

Antioxidant and Membrane Effects of Procyanidin Dimers and Trimers Isolated from Peanut and Cocoa

SANDRA V. VERSTRAETEN,[†] JOHN F. HAMMERSTONE,[‡] CARL L. KEEN,[§]
 CÉSAR G. FRAGA,^{*,§,||} AND PATRICIA I. OTEIZA^{†,§,⊥}

IQUIFIB-Department of Biological Chemistry (UBA-CONICET), School of Pharmacy and Biochemistry, University of Buenos Aires, Junin 956, 1113-Buenos Aires, Argentina, and Mars Incorporated, Hackettstown, New Jersey 07840, USA, and Department of Nutrition, University of California, One Shields Avenue, Davis, California 95616, USA, and Physical Chemistry-PRALIB, (UBA-CONICET), School of Pharmacy and Biochemistry, University of Buenos Aires, Junin 956, 1113-Buenos Aires, Argentina, and Department of Environmental Toxicology, University of California, One Shields Avenue, Davis, California 95616, USA

The antioxidant and membrane effects of dimer (Dim) and trimer (Trim) procyanidins isolated from cocoa (*Theobroma cacao*) (B- and C-bonded) and peanut (*Arachis hypogea* L.) skin (A-bonded) were evaluated in phosphatidyl choline liposomes. When liposomes were oxidized with a steady source of oxidants, the above dimers and trimers inhibited to a similar extent lipid oxidation in a concentration (0.33–5 μ M)-dependent manner. With respect to membrane effects, Dim A₁, Dim B, Trim A, and Trim C increased (Dim A₁ = Dim B and Trim A = Trim C), while Dim A₂ decreased, membrane surface potential. All of the procyanidins tested decreased membrane fluidity as determined by fluorescent probes at the water–lipid interface, an effect that extended into the hydrophobic region of the bilayer. Both dimers and trimers protected the lipid bilayer from disruption by Triton X-100. The magnitude of the protection was Dim A₁ > Dim A₂ > Dim B and Trim C > Trim A. Thus, dimers and trimers can interact with membrane phospholipids, presumably with their polar headgroup. As a consequence of this interaction, they can provide protection against the attack of oxidants and other molecules that challenge the integrity of the bilayer.

KEYWORDS: Flavonoids; polyphenols; procyanidins; antioxidants; lipid oxidation; membrane interactions; *Theobroma cacao*; *Arachis hypogea* L.

INTRODUCTION

The flavan-3-ols, (–)-epicatechin and (+)-catechin, together with their related oligomers, the procyanidins, are extensively distributed in nature. They are mainly synthesized in plants, and they can be present in high concentrations in certain plant-based foods and beverages (for a review see ref 1). The regular consumption of flavan-3-ol-containing foods has been associated with a reduced risk for vascular diseases (2–6) and certain cancers (2, 7–9). Supporting epidemiological findings, there is a number of studies both in vitro and in vivo indicating that flavanols and procyanidins have biological effects that can provide health benefits (10).

The skins of mature peanuts (*Arachis hypogea* L.) (11) and cocoa beans (*Theobroma cacao*) are particularly abundant in procyanidins. In cocoa beans, the procyanidins are primarily

of types B and C, in which the monomers are linked by 4 β →8 bonds as shown in **Figure 1I,III** for dimer B₂ (Dim B) and trimer C (Trim C), respectively. In contrast, peanut skin primarily contains A type procyanidins, in which the subunits are connected by both 4 β →8 carbon bonds and 2 β →O7 ether bonds as shown in **Figure 1II** for dimer A₂ (Dim A₂) and in **Figure 1IV** for trimer A (Trim A). This different bonding confers a different three-dimensional structure to these molecules. Recently, upon the basis of computational analysis, we suggested (12) that in the Dim B, the two (–)-epicatechin monomers can interact with each other, leading to a folded and packed molecule. In contrast, the additional rigidity conferred by the 2 β →O7 ether bond in the A procyanidins results in a more extended conformation. The differential folding of these two classes of procyanidins may also result in different physical interactions with biological molecules and structures. We and others have shown that a number of the properties associated with procyanidins are dependent in part on the number of monomers forming the molecule (13–15). Consequently, the major variables defining the procyanidin biological effects are the constituent flavanol, the length of the molecule, and the three-dimensional structure.

* To whom correspondence should be addressed. Tel: 530-754-6667. Fax: 530-752-8966. E-mail: cgfraga@ucdavis.edu.

[†] IQUIFIB.

[‡] Mars Inc..

[§] Department of Nutrition, University of California.

^{||} Physical Chemistry-PRALIB.

[⊥] Department of Environmental Toxicology, University of California.

