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Interaction of Antioxidant Biobased Epicatechin Conjugates with Biomembrane Models

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(−)-Epicatechin conjugates with sulfur-containing moieties are strong free radical scavengers with cell-protecting activities, which may be in part modulated by their capacity to bind to biological membranes. We present here a study of the interaction of these conjugates with membrane models such as multilamellar vesicles and a phospholipid-coated silica column (immobilized artificial membrane), monitored by differential scanning calorimetry and high-performance liquid chromatography, respectively. The nonpolyphenolic moiety significantly influenced the membrane behavior of the whole molecules. Bulky and hydrophobic conjugates clearly interacted with the phospholipids and may have a tendency to penetrate into the hydrophobic core of the vesicles. In contrast, the smaller cationic 4β-(2-aminoethylthio)epicatechin may be located at the outer interface of the lipid membrane. The outcomes from both experimental set-ups were in good agreement. The differences detected in the biological activities of the conjugates may be explained in part by their tendency to penetrate the cell membrane.

KEYWORDS: Antioxidants; catechins; flavonoids; liposomes; artificial membranes

INTRODUCTION

Natural polyphenols present in foods and supplements are widely appreciated for their putative health-promoting effects (*1*). They may be behind the low incidence of cancer and cardiovascular diseases of animals and humans following a diet rich in fruits and vegetables (*2*, *3*). Catechins and their oligomeric forms (proanthocyanidins) are the main polyphenolic components of nutraceutical preparations from grape or pine (*4*, *5*). We have described a new type of catechin conjugates with sulfur-containing compounds (*6*, *7*), which can be readily obtained from agrofood byproducts (*8*). The nonphenolic part of these molecules influences the antiproliferative and proapoptotic activities on skin and colon cancer cells (*9*), neuronal survival (*10*), and immunomodulation (*11*). Interestingly, the conjugates appear to protect cells against oxidative damage by maintaining the levels of endogenous antioxidant systems (e.g., glutathione) rather than just scavenging free radicals (*10*). Changes in the capacity to interact with biological membranes driven by the nonphenolic substituents may be partially responsible for the differences detected among the conjugates. In fact, we have shown that cationic derivatives penetrate the skin layers more efficiently than zwiterionic and underivatized catechins (*12*). To search for clues as to how the new molecules interact with biomembranes, simple membrane models such as liposomes and phospholipid-coated HPLC (high-performance liquid chromatography) columns are convenient tools.

Liposomes (e.g., multilamellar vesicles, MLVs) have been widely used as models for the interaction of small drugs and other biomolecules (proteins, peptides, DNA, and carbohydrates) with the cell membrane (*13*−*16*). In contrast, information about polyphenol interactions with membrane models is scarce. Simple catechins such as (−)-epicatechin (Ec) and (−)-epicatechin-gallate show some ability to penetrate into liposomes by a mechanism driven mainly by the capacity to establish hydrophobic interactions (*17*, *18*). The more hydrophobic gallate esters appear to penetrate more deeply into the bilayer (*18*). An alternative and more simple way to screen bioactive compounds for their interaction with biomembranes is the so-called immobilized artificial membrane (IAM). IAMs were introduced as HPLC column-packing materials by Pidgeon and Venkataram (*19*) and have proven to be useful tools to predict drug permeability in different biological systems (*20*, *21*). As far as we know, catechins and their derivatives have not been studied on IAM columns. The IAMs are prepared by covalently linking synthetic phospholipid analogues to silica-propylamine particles, in order to mimic the lipid environment of a fluid cell membrane on a solid matrix. Because of this chemical similarity, IAM columns are mainly used for the estimation of biomembrane transport properties. In previous studies, the retention factor on

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IAM columns correlated well with solute partition coefficients in fluid liposome systems (*22*, *23*).

We describe here the behavior of bioactive sulfur derivatives of Ec on MLV liposomes and a IAM column. Because some differences in the biological activities of the electrically charged catechin conjugates might be explained by a differential capacity to penetrate or somehow interact with biological membranes, these model systems may provide valuable information to help explain the biological properties of the new bioactive conjugates.

