydrocarbon layer in an egg lecithin bilayer is about 2.6 nm (Ivkov and Berestovsky 1981), and the interval between the regions of the glycerol residues is about 3.0—3.2 nm. In a probe molecule, the charge is delocalized over the chain of the conjugated bonds which is parallel to the lamellar plane. If it is located near the carbonyl groups of the hydrocarbon chains, i. e. at a distance of about 0.3 nm from the boundary between the polar and the hydrophobic layer, it is reasonable to assume $\Delta\varphi \simeq 0.1\Delta\psi$, where $\Delta\psi$ is the total transmembrane potential.

In principle, the thermodynamic approach allows an analysis of the potential-induced probe redistribution at any other probe location, e. g., when the hydrophobic probe is located in the centre of the lipid bilayer.

Partition coefficient changes induced by the action of the transmembrane potential

Let us analyse the probe distribution in membrane vesicles suspended in an aqueous medium (Fig. 1.) Three assumptions underlie the analysis: 1) the probe concentrations in the external and internal vesicular aqueous volumes are related by the Nernst law; 2) the probe shows an equilibrium distribution between each membrane monolayer and the aqueous medium in contact with it; 3) the partition coefficient is determined by the difference in the electrochemical potentials between the monolayer and the adjacent aqueous phase. The membrane under consideration is symmetrical. Thus, in the absence of transmembrane potential, the partition coefficients for both the external and internal membrane monolayers are identical. For vesicles of about 100 nm in diameter, the monolayer volumes are identical as well.

The constant of the interphase partition in the presence of an electrical field is

$$K = e^{-(\Delta\mu/RT + zF\Delta\varphi/RT)} = K_o e^{-zF\Delta\varphi/RT}, \tag{4}$$

where K_o is the partition constant without electrical field, and $\Delta\varphi$ is the difference in potentials between the monolayer and the aqueous medium in contact with it.

It is more convenient to express concentration in mole fractions. However, volume concentration is more frequently used, and we shall therefore adhere to its use. As noted in Introduction, the partition coefficient is proportional to the partition constant. It can thus be written:

$$K_p = K_{p,o} e^{-zF\Delta\varphi/RT} \tag{5}$$

The value of RT/F is about 25.5 mV at 20 °C.

The $\Delta\varphi$ value is determined by the distribution of the potential $\Delta\Psi$ across the membrane. The probe is charged positively and so the signs of the exponents in Eq. (4) and (5) thus depend on the sign of $\Delta\varphi$. When the transmembrane potential is inside-negative, $\Delta\varphi$ is negative for the external, and positive for the internal lipid monolayer of the membrane.

In this case it can be written:

$$K_{\mathrm{p,\,out}} = pK_{\mathrm{p,\,o}}, \qquad K_{\mathrm{p,\,in}} = \frac{1}{p} K_{\mathrm{p,\,o}} \tag{6}$$

where

$$p = e^{|zF\Delta\varphi/RT|} > 1.$$

With an inside-positive transmembrane potential, these relationships for K_p are inverse.

Inside-negative transmembrane potential

The distribution of a probe in a membrane suspension with no transmembrane potential can be described by simple equations:

$$\begin{aligned} n_p^o &= c_{\mathrm{p,\,o}}^a (V_{\mathrm{out}} + V_{\mathrm{in}}) + c_{\mathrm{p,\,o}}^m V_m \\ c_{\mathrm{p,\,m}}^m &= K_{\mathrm{p,\,o}} c_{\mathrm{p,\,o}}^a \end{aligned} \tag{7}$$

where n_p^o is the total quantity of the probe in the sample, $c_{\mathrm{p,\,o}}^a$ and $c_{\mathrm{p,\,o}}^m$ are the probe concentrations in the aqueous medium and membranes, respectively (in the absence of potential); V_{out} and V_{in} are the volumes of the aqueous medium outside and inside the vesicles, respectively; V_m is the volume of membranes.