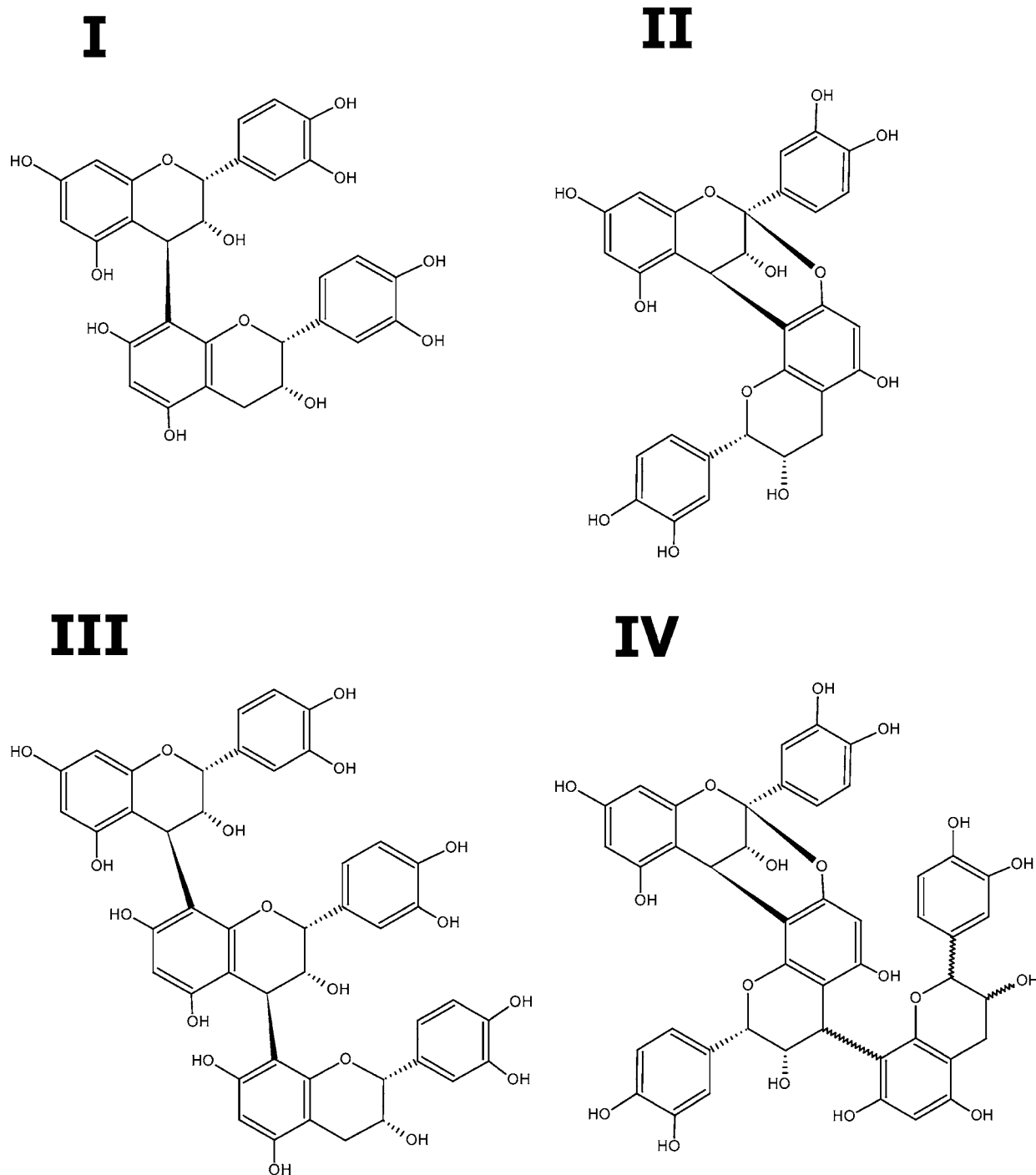


Figure 1. Chemical structures of dimer and trimer procyanidins. (I) Dimer B₂, epicatechin-(4 β -8)-epicatechin (cocoa); (II) dimer A₁, epicatechin-(4 β -8,2 β -07)-catechin (peanut skin); (III) trimer C₁ (cocoa); and (IV) trimer A (peanut skin).

In the present work, we compared the ability of A and B and C type procyanidins (dimers and trimers) to interact with membrane phospholipids. In contrast to previous experiments carried out using phosphatidyl choline (PC):phosphatidyl serine (60:40) liposomes (15), in this study, we used zwitterionic liposomes of PC, which lack the negative charge afforded by the presence of PS. The above reduces the possibility of electrostatic interactions, while allowing for dipole interactions. In this membrane model, we studied the effects of dimer and trimer fractions isolated from peanut (A type) and cocoa (B and C types) on (i) 2,2'-azobis(2,4-dimethylvaleronitrile) (AMVN)-mediated lipid oxidation, (ii) membrane surface potential and fluidity, and (iii) Triton X-100-mediated liposome disruption. On the basis of the present results, we conclude that

similar to what has been observed for the interaction between negatively charged vesicles and B type procyanidins (15), A type procyanidins have antioxidant and membrane effects. No significant differences were observed between A and B and C type procyanidins regarding antioxidant action and membrane disruption protection. The interactions of dimer and trimer procyanidins with membranes may result in a reduction in the ability of oxidants and other disturbing molecules to damage cell membranes.

MATERIALS AND METHODS

Chemicals. Dimer and trimer fractions were purified (11, 16, 17) and supplied by Mars Incorporated (Hackettstown, NJ). Fractions enriched with Dim B (mainly dimer B₂) or Trim C (mainly C₁) were

isolated from *Cocoapro cocoa*; fractions enriched with Dim A₁, Dim A₂ [dimer A₂ = epicatechin-(4β-8,2β-O7)-epicatechin], or Trim A were isolated from peanut skin. Bovine brain PC was purchased from Avanti Polar Lipids Inc. (Alabaster, AL). 1-Anilino-naphthalene-8-sulfonic acid (ANS) was obtained from Sigma Chemical Co. (St. Louis, MO). The fluorescent probes: 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-*s*-indacene-3-undecanoic acid (C₁₁-BODIPY), 16-(9-anthroyloxy)palmitic acid (16-AP), 6-(9-anthroyloxy)stearic acid (6-AS), and octadecyl Rhodamine B chloride were from Molecular Probes Inc. (Eugene, OR). AMVN was obtained from Wako Pure Chemical Ind. (Osaka, Japan).

