Interaction of the antioxidant flavonoid quercetin with planar lipid bilayers

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Abstract

Our capacitance and conductance measurements on reconstituted planar lipid bilayers (BLM) suggest an insertion of the flavonoid quercetin (QCT) in the membranes, which is concentration- and pH-dependent. Interaction of the flavonoid with the membrane has no impact on either structure or integrity of the lipid bilayer. The QCT molecules penetrate the lipid bilayer by intercalating between the flexible acyl chains of the phospholipids, the deepest insertion occurring in acidic medium, when QCT is neutral and completely liposoluble. Results indicated that aggregation of QCT within the hydrophobic core is accompanied by an increase of the transmembrane conductance following an alteration of the hydrophobic barrier for small electrolytes. By contrast, within alkaline media where QCT is deprotonated, the reaction site of the flavonoid is restricted to the hydrophilic domain of the membrane. This significantly changes the double layer capacitance as the negatively charged QCT molecules become sandwiched between polar headgroups at the bilayer surface. At highest alkaline pH, the transmembrane conductance was not affected, since QCT did not perturb the molecular packing of the hydrocarbonic acyl chains of the phospholipids. Results also demonstrated that changes in physical properties of the lipid bilayers following interstitial QCT embedding within either the hydrophobic domain or the polar headgroup domain may be related to both its lipophilic nature and interactions with the electric dipole moments of the polar headgroups of phospholipids. Data also demonstrated that translocation of QCT in the polar part of the lipid bilayer, at physiological pH and salt conditions, may be correlated with its optimized radical scavenging activity. This paper discusses the significance of the free radical scavenging capacity and antioxidant efficiency of QCT. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Bilayer lipid membranes; Quercetin; Bioflavonoid; Molecular interaction; Hydrophobic barrier

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Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag/AgCl</td>
<td>silver/silver–chloride electrode material</td>
</tr>
<tr>
<td>BLM</td>
<td>bilayer lipid membrane</td>
</tr>
<tr>
<td>C_m</td>
<td>membrane capacitance</td>
</tr>
<tr>
<td>CFTR</td>
<td>cystic fibrosis transmembrane conductance regulator</td>
</tr>
<tr>
<td>cis</td>
<td>cis half side of the BLM chamber</td>
</tr>
<tr>
<td>DMPC</td>
<td>dimiristoyl–phosphatidylcholine</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DPPC</td>
<td>dipalmitoyl–phosphatidylcholine</td>
</tr>
<tr>
<td>G_m</td>
<td>membrane conductance</td>
</tr>
<tr>
<td>HEPES</td>
<td>N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid]</td>
</tr>
<tr>
<td>3HF</td>
<td>3-hydroxyflavone</td>
</tr>
<tr>
<td>I</td>
<td>transmembrane current</td>
</tr>
<tr>
<td>PC</td>
<td>phosphatidylcholine</td>
</tr>
<tr>
<td>QCT</td>
<td>quercetin</td>
</tr>
<tr>
<td>Trans</td>
<td>trans half side of the BLM chamber (that is grounded)</td>
</tr>
<tr>
<td>U</td>
<td>applied transmembrane potential</td>
</tr>
</tbody>
</table>

1. Introduction

The flavonoids are a large and complex group of compounds that occur throughout the plant kingdom, providing color, flavor, anti-fungal and anti-bacterial activity. Quercetin (QCT) or so-called 3, 3', 4', 5, 7-pentahydroxyflavone is a strong anti-oxidant bioflavonoid product synthesized in many green and higher plants (Chen et al., 1996; Cao et al., 1997). This flavonoid may be a potential anti-cancer agent that promotes apoptosis of tumor cells (Takagi et al., 1998). The ability of QCT to scavenge free radicals and block lipid peroxidation raises the possibility that it may protect against cardiovascular diseases and cancer.

The free radical scavenging capacity and the anti-oxidative potential of QCT are determined by important structural determinants of this flavonoid (Fig. 1(A)): (1) radical target site located in the position of the dihydroxy (catechol) structure from 3' and 4', specifically for most of the anti-oxidants with a saturated 2,3-bond; (2) electron delocalization molecular ‘tool’ assured by the 2,3-double bond that is in conjugation with the 4-oxo function; (3) the presence of the adjacent 3- and 5-hydroxyl groups for maximizing the radical scavenging process as well as a higher radical absorption (Bors et al., 1990; Arora et al., 1998) (Fig. 1(B)).