MATERIALS AND METHODS

Chemicals. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was purchased from Avanti Polar Lipids Inc. (Alabaster, AL). Ec and 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) were obtained from Sigma Chemical Co. (St. Louis, MO). Chloroform and acetonitrile were from E. Merck (Darmstadt, Germany), and methanol was from Carlo Erba (Milano, Italy). Sodium dihydrogenphosphate monohydrate and disodium hydrogenphosphate were from E. Merck. All solvents and reagents used were of analytical grade. 4β-(*S*-Cysteinyl)epicatechin (Cys-Ec), 4β-(2-aminoethylthio)epicatechin (Cya-Ec), 4β-[*S*-(*O*-ethyl-cysteinyl)]epicatechin (ECys-Ec), and 4β-[*S*-(*N*-acetyl-*O*-methyl-cysteinyl)]epicatechin were synthesized in our laboratory by a previously published methodology (*7*, *24*). The structures of the molecules studied are depicted in **Figure 1**. Ultrapure water, produced by a Nanopure purification system coupled to a Milli-Q water purification system, with a resistivity of 18.2 MΩ cm, was used for the aqueous solutions.

Preparation of MLVs of DPPC. A standard solution of DPPC (4.4 mg/mL) in chloroform/methanol 1/1 v/v was prepared and aliquoted (100 μL) in glass tubes. This solution was evaporated to dryness under a nitrogen stream while the tube was rotated in order to obtain a thin film at the bottom of the tube walls. The residual solvent was removed by keeping the samples under high vacuum. MLVs were formed by hydrating the dried lipid with 100 μL of 5 mM oxygen-free HEPES buffer, pH 7.4, containing the corresponding amount of epicatechin or its derivatives, followed by five alternative cycles of sonication (2 min) and heating at 60 °C (2 min). Under these conditions, the polyphenolics remained intact as ascertained by reversed-phase HPLC before and after the treatment.

Differential Scanning Calorimetry (DSC). Calorimetric experiments were performed with a DSC 821E Mettler Toledo (Greifensee, Switzerland) calorimeter. DSC runs were carried out with fresh liposome preparations. Aluminum pans were loaded with 30 μL of DPPC MLV suspension (containing 0.13 mg of DPPC). Then, they were hermetically sealed. Three heating/cooling cycles were performed in a temperature range between 0 and 60 °C at a constant scanning rate of 5 °C/min. The data from the first scan were always discarded to avoid mixing artefacts. Experiments were carried out in triplicate.

HPLC. The retention data were acquired with an IAM.PC.DD2 column (100 mm × 4.6 mm i.d., 12 μm, Regis Technologies Inc., Morton Grove, IL). All measurements were performed with a Shimadzu liquid chromatograph (Kyoto, Japan) equipped with two Shimadzu LC-10AD pumps and a Shimadzu SPD-10AV detector. The eluent was 0.01 M phosphate aqueous buffer, pH 7, containing 20% CH_3CN. All compounds were dissolved in a mobile phase at a concentration of 0.1 mg/mL. The injection volume was always 10 μL. The detection wavelength was 214 nm. Isocratic conditions were always used at a flow rate of 1 mL/min. The column hold-up time was determined by using an aqueous solution of potassium bromide (0.1 mg/mL) as an unretained solute. Its detection was performed at 200 nm. Retention data were expressed by the logarithm of the capacity factor, log k, defined as $\log k = \log [(t_r - t_0)/t_0]$ where t_r and t_0 are the retention times of the solute and the unretained compound, respectively. All measurements were taken in triplicate.

RESULTS AND DISCUSSION

Lipid MLVs as a Membrane Model: DSC Studies. To evaluate the interaction between epicatechin derivatives (**Figure 1**) and lipid membranes, the phase transitions of DPPC MLVs with and without the solutes were evaluated by DSC. DSC thermograms of DPPC MLVs show a sharp transition at 41 °C and a pretransition at 35 °C, consistent with previously reported values (*25*, *26*). The pretransition corresponds to the transition between a gel phase and a ripple phase characterized by a tilt of the acyl chains caused by the bulky polar heads. The main transition takes place from an ordered gel state (P_β phase) at lower temperature to a more disordered liquid crystalline state (L_α phase) at higher temperature. In the gel state, the acyl chains are largely in trans conformation, while in the liquid state they tend more to gauche conformation. The average number of gauche conformers is related to the bilayer fluidity.