Liposome Preparation. An adequate amount of PC dissolved in chloroform was brought to dryness under high vacuum in a Buchi rotavapor for 15 min, followed by a 15 min under N₂. The dried PC was suspended (2.5 mM) in a 140 mM NaCl, 20 mM Tris-HCl buffer (pH 7.4) and incubated at 45 °C for 10 min. Large unilamellar vesicles were obtained by three cycles of 45 s sonication in a Branson 250 Sonifier (Branson Ultrasonics Corp., Danbury, CT) at 80 W. The final PC concentration was adjusted to 70 μM using the above buffer solution. The liposome average diameter was 120 nm as determined from steady state light scattering. To test the possibility that the dimers and trimers could induce vesicles aggregation, PC liposomes were incubated for 2 min at 37 °C in the presence of the procyanidins (0.33–5 μM), and the potential liposome aggregation was evaluated by measuring the increase in the light scattered by the samples (λ excitation and emission, 550 nm) in a Kontron SFM-25 spectrofluorometer (Kontron Instruments, sPA, Milan, Italy). Under the above conditions, the procyanidins tested in this work did not affect the light scattered by the liposome suspension (data not shown).

Evaluation of Lipid Oxidation. AMVN-induced lipid oxidation was evaluated using the fluorescent probe C₁₁-BODIPY (15, 18). PC liposomes (0.3 mL) containing 2 mol % of C₁₁-BODIPY were preincubated at 37 °C for 10 min with, or without, the addition of Dim A₁, Dim A₂, Dim B, Trim A, or Trim C (0.33–5 μM final concentration). After preincubation, 5 μL of an AMVN methanol stock solution (5 mM final concentration) was added and samples were further incubated for 90 min at 37 °C. AMVN is a lipid soluble azo compound that decomposes at a constant rate generating carbon-centered radicals that in the presence of oxygen yield peroxy radicals. The oxidation of the probe C₁₁-BODIPY was evaluated at two sets of excitation and emission wavelengths, as described by Drummen et al. (19). The emissions corresponding to the oxidized (λ_{excitation}, 488 nm; λ_{emission}, 520 nm) and reduced (λ_{excitation}, 545 nm; λ_{emission}, 590 nm) states of the probe were recorded. The oxidation of the liposomes was estimated by calculating the ratio between the fluorescence of the oxidized and the reduced states.

Evaluation of Membrane Surface Potential. Liposome membrane potential (Ψ₀) was evaluated as described by Winiski et al. (20) using the probe ANS. PC liposomes were incubated at 25 °C for 2 min in the absence or presence of Dim A₁, Dim A₂, Dim B, Trim A, or Trim C. One micromolar ANS was added to the samples, which were then incubated for 2 min. ANS fluorescence emission at 465 nm (λ_{excitation}, 380 nm) was measured, and changes in membrane surface potential were calculated from the equation:

$$\Delta\Psi_0 = \frac{RT}{F} \times \ln\left(\frac{I}{I_0}\right)$$

where *R* is the gas constant, *T* is the absolute temperature in K, *F* is the Faraday's constant, *I* is the ANS fluorescence intensity in liposomes incubated in the presence of the dimers or trimers, and *I*₀ is the fluorescence intensity in the liposomes incubated without additions.

Evaluation of Membrane Fluidity. A PC liposome stock suspension was divided in two aliquots; one was added with the probe 6-AS, and the other was added with the probe 16-AP, both probes at 0.1 mol % concentration. The resulting suspensions were incubated for 15 min at 37 °C to allow the incorporation of the probe into the bilayer. After the addition of Dim A₁, Dim A₂, Dim B, Trim A, or Trim C, samples were further incubated at 37 °C for 2 min with continuous stirring (300 rpm). Fluorescence polarization was calculated from the emission at 435 nm (λ_{excitation}, 384 nm) using the equation:

$$P = \frac{(I_{\parallel} - I_{\perp})}{(I_{\parallel} + I_{\perp})}$$

where *I*_∥ and *I*_⊥ are the fluorescence intensities measured with the emission polarizer oriented parallel and normal to the excitation polarizer, respectively.

Evaluation of Bilayer Transition to Micelle. PC liposomes containing 1.5 mol % of the fluorescent probe octadecyl rhodamine (15, 21) were incubated at 25 °C for 5 min, with or without the addition of variable amounts of Dim A₁, Dim A₂, Dim B, Trim A, or Trim C. The concentration of the probe was sufficient to produce the self-quenching of its fluorescence. A 10 μL aliquot of a 1% (v/v) Triton X-100 solution was added, and after a 2 min incubation under continuous stirring, the fluorescence emission at 580 nm (λ_{excitation}, 560 nm) was recorded. The addition of the detergent was continued until the achievement of a constant fluorescence emission that indicates the total disruption of the liposomes into micelles. The Triton X-100-dependent increase in fluorescence associated with the transition from bilayer to micelle follows a sigmoid behavior (15). The Triton X-100 concentration necessary to obtain the 50% of the maximal fluorescence intensity was defined as C₅₀, and this value was used to compare the effects of the dimers and trimers on membrane stability (15).

