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Effect of Dipole Modifiers on the Magnitude of the Dipole Potential of Sterol-Containing Bilayers

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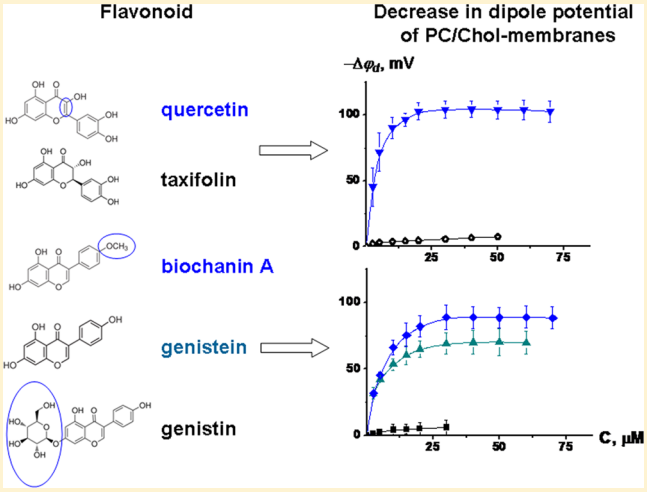
Supporting Information

ABSTRACT: The effects of various subclasses of flavonoids, Rose Bengal, and different styrylpyridinium dyes on the magnitude of the dipole potential of membranes composed of pure phospholipids and sterol-containing bilayers were investigated. Changes in the steady-state membrane conductance induced by cation−ionophore complexes were measured to examine the changes in the dipole potential of lipid bilayers. The characteristic parameters of the Langmuir adsorption isotherm for different flavonoids and Rose Bengal and the slope of the linear dependence of the dipole potential change on the aqueous concentrations of RH dyes were estimated. Chalcones (phloretin and phloridzin) and flavonols (quercetin and myricetin) strictly decrease the dipole potential of phospholipid- and sterol-containing membranes; the unsaturation of the C-ring and the hydrophobicity of the molecule contribute to the ability of the flavonoid to reduce the bilayer dipole potential. Rose Bengal decreases the magnitude of the bilayer dipole potential to a similar extent, but its affinity for membrane lipids is higher; the effects of RH dyes, chalcones, and phloroglucinol are determined by sterol concentration and type.



INTRODUCTION

The membrane dipole potential (φ_d) is an electric potential between the polar exterior of the lipid bilayer and its hydrocarbon interior. This potential drop originates from the specific orientation of polar lipid residues and water dipoles at the membrane−solution interface.[1,2] The dipole potential is related to the effective surface density of molecular dipoles, n, effective dipole moment normal to the interface, $\mu_\perp$, and the dielectric constant of the surroundings by the equation for a parallel-plate capacitor:

$$\varphi_d = \frac{\mu_\perp n}{\varepsilon_0 \varepsilon} \quad (1)$$

where ε_0 is the permittivity of free space.[3−5] The magnitude of the dipole potential depends on membrane lipid structure.[3,6−11]

The dipole potential affects peptide−lipid interactions in the membrane[12,13] and the activity of some membrane enzymes (phospholipase A2).[14] The dipole potential affects a number of drug interactions with cell membranes, in particular, the human HIV protease inhibitor saquinavir, the HIV-1 fusion inhibitor sifuvirtide, the antimicrobial agents berberine and benzalkonium, the bioactive compounds nicotinamide and picolinamide, the antibiotic bacitracin, and the anesthetic steroid pregnanolone.[15−20] There is evidence that φ_d of the blood-brain barrier determines its permeability to various substances.[21] The dipole potential regulates the conductance of membranes treated with ion carriers[22−25] and the activity of membrane ATPases, such as Na^+/K^+-ATPase and Ca^{2+}-ATPase.[26,27] The dipole potential influences the properties of ion channels formed by the antibiotics gramicidin, alamethicin and amphotericin B, as well as the properties of the *Helicobacter pylori* HP(2−20) antimicrobial peptide analogue HPA3 and the antifungal and antimicrobial lipopeptides syringomycin E and surfactin.[28−38]