The thermotropic parameters studied were the phase transition temperature (T_m) and the width at half-height of the heat absorption peak ($\Delta T_{1/2}$). The former relates to the stability of the membrane structure, and the latter carries information about

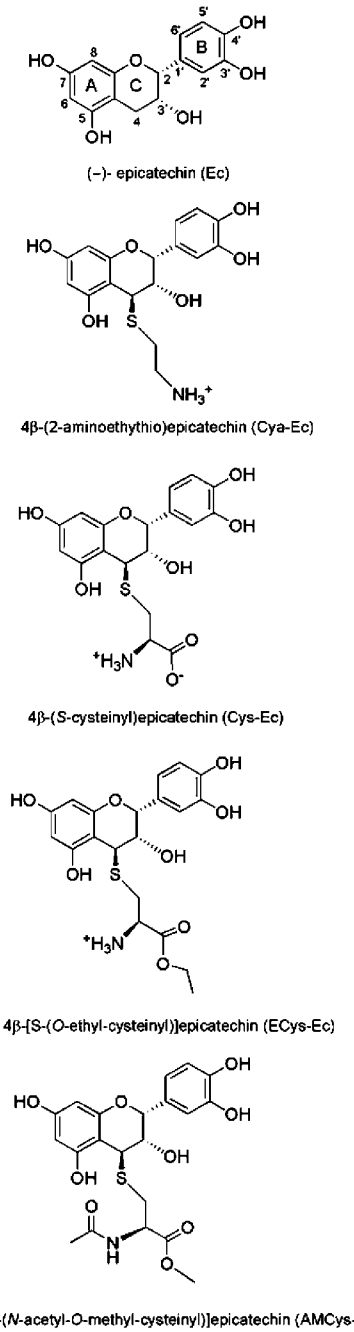

Figure 1. Structures of the epicatechin derivatives.

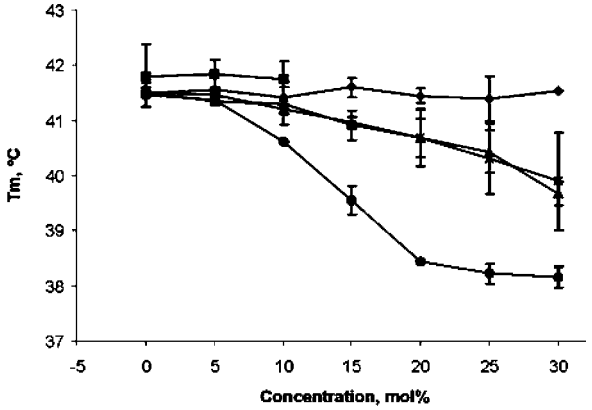

Figure 2. Phase transition temperature (T_m) of DPPC MLV liposomes as a function of the molar percentage of epicatechin derivatives in a concentration range between 0 and 30 mol %: Ec (×), Cya-Ec (♦), Cys-Ec (■), ECys-Ec (●), and AMCys-Ec (▲).

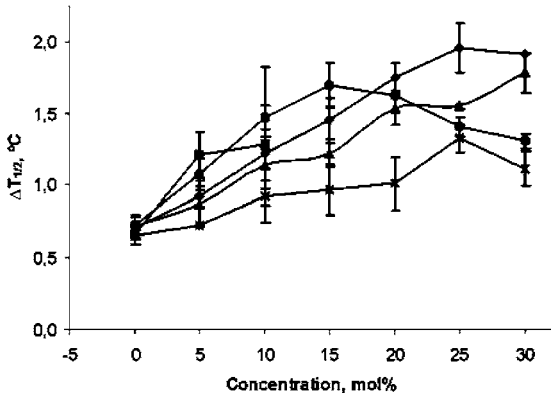

Figure 3. Transition width ($\Delta T_{1/2}$) of DPPC MLV liposomes as a function of the molar percentage of epicatechin derivatives in a concentration range between 0 and 30 mol %: Ec (×), Cya-Ec (♦), Cys-Ec (■), ECys-Ec (●), and AMCys-Ec (▲).

