Partitioning of δ-Tocopherol in Aqueous Mixtures of TAG and Isolated Muscle Membranes

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ABSTRACT: To study the uptake of δ-tocopherol by aqueous suspensions of membranes isolated from chicken muscle and the partitioning of the antioxidant in mixtures of chicken TAG and membranes, δ-tocopherol was added to suspensions of these components and they were centrifuged. In an aqueous suspension of membranes only, the tocopherol uptake increased linearly with tocopherol concentration, at about 50% of the added δ-tocopherol at all concentrations. The incorporation of tocopherol into the membranes was independent of incubation temperature in the range of 0–37°C. Studies with aqueous mixtures of membranes and TAG suggested a very low exchange of tocopherol between the different lipid fractions upon mixing when the tocopherol resided initially in one lipid fraction. Adding δ-tocopherol in ethanol favored incorporation of the antioxidant into the membranes; little antioxidant was incorporated into the membranes when it was added to the suspensions in oil. The results suggest that partitioning of exogenous δ-tocopherol between TAG and membrane lipids in muscle foods may be controlled in part by proper selection of the solvent in which it is added.

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Many foods are multiphase and multicomponent systems, containing various lipid-soluble substances that may be susceptible to oxidation. These include fats and oils (TAG) as well as mono- and diglycerides, sterols, lipid-soluble vitamins, phospholipids (PL), carotenoids (colorants), and others (1), some of which are surface active (e.g., mono- and diglycerides and PL) and can either promote or inhibit oxidation (2). In these systems, oxidation of lipids is considered to occur at the lipid–water interface (3–5). Thus, directing the added antioxidant to the location where oxidation is initiated or propagated may be of particular importance for the oxidative stability of a product.

The relationship between antioxidant partitioning and its efficiency as an antioxidant has been studied in various model or simple food systems such as butteroil–water (6), lipid or surfactant micellar systems (7,8), and liposomal or membrane systems (9–11). Furthermore, several studies have shown that partitioning and efficiency of an antioxidant are influenced by the charge of the antioxidant and the surface charge of these systems (8,10,12–14). Barclay and colleagues (10,11) showed that the partitioning of Trolox, a water-soluble derivative of tocopherol, into PL liposomes ranged from 10–25% depending on the type of surface charge. However, the activity of both ascorbic acid and Trolox was significantly enhanced in positively charged as compared to negatively charged liposomes. The results obtained in the aforementioned studies give valuable information on the partitioning behavior and efficiency of antioxidants in relatively simple systems. Direct extrapolation to more complex systems, such as those consisting of a combination of different lipids, may not be applicable in all cases, however. The goals of this study were to investigate the uptake of a lipid-soluble antioxidant (δ-tocopherol) by chicken muscle membranes in the presence or absence of added TAG, and also to examine the exchange of antioxidant between membranes and TAG upon mixing of the two.

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MATERIALS AND METHODS

Materials. HEPES buffer, BSA, nagarse type XXVII protease (7–14 units/mg solid), and δ-tocopherol (90% pure) were obtained from Sigma Chemical Co. (St. Louis, MO). High-purity α- (99%), γ- (99%), and δ-tocopherols (94%) for standard curve preparation were purchased from Supelco, Inc. (Bellefonte, PA). All reagents were of ACS grade and all solvents of HPLC grade.

Muscle sampling and treatment. Chickens were obtained from a local farm (Reading, MA) and sacrificed by asphyxiation using carbon dioxide. The muscles from thigh and leg were collected and stored (aged) whole at 0–4°C until used (10–14 h). Stored muscles were ground twice through 5-mm perforations in a KitchenAid model KSM90 grinder (300 W; KitchenAid Inc., St. Joseph, MI) at full speed. Depot fat from leg and breast muscles was also collected and stored at 0–4°C until used.

Isolation of TAG and membranes. Oil was obtained by mincing and heating depot fat for 30 min in a thermostated water bath at 45°C. The oil obtained was then centrifuged for 5 min at full speed at room temperature in an IEC clinical tabletop centrifuge (International Equipment Company, Needham Heights, MA) for better separation. The clear layer (primarily TAG) was collected and used for partitioning studies.