The adsorption of electroneutral molecules, which have a dipole moment and a preferred orientation on the boundary of the membrane and/or alter the water polarized at the lipid phosphates,[39,40] leads to a change in the magnitude of the membrane dipole potential. These molecules are called dipole modifiers. One can propose that dipole modifiers may contribute to $\mu_\perp$, n, and ε. Equation 1 assumes a linear dependence of the value of the dipole potential changes ($\Delta\varphi_d$) caused by adsorption of dipole modifiers on their concentration in the membrane, which in turn depends on the concentration of dipolar molecules in the bathing solution.[41] A linear dependence of the increase in φ_d on the concentration of styrylpyridinium dyes in a membrane bathing solution has been

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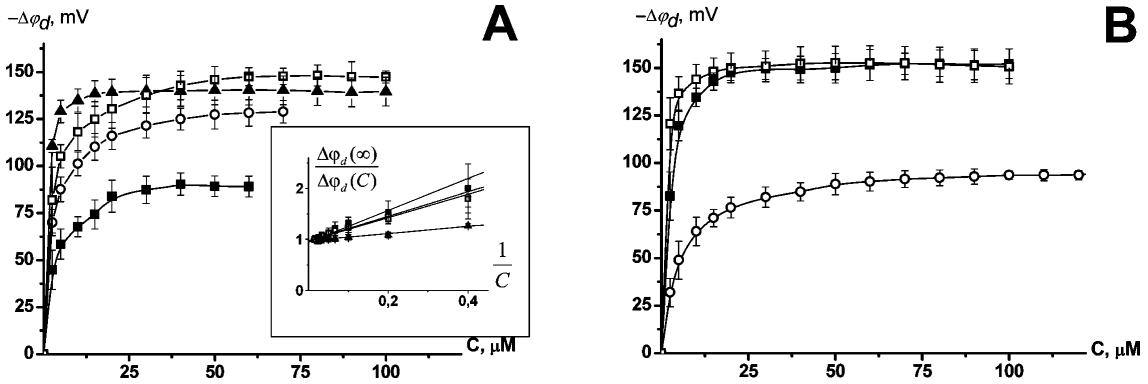

Figure 1. Dependence of the changes in the dipole potential of the membrane ($\Delta\varphi_d$) modified by NonA on the phloretin concentration in the membrane bathing solution. V = 50 mV. The bathing solution is 0.1 M KCl, pH 7.4. The membranes were made from (A) DOPS (■), DOPE (○), DOPC (▲), and DPPC (□); (B) DPPC:Chol (95:5 mol %) (●), DPPC:Chol (67:33 mol %) (○), and DPPC:Erg (67:33 mol %) (□). Inset: the dependence of $[\Delta\varphi_d(\infty)]/[\Delta\varphi_d(C)]$ on $1/C$ for bilayers made from DOPS (■), DOPE (○), DOPC (▲), and DPPC (□).

Table 1. Characteristic Parameters of the Langmuir Adsorption Isotherm for Phloretin on Membranes of Various Compositions

membrane composition	DOPS	DOPE	DOPC	DPPC	DPPC:Chol (95:5 mol %)	DPPC:Chol (67:33 mol %)	DPPC:Erg (67:33 mol %)
$-\Delta\varphi_d(\infty)$, mV	90 ± 6	128 ± 8	140 ± 8	147 ± 7	152 ± 8	95 ± 4	152 ± 9
K, μM	2.7 ± 0.8	2.2 ± 0.4	0.7 ± 0.2	2.0 ± 0.5	2.1 ± 0.4	5.0 ± 0.8	0.7 ± 0.3

reported.[26] However, the dependence of a decrease in φ_d on the concentration of the flavonoids phloretin and its glycoside phloridzin is close to linear only at low flavonoid concentrations and tends toward saturation at high flavonoid concentrations.[10,22,41,42] This relationship can be explained by the mutual interaction of the adsorbed dipoles as well as by the saturation of the binding sites on the surface of the membrane.[10,42] The Langmuir adsorption isotherm can be used to describe the adsorption of phloretin (phloridzin) to lipid bilayers as a first-order approximation.[42,43] According to ref 43:

$$\Delta\varphi_d(C) = \frac{\Delta\varphi_d(\infty)C}{C + K} \quad (2)$$

where $\Delta\varphi_d(C)$ is the dipole potential change at the C concentration of phloretin in the membrane bathing solution, $\Delta\varphi_d(\infty)$ is the maximum potential change, and K is the dissociation constant, which is a meaningful approximation of the affinity of the modifier for the lipid. Different methods give different values of the characteristic parameters of the Langmuir adsorption isotherm.[43,44] This discrepancy may be caused by the functional relationship between the parameters that determine φ_d, $\mu_\perp$, n, and ε.[43] It should be noted that the Langmuir adsorption isotherm neglects the important dipole−dipole interaction at the lipid surface. The change in φ_d caused by the adsorption of phloretin to membranes may strictly depend on the preexisting φ_d.[43]

The purpose of the work described here is to determine experimentally the effect of various dipole modifiers on the magnitude of φ_d. The changes in the steady-state membrane conductance induced by a complex of a cation with an ionophore (nonactin or valinomycin) were measured to estimate $\Delta\varphi_d$ of bilayers of various lipid compositions (pure phospholipids and cholesterol- or ergosterol-containing membranes) after the addition of dipole modifiers (flavonoids, RH dyes, and Rose Bengal) to a bathing solution. The parameters of the dependence of changes in the dipole potential of membranes on their concentration permit the calculation of the changes in φ_d at any concentration of these dipole modifiers in a membrane bathing solution.

■ RESULTS AND DISCUSSION

Figure 1 presents the effect of phloretin on φ_d of membranes of various compositions. The dependence of the magnitude of $\Delta\varphi_d$ of bilayers made from pure phospholipids on the phloretin concentration is shown in Figure 1A. Figure 1B shows the dependence of $\Delta\varphi_d$ of sterol-containing bilayers, made from DPPC:Chol (95:5 mol %), DPPC:Chol (67:33 mol %), or DPPC:Erg (67:33 mol %), on phloretin concentration. The inset of Figure 1A presents the dependence of $[\Delta\varphi_d(\infty)]/[\Delta\varphi_d(C)]$ on $1/C$ for bilayers made from pure phospholipids. The linearity of these dependences and the equality of the Y-intercepts to 1 clearly indicate the applicability of the Langmuir adsorption isotherm (2) for the approximation of the experimental data. A similar conclusion can be made for other experimental conditions in the presence of flavonoids and Rose Bengal (see Supporting Information). Table 1 presents the results of the approximation of the data presented in Figure 1 with the Langmuir adsorption isotherm (2). The magnitude of the maximum dipole potential change, $\Delta\varphi_d(\infty)$, depends on the membrane lipid composition and is smaller for negatively charged DOPS-bilayers than for uncharged DOPE-, DOPC-, and DPPC-membranes. According to ref 43, $\Delta\varphi_d$ caused by the adsorption of phloretin to membranes may strictly depend on the preexisting potential. Assuming that the dipoles of the adsorbed molecules can change their direction and their dipole moment, the dipoles should respond to the preexisting potential. In the case of DOPS-membranes, the electrostatic repulsions between negatively charged serine residues may contribute to the spacing between the lipid headgroups, thus producing a lower density of the dipoles responsible for φ_d compared to uncharged bilayers. However, according to ref 5, dimyristoylphosphatidylserine does not cause any major changes in the φ_d relative to dimyristoylphosphatidylcholine. This slight difference may be responsible for the varied ability of phloretin to decrease φ_d of charged and uncharged bilayers,