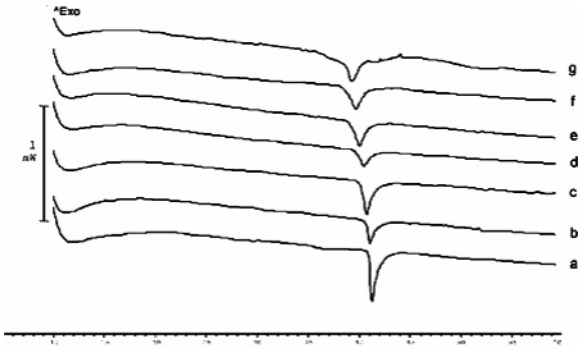

Figure 4. DSC curves obtained with DPPC-Ec. The molar percentages of Ec were a, 0% (i.e., pure DPPC); b, 5%; c, 10%; d, 15%; e, 20%; f, 25%; and g, 30%. The heating rate was 5 °C/min.

the cooperativity of the phase-transition process. Both parameters were plotted against the concentration of each product in the MLV preparation (0, 5, 10, 15, 20, 25, and 30 mol %). The results are summarized in **Figures 2** and **3**. **Figure 4** illustrates the endothermic transition peaks obtained in the presence of the parent polyphenol Ec. The effect of polyphenols such as Ec and other naturally occurring flavonoids on DPPC or 1,2-miristoyl-*sn*-glycero-3-phosphocholine membrane models has been described by other authors (*17*, *18*). The polyphenols abolish the pretransition and induce a decrease in the phase transition temperature and some broadening of this main transition peak. This behavior is common to other small organic molecules (*27*, *28*). In our hands, all of the compounds abolished the pretransition at 5 mol % whereas different effects on the main transition were recorded. Ec and the uncharged hydrophobic derivative AMCys-Ec behaved much like other small species. Both compounds caused a moderate decrease in T_m (**Figure 2**) meaning that they slightly affected the stability of the liposomes. Interestingly, a significant increase in $\Delta T_{1/2}$ was recorded for the bulkier hydrophobic derivative AMCys-Ec (**Figure 3**). This compound appears to raise the fluidity of the lipid bilayer much like other lipophilic molecules (e.g., short chain alkanes), which show a tendency to be located at the center of the hydrophobic core (*29*, *30*). Ec did not alter the membrane fluidity significantly. These results show that the introduction of a protected cysteine moiety may facilitate the incorporation of the polyphenol into the lipid. This may carry an improved capacity to protect membrane lipids from peroxidation. In fact, it has been suggested that similar hydrophobic sulfur-containing derivatives of no amino acidic nature are better antioxidants than more hydrophilic conjugates in a liposome lipid peroxidation model (*31*).

The electrically charged conjugates influenced the stability of the liposomes in different ways. Cationic ECys-Ec exerted the strongest perturbation of the liposome stability and fluidity recorded in this study. The temperature of the main transition (T_m) slumped until 25 mol % and then reached a plateau (**Figure 2**). This behavior indicates that the positively charged ECys-Ec interacts strongly with the phospholipid bilayer. In agreement with this, the $\Delta T_{1/2}$ suffered a steep increase until 15−20 mol %. At higher concentrations, the phase transition peaks are narrowed again. Under these conditions, the phospholipid molecules would be freed from the interaction with the catechin and participate in the phase transition again, increasing the cooperativity. This might be explained by the formation of clusters or self-aggregates of ECys-Ec. In fact, at high concentrations, $\pi-\pi$ stacking or cation$-\pi$ interactions might be relevant. The effects on the phase transition caused by the cationic derivatives may have points in common with those of cationic surfactants (*32*) and peptides (*33*).