Membranes were isolated from muscle with or without exogenous tocopherol added after enzyme treatment. Sodium ascorbate (0.2% w/w) and a proteolytic enzyme preparation

(0.05% w/w nagarse) were minced with ground muscle for 20 s in a Sunbeam® Oskar® Model 4817 food processor (Sunbeam-Oster Household Products, Hattiesburg, MS) followed by incubation for 60 min at 0–4ºC. The enzyme was added to break down muscle structural proteins and aid in the release of membranes (1,2). Membranes were then isolated using differential ultracentrifugation. Briefly, minced muscle was homogenized for 60 s in four volumes of 0.1 M HEPES buffer (pH 7.5) with 0.2% (wt/vol) ascorbate (hereafter simply referred to as "buffer"). This homogenate was centrifuged at $10,000 \times g$ for 20 min at 5–10ºC, and the resulting supernatant was centrifuged at $130,000 \times g$ for 30 min. The sediment was washed with 0.6 M KCl in the aforementioned HEPES buffer and recentrifuged at $130,000 \times g$ for 30 min. The resulting sediment was suspended in a small volume of the 0.1 M HEPES buffer with ascorbate. This membrane suspension contained 30–45 mg protein per mL in different preparations. The membrane suspension was used for partitioning studies as described below. Specific protein and lipid concentrations of the membrane suspensions are given in the appropriate tables and figures. Ascorbate was added to the buffers to protect the added tocopherol. TAG and membrane preparations were used immediately for partitioning studies and were stored on ice for the duration of the experiment unless otherwise noted.

Partitioning in membrane suspensions. To examine the effect of incubation temperature on tocopherol partitioning in membrane suspensions, duplicate 5-mL aliquots of the HEPES membrane suspensions were equilibrated on ice (~0–4°C), at room temperature (~20°C), and in a water bath at 37°C for 20 min. This was followed by the addition of 0.1 mL of ethanolic δ-tocopherol solution to the aliquots and vortexing for 30 s at 5-min intervals over an incubation period of 20 min. The samples were diluted with 50 mL of buffer and centrifuged at $130,000 \times g$ for 30 min. The resulting membrane sediment was suspended in buffer and homogenized in a Potter–Elvehjem tube, and specimens were taken for chemical analysis.

The effect of tocopherol concentration on uptake by suspended membranes was evaluated by adding increasing concentrations of δ-tocopherol in ethanol at a constant volume of 0.1 mL to 5 mL of membrane suspension in a 10-mL Potter–Elvehjem tube. Up to 4,000 $\mu g \cdot g^{-1}$ (ppm) tocopherol on a total lipid (TL) basis was added. A control membrane suspension to which only 0.1 mL ethanol was added was run simultaneously. After adding the tocopherol, the suspensions were vortexed for 30 s. This was followed by diluting the suspension with approximately 10 vol of buffer and centrifuging at $130,000 \times g$ for 30 min at refrigerated temperatures to separate any "free" tocopherol from the membranes prior to extraction of lipids. The resulting sediment was suspended in buffer and analyzed. Samples were kept on ice (0–4°C) during the experiment.

Partitioning in mixed membrane–TAG systems. For studies of antioxidant partitioning in model systems consisting of mixtures of TAG and membranes, 5 mL of warm (30–35°C) TAG were added to 15 mL of membrane suspensions in the HEPES buffer in a 55-mL Potter–Elvehjem tube. A control membrane suspension (15 mL), to which 5 mL of warm distilled deionized water was added, was run simultaneously. Samples were maintained at 30°C, the temperature at which the TAG are in liquid form, allowing for easier mixing of the lipids and subsequent separation.