On the other hand, QCT exhibits other potential physiological activities in the modulation of transport across different epithelial cell membranes (Gee et al., 1998; Walgren et al., 1998), and in the stimulation of either the sodium epithelial transport (Flonta et al., 1996) or the chloride secretion in intestinal epithelial cells (Cermak et al., 1998; Illek and Fischer, 1998). We found previously that the epithelial Na⁺ transport through the frog skin is stimulated in the presence of QCT within the apical side of the amphibian epithelium, but not within the basolateral side (Flonta et al., 1996). We therefore postulated that the scavenging activity of QCT may depend on its interaction with the lipid bilayer of cell membranes. To test this hypothesis, we have examined the ability of QCT to modify some physical properties of planar lipid bilayers, as there appears to be a basis for this elsewhere in the literature (Ikigai et al., 1993; Terao et al., 1994; Ioku et al., 1995; Saija et al., 1995a,b; Wojtowicz et al., 1996; Arora et al., 1998; Gordon and Roedig-Penman, 1998).
To investigate the mechanism of interaction between QCT and biological membranes, we pursued the effect of this biologically active flavonoid on reconstituted planar bilayer lipid membranes (BLM) using the method of 'painted bilayers'. The response of QCT was followed by simultaneously monitoring the transmembrane conductance and capacitance when the antioxidant flavonoid QCT was present within the bath, and then compared with the situation in its absence.

2. Materials and methods

QCT or so called 3, 3', 4', 5, 7-pentahydroxyflavone (Fig. 1(A)) was purchased from Sigma (St. Louis, MO) and used without further purification. QCT was dissolved in a very small amount of DMSO (3 μl), and then re-dissolved in the bathing electrolyte (100 mM NaCl, 20 mM Tris.HCl). The pH was adjusted with 0.1 M HCl or 1 M NaOH to the final value. For low-pH electrolyte solutions, we used a combination between Na2HPO4 (20 mM) and NaH2PO4 (20 mM) to give the final pH. All the bathing electrolytes were brought to the same osmolarity. For the preparation of the buffer solutions, Tris.HCl, NaH2PO4 and Na2HPO4 and NaCl (Sigma) were used. The electrolyte solution was stirred by magnetic bars (~ 1 min) immediately after QCT was added into the chamber. Stock solutions of QCT were added bi- (dose-response experiments, pH-dependent QCT insertion experiments) or unilaterally (kinetics of the QCT insertion).

2.1. Procedure of planar lipid bilayer reconstitution

Optically black lipid membranes with an area of 0.785 x 10^-2 cm² were formed using the
method of painted bilayers (Hanke and Schlue, 1993). Each of the two compartments of a Teflon cell was filled with 1.5 ml of electrolyte buffer. The trans chamber was grounded. The membrane-forming lipid solution contained 20 mg/ml phosphatidylcholine (Sigma) dissolved in n-decane. The aperture was pre-treated with the same lipid dissolved in hexane, in a concentration of 5 mg/ml. Phospholipids were also used without further purification treatment. Formation of the planar lipid bilayers was assigned by both criteria optically and electrically. Optically monitoring the formation of the membranes was followed through a close-focus multipurpose telescope (Pörschke, GmbH, Germany). Only the bilayers with electrical capacitance and conductance equal to 0.2 mF/cm² (at least) and 10–100 ns/cm², respectively, were accepted for experiments.

The transmembrane current was measured with Ag/AgCl electrodes connected to the bathing electrolyte via agar salt bridges (1% agar dissolved in 1 M KCl). The signals were amplified with a current amplifier with a gain of 10⁷ V/A, followed by an I/V converter and a voltage amplifier (with a gain and bandwidth equal to 100 and 1 kHz, respectively). The electrical hardware components (pulse generator, current-to-voltage converter, signal amplifiers) were designed in the workshop of the Max-Plank-Institut für Biophysik (Frankfurt/Main, Germany) and kindly provided by Dr Klaus Fendler and Professor Ernst Bamberg. Finally, the signal was filtered at 500 Hz and recorded with a digital oscilloscope (Hewlett Packard, USA). For further details regarding the procedure for the membrane formation see Fendler et al. (1985).