The effect of the positively charged Cya-Ec on the thermotropic behavior of DPPC liposomes set this compound apart from the rest of the series. The thermograms showed no change in T_m, while $\Delta T_{1/2}$ clearly increased over the interval between 0 and 25 molar percentage (**Figures 2** and **3**). The broadening of the main transition endothermic peak without any change in the melting temperature is characteristic of molecules localized in the outer cooperative zone of the bilayer. The same behavior has been described for peptide epitopes (*34*). The results are compatible with an interfacial location of Cya-Ec with some penetration of the more hydrophobic flavonoid ring into the hydrocarbon chain region closer to the polar head. In fact, it has been described that aromatic rings may penetrate the surfactant layers, making them fragile (*35*). This penetration might be driven by an effective interaction with the glycerol moiety connecting the acyl chains to the phosphocholine group. This would be supported by the observation that poly(oxyethylene) chains are highly soluble in aromatic hydrocarbons (*36*, *37*). In any case, Cya-Ec neither stabilized nor destabilized the liposomes, since the main transition happened at the same temperature at any concentration.

When liposomes were prepared in the presence of the zwitterionic Cys-Ec, the main transition was completely abolished already at 10 mol %. To obtain a more detailed description of its behavior, Cys-Ec was tested at a lower concentration range (0, 2, 4, 6, and 8 mol %). The plots obtained (**Figure 5**) show

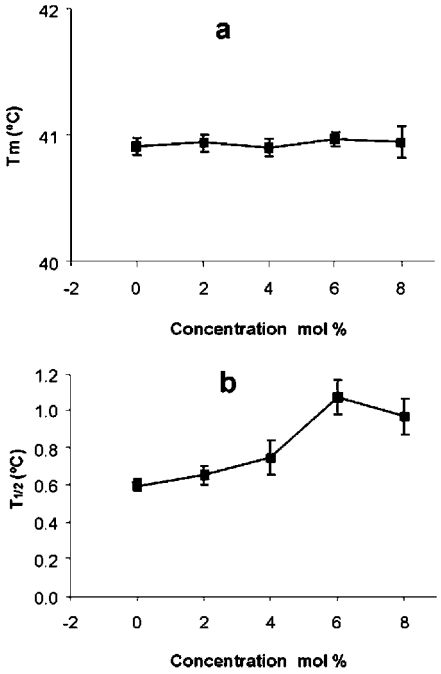

Figure 5. Phase transition temperature (T_m) (**a**) and transition width ($\Delta T_{1/2}$) (**b**) of DPPC MLV liposomes as a function of the molar percentage of Cys-Ec in a concentration range between 0 and 8 mol %.

Table 1. Retention of the Conjugates on the IAM Column[a]

compound	t_r	t_0	log *k*
ECys-Ec	1.21	19.65	1.183
AMCys-Ec	1.21	7.35	0.706
Ec	1.21	4.71	0.462
Cya-Ec	1.21	4.72	0.463
Cys-Ec	1.21	2.61	0.062

[a] Mobile phase, phosphate buffer, pH 7, 20% CH_3CN; *k* (retention factor) = $(t_r - t_0)/t_0$; experiments performed in triplicate.

no changes in T_m and a slight increase in $\Delta T_{1/2}$, meaning that the cooperativity of lipid−lipid interactions was only slightly affected. The liposomes appeared to be destroyed at concentrations of 10 mol % and above, while the lipid molecules remain in solution. The effect of this compound does not follow the most common tendency of membrane active substrates, which gradually affect the phase transition and cooperativity. At low concentrations (10% mol and below), Cys-Ec appeared to interact slightly with liposomes, probably by the same mechanism that Cya-Ec does. At higher concentrations, the lipidic structure was effectively disrupted.