In the presence of transmembrane potential, the probe distribution can be written as

$$\begin{aligned} n_p^o &= c_{\mathrm{p,\,out}}^a V_{\mathrm{out}} + c_{\mathrm{p,\,in}}^a V_{\mathrm{in}} + (c_{\mathrm{p,\,out}}^m + c_{\mathrm{p,\,in}}^m)\frac{V_m}{2} \\ c_{\mathrm{p,\,out}}^m &= K_{\mathrm{p,\,out}} c_{\mathrm{p,\,out}}^a, \qquad c_{\mathrm{p,\,in}}^m = K_{\mathrm{p,\,in}} c_{\mathrm{p,\,in}}^a \end{aligned} \tag{8}$$

where $c_{\mathrm{p,\,out}}^m$ and $c_{\mathrm{p,\,in}}^m$ are the probe concentrations in the external and internal membrane monolayer, respectively, $K_{\mathrm{p,\,out}}$ and $K_{\mathrm{p,\,in}}$ are determined by the expressions (6).

The probe concentrations in the external and internal aqueous volume are related by the Nernst equation:

$$c_{\mathrm{p,\,in}}^a / c_{\mathrm{p,\,out}}^a = e^{-zF\Delta\psi/RT} \tag{9}$$

where $\Delta\psi$ is the transmembrane potential. The signs of z and $\Delta\psi$ are opposite in our case, the exponent is thus positive. By analogy with (6), it can be written:

$$c^{a}_{p,\,in} = mc^{a}_{p,\,out} \tag{10}$$

where

$$m = e^{|zF\Delta\psi/RT|} > 1.$$

Taking into account Eqs. (6)—(10), the probe distribution without and with a potential are:

$$\begin{aligned} n^{o}_{p} &= c^{a}_{p,\,o}(V_{out} + V_{in} + K_{p,o}V_{m}) \\ n^{o}_{p} &= c^{a}_{p,\,out}\left[V_{out} + mV_{in} + \left(p + \frac{m}{p}\right)K_{p,o}\cdot\frac{V_{m}}{2}\right] \end{aligned} \tag{11}$$

respectively, and the concentrations in the external and internal membrane monolayers are:

$$c^{m}_{p,\,out} = c^{a}_{p,\,out}pK_{p,o}; \qquad c^{m}_{p,\,in} = c^{a}_{p,\,out}\cdot\frac{m}{p}K_{p,o} \tag{12}$$

The expressions in the brackets represent some effective volumes of the lipid-water system both in the absence and presence of a transmembrane potential.

In this case, the variable $K_{p,o}V_m$ is the effective volume of the membrane phase without a potential, and its ratio to the total effective volume of the sample determines the portion of the probe dissolved in membranes. The variables $pK_{p,o}\cdot\frac{V_m}{2}$ and $\frac{m}{p}K_{p,o}\cdot\frac{V_m}{2}$ are the effective volumes of the external and internal membrane monolayers in the presence of a transmembrane potential, respectively.

From equations (10) and (11), the probe concentration in the external and internal aqueous volume of the vesicle suspension with a potential are:

$$c^{a}_{p,\,out} = c^{a}_{p,\,o}\,.\,\beta, \qquad c^{a}_{p,\,in} = c^{a}_{p,\,o}\,.\,m\beta \tag{13}$$

respectively, where β is the ratio of the effective volumes both without and with a potential

$$\beta = \frac{V_{out} + V_{in} + K_{p,o}V_{m}}{V_{out} + mV_{in} + K_{p,o}\left(p + \frac{m}{p}\right)\frac{V_{m}}{2}} < 1 \tag{14}$$

i. e. under the action of a transmembrane potential, the effective volume increases $(1/\beta)$-fold. According to (7), (12) and (13), the respective probe concentrations in the external and internal monolayers are:

$$c^{m}_{p,\,out} = c^{m}_{p,\,o}\,.\,p\beta, \qquad c^{m}_{p,\,in} = c^{m}_{p,\,o}\cdot\frac{m}{p}\cdot\beta \tag{15}$$

Their ratio is:

$$\frac{c^{m}_{p,\,in}}{c^{m}_{p,\,out}} = \frac{m}{p^{2}} \tag{16}$$

This ratio can also be obtained using the difference in the electrochemical potentials. The voltage drop on the region of the membrane that separates the zones of the probe location is:

$$|\Delta\xi| = |\Delta\psi| - 2|\Delta\varphi| \tag{17}$$

Taking into account (6) and (10), the ratio of the concentrations can be written:

$$\frac{c^{m}_{p,\,in}}{c^{m}_{p,\,out}} = e^{|zF\Delta\xi/RT|} = e^{|zF\Delta\psi/RT|} \cdot e^{-2|zF\Delta\varphi/RT|} = \frac{m}{p^{2}} \tag{18}$$

i. e. a value analogous to (16) is obtained.

