JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2006,** *54,* 7287−7293 **7287**

Effects of Conjugated Linoleic Acid (CLA) Isomers on Oxygen Diffusion−Concentration Products in Liposomes and Phospholipid Solutions

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Conjugated linoleic acids (CLAs) are a group of octadecadienoic acids (18:2) that are naturally present in food products and may have beneficial health effects. Liposomes and ethanol solutions were prepared by mixing synthetic phosphatidylcholines (PCs) with *c*9,*t*11-CLA, *t*10,*c*12-CLA, and linoleic acid (LA) in the *sn*-2 position into natural PCs from soybean, egg yolk, rat brain, and rat heart at 5 mol %. The oxygen diffusion−concentration products were measured using electron spin resonance spin-label oximetry methods. Individual synthetic PCs, the phospholipid matrix, and the tested lipid systems all exhibited influence on oxygen diffusion−concentration products during lipid peroxidation. Incorporating 5 mol % PC(*c*9,*t*11-CLA) into soy and egg yolk PC increased oxygen consumption in liposome suspensions while it was decreased in rat heart and brain PCs. On the other hand, PC-(*t*10,*c*12-CLA) increased oxygen consumption in mixtures with egg yolk and rat heart PC but decreased it in soybean and rat brain PC. By comparison, PC(LA) decreased oxygen consumption in every case. In ethanol solutions, all of the synthetic PCs suppressed the capacity to generate peroxide radicals in the order of LA > *c*9,*t*11-CLA > *t*10,*c*12-CLA. In addition, PCs containing individual CLA isomers and LA differed in their capacities to react with and quench DPPH radicals in both ethanol solution and liposome, suggesting differences between CLA isomers and LA in DPPH radical−fatty acid interactions. Incorporation of CLA isomers and LA into dimyristyl-PC reduced the phase transition temperature from 23.6 to 23.1 and 23.3 °C, respectively. The results of this study provide evidence that the behavior of CLA isomers differs in the microenvironment of membranes possibly due to structural differences that affect the permeability of membranes to oxygen and lipid peroxidation.

KEYWORDS: Acid; CLA; ESR; free; linoleic; radicals

INTRODUCTION

Conjugated linoleic acids (CLAs) refer to a group of octadecadienoic acids (18:2) that contain conjugated, as opposed to methlyene interrupted, double bonds. The CLA isomers may be present as free fatty acids or esters. *cis*-9,*trans*-11-CLA (*c*9,*t*11-CLA) is the primary isomer naturally present in dairy and ruminant meat products that contributes to about 80% of the total dietary CLA intake in the human diet (*1*, *2*). In contrast, synthetic CLA preparations generally consist of two major CLA isomers, *trans*-10,*cis*-12-CLA (*t*10,*c*12-CLA) and *c*9,*t*11-CLA, and they are the major CLA isomers in supplemental products (*3*−*5*). CLAs have been reported to exhibit a wide range of promising beneficial effects in both in vitro cell culture and in vivo animal studies. These effects include, but are not limited to, anticancer and antiatherosclerosis effects, improvement of immunity, antioxidant effects, reduction of hypertension, and lower fat to lean muscle partitioning effects (*6*−*11*). The safety of synthetic CLA preparations of two isomers was demonstrated after feeding such a CLA mixture to rats at 1% of the diet for 18 months and observing no adverse effects (*6*). Similar results were recently reported in human subjects that received CLA supplements for 1 year (*12*). Although the mechanisms for the observed effects with the different CLA isomers are still not fully understood, it has been widely accepted that multimechanisms may be involved. This is supported by observations that individual CLA isomers show different responses to a number of biological activities (*9*, *13*−*16*). In CLA research, more work is still required to delineate its role in the attenuation of cancer

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10.1021/jf0610918 CCC: $33.50
Published on Web 08/22/2006

in animal study models and to discover its role in human nutrition in general.

The role of CLA in local oxidative status and stress has been investigated in a few models and biological systems since their influences on oxidative stress may explain some of the physiological responses (*16*−*21*). CLA inhibited linoleic acid (LA; *c*9,*c*12-18:2) oxidation (*22*), suppressed lipid peroxidation in mammary gland of rats (*18*), and directly reacted and quenched stable DPPH radicals (*7*). CLA also altered fatty acid oxidation in mitochondria, and the CLA isomers were more stable than LA under the experimental conditions used (*19*, *20*). Interestingly, one group suggested that CLA triggered apoptosis in Jurkat T cells through modulation of oxidative stress or levels of reactive oxygen species (*16*). These results indicate that it may be the capacity of CLA to alter the local oxidative status, and not simply by acting as antioxidants or prooxidants, that plays a critical role in their beneficial effects. In addition, growing evidence has indicated that individual CLA isomers might differ in their capacities to modulate local oxidative status (*15*, *19*, *20*, *23*). For example, we reported that *t*10,*c*12-CLA had greater initial velocity in CLA−DPPH radical reactions and *c*9,*t*11-CLA quenched more DPPH radicals when the CLA−radical reaction reached their equilibrium (*15*). Recently, increased *t*10,*c*12-CLA intake was found to accelerate peroxisomal and probably mitochondrial liver fatty acid oxidation that led to reduced triacylglycerol concentrations in liver and serum of hamsters fed CLA, whereas *c*9,*t*11-CLA intake had no significant effect under the same experimental conditions (*19*). In 1999, we reported that incorporation of a synthetic phosphatidylcholine (PC) with *c*9,*t*11-CLA in the *sn*-2 position at 5 mol % increased the oxygen diffusion−concentration products in soybean phospholipid bilayers, decreased the rate of oxygen depletion from the aqueous medium, and increased membrane swelling (*21*). This research suggested that the presence of CLA in phospholipid liposomes may increase the collision frequency of oxygen and other liposome components and/or promote the generation of reactive oxygen species. It is well-accepted that dietary CLA isomers incorporate into membrane phospholipids (*24*). It would therefore be of interest to see if different CLA isomers interact differently with molecular oxygen in cellular membranes and if this interaction alters the physicochemical properties of membranes. Such mechanism would provide an alternative explanation to biochemical mechanisms for the observed effects of CLA isomers in biological systems.