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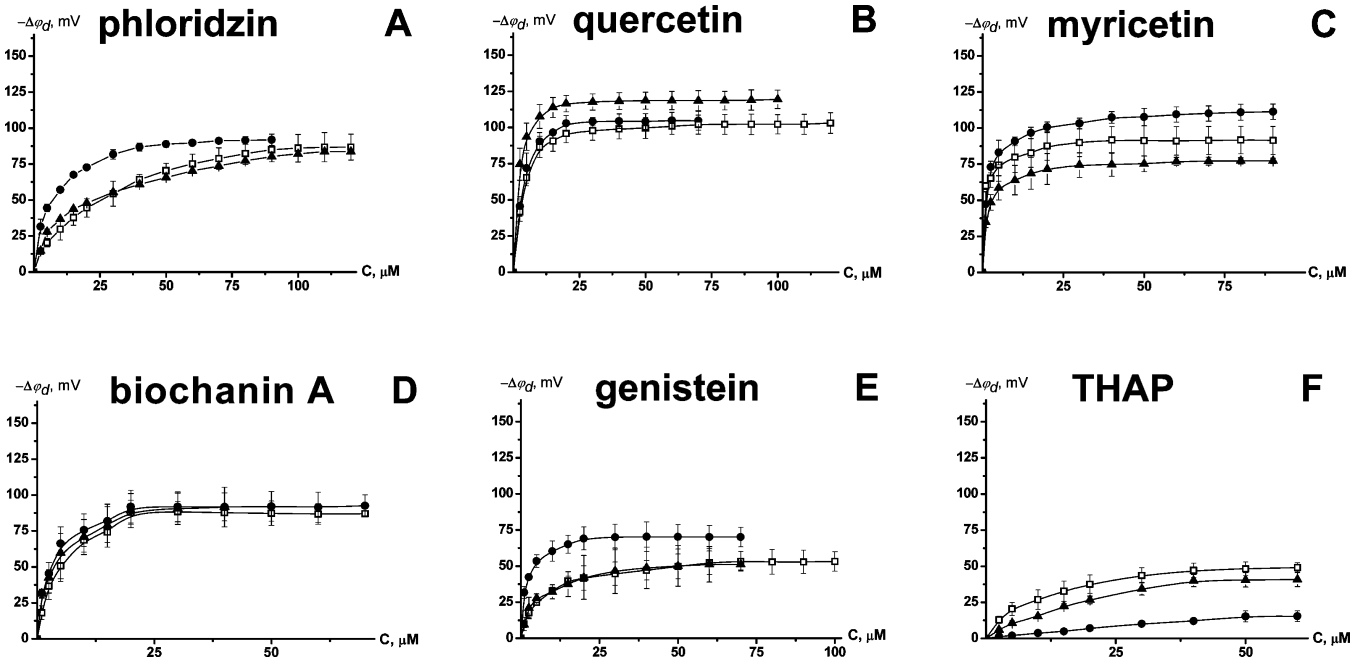

Figure 2. Dependence of the changes in the dipole potential of the membrane ($\Delta\varphi_d$) modified by NonA on flavonoid concentration in the membrane bathing solution: phloridzin (A), quercetin (B), myricetin (C); biochanin A (D), genistein (E), and THAP (F). The membranes were made from DPPC (●), DPPC:Chol (67:33 mol %) (□), and DPPC:Erg (67:33 mol %) (▲) and bathed in 0.1 M KCl, pH 7.4. V = 50 mV.

or both acyl chain unsaturation and the negative charge of DOPS contribute to the smaller preexisting dipole potential. Clarke and Lupfert demonstrated that certain anions reduce φ_d.[45] In this case, the adsorption of anions on the membrane should affect the effectiveness of phloretin to decrease φ_d. We investigated the effect of phloretin on φ_d of uncharged (DPPC) and negatively charged bilayers (50 mol % DOPS and 50 mol % DOPE) bathing in 0.1 M NaAsp. The values of $\Delta\varphi_d(\infty)$ and K are in a good agreement with those obtained in neutral and negatively charged bilayers bathing in 0.1 M KCl (data not shown). These data indicate that the adsorption of phloretin to the membranes slightly depends on the preexisting potential; it might depend on bilayer mechanical properties. It was recently shown by Warshaviak et al. that dipole-potential sensitive fluorescent probes are also sensitive to membrane tension.[46]