All the experiments were done at room temperature (24 ± 1°C). The transmembrane conductance was calculated by applying ±50 mV to the membrane and measuring the induced current (Gm = I/U).

2.2. The transmembrane capacitance components

As BLM in aqueous solution behaves as an excellent electrical insulator, and its specific capacitance (total capacitance divided by the membrane surface area) is related to the structure of the membrane (thickness, dielectric constant, and adjacent electric double layer) the impact of QCT under varying experimental conditions was investigated.

The bilayer set-up was equipped with a continuous system for recording the bilayer capacitance. Detailed descriptions are provided in elsewhere (Latorre and Alvarez, 1981; Alvarez, 1986; Schindler, 1989). A triangular pulse of 10 mV and the duration of 1 s was applied from a pulse generator to the bilayer membrane. The relation between the effective current across a capacitive membrane and the transmembrane capacitance (Cm) is the following:

\[ I = C_m \frac{dV}{dt} \]  

Accordingly, the transmembrane capacitance can be derived measuring the amplitude of the response signal (square-like wave).

Membrane capacitance is consisted of the contribution of the bilayer hydrophobic domain (denoted by the geometrical capacitance \( C_g \)) and both electric double layers adjacent to the bilayer surface (denoted by the double layer capacitance \( C_{dl} \)) (Everitt and Haydon, 1968; White, 1986). Because the three capacities are coupled in series:

\[ \frac{1}{C_m} = \frac{1}{C_g} + \frac{2}{C_{dl}} \]  

Accordingly:

\[ C_m = \frac{C_g C_{dl}}{2C_g + C_{dl}} \]  

The geometric capacitance indicates the characteristics of the thickness (\( d_{hc} \)) and dielectric coefficient of the phospholipid acyl chain domain (\( \epsilon_{hc} \)):

\[ C_g = \frac{\epsilon_{hc} d_{hc}}{d_{hc}} \]  

where the thickness of the lipid bilayer is given by the average thickness occupied by lipids and solvent molecules. Thus, if \( N_L \) and \( N_S \) are the numbers of phospholipids and solvent molecules per unit area of bilayer membrane, respectively, then the thickness can be expressed by the following expression:
\[ d_{hc} = N_1 V_{ac} + N_S V_S \]  
where \( V_{ac} \) and \( V_S \) denote the molecular volume of the two acyl-chains of the phospholipids and the molecular solvent volume, respectively.

The electrical double layer capacitance, for the case of zero membrane surface is given by the Gouy-Chapman expression (Everitt and Haydon, 1968):

\[ C_{dl} = \frac{\kappa}{4\pi} \]  
where

\[ \kappa = \frac{8\pi^2 e^2 n_0}{ikT} \]  
Here \( k, T, e, \varepsilon, z \) and \( n_0 \) denote the Boltzmann constant, the absolute temperature, the electronic charge, the dielectric constant, the valence of the electrolyte ions, and the average ionic concentration in the aqueous solution, respectively.

If the QCT molecules insert within the hydrophobic core of the lipid bilayer, these can contribute to the change of the membrane capacitance by modifying the geometrical capacitance. Thus, a new capacitance contribution due to the QCT embedding into the lipid bilayer \( C_{QCT} \) will couple in parallel with the geometrical capacitance. Therefore, in the presence of the QCT molecules inserted in the lipid bilayer, Eq. (3) will change to the following one:

\[ C_{m}^{QCT} = \left( C_m + \frac{C_{dl} C_{QCT}}{C_{dl} + 2C_g} \right) \times \frac{C_{dl} + 2C_g}{C_{dl} + 2C_g + 2C_{QCT}} \]  

Usually, for the case of zero membrane surface charge (such as neutral phospholipids) the electric double layer capacitance is comparable with the geometrical capacitance \( (C_{dl} \approx C_g, \text{ Läuger et al., 1967; Everitt and Haydon, 1968}) \). Even at very high QCT concentrations, the capacitance contribution due to the QCT embedding into the lipid bilayer \( (C_{QCT}) \) will be smaller than the other components \( (C_{dl} \text{ and } C_g) \), since the cross-sectional area of phospholipid-occupied domain is much higher than the cross-sectional area of the QCT-occupied domain.