This study was undertaken to compare high-purity synthetic PCs containing either *c*9,*t*11-CLA, *t*10,*c*12-CLA, or LA in the *sn*-2 position at 5 mol % on oxygen diffusion−concentration products and depletion rates in liposomes and ethanol solutions of natural PCs from soybean, egg, rat brain, and rat heart using electron spin resonance (ESR) spin-label oximetry methods. This study also examined and compared PC(*c*9,*t*11-CLA), PC(*t*10,*c*12-CLA), and PC(LA) for their reactions with the stable DPPH radicals in ethanol solutions and liposomes. The effects of the two CLA isomers on the phase transition temperature of liposomes were investigated and compared to that of LA. The results from this study may provide additional information to explain the potential mechanisms involved in the actions of various CLA isomers in biological systems.

MATERIALS AND METHODS

1-Stearoyl-2-octadec-*trans*10,*cis*12-dienoyl-*sn*-glycero-3-phosphorylcholine [PC(*t*10,*c*12-CLA)], 1-stearoyl-2-octadec-*cis*9,*trans*11-dienoyl-*sn*-glycero-3-phosphorylcholine [PC(*c*9,*t*11-CLA)], and 1-stearoyl-2-octadec-*cis*9,*cis*12-dienoyl-*sn*-glycero-3-phosphorylcholine [PC(LA)] were custom synthesized by Matreya, Inc. (Pleasant Gap, PA). 1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphorylcholine [PC(PO)], 1-palmitoyl-2-stearoyl-(16-doxyl)-*sn*-glycero-3-phosphocholine (16-PCSL), egg yolk PC (egg PC), soybean PC, rat heart PC, and rat brain PC were purchased from Avanti Polar Lipids Inc. (Alabaster, AL). The fatty acid composition of these natural PCs is shown in **Table 1**.

Table 1. Fatty Acid Composition of the Natural PCs Used in This Study[a]

	PC			
fatty acids	soy	egg	rat heart	rat brain
16:0	17	34	23	31
*c*9-16:1	ND	2	1	1
18:0	6	11	6	16
*c*9-18:1	13	32	13	39
*c*9,*c*12-18:2	59	18	43	1
*c*9,*c*12,*c*15-18:3	5	ND	1	ND
*c*11-20:1	ND	ND	ND	1
all *cis*-5,8,11,14-20:4	ND	3	6	5
all *cis*-4,7,10,13,16,19-22:6	ND	ND	ND	2
other	ND	ND	7	4

[a] The natural PCs were purchased from Avanti Polar Lipids Inc., and their purity was >99%. Fatty acid compositions in this table were expressed as g/100 g total fatty acids and are those provided by the supplier.

1,1-Diphenyl-1-picryhydrazyl radical, 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-1-yloxyl (CTPO), (4-pyridyl-1-oxide)-*N*-tetra-butylnitrone (4-POBN), and l-α-dimyristoylphosphatidylcholine (DMPC) were purchased from Sigma Chemical Co. (St. Louis, MO). 2,2′-Azobis (2-amino-propane) dihydrochloride (AAPH) and 2,2′-azobis(2,4-dimethylvaleronitrile (AMVN) were acquired from Polysciences (Warrington, PA).

Liposomes containing spin labels were prepared according to the protocol previously described (*21*). CTPO is water soluble and was used at a concentration of 0.14 mM in the oxygen consumption studies. A 0.5 mol % solution of spin-label 16-PCSL was used as a probe in the membranes for the phase transition temperature study. Fifty microliter glass capillaries were employed for the ESR oximetry measurements. The capillary tubes were placed inside the ESR Dewar insert, and a flow of nitrogen was used for controlling temperature.

Conventional ESR spectra were obtained using a Varian E-109 X-band spectrometer and a variable temperature controller accessory. ESR signals were obtained with 1 mW incident microwave power and 100 kHz field modulations of 1 (for phase transition), 0.05 (for oxygen consumption), or 2 G (for reactions with DPPH radicals) measurements. Spectra were recorded, stored, and manipulated using a special computer program (VIKING obtained from the National Biomedical ESR Center, MCW, Milwaukee, WI). A total of 1024 data points were taken by using a scan width of 100 (for phase transition) and 8 G (for oxygen consumption). Free radical generation was initiated by either AAPH or AMVN (*25*, *26*). In a nonstructural lipid system, the spin-trapping ESR method was used to detect the free radical generation during lipid peroxidation. All experiments were performed in duplicate.