DOPC is characterized by neutral curvature, while DOPE is characterized by negative spontaneous curvature due to the smaller size of the PE headgroup. PE headgroups might allow lipids to pack together more tightly within the bilayer relative to PC,[47] thus producing a higher density of the dipoles responsible for φ_d.[5] Such an effect had been suggested to explain the decrease in φ_d of monomyristoylphosphatidylcholine vesicles relative to dimyristoylphosphatidylcholine.[5] Clarke also observed that the value of φ_d decreases with increasing unsaturation of the hydrocarbon chains, which was explained by the effects of chain packing on the spacing between head groups.[48] Peterson et al. also found that the disruption of chain packing in lipids containing heteroatom-substituted acyl chains is comparable to that observed upon the introduction of a single double bond into a lipid with a saturated acyl chain.[2] The smaller preexisting dipole potentials of DOPE- and DOPC-bilayers may be responsible for the reduced ability of phloretin to decrease the φ_d compared to DPPC-membranes. This assumption is supported by the data of Lairion and Disalvo,[40] which demonstrated that the effectiveness of phloretin to change φ_d of monolayers differed when the monolayers were composed of dimyristoylphosphatidylcholine or eggPE. Contrary to expectations, the effects of lipid-negative spontaneous curvature (DOPE vs DOPC) and acyl chain unsaturation (DOPC vs DPPC) on $\Delta\varphi_d$ (∞) are negligible (Table 1). This means that mechanical properties of bilayers are slightly influenced on phloretin adsorption.

The concentration of Chol in the membrane-forming solution determines the magnitude of $\Delta\varphi_d(\infty)$ induced by phloretin (Table 1). The presence of substantial amounts of Chol (33 mol %) in the membrane leads to a significant decrease in $|\Delta\varphi_d(\infty)|$ compared to the absence of any sterols (DPPC) or the presence of a small amount of Chol (5 mol %) in the bilayers. Lairion and Disalvo obtained very similar results for PE monolayers in a fluid state, demonstrating that the effect of phloretin is lower when cholesterol is present in PE than in pure PE.[40] By contrast, the action of phloretin on φ_d of DMPE in the gel state was enhanced by the inclusion of Chol in monolayers. Andersen et al. also demonstrated that phloretin exhibited a 10-fold greater effect on K^+-NonA conductance in PE:Chol (20:80 mol %) membranes than in pure PE membranes.[22] According to Brockman,[39] for phase-separated multicomponent membranes the key factors controlling the size and shape of the lipid domains are line tension at the raft borders, which tends to make the domains compact and circular, and dipole repulsion, which arises because the dipoles are forced by virtue of the lipid amphipathic character to assume a parallel arrangement. The dependence of this balance on sterol type (Chol vs Erg), molar membrane sterol content, and sterol-induced dipole potential changes should be taken into account.[4,9,49−53] The preexisting heterogeneity of φ_d,[54] lateral distribution of phloretin between ordered and disordered lipid domains,[36] and the effect of phloretin on lipid packing and phase-separation in the membrane[10,56,57] may be responsible for the different action of phloretin on sterol-containing bilayers.

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Table 2. Characteristic Parameters of the Langmuir Adsorption Isotherm for Different Flavonoids on Membranes of Various Compositions

		flavonoid					
membrane composition	parameter	phloridzin	quercetin	myricetin	biochanin A	genistein	THAP
DPPC	$-\Delta\varphi_d(\infty)$, mV	92 ± 4	104 ± 7	111 ± 11	92 ± 11	70 ± 10	15 ± 4
	K, μM	5.1 ± 0.2	3.3 ± 0.5	3.3 ± 0.2	2.1 ± 0.3	1.3 ± 0.2	26.4 ± 5.6
DPPC:Chol (67:33 mol %)	$-\Delta\varphi_d(\infty)$, mV	102 ± 9	102 ± 7	91 ± 10	88 ± 9	53 ± 9	48 ± 5
	K, μM	13.3 ± 0.9	3.6 ± 0.5	1.5 ± 0.2	3.9 ± 0.8	4.4 ± 0.7	7.1 ± 1.7
DPPC:Erg (67:33 mol %)	$-\Delta\varphi_d(\infty)$, mV	86 ± 1	118 ± 7	77 ± 11	91 ± 14	51 ± 12	41 ± 5
	K, μM	11.8 ± 0.5	1.5 ± 0.5	3.1 ± 0.5	2.0 ± 0.4	4.3 ± 0.9	15.2 ± 1.9