After adding the TAG, the suspensions were homogenized mechanically (Wheaton overhead stirrer; Wheaton Instruments, Millville, NJ) for 30 s at room temperature at speed setting 4. Thereafter, approximately 2 vol of warm (30–35°C) buffer was added, and the suspension was centrifuged for 30 min at $130,000 \times g$ at 35–40°C to separate TAG and membranes. The TAG floating at the top of the centrifuge tube were collected, transferred to a test tube, and centrifuged in a tabletop centrifuge for 5 min at full speed for better separation. The recovery of the added TAG was estimated by weight, and its tocopherol content was determined. After collecting the TAG, the supernatant was discarded, and the sides of the centrifuge tubes were carefully wiped to remove any contaminating TAG. The membranes were then collected and suspended in buffer as described above, and specimens were taken for chemical analysis.

Chemical analysis. Protein contents of membrane suspensions were determined by means of the biuret reaction (15) using BSA as a standard.

TL of minced muscle and membrane suspensions were determined gravimetrically after extraction with organic solvents according to the method of Lee *et al.* (16). Briefly, for extraction of muscle lipids, 5 g of muscle sample was homogenized in a 250-mL Eberbach model 8580 blender (Ann Arbor, MI) for 60 s at moderate speed (setting 50 using a variable autotransformer) with 50 mL of cold 1:1 (vol/vol) chloroform/methanol solution. The homogenate was filtered through Whatman #4 filter paper into a separatory funnel, followed by the addition of 20 mL of 0.5% (wt/vol) NaCl solution to break the emulsion and facilitate phase separation. The organic phase was collected, and aliquots were taken to dryness on a hot plate at low setting to avoid excessive heating.

For extraction of membrane lipids from suspensions, approximately 5 mL of suspension was transferred to a separatory funnel and shaken for 60 s with 10 vol of cold 1:1 (vol/vol) chloroform/methanol solvent. This was followed by the addition of the appropriate volume of cold 0.5% (wt/vol) NaCl solution. The funnel was stored at refrigerated temperatures during phase separation, and the organic phase was collected and analyzed as described above. Myofibrillar protein contamination of membrane suspensions was estimated by SDS-PAGE analysis as described previously (17).

Phosphorus contents of lipid extracts were determined spectrophotometrically as phosphate according to the method of Anderson and Davis (18). Briefly, aliquots of the chloroform layer obtained after lipid extraction were transferred to test tubes and taken to dryness. The samples were then subjected to sulfuric acid hydrolysis at 155°C for 10 min, cooled, and oxidized with hydrogen peroxide at 155°C for 40 min.

Thereafter, 2 mL of distilled, deionized water and 0.8 mL of an ammonium molybdate–ascorbate solution (1:1 vol/vol) were added, and the mixture was heated for 7 min in a boiling water bath. After cooling, the absorbance was read at 797 nm against a blank. Sodium phosphate (dibasic) was used as a standard. A factor of 25 was used for converting lipid phosphorus to PL based on an average M.W. of PC divided by the atomic weight of phosphorus.

Tocopherol contents of lipid extracts and TAG isolates were determined by HPLC with fluorescence detection as described by Petillo *et al.* (19). Samples for tocopherol analysis were stored in amber HPLC vials at −40°C until injected onto the high-performance liquid chromatograph. All values reported are the average of measurements on at least duplicate samples.

Statistical analysis. Statistical analyses were carried out using the general linear model procedure (Jandel Scientific, San Rafael, CA). Least significant difference tests at $P < 0.05$ were used to separate means when significant differences were found.

RESULTS AND DISCUSSION

Partitioning of δ-tocopherol in aqueous membrane suspensions. (i) Effect of incubation temperature on uptake by membrane suspensions. Incubation temperatures in the range of approximately 4 to 37°C had no significant effect ($P < 0.05$) on tocopherol uptake by the membranes (Fig. 1) or on their chemical composition, including the PL-to-TL (PL/TL) or PL-to-protein (PL/protein) ratios of the suspensions (data not shown). Approximately 517 ± 4 and 362 ± 5 ppm δ-tocopherol, respectively, were added on a TL basis in the two experiments. The δ-tocopherol uptake by the membranes was on average 232 ppm in the first experiment and 179 ppm in the second experiment, corresponding to average uptakes of the added δ-tocopherol of 45 and 49%, respectively. The absence of a temperature effect may indicate either that the lipid in the chicken muscle membranes did not undergo a phase change over this temperature range or that the physical state of the membrane lipid bilayer did not affect tocopherol uptake. Kawamura *et al.* (20) showed that long-chain ω-phenylalkanols {$[C_6H_5(CH_2)_nOH]$, $n \geq 7$} and long-chain *n*-alkanols ($n \geq 10$) were incorporated into dipalmitoylphosphatidylcholine liposomes in both the liquid-crystalline or the gel (solid) phase. Barclay *et al.* (21) showed that cholesterol transfers directly into PC liposomes above or below the phase transition temperature.

