Preservation of polyunsaturated fatty acyl glycerides via intramolecular antioxidant coupling

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Ferulic acid and its esters are known to be effective antioxidants. Feruloyl di-γ-linolenoylglycerol was assessed for its ability to serve as an antioxidant for preventing the oxidation of its γ-linolenoyl polyunsaturated fatty acyl groups in model membrane phospholipid vesicles. The molecule was incorporated into single-lamellar vesicles comprised of 1,2-dioleoyl-sn-glycero-3-phosphocholine. Feruloyl di-γ-linolenoylglycerol was found to be highly resistant to 2,2′-azobisisobutyronitrile (AIBN)-initiated oxidation in comparison to di-γ-linolenoylglycerol. Analysis of the individual fatty acyl chains indicated that degradation of γ-linolenoyl groups from feruloyl di-γ-linolenoylglycerol proceeded much more slowly than loss of the entire molecule, indicating that the feruloyl moiety was preferentially oxidized. In vesicles incorporating di-γ-linolenoylglycerol and an equal amount (5 mol%) each feruloyl dioleoylglycerol, the extent of γ-linolenoyl protection was not as great as when the γ-linolenoyl groups were molecularly combined with a ferulate group. These findings indicate that the ferulate group of feruloyl di-γ-linolenoylglycerol expresses intramolecular antioxidant activity. Direct coupling of polyunsaturated fatty acids with phenolic antioxidants may improve the oxidative stability of sensitive fatty acids in food or topical uses.

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1. Introduction

The complex physical and chemical phenomena associated with polyunsaturated oil or fatty acid (PUFA) oxidation remain very active research topics due to their importance in health maintenance. Natural oil sources usually contain stabilizing phenolics, which can be supplemented with various antioxidants to further extend storage or use lifetime. Mechanisms by which phenolics protect lipids from oxidation include reduction of reactive oxygen, nitrogen or sulfur species and by hydride (H•) donation to oxidized lipids to interrupt propagation events (Catalá, 2009; Leopoldini et al., 2011).

While phenolic supplementation may be effective for bulk oil applications (e.g., frying), there are circumstances in which high-value polyunsaturated oils (e.g., krill, fish, and borage) may benefit from closer attention. Dermal delivery of polyunsaturated oils, particularly oils enriched in γ-linolenic acid (C18:3 Δ6,9,12), serves to restore hydration and function to aging skin (Brosche and Platt, 2000; Kawamura et al., 2011). Effective penetration through the skin’s stratum corneum layer can be obtained through the use of specially formulated microemulsions, liposomes or solid nanoparticles (Acosta et al., 2011; El Maghraby et al., 2008; Pawar and Babu, 2010). Due to partitioning, phenolics may be left behind on the skin’s upper surface as the delivery vehicle migrates through the stratum corneum to targeted epidermal and dermal layers (Lee et al., 2011). Separation from the antioxidant capabilities of the phenols molecule leaves the polyunsaturated oil unprotected. Thus the question arises as to whether direct molecular coupling of phenolic antioxidants with polyunsaturated fatty acids or oils provides a superior molecule that is not subject to loss of antioxidant capacity due to diffusion (dilution) or solubility differences. The attachment of marine PUFA to phenolics to make antioxidants that are lipophilic has been investigated (Mbatia et al., 2011; Sabally et al., 2007). Microemulsions of these materials demonstrated antioxidant capacity. However, it was not demonstrated whether the phenolics actually prevented oxidation of the PUFA to which they were attached. Similarly, while the antioxidant performance of ascorbyl CLA (conjugated linoleic acid) in o/w emulsions was examined it was not establish whether the ascorbyl group prevented oxidation of the CLA to which it was attached (Sorensen et al., 2011).