We tested the ability of different phloretin analogues to decrease φ_d of sterol-containing membranes and bilayers made of pure DPPC. For these purposes, we selected some representatives of the most common flavonoid subclasses: chalcones (phloretin and phloridzin); flavonols (quercetin and myricetin); isoflavones (biochanin A, genistein, genistin); the flavanol catechin, the flavanonol taxifolin, and the phloroglucinol THAP. Figure 2 presents the effects of phloridzin, quercetin, biochanin A, genistein, myricetin, and THAP on φ_d of membranes made from DPPC, DPPC:Chol (67:33 mol %) and DPPC:Erg (67:33 mol %). Table 2 presents the results of the approximation of the data presented in Figure 2 by the Langmuir adsorption isotherm (2) (see Supporting Information). The approximation of the data obtained with genistin, catechin, or taxifolin in DPPC-bilayers gives $\Delta\varphi_d(\infty) = -6 \pm 2$ mV and $K = 2.3 \pm 1.3$ μM. One can conclude that (i) the flavonols quercetin and myricetin strictly decrease φ_d of phospholipid- and sterol-containing membranes; (ii) the effectiveness of the chalcone phloridzin and the isoflavones genistein and biochanin A is slightly less than that of flavonols; (iii) the flavanol catechin and the flavanonol taxifolin have no significant influence on the magnitude of the membrane dipole potential; (iv) the increasing hydrophobicity of chalcones (phloridzin vs phloretin) and isoflavones (in order of increasing hydrophobicity: genistin, genistein, biochanin A) increases the effect on φ_d; (v) there is no pronounced dependence of $\Delta\varphi_d(\infty)$ induced by different flavonoids on membrane composition (sterol concentration and type), with the exception of phloretin (see above) and THAP, which demonstrates greater effectiveness for sterol-containing membranes relative to DPPC; (vi) the dissociation constants, K, of the two more hydrophilic analogues of phloretin, phloridzin and THAP, are significantly higher than that of other flavonoids. By consideration that K characterizes the inverse affinity of the flavonoid for the lipid, the last observations are in agreement with the data of Awiszus and Stark[58] that demonstrate that the partition coefficient of membrane/water interfaces of lecithin membranes for THAP is about 8 times lower than that for phloretin.

Our results clearly demonstrate that some rigid flavonoid molecules such as quercetin and myricetin can significantly alter φ_d nearly as well as phloretin. These data contradict the model proposed by Tarahovsky et al.[57] that the "hairpin" conformation of the flexible phloretin molecule is responsible for $\Delta\varphi_d$. According to Tarahovsky et al.,[57] the influence of quercetin on the mechanical properties of the bilayer is comparable that of cholesterol, while the influence of myricetin on lipid melting is low. The similar effect of quercetin and myricetin on φ_d suggests that they mainly contribute to $\mu_\perp$, rather than n. By comparing the structures of the flavonoids with their effects on φ_d, one can conclude that unsaturation of the C-ring and the hydrophobicity of the molecule contribute to the ability of the flavonoid to reduce φ_d. The first suggests that the partial negative charge of the flavonoid dipole is distributed somewhere in the region of the carbonyl group.

Kotova et al. assumed that the adsorption of Rose Bengal on the bilayer interface leads to a reduction of the dipole potential drop at the membrane−solution boundary, similar to the action of phloretin.[59] Our results obtained with Rose Bengal are in agreement with this proposal. Figure 3 presents the dependence of the decrease of the magnitude of the dipole potential of DPPC-bilayers on the concentration of Rose Bengal in the membrane bathing solution. The approximation of the data presented in Figure 3 by the Langmuir adsorption isotherm (see Supporting Information) gives $\Delta\varphi_d(\infty) = -121 \pm 9$ mV and $K = 0.16 \pm 0.05$ μM. Thus, Rose Bengal is the strongest dipole modifier among those noted above because it decreases the magnitude of the bilayer dipole potential as well as phloretin, but the affinity of Rose Bengal for the lipid is higher.