Statistics. Independent experiments were performed using fresh prepared liposomes. One-way analysis of variance followed by Dunnett's multiple comparison test and *t*-test were performed using routines available in StatView 5.0 (SAS Institute Inc., Cary, NC). Correlations were performed using the software GraphPad Prism (GraphPad Software, San Diego, CA). A *P* value lower than 0.05 was considered as statistically significant.

RESULTS

Effects of Dimer and Trimer Procyanidins on AMVN-Initiated Lipid Oxidation. The capacity of Dim A₁, Dim A₂, Dim B, Trim A, and Trim C to reduce the rate of the lipid oxidation initiated by AMVN was assessed in PC liposomes containing the fluorescent probe C₁₁-BODIPY. In the absence of additions, the ratio between the fluorescence corresponding to the oxidized and reduced states of the probe was 0.21 ± 0.01. After liposomes were incubated at 37 °C for 90 min in the presence of 5 mM AMVN, this ratio was increased to 9.0 ± 0.1 (*P* < 0.0001). When, prior to the addition of AMVN, the liposomes were preincubated for 10 min at 37 °C in the presence of the different dimers or trimers, a significant decrease in the ratio of C₁₁-BODIPY fluorescences was observed at all of the concentrations tested (Figure 2). The extent of the decrease in AMVN-induced C₁₁-BODIPY oxidation depended on the amount of dimers and trimers present in the incubation media (Figure 2A,B), reaching the maximal effect at a concentration of 2.5 μM. No significant differences were noted between the dimers and the trimers with respect to their protective effects against AMVN-mediated C₁₁-BODIPY oxidation (paired *t*-test for each concentration). The effect of Dim A₂ was lower (~25% at 1.65 μM; *P* < 0.05) than the effects of the other dimers and trimers.

Effect of Dimer and Trimer Procyanidin on Membrane Surface Potential. Whether the interaction of the procyanidin dimers and trimers with phospholipids could affect the liposome surface potential was investigated using the fluorescent probe ANS. Both Dim A₁ and Dim B induced a significant (*P* < 0.005) increase in liposome ΔΨ₀ (Figure 3A). This effect was influenced by dimers concentration reaching a maximal effect at 2.5 μM dimer. Dim A₂ had a biphasic effect on membrane surface potential. Between 0.33 and 1.25 μM, Dim A₂ induced a concentration-dependent increase of ΔΨ₀, and the magnitude of this effect was similar to that observed for Dim A₁ and Dim B. At concentrations higher than 2.5 μM, the liposome ΔΨ₀

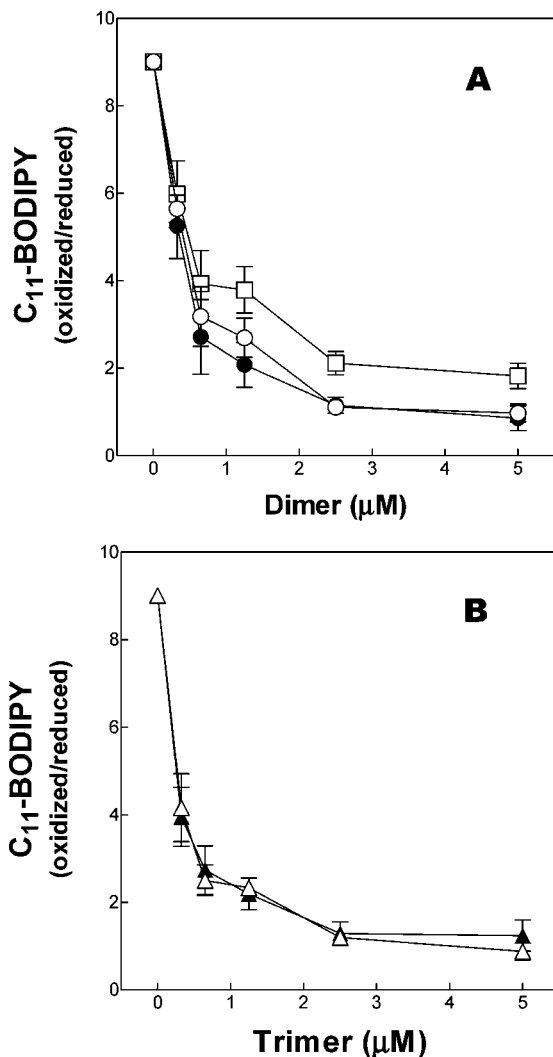


Figure 2. Antioxidant effects of dimer and trimer procyanidins against AMVN-mediated C_{11} -BODIPY oxidation. PC liposomes containing the fluorescent probe C_{11} -BODIPY were incubated in the presence of different concentrations of (A) Dim A₁ (○), Dim A₂ (□), and Dim B (●) or (B) Trim A (△) or Trim C (▲). Lipid oxidation was initiated by the addition of 5 mM AMVN. After a 90 min incubation at 37 °C, C_{11} -BODIPY oxidation was evaluated from the ratio between the fluorescence of the oxidized and reduced form, as described in the Materials and Methods. Results are shown as means \pm SEM of four independent experiments.

decreased as Dim A₂ concentrations increased, returning to baseline values at 5 μM concentration (Figure 3A). Both Trim A and Trim C increased $\Delta\Psi_0$ to a similar extent (2.8 mV) over the concentration range of 0.33–5 μM (Figure 3B). Consistent with their antioxidant effects, dimers and trimers had their maximal effects on $\Delta\Psi_0$ at concentrations close to 2.5 μM .