3. Results

We obtained in our experimental bilayer membrane set-up stable BLMs (they are maintained for at least 3 h) with an average capacitance in a range of 0.2–0.5 \( \mu F/cm^2 \) and a conductance of 10–100 nS/cm². These parameters are consistent with previous studies on the physical properties of the reconstituted bilayer lipid membranes (White and Thompson, 1973; Fuks and Homblé, 1994). Because the membrane capacitance \( (C_m) \) is also dependent on the square of the applied voltage \( (V_m) \), we used the same voltage amplitude of the pulses (10 mV) throughout this work to have a meaningful comparison of the experimental data (White and Thompson, 1973).

3.1. Dose-response experiments of QCT on the physical properties of the BLM

We carried out dose-response experiments (between 0.3 and 300 \( \mu M \)) to investigate the effect of QCT on physical characteristics of the phospholipid bilayer membranes. After adding QCT on both sides of the BLM chamber, we noticed concentration dependent increases of the bilayer conductance (Fig. 2). Dose-response experiments were carried out with buffers at physiological salt concentrations and neutral pH (100 mM NaCl, 20 mM Tris.HCl). Additionally, we performed control experiments with the BLM alone in the pres-
Fig. 2. Normalized transmembrane conductance changes due to bilateral QCT addition are illustrated by dose-response family of experimental curves (for QCT concentrations of 0.3, 3, 30 and 300 μM). The experiments were performed at physiological salt concentration (100 mM NaCl and 20 mM Tris.HCl) and neutral pH. The plot presents the normalized values.

This means that QCT is active in this concentration range. Comparable saturation conductance states were recorded for 3 and 30 μM QCT (a 120–150% increase). Experiments were performed for long recording duration (~ 3 h). After QCT addition in the bathing fluids, a complete steady state is reached in 30–40 min. Interestingly, at a high QCT concentration (300 μM), a large but slow transmembrane conductance increase was noticed (Fig. 2), steady state conditions being reached at 200 min into the experiment.

By contrast with the experiments performed at relatively low QCT concentration, the profile of the transmembrane conductance increase for 300 μM, was surprisingly found as non-monotonic one, namely a zig-zag way. This behavior may be a result of competition between two distinct processes that take place at very high drug concentrations. Firstly, the QCT molecules may aggregate and segregate within cluster micro-domains (Popescu et al., 1997). Secondly, QCT may exhibit the capacity to leave the lipid bilayer membrane with time by a simple passive diffusion process, resulting in competing processes relative to perturbation of the hydrophobic barrier of the lipid bilayer.

Results also demonstrated the normalized transmembrane capacitance change occurring in the dose-response experiments performed for the same QCT concentration range (Fig. 3). The transmembrane capacitance increased due to interstitial intercalation of QCT molecules between the acyl chains inside the hydrophobic bilayer domain implying that majority of QCT molecules are present in the bathing fluid, resulting in more aggregated QCT micro-domains and larger interstitial QCT segregation inside the hydrophobic core.

Comparing Figs. 2 and 3, we can simply see that the kinetics of the transmembrane capacitance change due to the QCT insertion in the lipid bilayer is much slower than that of the transmembrane conductance change. Furthermore, for 300 μM QCT, the conductance profile is more sensitive than the transmembrane capacitance in terms of a non-monotonic behavior. These findings can be explained by taking into account the physical processes that are probed by the two parameters.
Firstly, the transmembrane capacitance that corresponds to 300 μM QCT exhibits an almost monotonic increase, due to a continuous increase of the number of the aggregated QCT micro-domains. This capacitance increase makes sense if we consider the equation (10). As discussed in Section 2.2, for high QCT concentrations $C_{QCT} < C_{dl} \approx C_g$ (Läuger et al., 1967; Everitt and Haydon, 1968). In these conditions:

$$\frac{C_{dl} + 2C_g}{C_{dl} + 2C_g + 2C_{QCT}} \approx 1 \Rightarrow C_{mQCT}$$

$$C_m + \frac{C_{dl}C_{QCT}}{C_{dl} + 2C_{QCT}} \quad (11)$$

Therefore, at higher QCT concentrations the membrane capacitance increases. As Eq. (11) looks like, the enhancement of the membrane capacitance following high QCT concentration treatment is not very high, that is consistent with the experimental capacitance profile from Fig. 3 (not more than 20%). Indeed, the second term of Eq. (11) is less than $C_m$, since $C_{QCT}$ is normally smaller than $C_g$ (see Eq. (3)).