Ferulic acid (3-(3’-methoxy-4’-hydroxyphenyl)-propenoic acid) is a common and plentiful phenolic dietary constituent found in various edible plant sources (Clifford, 1999; Zhao and Moghadasian,...
2008). It is present as both the free acid and in a variety of ester forms. The esters are found predominately as architectural components of the hemicellulose and lignin fractions of plant cell walls, as well as in suberin and cutin waxy surfaces of leaves and roots. Ferulic acid is found esterified to phytosterols in corn and rice bran oils (Wang et al., 2002), and as esters of ω-hydroxylalkanoic acids and long-chain alkan-1-ols in potato periderm (Gaça and Pereira, 2000; Yunoki et al., 2004). Natural feruloyl glycerols have been identified in potato, grasses and lilies (Cooper et al., 1978; Gaça and Pereira, 2000; Shimomura et al., 1987). Feruloylated vegetable oils have been synthesized and commercialized (Laszlo et al., 2003).

The antioxidant capacity of ferulic acid and its esters has been extensively investigated (Kikuzaki et al., 2002; Nenadis et al., 2003). Water-soluble ferulic acid and its lipophilic esters have antioxidant capacities typical of simple phenols, which as a class of compounds are generally less potent than polycyclic polyphenols (e.g., rutin) (Leopoldini et al., 2011). Yet the simplicity and ready availability of ferulic acid makes it an excellent choice for preparing model lipophilic antioxidants. Feruloyl glycerols, such as feruloyl dioleoylglycerol, have been shown to be good antioxidants in various in vitro systems including liposomes (Choo and Birch, 2009; Laszlo et al., 2010).

For the present work, we examined the hypothesis that a phenolic antioxidant intramolecularly coupled to PUFA provides better protection than the same antioxidant present as a co-solute in a vesicular system. Specifically, the sensitivity to oxidation of the PUFA in feruloyl di-γ-linolenoylglycerol (Fig. 1) was compared to that of a di-γ-linolenin/feruloyl dioleoylglycerol admixture in liposomes.

2. Materials and methods

2.1. Reagents and materials

Ethyl ferulate was obtained from Sinova Corporation (Ningbo, China). Tri-γ-linolenin, di-γ-linolenin, and ethyl γ-linolenate were supplied from Nu-Chek-Prep (Elysian, MN). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) and p-toluenesulfonic acid were purchased from Sigma-Aldrich (St. Louis, MO). Novozym 435 was purchased from Novozymes through Brenntag Great Lakes (Chicago, IL). 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) was bought from Avanti Polar Lipids (Alabaster, AL). Feruloyl dioleoylglycerol (FDGO) was prepared as described previously (Laszlo et al., 2010).

2.2. Feruloyl di-γ-linolenoylglycerol (feruloyl di-γ-linolenin or FDIG) preparation

Ethyl ferulate (2.4 g, 0.011 mol), tri-γ-linolenin (10 g, 0.011 mol) and immobilized Candida antarctica lipase B (1.25 g of Novozym 435) were combined and shaken at 60 °C under N2 for one week. The Novozym 435 was removed by filtration (nylon filter). The reaction product was subjected to flash chromatography using a C18 reverse phase column and gradient elution starting at 100% acetonitrile progressing to 70:30 v/v acetonitrile/acetone, with UV detection at 340 nm. The FDIG containing peak was subjected to further purification by HPLC using a semi-preparative Luna phenyl-hexyl column (250 mm × 10 mm, 10 μm; Phenomenex, Torrance, CA) developed with methanol at a flow rate of 5 mL/min. The FDIG product was a yellow oil at room temperature. 1H NMR (d6-acetone): δ (ppm) 7.65 (1H, d, CH−Ar), 7.37 (1H, s, Ar−H), 7.16 (1H, d, Ar−H), 6.89 (1H, d, Ar−H), 6.43 (1H, d, CH=CH−Ar), 5.36 (12H, m, CH2CH=CHCH2), 4.1–4.5 (4H, m, CH2O), 3.95 (3H, s, OCH3), 2.35 (4H, m, CH2CH2CO2), 2.04 (m, CH3CH2CH=CH), 1.63 (m, CH2CH2CH3), 1.38 (m, CH2CH2CH2), 0.90 (6H, m, CH3). 13C NMR (d6-acetone): δ (ppm) 172.3 (CH2CH2CO2), 166.2 (CH=CHCO2), 149.4 (Ar), 148.0 (Ar), 145.7 (Ar=CH=), 130.0 (CH2CH=CHCH2), 126.5 (Ar), 123.3 (Ar), 115.2 (Ar), 114.1 (CH=CHCO2), 110.4 (Ar), 69.2 (CH2CH2O−2), 61.9 (CH2CH2O−2), 55.6 (CH3O), 33.7 (CH2CH2CO2), 31.6 (CH2CH2CH3), 29.1 (aliphatic), 27.0 (CH2CH=CHCH2), 24.8 (CH2CH2CH=CHCH2), 22.7 (CH3CH2−), 13.5 (CH3−). Electrospray ionization mass spectroscopy (ESI-MS): 787.517 [M−H]+ (calcld. C49H71O2: 787.515).