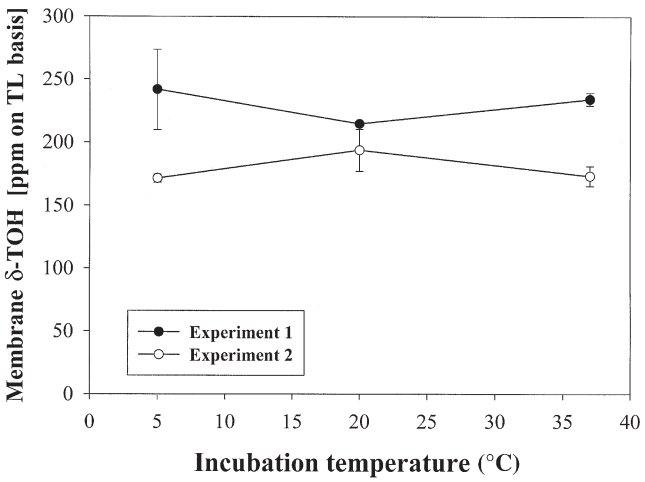

FIG. 1. Effect of incubation temperature on δ-tocopherol (δ-TOH) uptake by membrane suspensions. Samples were thermally equilibrated for 20 min prior to the addition of an ethanolic δ-tocopherol stock. The suspensions contained approximately 30–35 mg·mL^{-1} protein and 17–18 mg·mL^{-1} total lipids (TL). The results of two separate experiments are shown.

(ii) Effect of adding increasing amounts of tocopherol in ethanol on uptake by membrane suspensions. Up to approximately 4,000 ppm of ethanolic δ-tocopherol on a TL basis was added to the membrane suspensions. This amount is over 10 times the tocopherol concentration permitted by the U.S. FDA (300 ppm) and nearly 20 times the endogenous tocopherol concentration found in muscle membrane lipids (22). The PL/TL and PL/protein ratios for the original membrane suspension prior to adding tocopherol were 0.87 and 0.40, respectively. The ratios for the control, to which only ethanol was added, were 0.92 and 0.49, respectively. The ratios for sample membrane suspensions after the tocopherol was added and centrifuged were on average 0.88 ± 0.04 for PL/TL and 0.45 ± 0.02 for PL/protein, respectively. No significant differences ($P < 0.05$) were observed between the PL/TL ratios of the original membrane suspension and the samples. However, a slight increase in the PL/protein ratio was observed between the original membrane suspension and the samples, suggesting that approximately 14% of the proteins were removed owing to the additional washing with buffer and centrifuging after the tocopherol was added. SDS-PAGE analysis of the membrane suspensions indicated no detectable contamination by myosin before or after the additional wash.

The tocopherol uptake by the membranes was linearly dependent ($y = 28.09 + 0.43x$; $R^2 = 0.945$) on the concentration of tocopherol added over the range tested (Fig. 2). Of the δ-tocopherol added, 35 to 51% was taken up by the membrane preparation under the conditions of these experiments. No saturation effect was obvious over the concentration range tested. It is possible that saturation may be reached at higher tocopherol concentrations. Kagan *et al.* (23) found no saturation of multi- or monolamellar liposomes with α-tocopherol at molar ratios of tocopherol/PL ranging from 1:100 to 1:1000. The tocopherol concentrations they added corresponded to approximately 60 to 600 ppm on a lipid basis using an average M.W. of PL of 750 Da. The results obtained may partly be explained by a time-dependent incorporation of tocopherol into lipid structures. Barclay (24) and Barclay and colleagues (25) showed that α-tocopherol was incorporated very slowly into aqueous suspensions of PL liposomes, requiring several (8–12) hours of incubation when introduced either directly into the aqueous phase or when transferred from donor liposomes. Cholesterol, another membrane component, was shown to partition directly into PC bilayers when