IAM. Ec and its sulfur derivatives were tested on an IAM column using a mobile phase buffered at pH 7 containing 20% CH_3CN. The conditions inside the column, with a continuous flow of fresh solvent, may be comparable to the situation on the MLV model at low molar solute concentrations. The retention factors obtained are summarized in **Table 1**. The results are in good agreement with those obtained with MLV vesicles. ECys-Ec was the most retained solute followed by AMCys-Ec and Ec. The observation that AMCys-Ec was more efficiently retained than Ec on the column may be explained by the tighter interaction of the former compound with the hydrophobic part of the lipid as shown by its effect on the liposome fluidity (**Figure 3**). Cya-Ec and Ec eluted at the same time. In this case, while Ec appears to be able to establish hydrophobic contact with the lipid (*18*), Cya-Ec might be mainly interacting with the polar outer interface of the lipid, as suggested by the observations on the liposome model. The IAM column is not able to discriminate between the two kinds of interaction. Finally, Cys-Ec was not retained by the IAM stationary phase, in agreement with the plots obtained with liposomes (**Figure 5**), which indicate that no perturbation of the vesicle structure occurs at low concentrations.

The retention of epicatechin derivatives on an IAM relates well with their behavior on MLVs. In agreement with previous results for other compounds (*21*, *38*), IAM chromatography proved to be a convenient way to quickly test polyphenolic derivatives for their putative interaction with membranes. On the other hand, IAM does not tell us about the kind of interaction (polar, hydrophobic) involved or where the solute may be located. More detailed information about the nature of the interaction of the active molecules can be obtained from experiments on vesicles using DSC. This is particularly important for compounds such as Cya-Ec, which appears to bind to the lipids by both polar and hydrophobic interactions, with a possible biologically significant tendency to be located in the outer parts of the membrane.

The results presented here show that the incorporation of a nonpolyphenolic moiety onto the epicatechin structure may significantly influence its interaction with model membranes. Particularly, while cationic Cya-Ec may have a tendency to be located at the outer interface of the lipid membrane, ECys-Ec, also cationic with a bulkier nonpolyphenolic moiety, appears to efficiently penetrate into the inner zones of the liposome. The uncharged AMCys-Ec may penetrate into the core lipids mostly by hydrophobic interactions. We have shown that some biological properties of the epicatechins are influenced by the nonpolyphenolic part of the molecules and the present results clearly show that the same modifications influence the capacity of the conjugates to interact with biological membrane models. So far, while no clear-cut relation seems to arise from the comparison between the biological and the physicochemical behavior of the conjugates, the results suggest that the nonphenolic part of the molecules might determine whether the phenolics are available for action on surface receptors, inside the membrane, or even intracellularly. The information gained about the behavior of the cell-protecting antioxidant thioconjugates in membrane environments may contribute to elucidate their mechanisms of action and open new avenues for the valorization of agrofood byproducts.

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LITERATURE CITED

(1) Shahidi, F.; Naczk, M. In *Phenolics in Food and Nutraceuticals*; CRC Press: Boca Ratón, FL, 2004.
(2) Nichenametla, S. N.; Taruscio, T. G.; Barney, D. L.; Exon, J. H. A review of the effects and mechanisms of polyphenolics in cancer. *Crit. Rev. Food Sci. Nutr.* **2006**, *46*, 161−183.
(3) Manach, C.; Mazur, A.; Scalbert, A. Polyphenols and prevention of cardiovascular diseases. *Curr. Opin. Lipidol.* **2005**, *16*, 77−84.
(4) Ray, S.; Bagchi, D.; Lim, P. M.; Bagchi, M.; Gross, S. M.; Kothari, S. C.; Preuss, H. G.; Stohs, S. J. Acute and long-term safety evaluation of a novel IH636 grape seed proanthocyanidin extract. *Res. Commun. Mol. Pathol. Pharmacol.* **2001**, *109*, 165−197.
(5) Rohdewald, P. A review of the French maritime pine bark extract (Pycnogenol (R)), a herbal medication with a diverse clinical pharmacology. *Int. J. Clin. Pharmacol. Ther.* **2002**, *40*, 158−168.