At high drug concentrations, the fluidity of the BLM is modified laterally (Popescu et al., 1997). The transmembrane capacitance probes an average of the extent of these lateral aggregated QCT micro-domains.

Secondly, the transmembrane conductance obeys changes due to a modification of the hydrophobic barrier of the BLM for monovalent ions present in the bath. At the QCT domain – phospholipid bilayer interfaces, the QCT molecules may induce specific kink-like conformational changes to intermediate acyl chains of phospholipids, thus modifying the hydrophobic barrier for ions from the bath (Xiang et al., 1998). Therefore, the passive permeability for monovalent ions from the bathing fluid may be impaired, as structural changes of the QCT domains in the bilayer interfaces are governed by the two competitive processes mentioned above.

Surprisingly enough, at very low QCT concentration (0.3 μM) and neutral pH, we found that the transmembrane capacitance slightly decreased (Fig. 3, bottom curve). Taking into consideration that, at this QCT concentration, the transmembrane conductance increased, the capacitance profile can be explained exclusively by the presence of a fraction of QCT molecules near the polar headgroup region, and thus affecting indirectly the electrical double layer capacitance (Eq. (6)). Therefore, the result is a complex contribution coming from both a capacitance increase due to perturbation of hydrophobic barrier by a certain fraction of QCT molecules and a capacitance decrease of the electrical double layer via close distribution of the QCT molecules between polar groups of phospholipids. Everitt and Haydon (1968) showed that the electric double layer capacitance have a decrease upon the enhancement of the cross-section area per ion (Fig. 2) For a certain decrease of the double layer capacitance $C_{dl}$, the second term of the parentheses becomes smaller. Since both $C_{QCT}$ (due to a very low QCT concentration) and $C_{dl}$ are small, then the second term of the parenthesis from Eq. (10) may be neglected. Therefore, $C_{mQCT}$ becomes slightly smaller than $C_m$, as the remaining fraction is near, but slightly smaller than 1. This theoretical formalism of the balance of these capacitance components for the extreme QCT concentrations fit very well with our experimental findings. However, for other QCT concentrations, it is very difficult to have interpretations of the experimental capacitance profile via Eq. (10).

3.2. Kinetics of the QCT insertion in the membrane is sensitive to a possible asymmetry of the lipid bilayer

We also studied the QCT effect on the physical properties of lipid bilayer, when added unilaterally within the bathing fluid (Figs. 4 and 5). These experiments were carried out at physiological salt concentration and neutral pH. Surprisingly enough, the addition of 30 μM QCT in one side of the BLM chamber modifies the transmembrane capacitance in a different way. A sudden jump in the transmembrane capacitance was noticed. This capacitance increase is higher when QCT is present in the cis side of the bilayer, rather than the situation of the QCT treatment in the trans side (Fig. 5). This result suggests the presence of an asymmetry, which influences the insertion abil-
ity of QCT in the BLM. For instance, the bilayer formed by the painting method might not be perfectly flat, but presumably exhibits a spontaneously appeared curvature.

The response in capacitive current is certainly dependent on the molecular packing of the phospholipids in both leaflets of the bilayer. A certain curvature of the BLM induces differences between the surface tension and packing of the lipids in both monolayers of the bilayer. Secondly, the transmembrane capacitance change is dependent on the surface area as well as the location of this possible curvature. For clarifying the cause of this asymmetry, further experimentation is necessary in a future work. On the other hand, comparing the results from unilateral and bilateral QCT treatment in the BLM chamber, we can observe that the steady state of the conductance and capacitance measurements is reached faster for the first case, rather than for the latter case. We conclude that the QCT insertion in BLM is faster when QCT is present in one side of the BLM chamber only. The transmembrane QCT concentration gradient and the asymmetrical osmotic pressure conditions could play an important role in the kinetics of the flavonoid entrapping within the lipid bilayer.