2.3. Lipid preparation and formation of unilamellar phospholipid vesicles

Phospholipid vesicles were prepared following the classic rehydration/extrusion protocol (MacDonald et al., 1991) with some minor modifications. DOPC in chloroform and di-γ-linolenin, FDGO or FDIG (at 5 mol% of total lipids) in ethanol were added to amber vials and then gently mixed. Chloroform and ethanol were removed by blowing argon into the vials for 30 min until a dried lipid film was present. Residual solvent was removed by keeping the dried films under vacuum for 6 h at room temperature. The dried lipid films were stored under argon at −20 °C until needed.

To form phospholipid vesicles, the dried lipid films were allowed to equilibrate to ambient temperature, rehydrated in buffer (20 mM Tris HCl, pH 7.4) at a total lipids concentration of 40 mM, and then mixed periodically over an hour. The multilamellar vesicles formed from the rehydration step were passed 11 times through two (stacked) polycarbonate 100-nm filters in a Lipex extruder (Lipex Northern Extruder, Burnaby, Canada) under N2 (1.7 MPa) to form unilamellar vesicles (approx. 100 nm in diameter). Vesicles were covered with argon, protected from light and stored at 4 °C until used.

2.4. Vesicle peroxyl radical treatment

Lipid peroxidation was initiated by addition of AAPH (10 mM final concentration) to a buffered solution containing vesicles.
Vesicles were treated at 37 °C in the dark with continuous shaking. The reaction mixture was periodically sampled (0.5 mL) to determine the extent of lipid (di-γ-linolenin, FDOG or FDIG) oxidation by HPLC analysis as summarized in Scheme 1. All tests were run in triplicate and averaged.

Vesicle samples were diluted with ethyl acetate (1:2 by vol) to extract di-γ-linolenin, FDOG or FDIG. Following 2 min of centrifugation (13,000 × g) to facilitate phase separation, the organic phase was filtered (Acrodisc LC 13 mm syringe filter with 0.45 µm PVDV membrane, Pall Corporation) and immediately subjected to HPLC analysis for the measurement of residual di-γ-linolenin, FDOG or FDIG.

2.5. Lipid transesterification

In order to determine the extent of oxidation of individual γ-linolenin chains (Scheme 1), residual di-γ-linolenin present in the ethyl acetate extracts of AAPH-treated vesicles were converted to ethyl γ-linolenate by treatment with Novozym 435 (30 mg/mL) for 3 h at 37 °C. Enzyme was removed by filtration (Acrodisc syringe filter). FDOG was negligibly transesterified by the enzyme under these conditions. FDIG from ethyl acetate extracts of AAPH-treated vesicles were converted to ethyl γ-linolenate by treatment with p-toluenesulfonic acid (90 mg/mL) for 24 h at 37 °C.

2.6. HPLC analysis

Di- and triacylglycerols extracted from phospholipid vesicles were analyzed with a Shimadzu liquid chromatograph equipped with a UV detector and a C18 (Phenomenex) reverse phase column (50 mm × 4.6 mm, 5.0 µm bead) housed in a column heater at 30 °C. For analysis of ethyl γ-linolenin, di-γ-linolenin and FDIG, the column was developed at 1.0 mL/min with an acetonitrile/water mixture over 12 min (isocratically initially with 90:10 v/v acetonitrile/water for 4 min, then progressing to 100% acetonitrile in a two-min linear gradient). FDOG was chromatographed isocratically employing a solvent consisting of 70:30 v/v acetonitrile/acetic acid. Detector response at 206 nm was used for ethyl γ-linolenin and di-γ-linolenin quantification, while 325 nm was used for FDOG and FDIG analysis.