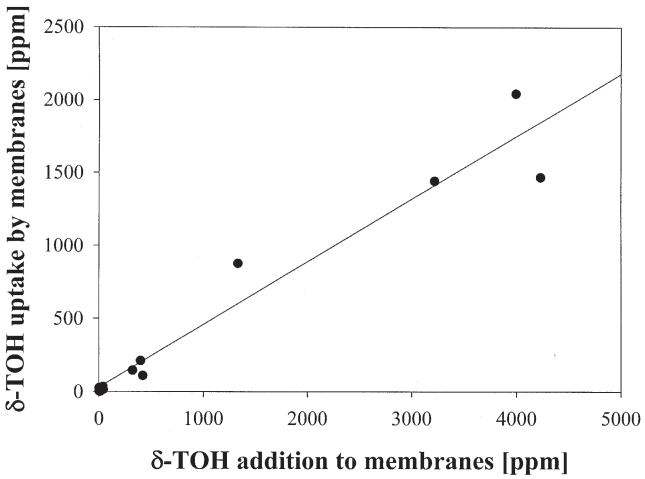

FIG. 2. δ-Tocopherol (δ-TOH) uptake by membranes in relation to concentration of ethanolic tocopherol added. Results are expressed as ppm tocopherol on a suspension TL basis ($\mu g \cdot g^{-1}$). The suspensions contained approximately 35–45 $mg \cdot mL^{-1}$ protein and 17–25 $mg \cdot mL^{-1}$ TL. The results from three separate experiments are shown. Samples were incubated on ice (0–4°C). For abbreviation see Figure 1.

the two were shaken together at 37°C (21). Furthermore, Serbinova and Packer (9) reported an 84–90% incorporation when mitochondrial or microsomal membrane suspensions were incubated with ethanolic α-tocopherol solutions for 20 min at 25°C, followed by centrifuging for 60 min at 105,000 × *g* at 4°C to remove "free" tocopherol. The tocopherol concentration corresponded to approximately 34 µg tocopherol per mg mitochondrial or microsomal protein. Similarly, the incubation of rat liver mitochondrial suspensions with 100 µM [^{14}C]-α-tocopherol in the homogenization buffer (0.25 M sucrose, 1 mM EGTA, 5 mM Tris-HCl, pH 7.4; approximately 22 µg tocopherol·mg mitochondrial protein^{-1}) at room temperature for 30 min, followed by isolation of mitochondria by differential centrifugation, resulted in incorporation of approximately 98% of the tocopherol added (26). Interestingly, Bieri *et al.* (27) found that isolated rat red blood cell lipids incorporated approximately 51% of α-tocopherol added in an aqueous BSA solution (pH 7.4), and 42% of the more polar γ-tocopherol, after incubation for 4 h at 37°C in a Dubnoff shaking incubator. The lower incorporation of γ-tocopherol, as compared to that of α-tocopherol, raises the possibility that the slightly more polar δ-tocopherol would be incorporated to an even lesser extent into membranes.

The hydrophobicity of a compound has been shown to affect its partitioning into lipids. The incorporation of the lipid-soluble ubiquinone (UQ_{10}, chain of 10 isoprenoid units) from an aqueous medium into membranes has been shown to be higher than for its homologs of shorter chain length, e.g., UQ_3, which has an isoprenoid chain length similar to that of tocopherol (28,29). Similarly, the fluorescence studies of Kagan *et al.* (23) showed that the degree of both binding and incorporation of α-tocopherol (C_{16}) to microsomal fractions of rat liver cells was higher compared to its homologs of shorter chain lengths (C_{11}, C_6, and C_1). Approximately 84% of the added α-tocopherol (C_{16}) became associated with the microsomal fraction, based on determinations of the tocopherol concentrations in the aqueous and lipid phases. Their fluorescence studies also indicated that some of the α-tocopherol associated with the microsomal fraction was not completely incorporated into the hydrophobic zone of the membrane bilayer.