RESULTS AND DISCUSSION

Effects of Individual CLA Isomers on Oxygen Diffusion−Concentration Products. Liposomes prepared from mixtures of soy, egg yolk, rat brain, and rat heart PC and 5 mol % synthetic PCs containing *c*9,*t*11-CLA, *t*10,*c*12-CLA, or LA in the *sn*-2 position were examined for their oxygen diffusion−concentration products using ESR spin-label oximetry methods. The natural PCs were chosen because of their diverse fatty acid compositions that differed in degree of unsaturation and chain length. The oximetry method determines the oxygen concentration by measuring the superhyperfine structural changes at the low field line of the ESR spectrum using CTPO as the spin label (*27*−*29*). This superhyperfine structural change is proportional to the local oxygen concentration in liposome. Loss

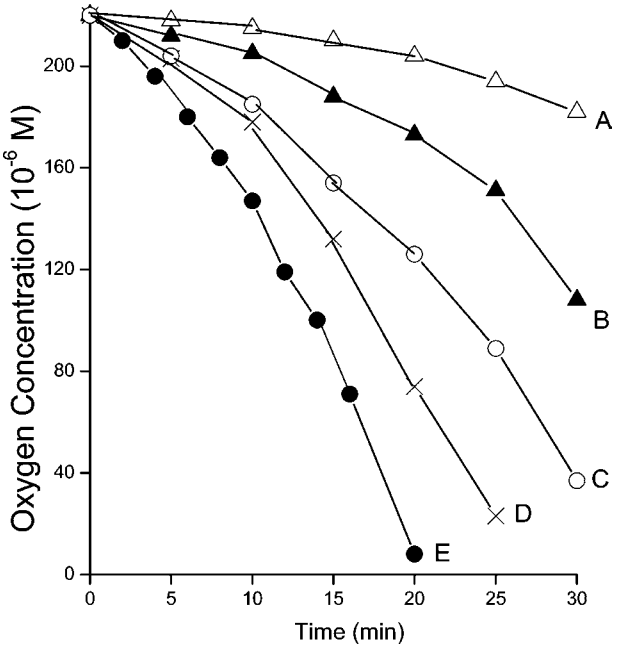

Figure 1. Oxygen consumption kinetics of liposomes prepared using soy PC. Oxygen consumption was measured in closed capillary tubes with individual test systems that included (**A**) 5 mol % PC(*c*9,*t*11-CLA) in DMPC, (**B**) 5 mol % PC(LA) in soy PC, (**C**) 5 mol % PC(*t*10,*c*12-CLA) in soy PC, (**D**) soy PC alone, and (**E**) 5 mol % PC(*c*9,*t*11-CLA) in soy PC. Aqueous liposome suspensions (30 mg/mL) were prepared at 37 °C in 25 mM HEPES buffer (pH 7.2) containing CTPO (0.14 mM). Aliquots were taken for ESR determination from the (aerated) reaction mixture that had been incubated at 37 °C for 45 min.

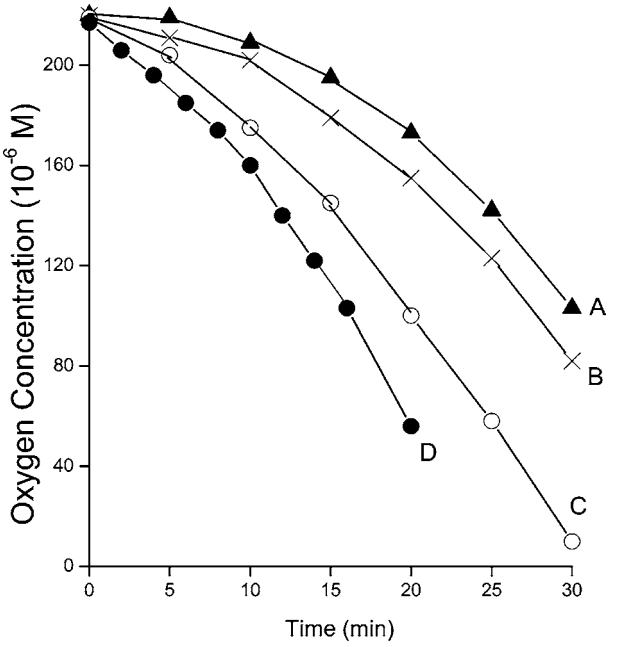

Figure 2. Oxygen consumption kinetics of liposomes prepared using egg yolk PC. The individual test systems included (**A**) 5 mol % of PC(LA) in egg PC, (**B**) egg PC alone, (**C**) 5 mol % PC(*t*10,*c*12-CLA) in egg PC, and (**D**) 5 mol % PC(*c*9,*t*11-CLA) in egg PC. For detailed preparation, see **Figure 1**.

of molecular oxygen in closed capillaries containing liposomes has been used as a diagnostic indicator of lipid oxidation (*21*). The oxygen consumption of the aqueous liposomes during 30 min is presented in **Figures 1**−**4**. The order of oxygen consumption rates in the liposome prepared from soy PC is as follows: 5 mol % PC(*c*9,*t*11-CLA) in soy PC > soy PC alone > 5 mol % PC(*t*10,*c*12-CLA) in soy PC > 5 mol % PC(LA) in soy PC (**Figure 1**). The order of oxygen consumption rates in the liposome prepared from egg yolk PC (egg PC) is as follows: 5 mol % PC(*c*9,*t*11-CLA) in egg PC > 5 mol % PC-(*t*10,*c*12-CLA) in egg PC > egg PC alone > 5 mol % PC(LA) in egg PC (**Figure 2**). Incorporation of 5 mol % *c*9,*t*11-CLA PC in both soy and egg PCs increased oxygen consumption in the corresponding liposome suspensions, while incorporation of same amount of LA decreased oxygen consumption in liposomes prepared from both PCs. On the other hand, *t*10,*c*12-CLA PC decreased oxygen consumption in the aqueous liposomes containing soy PC but increased oxygen consumption in egg yolk PC liposomes (**Figures 1** and **2**). These results indicate that the CLA isomers differ in their effects on oxygen diffusion−concentration products in liposomes and compared to LA. Furthermore, the *c*9,*t*11-CLA and *t*10,*c*12-CLA isomers differ in their influence on lipid peroxidation in liposomes depending on physicochemical properties of the phospholipid matrix and its fatty acid composition. In other words, the effect of individual CLA isomers on lipid peroxidation depends on the chemical structures of the phospholipid liposomes.