2.7. LC high resolution mass spectroscopy analysis (LCMS)

To confirm the structure of FDIG, its mass was determined with an Thermo Scientific Accela UHPLC system (auto injector, PDA detector and a 1250 quaternary pump) coupled with a LTQ Orbitrap Discovery Mass Spectrometer (MS); a high precision linear electrostatic ion trap (LTQ XL), with an Ion Max electrospray ionization (ESI) source, all running under Thermo Scientific Xcalibur LC–MS software. The MS was calibrated at least weekly with a standard calibration mixture recommended by Thermo Scientific and the signal detection was optimized by running the autotune software feature as needed. The MS was run with the ESI probe in the negative mode. The source inlet temperature was 350 °C, the sheath gas rate was set at 10 arbitrary units, the auxiliary gas rate was set at 2 arbitrary units and the sweep gas rate was set at two arbitrary units. The maximal mass resolution was set at 30,000, the spray voltage was set at 3.0 kV, and the tube lens was set at –100 V. Other parameters were determined and set by the calibration and tuning process. An Inertsil ODS-3 reverse phase C18 column (150 mm × 3.0 mm, 3.0 µm bead) from Varian was used. The column was developed isocratically with 60:40:0.4 v/v acetonitrile/acetone/acetic acid at a flow rate of 0.25 mL/min. The effluent was monitored at 325 nm on the PDA.

3. Results

3.1. Lipid oxidation mechanism and kinetics

Free radicals such as alkyl, alkoxyl, and peroxy radicals (R•, RO•, and ROO•, respectively) are formed during the oxidation of fats and oils. This oxidation can lead to rapid degradation of lipids, particularly so for polyunsaturated fatty acids. Oxidation steps are typically formulated as follows (Huang et al., 2005):

Initiation:
ROO• + LH → ROOH + L•

Propagation:
L• + O2 → LOO•

Inhibition:
ROO• + AH → ROOH + A•

Termination:
A• → nonradical products

An initial peroxyl radical (ROO•) may spontaneously form via reaction of a carbon radical with O2, which subsequently abstracts hydrogen (H•) from a polyunsaturated lipid (LH). The generated lipid radical (L•) in turn reacts with O2 to propagate additional peroxy radicals (LOO•) that continue the process. Termination of the oxidation steps occurs when radicals combine to form non-radical products, or through inhibition by a quencher (antioxidant) molecule (AH) that does not generate more lipid radicals. Due to the propagation steps, a small amount of initiator can thus lead to the destruction of a large amount of polyunsaturated lipid in the absence of a quencher. The effectiveness of the quencher (antioxidant) hinges on its ability to interact with the free radical initiator or the oxidized lipid.

For the present work, AAPH served as the initiator and glycerol esters of γ-linolenate were the lipids subjected to oxidation. AAPH decomposes to form radicals (presumptively ROO•) in oxygenated aqueous media at a known rate (k) of 0.00396 h−1 (t½ 175 h) at 37 °C (Niki, 1990). ROO• attack polyunsaturated lipids within a membrane, even lipids that are deeply buried in an intact liposomal bilayer (Laszlo et al., 2012), leading to lipid oxidation and degradation. However, ROO• would not be expected to oxidize the oleoyl chains of the phospholipid (DOPC) used to form liposomes (Yamamoto et al., 1984).
Two radicals are formed from each AAPH molecule that thermally decomposes. Given this stoichiometry, the AAPH-derived radical concentration is calculated from:

\[ [\text{Radical}] = 2 \times (1 - \exp(-kt))) \times [\text{AAPH}]_{t=0} \tag{8} \]

AAPH-derived radicals may disproportionate or recombine, lessening the expected concentration of radicals (Werber et al., 2011). However, the AAPH-derived radical concentration shown in Figs. 2–4 was estimated from Eq. (8) without regard to the extent of radical disproportionation or recombination. Note as well that the radicals derived from AAPH thermal decomposition are referred to as peroxyradicals, as is conventionally done, although the non-transient existence of such radicals has not been confirmed (Werber et al., 2011).