As noted earlier, for large vesicles used in the experiments, the volumes of both the external and internal membrane monolayers are identical. Thus, the average probe concentration in the membrane can be expressed as the arithmetic mean:

$$c^{m}_{p,\,av} = \frac{c^{m}_{p,\,out} + c^{m}_{p.\,in}}{2} = \frac{1}{2} c^{m}_{p,\,o} \beta \left(p + \frac{m}{p} \right) \tag{19}$$

Quatitative estimations can be made provided that both the initial probe concentration in the sample and the membrane concentration as well as the value of the transmembrane potential are known. As shown in previous papers (Ivkova et al. 1982; Ivkova et al. 1983a), the partition coefficient can be calculated from the data on back fluorimetric titration. Lipid concentration is expressed as a rule, in weight units (e. g. mg/ml). Membrane volume can easily be estimated if the partial specific density of the lipid is known. This value is about 1 $g.cm^{-3}$ for most phospholipids and total natural membrane lipids.

To our regret, the exact location of the probe in membranes is unknown. However, reasonable estimations can be made on the assumption that approximately 1/10 of the transmembrane potential falls on the region between the zone of the probe location and the adjacent aqueous medium. In our experiments on egg lecithin liposomes, a potential of -130 mV was used. It was generated using K^{+}-valinomycin. In this case, $m = 200$ and $p \simeq 1.7$. The partition coefficient of diS-C_3-(5) for egg lecithin membranes in aqueous media containing 0.2 mol KCl or NaCl is $(5.70 \pm 0.95).10^{4}$ (Ivkova et al. 1983b). The internal volume, V_{in} of vesicles, 100 nm in diameter and about 5 nm in thickness, is about 3 V_m.

Quantitative estimations of the probe distribution in membrane suspensions for three different lipid concentrations are given in Table 1. They allow a comparison of the probe concentrations in the presence of a transmembrane potential with those without potential. It is clear that the action of the potential results in a sharp increase in the effective volume, due first of all, to the increase in

the effective volume of the membrane phase. This evokes a redistribution of the probe between the aqueous and the lipid phase, and thus an increase in the average probe concentration in membranes. The second process is the redistribution of the probe between the external and the internal membrane monolayer, which leads to a sharp increase in the dye concentration in the internal and to a decrease in the external monolayer.

These effects are more pronounced within a concentration range the values V_{out} and $K_{p,o}V_m$ being of the same order. If a charged hydrophobic probe is located in the centre of the hydrocarbon region, the probe concentrations can be calculated from the same formulae (15), taking into account that $|\Delta\varphi| = |\Delta\psi/2|$ and $m = p^2$. In this case, the dye concentration is

$$c^m_{p,av} = c^m_{p,out} = c^m_{p,in} = c^m_{p,o} \cdot p\beta \tag{20}$$

At $m = 200$, the values of the coefficient $p \simeq 14$ and those of β values are 0.072; 0.082 and 0.173 at lipid concentrations of 1 mg/ml; 0.1 mg/ml and 0.01 mg/ml, respectively; and at $K_{p,o} = 5.7 \times 10^4$. The ratios $c^m_{p,av}/c^m_{p,o}$ are about 1.015; 1.15 and 2.44 at the above lipid concentrations, i. e. the probe concentration in membranes increases under the action of a transmembrane potential. Processes of probe redistribution are schematically shown in Fig. 1 (*c* and *d*).