The order of oxygen consumption rates differed when the synthetic PCs were incorporated into the liposomes of rat heart and brain PCs. The order for rat heart PC was 5 mol % PC-(*t*10,*c*12-CLA) > rat heart PC alone > 5 mol % PC(*c*9,*t*11-CLA) > 5 mol % PC(LA) in rat heart PC (**Figure 3**), while that for rat brain PC was rat heart PC alone > 5 mol % PC-

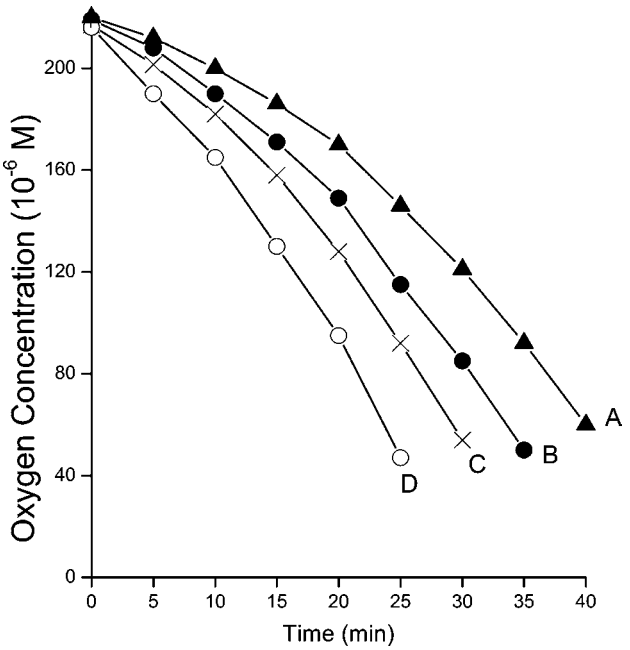

Figure 3. Oxygen consumption kinetics of liposomes prepared using rat heart PC. The individual test systems included (**A**) 5 mol % of PC(LA) in rat heart PC, (**B**) 5 mol % of PC(*c*9,*t*11-CLA) in rat heart PC, (**C**) rat heart PC alone, and (**D**) 5 mol % PC(*t*10,*c*12-CLA) in rat heart PC. For detailed preparation, see **Figure 1**.

(*t*10,*c*12-CLA) > 5 mol % PC(*c*9,*t*11-CLA) > 5 mol % PC-(LA) in rat brain PC (**Figure 4**). Incorporation of PC(*t*10,*c*12-CLA) increased oxygen consumption in the liposome suspensions prepared from rat heart PC (**Figure 3**) but reduced the lipid oxidation in rat brain PC liposomes (**Figure 4**). In contrast to the observations for both soy and egg yolk PCs, the liposomes of both rat heart and brain PCs containing 5 mol % *t*10,*c*12-CLA consumed more oxygen in the assay system than that containing 5 mol % *c*9,*t*11-CLA, clearly demonstrating that the

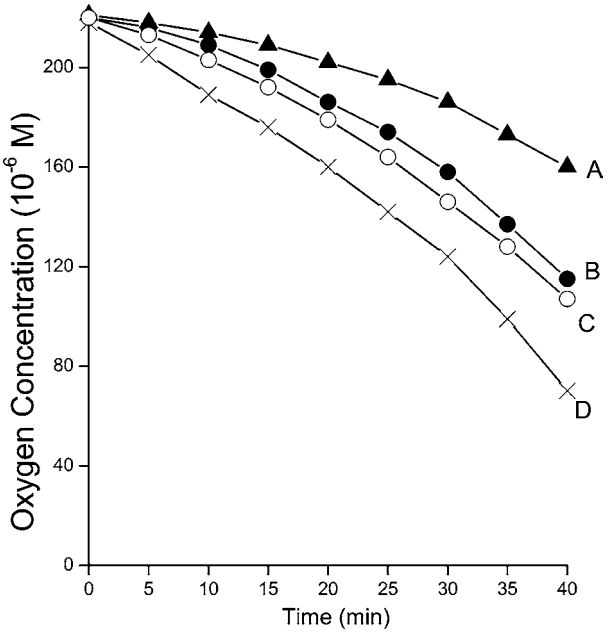

Figure 4. Oxygen consumption kinetics of liposomes prepared using rat brain PC. The individual test systems included (**A**) 5 mol % of PC(LA) in rat brain PC, (**B**) 5 mol % of PC(*c*9,*t*11-CLA) in rat brain PC, (**C**) 5 mol % of PC(*t*10,*c*12-CLA) in rat brain PC, and (**D**) rat brain PC alone. For detailed preparation, see **Figure 1**.