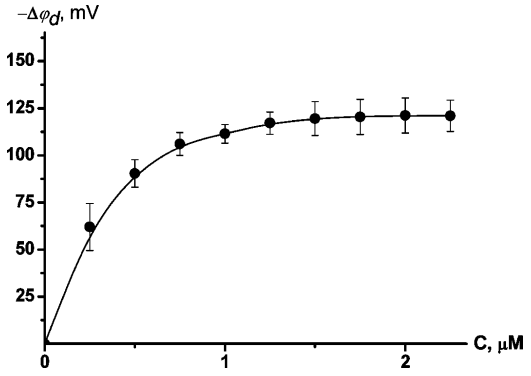

Figure 3. Dependence of the changes in the dipole potential ($\Delta\varphi_d$) of the DPPC-membranes modified by NonA on the concentration of Rose Bengal in the membrane bathing solution. The membranes were bathed in 0.1 M KCl, pH 7.4. V = 50 mV.

As noted in the Introduction, a linear dependence of the increase in φ_d of PC-bilayers on the concentration of styrylpyridinium dyes was observed by Malkov and Sokolov.[26] We have extended this finding to sterol-containing bilayers. Table 3 presents the results of the linear approximation of the data using the expression $\Delta\varphi_d(C) = \beta C$, where β is the constant, which characterizes the effectiveness of the RH dye to increase φ_d. RH dyes differ from each other in the lengths of their "tails" and the polyene fragment between the rings. One can observe from Table 3 that (i), among these dyes, RH 421 has the strongest effect, RH 237 has an intermediate effect and RH 160 has the smallest effect on increasing φ_d of membranes made from pure DPPC; (ii) the effectiveness of RH 421 in increasing φ_d decreases with increasing sterol concentration in

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Table 3. Slope of the Linear Dependence (β, mV/μM) of the Increase in the Dipole Potential of Membranes of Various Compositions on the Concentration of Styrylpyridinium Dyes[a]

	styrylpyridinium dyes		
membrane composition	RH 421	RH 237	RH 160
DPPC	26.1 ± 4.2	9.2 ± 0.7	6.5 ± 0.3
DPPC:Chol (95:5 mol %)	13.2 ± 1.6	–	–
DPPC:Chol (67:33 mol %)	8.7 ± 0.9	14.6 ± 1.8	10.0 ± 0.5
DPPC:Erg (67:33 mol %)	12.9 ± 2.2	15.4 ± 2.3	9.1 ± 0.1

[a]Membranes were modified by Val and bathed in 0.1 M KCl, pH 7.4.

the membrane; (iii) the effectiveness of RH 237 and RH 160 in increasing φ_d is slightly larger for sterol-containing bilayers than for membranes made from pure DPPC. The data for the action of RH dyes on DPPC-membranes are in agreement with the data of Malkov and Sokolov.[26] According to Passechnik and Sokolov,[60] the depth of the adsorption plane inside the bilayer increases in the order RH 160, RH 421, and RH 237. RH 237 may have had the strongest effect on sterol-containing bilayers among the tested dyes due to the maximum depth of its adsorption plane inside the membrane and potential interaction with sterol molecules. Literature data indicate that styrylpyridinium dyes may contribute not only via electrostatic repulsions, but also via membrane elasticity changes.[61]

The characteristic parameters of the Langmuir adsorption isotherm for different flavonoids and Rose Bengal and the parameters of the linear dependence of $\Delta\varphi_d$ of phospholipid- and sterol-containing membranes on the aqueous concentration of RH dyes were estimated. Flavonols and Rose Bengal were found to strictly decrease φ_d; the unsaturation of the C-ring and the hydrophobicity of the flavonoid molecule contribute to the ability of the flavonoid to reduce φ_d. The effects of chalcones and styrylpyridinium dyes are determined by the sterol composition of the membranes.

■ ASSOCIATED CONTENT

Supporting Information

S1 contains Materials and Methods section. Supporting Information S2 contains additional figures presenting the dependences of $[\Delta\varphi_d(\infty)]/[\Delta\varphi_d(C)]$ on inverse flavonoid or Rose Bengal concentration and dependences of the increase in the membrane dipole potential on the styrylpyridinium dye concentration in different lipid conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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