Effect of Dimer and Trimer Procyanidin on Membrane Fluidity. Considering the results on $\Delta\Psi_0$, it can be inferred that the dimers and trimers interact with the membrane surface. We further characterized this interaction by measuring the effects of the dimers and trimers on membrane fluidity using 6-AS and 16-AP. When PC liposomes were incubated at 37 °C in the presence of Dim A₁, Dim A₂, or Dim B, a significant and concentration-dependent increase in the fluorescence polarization of the probe 6-AS was observed (Figure 4A). Similar results were obtained for Trim A and Trim C (Figure 4B). Using the probe 16-AP, a differential effect of the dimers was observed (Figure 4C). While all three dimers significantly increased 16-

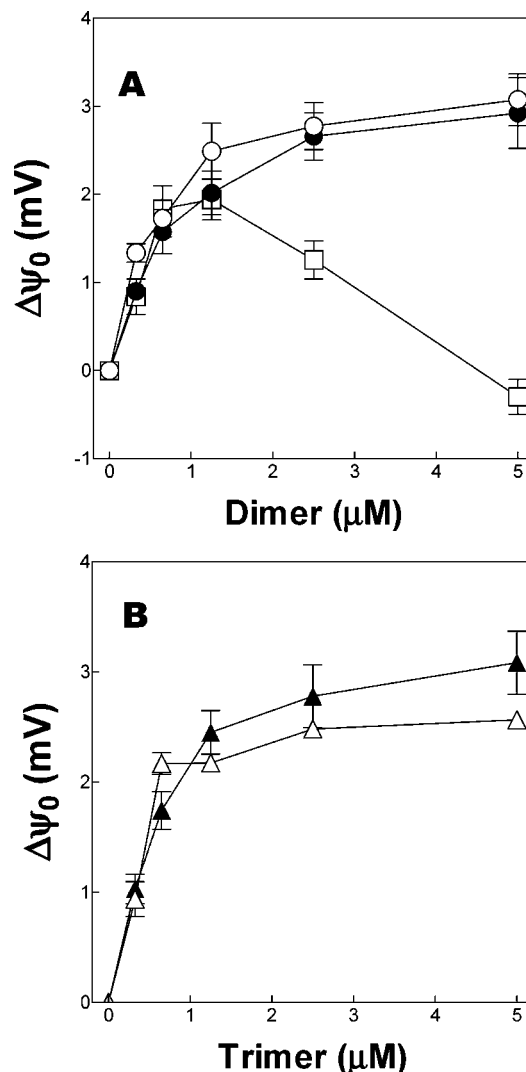


Figure 3. Effects of dimer and trimer procyanidins on membrane surface potential. PC liposomes were incubated for 2 min at 25 °C in the presence of different concentrations of (A) Dim A₁ (○), Dim A₂ (□), and Dim B (●) or (B) Trim A (△) or Trim C (▲) and then added with the probe ANS. Changes in liposome surface potential ($\Delta\Psi_0$) were calculated as described in the Materials and Methods. Results are shown as means \pm SEM of four independent experiments.

AP fluorescence polarization, the magnitude of the effects differed (Dim A₂ > Dim A₁ > Dim B). Using the same probe, Trim C showed a biphasic effect. At concentrations lower than 1 μM , it induced a decrease in the fluorescence polarization of 16-AP (Figure 4D); at concentrations higher than 1 μM , it induced a significant increase in the fluorescence polarization of 16-AP. Significant correlations were found between the effects of dimers and trimers on surface membrane fluidity as evaluated with the probe 6-AS, and the ability of these molecules to reduce the rate of AMVN-mediated C_{11} -BODIPY oxidation [Dim A₁ ($r^2 = 0.83$, $P < 0.05$), Dim A₂ ($r^2 = 0.86$, $P < 0.05$), Dim B ($r^2 = 0.93$, $P < 0.01$), Trim A ($r^2 = 0.95$, $P < 0.01$), and Trim C ($r^2 = 0.89$, $P < 0.05$)].

Effect of Dimer and Trimer Procyanidins on the Bilayer Transition to Micelle. To evaluate the possibility that dimers and trimers can reduce the accessibility of hydrophobic molecules to the membrane, we investigated the transition of liposome bilayer to a micelle structure mediated by the successive incorporation of a nonionic detergent among PC moieties. The interaction of the dimers and trimers with