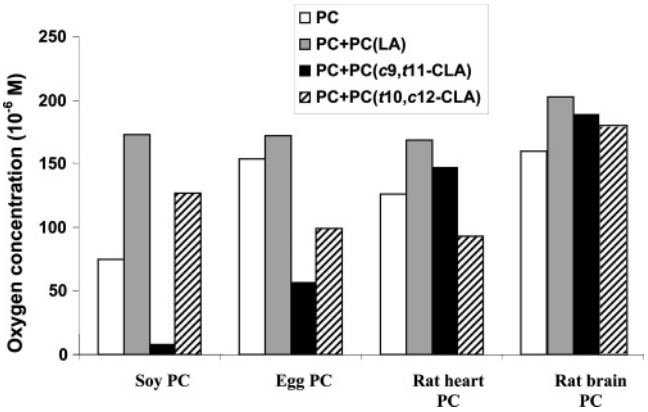

Figure 5. Effect of LA and CLA isomers on lipid peroxidation in liposomes. This figure represents a one time snapshot of the different peroxidation rates of all of the different fatty acid/phophatidylcholine (FA/PC) mixtures obtained after 20 min of reaction using the same liposome model (**Figures 1**–**4**). Each value represents the mean of only two determinations per FA/PC mixture after 20 min. The four natural PCs from soybean, egg yolk, rat heart, and rat brain were compared to themselves and to mixtures containing additions of the three synthetic PCs with LA, *c*9,*t*11-CLA, or *t*10,*c*12-CLA in the *sn*-2 position at a level of 5 mol %.

chemical compositions of the phospholipids significantly altered the effect of CLA isomers on oxygen diffusion−concentration products in liposome suspensions. As compared to the synthetic PCs containing different CLA isomers, incorporation of PC-(LA) in rat heart and brain phospholipids decreased the oxygen consumption in all four types of liposome suspensions evaluated (**Figures 1**−**4**), which is associated with reduced lipid peroxidation. The oxygen concentrations in the individual liposome samples prepared from each synthetic PC (LA, *c*9,*t*11-CLA, or *t*10,*c*12-CLA) after 20 min of incubation in the sealed capillaries are compared in **Figure 5**. Data in this figure clearly demonstrated that the oxygen consumption was generally greater in liposomes prepared from natural PCs with more long-chain

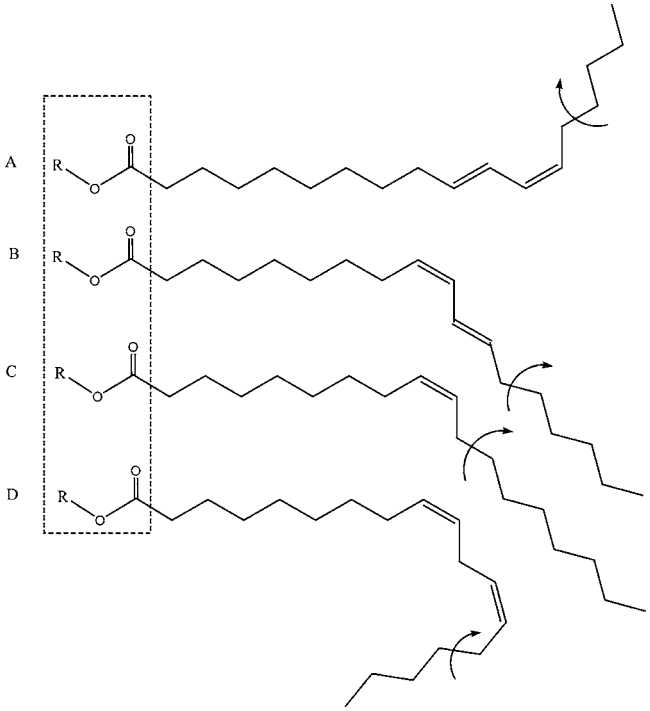

Figure 6. Two-dimensional structure of (**A**) *t*10,*c*12-CLA, (**B**) *c*9,*t*11-CLA, (**C**) oleic acid(*c*9-18:1), and (**D**) LA(*c*9,*c*12-18:2). The carbonyl functional group and the first eight methylene carbons of each of the fatty acids are drawn in the same plane. The arrow indicates the occurrence of first single carbon−carbon bond in the molecule that is free to rotate after the double bond system(s).

PUFAs and that incorporation of the same amount of synthetic PC containing LA, *c*9,*t*11-CLA, or *t*10,*c*12-CLA into the natural PCs markedly influenced the resultant oxygen consumptions under the experimental conditions used (**Figure 5**). The differences in oxygen consumption reflect different levels of lipid oxidation or different local oxidative status/stress.

The differences in the rate of oxygen diffusion in the different lipid systems are not well-understood. They appear to be related to the structure of the fatty acids in the synthetic PCs added as well as to the phospholipid matrix. The individual fatty acids used in the synthetic PCs are compared in **Figure 6** in which common structural features (i.e., the carbonyl functional group and the methylene groups from C2 to C8) are presented adjacent to the structural differences due to the number of double bonds and their position and geometry. An arrow is used to show the first bond to have free rotation after the double bond system in each molecule. All carbon−carbon single bonds in **Figure 6** can undergo rotation and represent low-energy states, except the single bonds directly attached to a double bond whether in conjugated (Δ9-Δ10, Δ11-Δ12, and Δ13-Δ14 in *t*10,*c*12-CLA or Δ8-Δ9, Δ10-Δ11, and Δ12-Δ13 in *c*9,*t*11-CLA) or methylene interrupted fatty acids (Δ8-Δ9, Δ10-Δ11, Δ11-Δ12, and Δ13-Δ14 in LA). These single bonds are most stable when they are coplanar with the double bonds in the molecule. Both CLA isomers can therefore occur as two isoforms as demonstrated in **Figure 7** for the *t*10,*c*12-CLA isomer. The isoforms are *cisoid* or *transoid* representing stable energy minima. To our knowledge, it is not known what geometries are presented at physiological temperature for any *trans*,*cis* or *cis*,*trans* (*c*/*t*) CLA isomers.