### 3.2 Di-\(\gamma\)-linolenin oxidation

AAPH-initiated rapid oxidation of di-\(\gamma\)-linolenin incorporated into large (100 nm), unilamellar liposomes is shown in Fig. 2. The molecule degraded with a pseudo first-order kinetic \(\exp(-kt))\). Rate constants and half-life values are given in Table 1. The individual acyl chains of the molecule, analyzed as their ethyl esters, decayed at a slightly slower rate than that of the whole molecule. As no distinct intermediary decay products were observed by HPLC, such as those that might be produced with one intact \(\gamma\)-linolenate acyl chain and one chain-shortened acyl group, it is likely that oxidation of one acyl chain led to a very rapid destruction of both acyl chains within a molecule. This inference was supported by the observation that ethyl \(\gamma\)-linolenate treated under the same conditions (5 mol% in DOPC liposomes) decayed more slowly \((t_{1/2} 4.3 \text{ h}, \text{ data not shown})\) than that observed for the individual acyl chains of di-\(\gamma\)-linolenin \((t_{1/2} 2.0 \text{ h}, \text{ Table 1})\). Radical propagation was clearly
Table 1

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Decay rate (h⁻¹)</th>
<th>Half-life (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Di-γ-linolenin</td>
<td>0.458 ± 0.002</td>
<td>1.5</td>
</tr>
<tr>
<td>Ethyl γ-linolenate</td>
<td>0.34 ± 0.01</td>
<td>2.0</td>
</tr>
<tr>
<td>FDOGc and di-γ-linolenin</td>
<td>0.022 ± 0.003</td>
<td>31</td>
</tr>
<tr>
<td>FDOG</td>
<td>0.018 ± 0.002</td>
<td>39</td>
</tr>
<tr>
<td>Ethyl γ-linolenate</td>
<td>0.013 ± 0.002</td>
<td>53</td>
</tr>
<tr>
<td>FDOMc</td>
<td>0.020 ± 0.001</td>
<td>35</td>
</tr>
<tr>
<td>Ethyl γ-linolenate</td>
<td>0.0056 ± 0.0002</td>
<td>1.2 × 10⁴</td>
</tr>
</tbody>
</table>

* Total lipid (phospholipid plus diglyceride or triglyceride) concentration was 40 mM in 20 mM Tris HCl, pH 7.4, with 10 mM AAPH present. Treatments were conducted at 37 °C in the dark.

β Di-γ-linolenin concentration was 2.0 mM. Subsequent to AAPH treatment, individual acyl chains of di-γ-linolenin were converted to ethyl γ-linolenate by transesterification with ethyl acetate catalyzed by Novozym 435. The sensitivity of di-γ-linolenin to oxidation is a characteristic shared by many polysaturated lipids.

3.3. FDOG/di-γ-linolenin oxidation

The antioxidant activity of a phenolic compound is principally attributable to its scavenger activity toward free radicals via electron or hydrogen atom transfer (Leopoldini et al., 2011). Ferulate groups act by such mechanisms (Kancheva, 2009). We have previously demonstrated the antioxidant capability of the feruloyl lipid FDOG (Fig. 1) in liposomes (Laszlo et al., 2010). In that work, FDOG was shown to be similar to Trolox, a vitamin E analog, in its ability to delay the AAPH-initiated oxidation of a liposome-embedded fluorogenic reporter group.