Some of the tocopherol added to membrane suspensions in this study may aggregate and form micellar or other structures in the aqueous phase (30,31), possibly emulsified with proteins and small amounts of membrane and/or neutral lipids. The studies of Kagan and colleagues (23) and Kagan and Quinn (32) showed that α-tocopherol and its homologs are capable of aggregating in water. Approximately 16% of α-tocopherol that was added to aqueous suspensions of rat liver microsomes was found to exist in clusters in the aqueous phase. Packer *et al.* (33) suggested that *in vitro* addition of α-tocopherol (0.3 M ethanolic stock) to subcellular membrane suspensions results primarily in the formation of an emulsion of tocopherol droplets, with perhaps little incorporation into the membranes. The tocopherol content of the supernatant obtained after centrifugation of membrane suspensions was not determined in our studies. However, our studies with tocopherol suspended in buffer only (up to approximately 700 µg mL^{-1}) indicated that any "free" tocopherol (i.e., tocopherol not associated with lipids in the membrane suspensions) would coalesce and rise during centrifugation. This is expected since the density of the tocopherol is less than that of the buffer. In the samples with the highest tocopherol concentrations, the upper parts of the centrifuge walls were smeared with tocopherol.

Exchange of δ-tocopherol between TAG and membranes. (i) Mixing of membranes containing δ-tocopherol with TAG free of δ-tocopherol. Membranes containing δ-tocopherol were isolated from minced chicken leg muscle to which approximately 500 ppm δ-tocopherol had previously been added. TAG free of δ-tocopherol were mixed with suspended membranes containing δ-tocopherol followed by separation, as described in the Materials and Methods section. The tocopherol concentration in the lipids before and after separation is shown in Table 1. The concentration of δ-tocopherol in the membranes prior to adding the TAG was approximately 460–480 ppm. δ-Tocopherol was not detected in the TAG added to the membranes. After mixing and separating the two lipid phases, the estimated amount of the added TAG recovered after centrifugation was on average 82 and 81%, respectively, for the two experiments. The recovery was estimated based on gravimetric comparison of the TAG added to the TAG recovered after separation of the lipid fractions. It is therefore reasonable to assume that the TAG recovered are a good representation of the total TAG in this model system. The TAG obtained after mixing and separation contained little or no δ-tocopherol. There was a 12.7% reduction in membrane-associated tocopherol in Experiment 1, whereas no significant change ($P < 0.05$) was observed in Experiment 2.

TABLE 1
Changes in δ-Tocopherol Concentration (n = 4) of Membranes Containing δ-Tocopherol on Addition of TAG[a]

	δ-Tocopherol (ppm)	
Sample	Experiment 1	Experiment 2
TAG	ND	ND
Membranes[b]	457 ± 8	477 ±16
Membranes after TAG addition	399 ± 10	499 ± 47
TAG after separation	0.5 ± 0.1	<0.01

[a]Membrane suspensions contained 35–36 mg·mL^{-1} protein and 16–19 mg·mL^{-1} membrane lipids prior to addition of TAG (4.5 g). The ratio of TAG to membrane lipids was approximately 18:1 (w/w) for the two experiments. Samples were maintained at 30°C. ND, not detected.
[b]Prior to the addition of TAG.