The first structure shown in **Figure 6** is that of the *t*10,*c*12-CLA that is characterized by a "bend" in direction away from a linear cylindrical structure beginning with the Δ13 carbon.

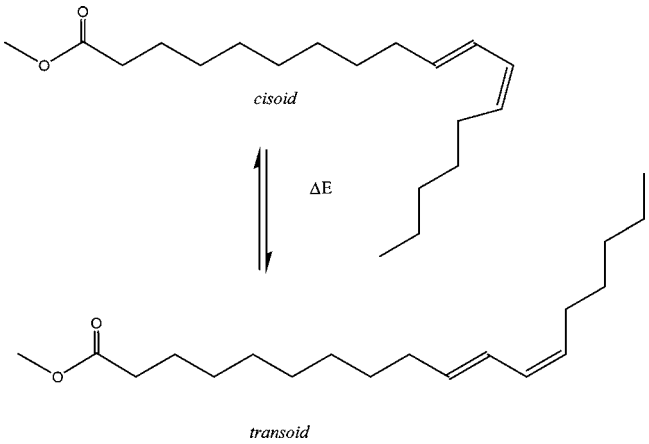

Figure 7. Two-dimensional structure of the two possible configurations of *t*10,*c*12-CLA representing minimum energy states, i.e., in a *cisoid* or *transoid* configuration. Single carbon–carbon bonds can freely rotate in the *t*10,*c*12-CLA molecule except for the single bond in the conjugated functional moiety. The molecule is most stable when both double bonds and the single bond are in the same plane. Exchange between the two geometric configurations involves an expenditure of energy.

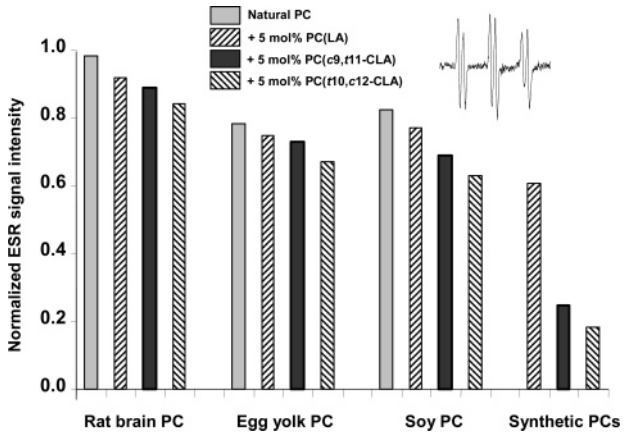

Figure 8. Lipid peroxidation of three natural PC matrices mixed with different synthetic PCs containing specific fatty acids in ethanol solutions. The lipid peroxide radicals generated during lipid peroxidation were determined using 4-POBN as the spin trapping agent. The normalized ESR signal of the peroxide radical–POBN spin adduct was recorded with 15 mW incident microwave and 100 kHz field modulation of 1 G at 37 °C. Each reaction mixture contained 30 mg/mL total lipid, 30 mM AMVN, and 30 mM 4-POPB spin trap in ethanol solution. This reaction mixture was incubated at 50 °C for 2.5 h before it was transferred to the capillary tube for ESR examination. Spectra were recorded after 5 min of equilibration. The inset illustrates the ESR signal of the peroxide radical–POBN spin adduct. The natural PCs from soy, egg, and rat brain were analyzed individually and as mixtures with synthetic PCs containing LA, *c*9,*t*11-CLA, and *t*10,*c*12-CLA at 5 mol % solutions in ethanol. The last comparison was that of the three individual synthetic PCs.

The first bond that has free rotation after the bend in oleic acid (*c*9-18:1), *c*9,*t*11-CLA, and LA occurs after Δ11, Δ13, and Δ14, respectively. This general model is equally applicable in liposomes as in cellular membranes. The number of carbon atoms involved in the double bond system that imparts certain rigidity to the molecule also differs between these fatty acids. There are four, six, and seven carbons involved in oleic acid, in both CLA isomers, and in LA, respectively. The length of the terminal end that is free to rotate in each of these fatty acids also differs. These differences in fatty acid structures influence the packing in artificial as well as cell membranes. We previously reported that the oxygen consumption rate of the liposomes containing PC(*c*9,*t*11-CLA) was significantly greater than that with PC(LA), suggesting that the *c*9,*t*11-CLA content in membranes might account for the increased membrane permeability to oxygen diffusion (*21*).