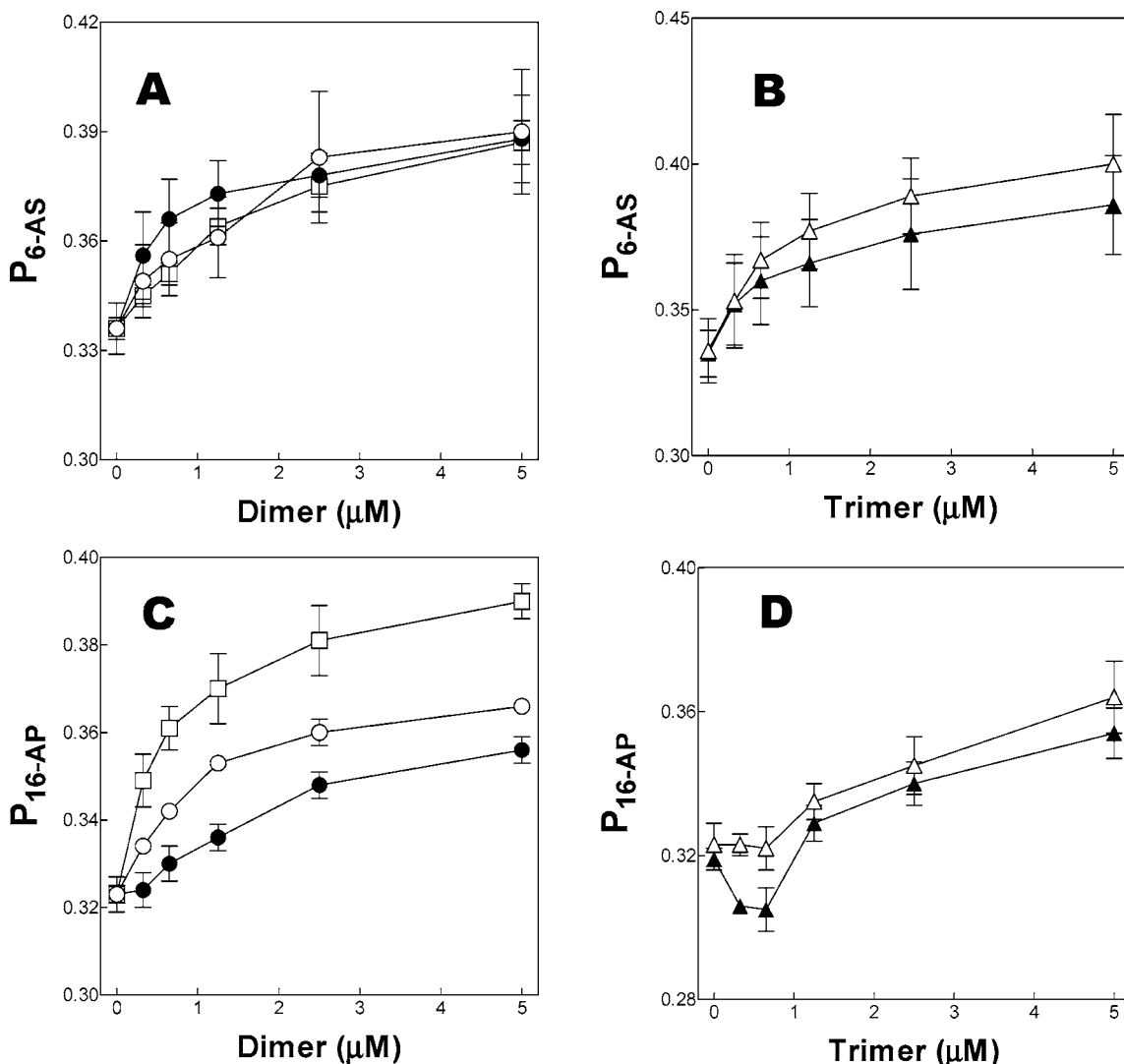


Figure 4. Effects of dimer and trimer procyanidins on membrane fluidity. PC liposomes containing the fluorescent probes 6-AS (A,B) or 16-AP (C,D) were incubated in the presence of Dim A₁ (○), Dim A₂ (□), Dim B (●), Trim A (△), or Trim C (▲) (0.33–5 μM). The fluorescence polarizations of the probes 6-AS (P_{6-AS}) and 16-AP (P_{16-AP}) were measured as described in the Materials and Methods. Results are shown as means \pm SEM of four independent experiments.

liposomes resulted in a displacement of the titration curves toward higher Triton X-100 concentrations. **Figure 5** shows that the displacement (increase in C_{50}) was dependent on the concentration of dimers and trimers. The largest effects were observed when liposomes were incubated in the presence of Dim A₁ (**Figure 5A**). The magnitude of the effect was significantly higher than that observed for Dim A₂ and Dim B at concentrations up to 2.5 μM ($P < 0.01$, paired t -test). Similarly to the dimers, both Trim A and Trim C induced a concentration-dependent increase in membrane protection (**Figure 5B**). The increases in C_{50} were higher for Trim C than for Trim A at concentrations beyond 0.65 μM ($P < 0.05$, paired t -test). Significant positive correlations were found between the increase in C_{50} induced by the dimers and trimers and their effect on membrane fluidity as evaluated with the probe 6-AS [Dim A₁ ($r^2 = 0.91$, $P < 0.005$); Dim A₂ ($r^2 = 0.94$, $P < 0.005$); Dim B ($r^2 = 0.94$, $P < 0.005$); Trim A ($r^2 = 0.86$, $P < 0.01$); and Trim C ($r^2 = 0.82$, $P < 0.01$)].