Inside-positive transmembrane potential

Calculation of the redistribution of a positively charged probe in a suspension of closed vesicles under the action of an inside-positive transmembrane potential can

Table 1. Quantitative estimations of probe redistribution in an egg lecithin vesicle suspension under the action of an inside-negative transmembrane potential. The initial volume of the aqueous medium was 1 ml. 5×10^{-6} ml of the liposome suspension in 0.2 mol/l KCl were added to the medium containing 0.2 mol/l NaCl, $V_{out} = 1$ ml, $K_{p,o} = 5.7 \times 10^4$, $m = 200$, $p = \sqrt[10]{m} \simeq 1.7$, $m/p^2 = 69$, $m/p = 118$. The probe is located near the boundary between the polar and the hydrocarbon region.

Probe concentration: Volumes and ratios	Lipid concentration (mg/ml)		
	1.0	0.1	0.01
V_m (ml)	10^{-3}	10^{-4}	10^{-5}
V_{in} (ml)	3×10^{-3}	3×10^{-4}	3×10^{-5}
β	0.0171	0.0194	0.0450
$c^a_{p,out}/c^a_{p,o}$	0.0171	0.0194	0.0450
$c^a_{p,in}/c^a_{p,o}$	3.42	3.88	9.00
$c^m_{p,out}/c^m_{p,o}$	0.029	0.033	0.077
$c^m_{p,in}/c^m_{p,o}$	2.015	2.28	5.3
$c^m_{p,av}/c^m_{p,o}$	1.02	1.16	2.68

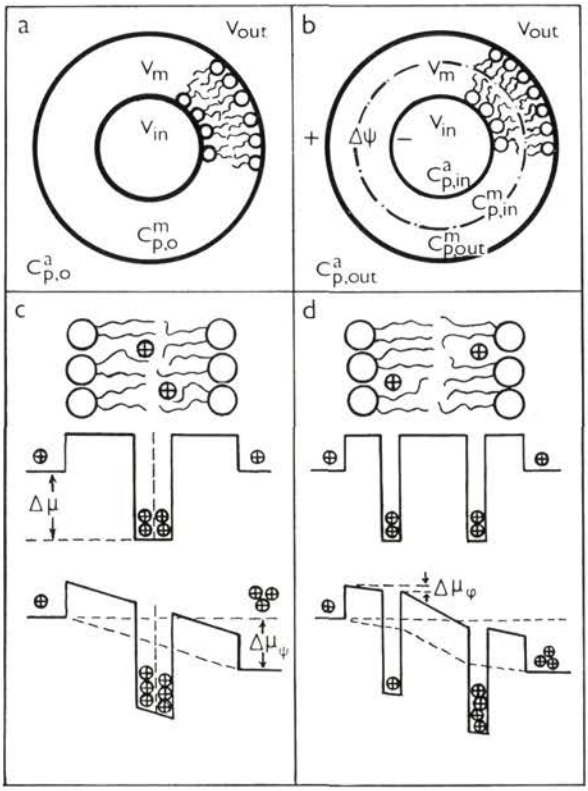

Fig. 1. Schematic representation of a positively charged probe distribution in membrane vesicle suspensions: *a*) without transmembrane potential; *b*) with a potential; *c*) transmembrane potential profile and its change under the action of the electric field, the probes are located in the centre of the hydrocarbon layer; *d*) the same as in (*c*), except that the probes are located near the boundary between the polar and the hydrocarbon region.

be made in the same way. The same expressions can be used for m and p:

$$p = e^{|zF\Delta\varphi/RT|} > 1 \quad \text{and} \quad m = e^{|zF\Delta\psi/RT|} > 1$$

The partition coefficients in the presence of a transmembrane potential are

$$K_{p,\,out} = \frac{1}{p} K_{p,\,o}, \qquad K_{p,\,in} = pK_{p,\,o} \tag{21}$$

and the ratio of the concentrations is

$$c^a_{p,\,out} / c^a_{p,\,in} = m \tag{21a}$$

The respective probe distribution without and with the potential can be written

$$n_p^o = c_{p,o}^a (V_{out} + V_{in} + K_{p,o} V_m)$$
$$n_p^o = c_{p,out}^a \left[V_{out} + \frac{V_{in}}{m} + \left(\frac{1}{p} + \frac{p}{m}\right) K_{p,o} \cdot \frac{V_m}{2} \right] \quad (22)$$

and the respecive concentrations of the probe in the external and internal membrane monolayers are:

$$c_{p,out}^m = c_{p,out}^a \cdot \frac{1}{p} K_{p,o}$$
$$c_{p,in}^m = c_{p,out}^a \cdot \frac{p}{m} K_{p,o} \quad (23)$$