The ability of FDOG to protect di-γ-linolenin from AAPH-initiated oxidation is revealed in Fig. 3. Equal amounts of FDOG and di-γ-linolenin (5 mol% each of total liposomal lipids) were incorporated into vesicles. The half-life of di-γ-linolenin increased from 2.0 h in the absence of FDOG to 39 h with FDOG present (Table 1), representing a 26-fold increase in the oxidative stability of di-γ-linolenin in liposomes. FDOG was consumed at a pace slightly faster than that of di-γ-linolenin, reflecting its ability to quench free radical propagation through self-destruction. The extent of combined FDOG and di-γ-linolenin consumption was comparable with that of AAPH-derived peroxide radical production (Fig. 3 inset). Thus, unlike the situation in which radical propagation went unchecked with di-γ-linolenin alone in the vesicles (cf. Figs. 2 and 3 inserts), propagation oxidation events were suppressed with FDOG present. FDOG increased the longevity of the individual di-γ-linolenin acyl chains to the same extent (27-fold) as it did for the entire molecule, implying intramolecular di-γ-linolenin oxidation was not suppressed fully.

3.4. LCMS oxidation

LCMS analysis of FDIG (not shown) detected the presence of two peaks of identical mass, reflecting the presence of both the sn-1(3)-feruloyl-di-γ-linolenoylglycerol (depicted in Fig. 1) and the sn-2-feruloyl-di-γ-linolenoylglycerol species in the sample. Based on the UV absorbance of the peaks, the isomers were in a 4:1 ratio. Prior NMR analysis of FDOG indicated that of its two isomers the sn-1-feruloyl species was predominant (9:1) (Laszlo et al., 2010), reflecting the sn-1,3 selectivity of the enzyme used to make FDOG and FDIG. Therefore it was assumed that the sn-1(3)-feruloyl-di-γ-linolenoylglycerol is the predominant isomer of FDIG as well. Both FDIG isomers were consumed at equal rates in the AAPH-initiated reaction, therefore they were treated as a single entity for the reported decay rates (Table 1).

Two components of FDIG, the feruloyl group and the fatty acyl chains, are both subject to oxidation. Therefore, it was necessary to deconstruct the molecule into its components following AAPH-induced oxidation of liposomes containing FDIG to ascertain the extent of fatty acyl chain oxidation. The extent of loss over time of FDIG and its γ-linolenin acyl chains is shown in Fig. 4. The half-life of FDIG as a whole in AAPH-treated vesicles (Table 1) was slightly greater than that observed with FDOG (with di-γ-linolenin present; see Section 3.3). However, the half-life of the γ-linolenic acyl chains of FDIG was 60-fold longer than the half-life of acyl chains of di-γ-linolenin in the absence of antioxidant and, most significantly, 2.2-fold greater than that of FDOG-protected di-γ-linolenin acyl chains (Table 1). The amount of FDIG consumed over the course of the reaction was significantly less than the amount of AAPH peroxo radicals generated (Fig. 4, inset) with antioxidant potential) for more than one free radical (Shahidi and Zhong, 2011). These findings demonstrate that the attached feruloyl group of FDIG is more protective of γ-linolenic acid chain oxidation than an equivalent amount of extramolecular ferulate (FDOG).

4. Discussion

PUFAs such as γ-linolenic acid are readily oxidized from contact with free radicals or auto-oxidation with oxygen. Countermeasures can involve shielding from light, inert atmospheres, and metal chelants, but antioxidants have a dual capability. An antioxidant can prevent lipid oxidation by reacting with initiating radicals (e.g., ROO•), or it can diminish peroxidation by reacting with lipid peroxyl radicals (LOO•) and thus prevent propagation of oxidizing events. Water-soluble antioxidants (e.g., ascorbic and uric acids) likely suppress lipid oxidation by reducing ROO• in the aqueous phase. Lipid-soluble antioxidants (e.g., α-tocopherol) can intercept ROO• at an aqueous/oil interface as well as react with LOO• in an oil droplet or membrane bilayer. The radicals produced from AAPH decomposition, although positively charged, are able to reach membrane probes buried well within the hydrophobic core of liposomes (Laszlo et al., 2012). Although the use of hydrophobic antioxidants to suppress lipid oxidation would thus seem preferable to hydrophilic agents, observations of the ‘polar paradox’ in which the reverse is true speaks to the complexity of events in multi-phase systems (Laguerre et al., 2010; Shahidi and Zhong, 2011). The ‘polar paradox’ may in part arise from dilution effects of hydrophobic antioxidants in a bulk oil phase, as opposed to a high concentration of hydrophilic antioxidant at the aqueous/oil interface. The deleterious influence of antioxidant dilution is difficult to overcome. The bi-molecular nature of antioxidant–radical reactions (Eqs. (4) and (5)) dictates that proximity (along with molecular orientation, bond dissociation enthalpy, and ionization potential) influences reaction rate. It would seem therefore obvious that the ubiquity of an intramolecular antioxidant should be more efficacious than an antioxidant not attached to a PUFa, ceteris paribus, yet this does not appear to have been previously substantiated. Deuteration appears to be an effective alternative for lowering PUFa oxidizability (Hill et al., 2011).