(6) Torres, J. L.; Lozano, C.; Juliá, L.; Sánchez-Baeza, F. J.; Anglada, J. M.; Centelles, J. J.; Cascante, M. Cysteinyl-flavan-3-ol conjugates from grape procyanidins. Antioxidant and antiproliferative properties. *Bioorg. Med. Chem.* **2002**, *10*, 2497−2509.
(7) Torres, J. L.; Bobet, R. New flavanol-derivatives from grape (*Vitis vinifera*) byproducts. Antioxidant aminoethylthio-flavan-3-ol conjugates from a polymeric waste fraction used as a source of flavanols. *J. Agric. Food Chem.* **2001**, *49*, 4627−4634.
(8) Selga, A.; Torres, J. L. Efficient preparation of catechin thio conjugates by one step extraction/depolymerization of pine (Pinus pinaster) bark procyanidins. *J. Agric. Food Chem.* **2005**, *53*, 7760−7765.
(9) Lozano, C.; Torres, J. L.; Julia, L.; Jiménez, A.; Centelles, J. J.; Cascante, M. Effect of new antioxidant cysteinyl-flavanol conjugates on skin cancer cells. *FEBS Lett.* **2005**, *579*, 4219−4225.
(10) Torres, J. L.; Lozano, C.; Maher, P. Conjugation of catechins with cysteine generates antioxidant compounds with enhanced neuroprotective activity. *Phytochemistry* **2005**, *66*, 2032−2037.
(11) Mitjans, M.; Del Campo, J.; Abajo, C.; Martínez, V.; Selga, A.; Lozano, C.; Torres, J. L.; Vinardell, M. P. Immunomodulatory activity of a new family of antioxidants obtained from grape polyphenols. *J. Agric. Food Chem.* **2004**, *52*, 7297−7299.
(12) Alonso, C.; Notario, J.; Ramón, E.; Lozano, C.; Parra, J. L.; Torres, J. L.; Coderch, L. Percutaneous absorption of flavan-3-ol conjugates from plant procyanidins. *Drug. Exp. Clin. Res.* **2004**, *30*, 1−10.
(13) Busquets, M. A.; Alsina, M. A.; Haro, I. Peptides and liposomes: From biophysical to immunogenic studies. *Curr. Drug Targets* **2003**, *4*, 633−642.
(14) Ohtake, S.; Schebor, C.; De Pablo, J. J. Effects of trehalose on the phase behavior of DPPC-cholesterol unilamellar vesicles. *Biochim. Biophys. Acta* **2006**, *1758*, 65−73.
(15) Pignatello, R.; Intravaia, V. D.; Puglisi, G. A calorimetric evaluation of the interaction of amphiphilic prodrugs of idebenone with a biomembrane model. *J. Colloid Interface Sci.* **2006**, *299*, 626−635.
(16) Boija, E.; Johansson, G. Interactions between model membranes and lignin-related compounds studied by immobilized liposome chromatography. *Biochim. Biophys. Acta* **2006**, *1758*, 620−626.
(17) Saija, A.; Bonina, F.; Trombetta, D.; Tomaino, A.; Montenegro, L.; Smeriglio, P.; Castelli, F. Flavonoid-biomembrane interactions: A calorimetric study on dipalmitoylphosphatidylcholine vesicles. *Int. J. Pharm.* **1995**, *124*, 1−8.
(18) Caturla, N.; Vera-Samper, E.; Villalain, J.; Mateo, C. R.; Micol, V. The relationship between the antioxidant and the antibacterial properties of galloylated catechins and the structure of phospholipid model membranes. *Free Radical Biol. Med.* **2003**, *34*, 648−662.
(19) Pidgeon, C.; Venkataram, U. V. Immobilized artificial membrane chromatography: Supports composed of membrane lipids. *Anal. Biochem.* **1989**, *176*, 36−47.
(20) Yang, C. Y.; Cai, S. J.; Liu, H.; Pidgeon, C. Immobilized artificial membranes—Screens for drug-membrane interactions. *Adv. Drug Delivery Rev.* **1997**, *23*, 229−256.
(21) Lázaro, E.; Ràfols, C.; Abraham, M. H.; Rosés, M. Chromatographic estimation of drug disposition properties by means of immobilized artificial membranes (IAM) and C18 columns. *J. Med. Chem.* **2006**, *49*, 4861−4870.