It is obvious that the action of a transmembrane potential results in a decrease in the effective volume of the two-phase system, and in an increase in the probe concentration in the external aqueous medium, $c_{p,out}^a$:

$$c_{p,out}^a = c_{p,o}^a \cdot \beta, \qquad c_{p,in}^a = c_{p,o}^a \cdot \frac{\beta}{m} \quad (24)$$

where β is the ratio of the respective effective volume without and with a potential:

$$\beta = \frac{V_{out} + V_{in} + K_{p,o} V_m}{V_{out} + \frac{V_{in}}{m} + K_{p,o}\left(\frac{1}{p} + \frac{p}{m}\right)\frac{V_m}{2}} > 1 \quad (25)$$

The ratio of the respective probe concentrations in the internal and external membrane monolayer is

$$\frac{c_{p,in}^m}{c_{p,out}^m} = \frac{p^2}{m} \quad (26)$$

i. e. the reciprocal of the concentration under an inside-negative potential (16). Taking into account (23) and (24), these concentrations can be written as

$$c_{p,out}^m = c_{p,o}^m \frac{\beta}{p}, \qquad c_{p,in}^m = c_{p,o}^m \cdot \frac{p}{m} \cdot \beta \quad (27)$$

The average dye concentration in membranes is

$$c_{p,av}^m \simeq \frac{c_{p,out}^m + c_{p,in}^m}{2} = \frac{1}{2} c_{p,o}^m \cdot \beta \left(\frac{1}{p} + \frac{p}{m}\right) \quad (28)$$

Quantitative estimations can be made for the same conditions as in the case of an inside-negative transmembrane potential (Table 2). Absolute values of positive and negative potentials are identical.

Table 2. Quantitative estimation of probe redistribution in an egg lecithin vesicle suspension under the action of an inside-positive transmembrane potential. The conditions are the same as in Table 1. $V_{out} = 1$ ml, $K_{p,o} = 5.7 \times 10^4$, $m = 200$, $p = 1.7$. The probe is located near the boundary between the polar and the hydrocarbon region.

Probe concentration: Volumes and ratios	Lipid concentration (mg/ml)		
	1.0	0.1	0.01
V_m (ml)	10^{-3}	10^{-4}	10^{-5}
V_{in} (ml)	3×10^{-3}	3×10^{-4}	3×10^{-5}
β	3.2	2.5	1.34
$c^a_{p,out}/c^a_{p,o}$	3.2	2.5	1.34
$c^a_{p,in}/c^a_{p,o}$	0.016	0.0125	0.0067
$c^m_{p,out}/c^m_{p,o}$	1.88	1.47	0.79
$c^m_{p,in}/c^m_{p,o}$	0.027	0.021	0.0114
$c^m_{p,av}/c^m_{p,o}$	0.95	0,74	0.40

The analysis of the data given in Table 2 shows that, at a lipid concentration of about 0.01 mg/ml, an inside-positive potential can evoke an increase in the fluorescence or no optical response at all. At a lipid concentration of about 1 mg/ml a fluorescence quenching due to a sharp increase in the probe concentration in the external monolayer may be observed.

When the probe is located in the centre of the hydrocarbon layer ($m = p^2$), its concentration in membranes decreases due to the presence of the transmembrane potential.

Conclusion

In the present work the hypothetical mechanism for redistribution of a positively charged probe in a suspension of closed membrane vesicles under the action of a transmembrane potential have been analysed. With known local changes of the probe concentrations in such a system, and the concentration dependences of fluorescence intensity, the value of the optical response can in principle be calculated.

It should be remembered that this approach can only be used if the probe solutions in both the aqueous medium and membranes are diluted enough. In principle, this method of analysis can not only be applied for fluorescent probes but for various small charged hydrophobic and amphiphilic molecules in a membrane suspension as well.

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