(ii) Mixing membranes free of δ-tocopherol with TAG containing δ-tocopherol. Membranes free of δ-tocopherol were isolated from minced chicken leg muscle. TAG containing δ-tocopherol were obtained by vortexing TAG with 0.1 mL of ethanolic δ-tocopherol solution at 30°C for 15 s at 5-min intervals over a period of 20 min. This was followed by centrifugation for 5 min at full speed in a tabletop centrifuge to remove any air bubbles formed during the mixing. TAG containing δ-tocopherol were mixed with suspended membranes free of δ-tocopherol followed by separation as described in the Materials and Methods section. The tocopherol concentrations in the TAG and membranes before and after separation are shown in Table 2. No significant change ($P < 0.05$) was observed in the PL/protein ratio after mixing and separation of the two lipid fractions. The ratios before and after mixing were 0.36 for both samples in the first experiment, and 0.43 and 0.42 in the second experiment. However, mixing significantly ($P < 0.05$) decreased the PL/TL ratio of the membranes obtained after separation, possibly due to a contamination of the membrane preparation with TAG. The PL/TL ratio decreased from 0.77 to 0.70 and from 0.85 to 0.73, respectively, for the two experiments performed. The TAG added to the membrane suspension contained 230–260 ppm δ-tocopherol on a TAG basis. With the assumption that this change in the PL/TL ratio was entirely due to uptake of TAG by the membrane, this corresponded to an incorporation of 88–154 mg of TAG/g of phospholipid into the membranes. Based on the δ-tocopherol concentration of the TAG prior to mixing (Table 2), this incorporation of TAG-associated tocopherols could result in a tocopherol concentration in the membrane fraction of approximately 30–40 ppm. Thus, the measured tocopherol concentration in the membranes could be entirely accounted for by incorporation of TAG-associated tocopherol.

TABLE 2
Incorporation of δ-Tocopherol by Membranes on Addition of TAG Containing δ-Tocopherol (n = 4)[a]

	δ-Tocopherol (ppm)	
Sample	Experiment 1	Experiment 2
TAG[b]	256 ± 2	226 ± 6
Membranes[c]	0	0
Membranes after TAG addition[d]	9 ± 6	9 ± 5
TAG after separation	251 ± 13	224 ± 6

[a]Membrane suspensions contained 32–36 mg·mL^{-1} protein and 16–18 mg·mL^{-1} membrane lipids prior to addition of TAG (4.5 g). The ratio of TAG to membrane lipids was 25–28:1 (w/w) for the two experiments. Samples were maintained at 30°C.
[b]To which δ-tocopherol had been added.
[c]Prior to addition of TAG.
[d]After separation of TAG and membranes.

δ-Tocopherol was not detected in the membranes isolated from the minced enzyme-treated muscle. The estimated amount of the added TAG accounted for after separation of the lipids was on average 84 and 80%, respectively, in the two experiments. The tocopherol concentration of the membranes obtained after separation was approximately 9 ppm on a membrane lipid basis. This corresponds to only 4–8 μg δ-tocopherol (0.5% w/w) being associated with the membranes out of a total of 1,000–1,150 μg δ-tocopherol originally in the TAG added to the membrane suspension.

(iii) Partitioning of δ-tocopherol in aqueous mixtures of TAG and membranes—effect of the carrier. TAG and suspended membranes, both free of δ-tocopherol, were mixed and mechanically homogenized. Then, 0.1 mL of δ-tocopherol dissolved in ethanol was added to duplicate samples while the same volume of δ-tocopherol dissolved in corn oil was added to another sample. The samples were again mixed mechanically for 30 s followed by the addition of approximately 2 vol of warm (30°C) buffer and centrifugation to separate the lipid fractions. The tocopherol concentrations (ppm) and absolute amounts (μg) in the lipids after separation are shown in Table 3. δ-Tocopherol was not detected in the original TAG, or in the original membrane suspension to which the TAG were added. The δ-tocopherol added in the carriers to the model system ranged from approximately 170 to 200 ppm on a total lipid basis of the system (TAG + membrane lipids). The yields of TAG obtained after separation of the lipids in the two experiments were 91 and 90%, respectively. The addition to the model system of δ-tocopherol dissolved in corn oil resulted in only a small amount of tocopherol (6–8 ppm) being associated with the membrane fraction. Approximately 190–200 ppm δ-tocopherol were determined in the TAG, indicating little change in distribution. There was a considerable increase in tocopherol concentration in the membrane fraction when the antioxidant was added in ethanol. In one case the concentration went from 197 to 328 ppm, and in another from 167 to 499 ppm. In contrast, as shown in both experiments in Table 3, the concentration of tocopherol in the TAG was lower than what was originally added, as might be expected since a higher concentration was present in the membrane fraction.