3.3. Acidic medium favors a deeper embedding of the QCT molecules within the hydrophobic core of the BLM

We found that the QCT insertion in BLM is strongly pH-dependent. Looking at Fig. 1(b), we can see that at higher pH the 4’- and 7-hydroxyl groups may lie in a deprotonated state, when QCT is dissociated (see details in Bors et al., 1990). In the vice-versa situation, an acidic pH may cause a significant enhancement in the
transmembrane capacitance (Fig. 6), whereas the transmembrane conductance obeys to a three-fold enhancement (Fig. 7). Accordingly, the QCT insertion takes place deeper in the hydrophobic core of the lipid bilayer. Therefore, the perturbation of the hydrophobic barrier for monovalent ions (e.g. passive permeability) following the intercalation of the QCT molecules between the intermediate acyl chains of phospholipids is significant at lower pH. In this situation, the QCT molecules are not dissociated, but completely liposoluble, and they insert deeper into the hydrophobic core than at neutral or alkaline pH (Figs. 6 and 7). We conclude that, at acidic pHs, the QCT insertion induces an increase of the transmembrane capacitance due to an augmentation of the cross-sectional BLM surface.

By contrast, at pH 8, we found no change in the transmembrane conductance, but a relatively rapid decrease in the transmembrane capacitance. This finding supports no insertion of the QCT molecules inside the hydrophobic core of the BLM, excepting a small hydrophobic moiety of this flavonoid. We believe that, at alkaline pH, most of the QCT molecules are either sandwiched between the polar headgroups of phospholipids with a small hydrophobic moiety intercalated between acyl chains or exclusively in the polar domain and partly embedded in the aqueous solution near the bilayer surface. The lack of the transmembrane conductance change for alkaline pHs (Fig. 7) might explain the fact that the strong hydrophobic barrier for ions and the very low passive ionic permeability are not impaired for this case. Secondly, a decrease in the transmembrane capacitance is mostly a contribution coming from the electric double layer capacitance (see Eqs. (6) and (7)) following adsorption of the QCT molecules at the bilayer surface.

At an alkaline pH, the QCT molecule is negatively charged, and thus it affects the complete separation between the charges at the bilayer surface (re-distribution of the counterions at the bilayer surface). Consequently, the electric double layer capacitance $C_{dl}$ decreases, whereas the geometric capacitance remains almost constant. Also, an electrostatic interaction between the charged QCT sites and the polar headgroups can induce the local changes of the dipole potential. Overall, it is expected that the interstitial distribution of the QCT molecules in the polar domain changes the membrane potential profile.

Our results suggest strongly that QCT is inserted in the lipid bilayer. The insertion is deeper at a more acidic pH, whereas an intercalation of QCT between the polar headgroups of phospholipids takes place in alkaline aqueous solution. We rule out that QCT lies in aqueous medium like in the case of quercetin glucosides (Ioku et al., 1995), since the latter ones are more hydrophilic flavonoids.

4. Discussion and conclusions

Numerous studies regarding the effects of the antioxidant QCT on liposomes (Ikigai et al., 1993; Terao et al., 1994; Wojtowicz et al., 1996; Arora et al., 1998; Gordon and Roedig-Penman, 1998) have shown that the embedding site as well as the membrane domain site for free diffusion of the radicals should be taken into consideration, as these contribute to its efficiency as an antioxidant. The key object of the present investigation was to examine whether QCT molecules deeply bury themselves in the hydrophobic domain, or...
whether they bind to the polar headgroup region of the bilayer and only penetrate the acyl chains via insertion of a certain hydrophobic moiety. Secondly, we were interested to see whether QCT might change the fluidity of the membranes, in such a way to promote the breakdown of the lipid bilayer or the appearance of statistical pores via the changes in the elasticity of the hydrophobic region of membrane.