In addition, the two protective effects of the procyanidins were associated as dictated when correlating the effect of dimers and trimers on the prevention of Triton X-100-mediated liposome to micelle transition and the reduction of the rate of AMVN-mediated C₁₁-BODIPY oxidation [Dim A₁ ($r^2 = 0.90$,

$P < 0.005$); Dim A₂ ($r^2 = 0.82$, $P < 0.05$); Dim B ($r^2 = 0.82$, $P < 0.05$); Trim A ($r^2 = 0.99$, $P < 0.0001$); and Trim C ($r^2 = 0.98$, $P < 0.001$)].

DISCUSSION

In this work, we characterized procyanidins with differing oligomer chain lengths (dimers and trimers) and configurations with respect to their antioxidant and membrane effects. We assayed procyanidin fractions of Dim A₁, Dim A₂, and Trim A isolated from peanut skin and fractions of Dim B and Trim C isolated from cocoa. The selection of these two groups of procyanidins was based on the hypothesis that an alteration in the three-dimensional structure dictated by the extra 2 β -O7 ether bond between the monomer units in the A type procyanidins would affect their interaction with membrane components.

We first investigated the *in vitro* antioxidant capacity of cocoa and peanut dimer and trimer fractions on liposomes containing the fluorescent probe C₁₁-BODIPY. This probe is oxidized by peroxyl radicals (18, 19) generated during the AMVN-induced lipid oxidation. In the concentration range assessed (0.33–5 μM), both the dimers and the trimers displayed a concentration-dependent prevention of C₁₁-BODIPY oxidation initiated by 5

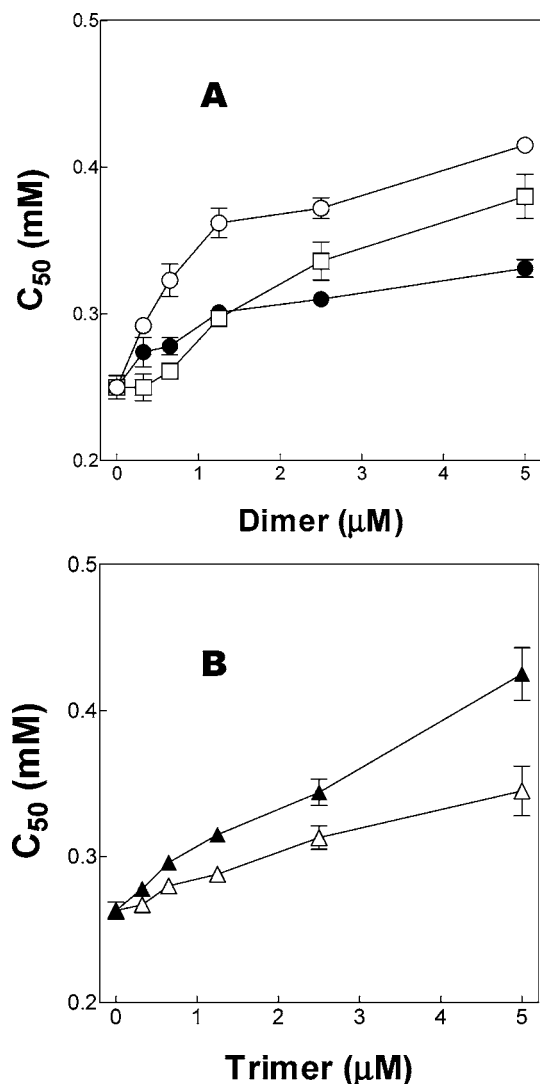


Figure 5. Effects of dimer and trimer procyanidins on bilayer transition to micelles. PC liposomes containing the fluorescent probe octadecyl rhodamine were incubated for 5 min at 25 °C in the presence of different concentrations of (A) Dim A₁ (○), Dim A₂ (□), and Dim B (●) or (B) Trim A (△) or Trim C (▲). After incubation, Triton X-100 (0–1.5 mM) was added and the increase in fluorescence emission was recorded. C₅₀ = concentration of Triton X-100 necessary to achieve the 50% of the maximal fluorescence intensity. Results are shown as means ± SEM of four independent experiments.

mM AMVN. When experiments were carried out in the presence of lower AMVN concentration (2 mM), all of the procyanidins tested almost completely abolished C₁₁-BODIPY oxidation (data not shown). At 5 mM AMVN, the maximal inhibitory effect for the dimers and trimers was achieved at 2.5 µM. The magnitude of the protective effect against AMVN-mediated C₁₁-BODIPY oxidation depended on the number of flavanol units present in the procyanidins. For example, at 0.33 µM trimers (0.99 µM flavanol units), the amount of probe oxidized was decreased by a 45%, a value similar to that observed with dimers at a concentration of 0.65 µM (1.30 µM flavanol units). These results are in agreement with those previously reported (15) showing a dependence of the protection of lipid oxidation with the number of monomers present in the B and C type procyanidins. When comparing the antioxidant effects of dimers, no significant differences were observed, as well as when comparing the effects of the trimers.