Covalent attachment of phenolic acids to plant- or animal-derived oils is readily achieved (Laszlo et al., 2003; Sabally et al., 2012).
2007), thereby creating hydrophobic antioxidants. FDOG (Fig. 1) is representative of this class of molecules. Its function would be to prevent oxidation of other lipids, as demonstrated previously with model lipid probes (Laszlo et al., 2010) and herein with the PUFA of δ-γ-linolenin (Fig. 3). Presumably FDIG (Fig. 1) could serve that same role, but that is not the question under consideration. Does the covalent attachment of a phenolic group to δ-γ-linolenin enhance the oxidative stability of the PUFA relative to that which can be achieved with an equivalent amount of antioxidant present in the membrane? The observation that the acyl chains of FDIG persist for a 2.2-fold longer time than those of δ-γ-linolenin in the presence of FDOG (Table 1) provides affirmative support to this question. This increased longevity must be ascribed to the feruloyl group because the FDIG molecule as a whole is consumed at a rate 3.4-fold faster than its constituent fatty acid acyl groups (Table 1).

The known rate of AAPH thermal degradation provides a useful guide to understanding the rate at which PUFA oxidize in the presence of free radicals. Although the rate of AAPH degradation is known, that rate at which stable free radicals are formed is not evident. Therefore, the expression for the rate of AAPH-derived radical formation (Eq. (8)) represents an upper limit of the free radical production. Recombination and disproportionation diminish the available free radicals (Werber et al., 2011). Despite this uncertainty, a comparison of the extent of radical production with PUFA oxidation demonstrated that (1) without antioxidant, PUFA oxidation events exceeded free radical production (Fig. 2, inset); (2) with antioxidant present the sum of the antioxidant oxidized and PUFA oxidized was approximately the same as the amount of free radical generated (Fig. 3, inset); and (3) with an antioxidant attached to the PUFA the extent of oxidant is less than the apparent level of free radical (Fig. 4, inset). This can be rationalized by considering that free radical propagation in the absence of a quencher will oxidize more PUFA than the amount of AAPH-generated radicals. Radical propagation is not completely suppressed with added antioxidant present because PUFA oxidation continues, albeit significantly more slowly than in the absence of antioxidant. This reflects the mobility limitations imposed on the antioxidant in a bilayer. With an attached antioxidant, the antioxidant is preferentially oxidized and radical propagation is abolished. That FDIG oxidation was significantly slower than the generation of radicals from AAPH may reflect a higher than 1:1 stoichiometric quenching by feruloyl groups as well as the lower than 1:2 ratio of AAPH decomposition to successfully spawned free radicals. Thus, the feruloyl moiety in FDIG is sacrificed to prevent PUFA destruction.

Effective (epi)dermal delivery of PUFA requires permeation past the skin's corneum stratum barrier. Liposomes of various form and composition are capable of making this transit, although on the time scale of many hours (Chen et al., 2010). The protection afforded by phenolic acid-modified PUFA glycercides ensures delivery of undegraded PUFA to the cells would be dependent on an esterase activity capable of separating the PUFA groups from the glyceride. Human skin has esterase activity (Gelo-Pujic et al., 2008; Shibayama et al., 2008; Zhu et al., 2007), but it is not known how reactive these enzymes may be with such substrates as FDIG.

In conclusion, robust protection of oxidatively sensitive lipids is afforded by covalent association with an antioxidant such as ferulic acid. An intramolecular antioxidant is not impeded by diffusion in preventing attached lipid oxidation.

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References


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