On the subject of QCT insertion into BLM, we found that a very high QCT concentration into the bathing fluid (300 \( \mu \)M) promotes a significant penetration of the planar lipid bilayer. Simultaneously with the accumulation of the QCT molecules in the interfaces, the QCTs are able to modify the structural features of the interfaces by promoting the kink-like acyl conformations of the phospholipids near the interfaces, similarly with the drug action molecules or anaesthetics. Thus, the lipid bilayer becomes much more leaky to small electrolytes implying that the transmembrane conductance increases as more QCT molecules perturb the bilayer (Fig. 2). Our results are in good agreement with the study of Ikigai et al. (1993). Thus, these results are not without precedent. However, the apparent step-wise increases in membrane conductance may now have been observed for the first time. This suggests that the increase of the lipid bilayer conductance is not caused by the formation of ionic pores or ionophore-like structures. This finding rules out the idea that the stimulation in the short-circuit current recorded in the frog skin epithelium (almost exclusively assigned to the Na\(^+\) influx) following the apical QCT addition (Flonta et al., 1996) is caused by the insertion of an ionophore-like molecular assembly in the epithelial cell membrane.

From our conductance and capacitance measurements, it seems that the interaction between QCT and the lipid bilayer is similar to that exhibited by the local anaesthetics and drugs. Local anaesthetics induce the bilayer fluctuations and passive permeability changes due to their presence in membrane by interstitial intercalation in the lipid bilayer matrix. There is a wide variety of molecules in this category. Mouritsen and co-workers developed an interaction molecular model of foreign molecules (drugs, anaesthetics, etc.) with the lipid bilayer matrix (Jorgensen et al., 1993; Mouritsen et al., 1995). The bilayer, although is generally characterized by low permeability to small cations such as Na\(^+\) and K\(^+\), becomes much more leaky in the presence of such drugs or anaesthetics.

It is noteworthy that procaine, a tertiary amine anaesthetic, stimulates the Na\(^+\) transport across the frog skin epithelium (Flonta et al., 1988). On the other hand, procaine enhances the Na\(^+\) permeability of DMPC bilayers, especially for temperatures below the melting temperature (Tsong et al., 1977). Therefore, part of the Na\(^+\) influx increase in the amphibian epithelium is accounted for a permeabilization of the lipid bilayer for the Na\(^+\) ions following its interaction with procaine. Also, the Na\(^+\) permeability of the DPPC bilayers increased as more procaine was incorporated (Tsong et al., 1977). It seems that the QCT case is similar to that of the anaesthetic procaine. Discrimination of the passive Na\(^+\) flux across the lipid bilayer itself and the passive Na\(^+\) flux through the Na\(^+\) channels might be assessed quickly by a recently developed method (Movileanu, 1999).

The finding from the current investigation that QCT penetrates the lipid bilayer increasing its capacitance agrees well with those of others (Fischer et al., 1998), who found a direct dependence of the capacitance increase of the lipid bilayer on the QCT-activated CFTR-mediated transmembrane current.

Findings from the present investigation that QCT interaction with the lipid bilayer may be mediated by distribution of charged and uncharged polar headgroups oriented into the hydrophilic region, the acyl chain length and the degree of saturation in the hydrophobic core may be in good agreement with the work of others (Jain, 1988; Movileanu and Popescu, 1995; Movileanu et al., 1998).

Our work demonstrates, that QCT molecules penetrate deeply into the hydrophobic core of the membrane when BLM is reconstituted at acidic pH. As to the role of DMSO, it is noteworthy that DMSO concentrations used in this work did not induce any effect on the physical properties of
lipid bilayers, implying that the liposoluble QCT modifies the phospholipid molecular area and affects its surface geometry and electrostatics. These results correlate with those for other flavonoids such as hesperetin and naringenin (Saija et al., 1995a,b).

The present experimental data indicate that a higher pH of the aqueous solution induces: (1) a closer distribution of the negatively charged sites of QCT to the bilayer surface, thus affecting the electrical double layer capacitance; (2) a less possibility that the hydrophobic barrier for small electrolytes be perturbed. Summarizing, results presented in the present investigation suggest QCT interacts and penetrates lipid bilayer giving rise to its maximized antioxidant capacity. This agrees well with other work that suggest, at physiological pH, QCT (although a rather lipophilic molecule) interacts with the polar head of phospholipids by locating itself at the membrane surface (Ioku et al., 1995). The results are also consistent with those observed for liposomes (Terao et al., 1994; Gordon and Roedig-Penman, 1998).

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