Recently, we demonstrated that certain B and C type procyanidins purified from cocoa can decrease the membrane potential at the water–lipid interface in liposomes composed of PC and phosphatidyl serine (15). In PC liposomes, with the exception of Dim A₂, which showed a bimodal effect, all of the procyanidins tested increased the surface membrane potential. The differential effects observed comparing the two liposome systems may be due to the different lipid composition rather than to differences in the behavior of the procyanidins per se. In PC liposomes, the distance between their headgroups may allow the dimers/trimers to penetrate deeper into the bilayer surface generating an apparent increase in the surface potential. In contrast, a low penetration into the bilayer caused by a tighter packing of the lipid headgroups occurs in PC:PS liposomes determining a shallow interaction with the phospholipids. In that case, the procyanidin–phospholipid interaction should result in a decrease in the surface potential, as we observed in this work for Dim A₂ and previously for the longer procyanidins (15).

The influence of procyanidin dimers and trimers on membrane fluidity was investigated to further understand the interaction of the procyanidins with membranes and to examine possible relationships with procyanidin antioxidant actions. For these experiments, two fluorescent probes were used, 6-AS and 16-AP. In these molecules, the anthroyloxy group that is capable of sensing the fluidity of the lipid environment is situated at different depths in the bilayers. Kaiser and London (22) calculated that in 6-AS the fluorophore is approximately at 7 Å from the membrane surface, while for the 16-AP this distance is estimated at 18 Å. These differences allow us to evaluate the impact of the procyanidin–membrane interactions on lipid fluidity both at the surface level and in the hydrophobic core of the membrane. Dimers and trimers decreased membrane fluidity to a similar degree. The lack of a differential effect could be due to the fact that the membrane–procyanidin interactions are through a zone of the procyanidin moiety that is independent of the molecules size (dimer vs trimer) and that is not affected by the folding of the procyanidins (A type vs B and C types). Moreover, the fact that the phospholipid headgroups are already highly ordered determines that their interaction with an externally added molecule would only impart a slight additional effect on their ordering.

A different behavior of the procyanidins on liposomes fluidity was observed with the probe 16-AP that senses the membrane core. In this region of the membrane reside the acyl chains of phospholipids that in the particular case of natural phospholipids have a great diversity of fatty acids chain lengths and unsaturation degrees. Consequently, the ordering of the hydrophobic environment in the liposomes is low and more prone to be altered by external molecules. In the current system, both dimers and trimers promoted the rigidification of the hydrophobic core. However, while at 5 µM concentration, Dim A₁, Dim B, and the trimers displayed a similar maximal effect (9–14% change), and Dim A₂ had the highest effect (22% change) in promoting the loss of membrane fluidity. Again, these results support the hypothesis that Dim A₂ interacts differently with the bilayer causing a more profound impact on membrane rheology than the other dimers and trimers studied.

Accordingly to the above-discussed results, a significant and positive correlation was found between the effects of procyanidin dimers on the fluorescence polarization of the probes 6-AS and 16-AP (data not shown). The slopes of the lineal regression were similar for Dim A₁ (0.69 ± 0.09) and Dim B (0.70 ± 0.10). The fact that these slopes are lower than one

suggests that these molecules primarily affect membrane fluidity in the hydrophilic region of the bilayer. On the other hand, the slope for Dim A₂ was 1.081 ± 0.009 , indicating that Dim A₂ had the strongest action propagating its rigidifying effect from the water–lipid interface of the bilayer to the hydrophobic core.

Finally, we investigated whether the A type procyanidins could prevent the disruption of the membrane integrity. We previously demonstrated that B and C type procyanidins (monomer to hexamer) isolated from cocoa reduced the incorporation of Triton X-100 into the bilayer (15). In the current work PC liposome system, Dim A₁ and Dim A₂ had a higher protective effect on Triton X-100-mediated liposome disruption than did Dim B and Dim C. This differential effect could be attributed to a different folding of the molecules belonging to these two series. Mackenzie et al. (12) reported that Dim B subunits are stacked, establishing internal hydrogen bonds. Because both Dim A₁ and Dim A₂ subunits are connected by two covalent bonds (Figure 1), the subunits should not be able to fold over themselves; thus, they will have a more elongated structure than in Dim B. If this concept is correct, Dim A₁ and Dim A₂ should have a higher number of contact sites with the membrane, thus covering a bigger membrane surface per dimer molecule. The above should translate into a more efficient protection of bilayer from Triton X-100-induced disruption.

Collectively, the present results indicate that dimers and trimers isolated from peanut and cocoa interact with lipid membranes and thus can modulate membrane fluidity. The alteration of membrane fluidity can per se affect numerous cellular processes influencing, among others, the functionality of membrane-associated enzymes (23–25) and certain intracellular transport mechanisms (26, 27) and membrane receptors (28, 29). In addition, procyanidins may reduce the incorporation into the bilayer of hydrophobic compounds that can affect, either directly or indirectly, the integrity of the membrane.

With respect to the physiological relevance of dimers and trimers, it has been reported that dietary procyanidins are relatively stable during gastric transit (30); thus, high micromolar concentrations of both dimers and trimers can be predicted to occur throughout the gastrointestinal tract following the consumption of procyanidin-rich foods. While absorption of trimers from the gut is thought to be very limited, monomers and dimers can be readily detected in the plasma pool within 2 h after the consumption of flavonoid rich foods or beverages. Thus, while the potential protective effects of dimers and trimers that are described in this paper are probably most relevant for the gastrointestinal track, the dimers may also provide some protection to cells in circulation, as well as vascular endothelial cells.

In summary, the present results underscore the relevance of defining procyanidin chemical properties and their interactions with biological molecules when considering to characterize a potential health effects.

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