The effects of PCs containing LA or different CLA isomers on oxygen diffusion−concentration products were also investigated in ethanol solutions with the four natural PCs (soy, egg yolk, rat heart, and brain) using ESR and POBN as the spin-trapping agent. This ESR approach measured the peroxide radical−POBN adducts, in which the ESR signal intensity positively associated with the concentration of the radical−POBN adduct. The peroxide radicals are intermediates of lipid peroxidation, and their level is proportional to the degree of lipid peroxidation. Incorporation of synthetic PC with LA, *c*9,*t*11-CLA, and *t*10,*c*12-CLA reduced lipid peroxidation in the ethanol solutions of soy, egg yolk, and rat brain PCs (**Figure 8**). It was of interest to note that both *c*9,*t*11-CLA and *t*10,*c*12-CLA showed a stronger capacity than LA to suppress the generation of peroxide radicals in the ethanol solutions in all of the phospholipid matrices examined. Incorporation of *t*10,*c*12-CLA into the phospholipids exhibited the strongest inhibitory capacity on the formation of peroxide radicals in the ethanol solutions (**Figure 8**). Each of the individual synthetic PCs (LA, *c*9,*t*11-CLA, and *t*10,*c*12-CLA) by themselves also showed a stronger capacity to inhibit peroxide formation in ethanol solution than a mixture of 5 mol % with soy, egg yolk, or rat brain PCs under the same experimental condition (**Figure 8**).

The results clearly indicate differences in response for each of the synthetic PCs when incorporated into liposomes (**Figures 1**−**5**) or suspended in ethanol solutions (**Figure 8**) and reflect altered oxygen diffusion−concentration products and lipid peroxidation in these two lipid systems. In the ethanol solution, the PC molecules are randomly dispersed unlike liposomes, where the PCs occur in an ordered structural alignment. The synthetic PCs containing LA, *c*9,*t*11-CLA, and *t*10,*c*12-CLA have different configurations and conformations as shown in **Figures 6** and **7** that impact the alignments of the natural PCs, consistent with previous results (*21*). Incorporating the synthetic PCs in natural egg yolk, soybean, rat heart, and rat brain PC liposomes caused significant structural and functional changes as evidenced by different oxygen diffusion−concentration products. These data suggest that changes in lipid geometry caused by the incorporation of *c*9,*t*11-CLA, *t*10,*c*12-CLA, or LA contributed to the overall lipid peroxidation and the oxygen diffusion−concentration products in the selected testing lipid systems.

DPPH Radical−CLA Isomer Reactions in Different Lipid Systems. The synthetic PCs were evaluated for their reactions with the stable DPPH radical in ethanol solution and in liposome suspensions using ESR. The intensity of the ESR signals is proportional to the concentration of DPPH radicals (**Figure 9**). Each of the synthetic PCs reacted with DPPH radicals in either ethanol or liposome suspensions. Incorporating the synthetic PCs at 5 mol % into DMPC reduced the ESR signal intensity in ethanol solutions and aqueous liposome suspensions (**Figure 9A**,**B**), suggesting that LA and the CLA isomers may directly react with the DPPH radical in ethanol and in liposome suspensions under our experimental conditions. Furthermore, data from the present study also indicated that LA, *c*9,*t*11-CLA, and *t*10,*c*12-CLA exhibited different reactivities with DPPH radicals. These observations are in agreement with previous reports that free fatty acids of LA and CLA isomers differed in their capacity to directly react with and quench DPPH radicals in ethanol and toluene solutions (*7*, *15*). In ethanol solutions,