(22) Ong, S.; Liu, H.; Pidgeon, C. Immobilized-artificial-membrane chromatography: Measurements of membrane partition coefficient and predicting drug membrane permeability. *J. Chromatogr. A* **1996**, *728*, 113−128.
(23) Taillardat-Bertschinger, A.; Martinet, C. A. M.; Carrupt, P. A.; Reist, M.; Caron, G.; Fruttero, R.; Testa, B. Molecular factors influencing retention on immobilized artificial membranes (IAM) compared to partitioning in liposomes and *n*-octanol. *Pharm. Res.* **2002**, *19*, 729−737.
(24) Lozano, C.; Juliá, L.; Jiménez, A.; Touriño, S.; Centelles, J. J.; Cascante, M.; Torres, J. L. Electron transfer capacity of catechin derivatives and influence on the cell cycle and apoptosis in HT29 cells. *FEBS J.* **2006**, *273*, 2475−2486.
(25) Lo, Y.-L.; Rahman, Y.-E. Protein location in liposomes, a drug carrier: A prediction by differential scanning calorimetry. *J. Pharm. Sci.* **1995**, *84*, 805−814.
(26) Ramaswami, V.; Haaseth, R. C.; Matsunaga, T. O.; Hruby, V. J.; O'Brien, D. F. Opioid peptide interactions with lipid bilayer membranes. *Biochim. Biophys. Acta* **1992**, *1109*, 195−202.
(27) Lohner, K. Effects of small organic molecules on phospholipid phase transitions. *Chem. Phys. Lipids* **1991**, *57*, 341−362.
(28) Jutila, A.; Söderlund, T.; Pakkanen, A. L.; Huttunen, M.; Kinnunen, P. K. J. Comparison of the effects of clozapine, chlorpromazine, and haloperidol on membrane lateral heterogeneity. *Chem. Phys. Lipids* **2001**, *112*, 151−163.
(29) McIntosh, T. J.; Simon, S. A.; MacDonald, R. C. The organization of *n*-alkanes in lipid bilayers. *Biochim. Biophys. Acta* **1980**, *597*, 445−463.
(30) Pope, J. M.; Dubro, D. W. The interaction of *n*-alkanes and *n*-alcohols with lipid bilayer membranes: A 2H-NMR study. *Biochim. Biophys. Acta* **1986**, *858*, 243−253.
(31) Tanaka, T.; Kusano, R.; Kouno, I. Synthesis and antioxidant activity of novel amphipathic derivatives of tea polyphenol. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1801−1806.
(32) Castillo, J. A.; Pinazo, A.; Carilla, J.; Infante, M. R.; Alsina, M. A.; Haro, I.; Clapés, P. Interaction of antimicrobial arginine-based cationic surfactants with liposomes and lipid monolayers. *Langmuir* **2004**, *20*, 3379−3387.
(33) Abrunhosa, F.; Faria, S.; Gomes, P.; Tomaz, I.; Pessoa, J. C.; Andreu, D.; Bastos, M. Interaction and lipid-induced conformation of two Cecropin-Melittin hybrid peptides depend on peptide and membrane composition. *J. Phys. Chem. B* **2005**, *109*, 17311−17319.
(34) Sospedra, P.; Mestres, C.; Haro, I.; Muñoz, M.; Busquets, M. A. Effect of amino acid sequence change on peptide-membrane interaction. *Langmuir* **2002**, *18*, 1231−1237.
(35) Kunieda, H.; Yano, N.; Solans, C. The stability of gel-emulsions in a water/non-ionic surfactant/oil system. *Colloids Surf.* **1989**, *36*, 313−322.
(36) Christenson, H.; Friberg, S. E. Spectroscopic investigation of the mutual interactions between nonionic surfactant, hydrocarbon, and water. *J. Colloid Interface Sci.* **1980**, *75*, 276−285.
(37) Bailey, F. E.; Koleske, J. V. *Nonionic Surfactants. Physical Chemistry*; Marcel Dekker: New York, 1987; pp 927−969.
(38) Vrakas, D.; Giaginis, C.; Tsantili-Kakoulidou, A. Different retention behavior of structurally diverse basic and neutral drugs in immobilized artificial membrane and reversed-phase high performance liquid chromatography: Comparison with octanol-water partitioning. *J. Chromatogr. A* **2006**, *1116*, 158−164.

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