The results in Table 3 are also expressed as absolute values (μg) in each lipid fraction. Recovery of added δ-tocopherol was estimated based on the assumption that the tocopherol concentration of the lipids obtained after separation was the same as that of the lipids not collected (approximately 10–15% of the TAG and 15–20% of the membrane lipids that are lost during sample handling). On an absolute weight basis, essentially all the added δ-tocopherol was recovered when

TABLE 3
Partitioning of Added δ-Tocopherol in a Mixed TAG and Membrane System as Affected by the Tocopherol Carrier (n = 4)[a]

	Tocopherol carrier			
	Experiment 1		Experiment 2	
Sample	Corn oil	Ethanol	Corn oil	Ethanol
Tocopherol added[b]	185 ± 8	197 ± 2	181 ± 6	167 ± 3
	(887 ± 27)	(945 ± 10)	(880 ± 30)	(800 ± 8)
TAG after separation	202 ± 6	144 ± 5	190 ± 4	97 ± 4
	(930 ± 25)	(648 ± 23)	(872 ± 18)	(426 ± 18)
Membranes after separation	8 ± 1	328 ± 46	6 ± 0	499 ± 88
	(2 ± 0)	(97 ± 14)	(2 ± 1)	(163 ± 29)
Recovery[c] (%)	105	79	99	74

[a]Results are expressed as ppm tocopherol on a lipid basis. Absolute amounts (μg) of tocopherol are given in parentheses. Membrane suspensions contained 33–34 mg·mL^{-1} protein and 15–17 mg·mL^{-1} membrane lipids to which 4.5 g TAG were added. The ratio of TAG to membrane lipids was 15–16:1 (w/w) for the two experiments. Samples were maintained at 30°C.
[b]Concentration [ppm] based on the total lipid content of the model system.
[c]Tocopherol recovery is calculated based on absolute tocopherol amounts.

added in the corn oil. In contrast, recoveries were only 74–79% when the tocopherol was introduced in ethanol. Some of the unrecovered tocopherol may have been destroyed, e.g., by pro-oxidants such as heme compounds and low M.W. iron compounds, upon entering the aqueous phase or by preformed lipid peroxides upon entering either lipid fraction. Also, some of the tocopherol possibly formed relatively stable aggregates within the aqueous phase (23). Adding some support to this theory is the fact that when tocopherol was added in ethanol to membrane suspensions alone, only approximately 50% was estimated to have gone into the lipid fractions.

The partitioning of tocopherol between aqueous mixtures of membranes and TAG depended on the polarity of the tocopherol carrier. Therefore, introducing a lipid-soluble antioxidant into the system in a carrier that aids in its transfer across the aqueous–lipid interfacial barrier is important if you wish to selectively direct the antioxidant into the membrane lipids. The membrane surface tension may play an important role in this process. The adsorption of the antioxidant to the lipids is also affected by the viscosity of the aqueous phase, the antioxidant concentration, the lipid surface area, and the affinity of the antioxidant for the different lipids (34). In this context, it is worth remembering that the surface area of membrane lipids is some two orders of magnitude larger than that of the same amount of TAG (35).

The results obtained in this study demonstrate the important effect the polarity of the tocopherol carrier has on the partitioning of the antioxidant between the different lipids in our model system. The introduction of the antioxidant to the system in a water-miscible carrier disperses the antioxidant in the aqueous phase, from which it will partition between the lipids. Furthermore, the results suggest that lipid-soluble antioxidants such as tocopherols can be directed to the less stable membrane lipids of muscle foods by introduction in an appropriate water-miscible solvent. This preferential direction of the antioxidant to the less stable membrane lipids may result in better oxidative stability of the membrane lipids, thus leading to an increase in shelf life of the product or allowing manufacturers to use lower amounts of the required antioxidants to achieve the desired shelf life.

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