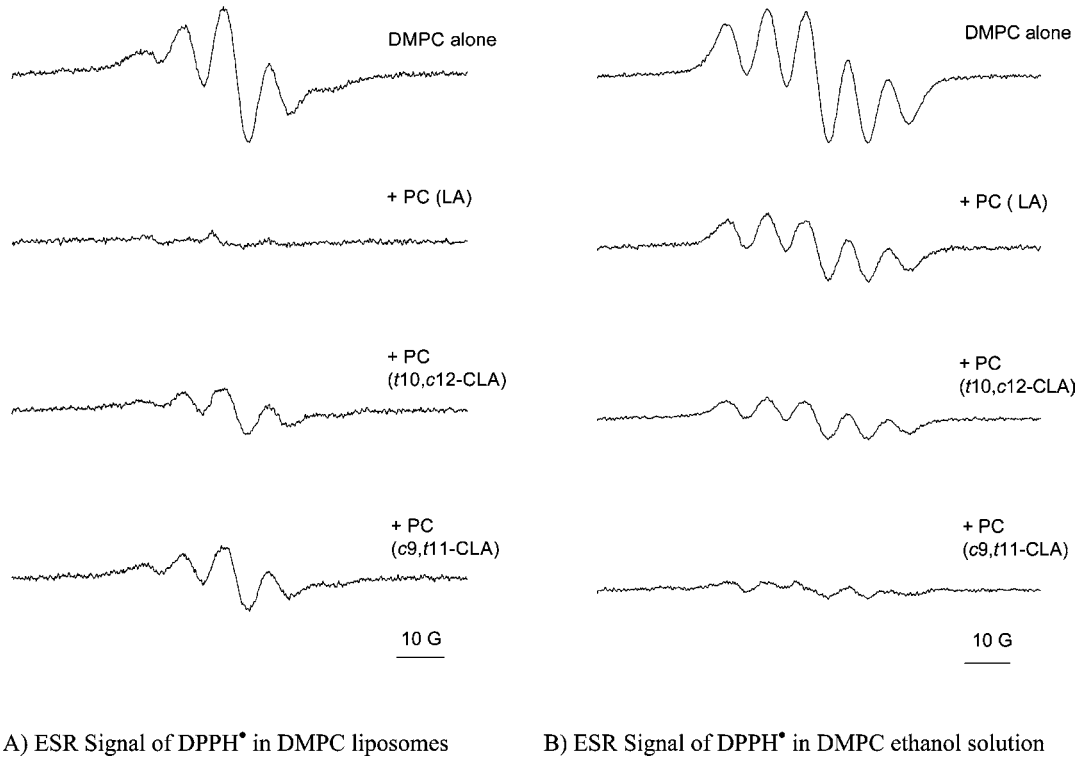

Figure 9. DPPH radical–fatty acid reactions in the DMPC testing system. ESR signals were recorded with 10 mW incident microwave and 100 kHz field modulation of 2 G at 37 °C. The initial concentration was 0.25 mM for DPPH radicals and 5 mol % synthetic PCs with CLA isomers or LA in the *sn*-2 position in all reaction mixtures. The reaction mixtures were incubated at 60 °C for 60 min, and ESR experiments were performed at 37 °C. The intensity of DPPH radical signals was measured in liposomes (**A**) and in ethanol solution (**B**). Pure DMPC and 5 mol % mixtures of PC(LA), PC(*c*9,*t*11-CLA), and PC(*t*10,*c*12-CLA) in DMPC were measured.

DMPC mixed with 5 mol % PC(*c*9,*t*11-CLA) had the strongest capacity to scavenge DPPH radicals in this system, while 5 mol % PC(LA) had the least ability to react with DPPH radicals (**Figure 9A**). Interestingly, DMPC mixed with 5 mol % PC-(LA) had the greatest capacity to scavenge radicals in the aqueous liposome suspensions, which is much stronger than that of either CLA isomer (**Figure 9B**). These results showed the potential effects of lipid systems and microenvironmental conditions on fatty acid−radical reactions.

Effects of LA, *c*9,*t*11-CLA, and *t*10,*c*12-CLA on Phase Transition Temperature of Phospholipid Bilayer Systems. Liposomes have been used as a model for investigating cellular membranes. It is widely accepted that very small changes in fatty acid composition may result in measurable changes in liposomal properties using ESR techniques. For instance, the intensity of the center line in the ESR signal of 16-PCSL may be normalized and plotted as a function of temperature to determine the phase transition temperature. Using this technique, the effect of adding 5 mol % PC(LA), PC(*c*9,*t*11-CLA), or PC-(*t*10,*c*12-CLA) on the phase transition temperature (T_m) of DMPC liposome was evaluated (**Figure 10**). The signal intensity changed dramatically at the T_m of the DMPC liposome; more than 50% of the ESR signal was reduced in this cooling experiment. The T_m changes as the result of incorporating 5 mol % of PC(LA), PC(*c*9,*t*11-CLA), or PC(*t*10,*c*12-CLA) included changes in both the phase transition temperature and the sensitivity of phase transition to temperature change (**Figure 10**). The T_m for DMPC alone was estimated to be 23.6 °C. When DMPC was spiked with the synthetic PC of either CLA isomers, the T_m was 23.3 °C, as compared to 23.1 °C when DMPC was spiked with 5 mol % PC(LA). The width of transition ($T_{1/2}$) was the largest for LA, followed by that for *c*9,*t*11-CLA > *t*10,*c*12-CLA > DMPC. These data suggest that incorporation of LA and individual CLA isomers into phospholipid bilayers altered the physical property that may lead to the altered membrane properties and functions. More research is required to further understanding of behavior of individual CLA isomers in the cellular membrane using artificial phospholipid bilayers, cultured cells, and animal models.

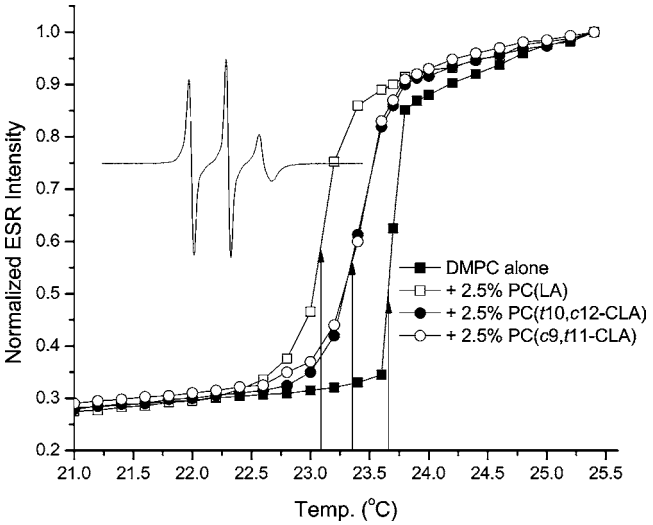

Figure 10. Phase transition curve of different DMPC liposomes. The intensity of the centerline of the ESR signal of 16-PCSL was normalized and plotted as a function of temperature. Pure DMPC and 5 mol % mixtures of PC(LA), PC(*c*9,*t*11-CLA), and PC(*t*10,*c*12-CLA) in DMPC were measured. The inset illustrates the ESR signal of the 16-PCSL spin label.

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Received for review April 18, 2006. Revised manuscript received June 30, 2006. Accepted July 17, 2006. This research was partially supported by a grant from the U.S. Department of Agriculture-CSREES National Research Initiatives with federal Grant 20043550314852. Contribution S267 from Food Research Program, Agriculture and Agri-Food Canada.

JF0610918