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Conjugated Linoleic Acid Isomers Differ in Their Free Radical Scavenging Properties

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Conjugated linoleic acid (CLA) isomers were investigated for free radical scavenging properties against the stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH•) by electron spin resonance (ESR) spectrometry and spectrophotometric methods. ESR measurements confirmed that both c9,t11-CLA and t10,c12-CLA directly reacted with and quenched DPPH radicals, whereas spectrophotometric analysis demonstrated that c9,t11-CLA and t10,c12-CLA differed in their kinetic and thermodynamic properties in reacting with DPPH radicals. t10,c12-CLA was shown to exhibit a greater initial velocity in CLA−DPPH radical reactions at levels of 2.5−80 mg/mL, and c9,t11-CLA scavenged more DPPH radicals at steady state. Similar dose and time relationships were observed for both isomers. In addition, a mixture of c9,t11- and t10,c12-CLA isomers demonstrated a greater initial velocity in quenching DPPH radicals than either isomer alone on the same concentration basis, suggesting that a synergistic effect between CLA isomers existed in their reactions with DPPH radicals. These results support the conclusion that individual CLA isomers differ in their biological actions and indicate that interaction(s) between isomers may contribute to their beneficial effects.

KEYWORDS: Conjugated linoleic acids; antioxidant; free radical scavenging activity; electron spin resonance; c9,t11-CLA; t10,c12-CLA

INTRODUCTION

Conjugated linoleic acids (CLA) refer to a group of octadecadienoate derivatives, which contain conjugated double bonds at different positions and in different geometrical configurations. CLA have been receiving more attention in the past decade because they are natural food components that may have important health benefits. These beneficial effects include anticarcinogenesis (*1*−*4*), antiatherosclerotic and plasma lipid-lowering effects (*5*, *6*), reducing body fat (*7*), and free radical scavenging and antioxidant effects (*8*, *9*). Antioxidant properties, including free radical scavenging capacity, may contribute to their beneficial effects, although the mechanism(s) involved in the biological actions of CLA are still not well understood (*8*, *9*).

Many CLA isomers were detected in food products and lipids isolated from cell or animal tissue samples enriched with CLA (*10*−*12*). *cis*-9,*trans*-11-CLA (c9,t11-CLA) is believed to be the primary form of CLA naturally present in food products including animal foods, whereas c9,t11-CLA and *trans*-10,*cis*-11-CLA (t10,c12-CLA) are the two major isomers detected in synthetic CLA preparations at similar levels (*13*). Growing evidence has indicated that individual CLA isomers might act differently in the biological systems and contribute differently in their beneficial or potential side effects (*4*). Moya-Camarena and collaborators (*14*) found that c9,t11-CLA is the most efficacious peroxisome proliferator-activated receptor α (PPARα) activator with the greater affinity. The IC_{50} values of human PPARα in the competitive binding scintillation proximity assay were 140 and 200 nM for c9,t11-CLA and t10,c12-CLA, respectively (*14*), but a CLA isomer mixture, containing both c9,t11-CLA and t10,c12- CLA at equal levels, failed to induce hepatic peroxisome proliferation in Sprague−Dawley rats (*14*). Limited evidence also indicated that c9,t11-CLA may more effectively enhance the growth of young rodents, whereas t10,c12-CLA may interfere with the efficacy of c9,t11-CLA (*4*). In addition, c9,t11-CLA was preferred over t10,c12-CLA in incorporation into tissue lipids (*15*) and cellular lipid fractions (*11*).

In contrast, t10,c12-CLA was observed to be more effective than c9,t11-CLA in changing the body composition in a mice-feeding study (*16*). In another study, the influence of CLA on the growth and differentiation of 3T3-L preadipocytes was evaluated (*17*). t10,c12-CLA showed a greater capacity than the CLA isomer mixture in inducing apoptosis and reducing incorporation of fatty acids into cell triglycerides. The results from this study demonstrated that t10,c12-CLA is a major antiadipogenic isomer of CLA. Recently, Lin and coauthors (*18*) reported that t10,c12-CLA is more effective than c9,t11-CLA in suppressing hepatic triglycerides secretion in Hep G2 cell cultures. In addition, t10,c12-CLA, but not c9,t11-CLA, decreased the expression of stearoyl-CoA desaturase in cultured

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3T3-Li adipocytes (*4*). Leung and Liu (*9*) reported that t10,c12-CLA (>98% pure), at levels of 2 and 20 μM, was a stronger antioxidant than c9,t11-CLA (>98% pure) or α-tocopherol. Furthermore, t10,c12-CLA at a level of 200 μM was a weaker antioxidant than α-tocopherol, whereas c9,t11-CLA showed a strong pro-oxidant activity at 200 μM. More information about the different physiological effects of c9,t11-CLA and t10,c12-CLA was discussed in a minireview prepared by Pariza and co-workers in 2000 (*4*).

In addition to the different biological activities of individual CLA isomers, it was also observed that a mixture of CLA isomers might have greater biological activity than a single isomer. In 1999, the effects of individual CLA isomers on human platelet aggregation and arachidonic acid metabolism were investigated (*19*). A mixture of CLA isomers (containing 44% c9,t11-CLA, 46% t10,c12-CLA, 4% other CLA isomers, 4% linoleic acid, and 2% oleic acid) had slightly higher antiaggregatory potencies than either c9,t11-CLA or t10,c12-CLA against collagen or arachidonic acid induced human platelet aggregation; moreover, t10,c12-CLA was more effective than c9,t11-CLA in inhibiting arachidonic acid induced platelet aggregation (*19*). It was also observed that t10,c12-CLA was a stronger inhibitor for platelet cyclooxygenase than c9,t11-CLA as measured by [^{14}C]thromboxane B_2 formation, and the CLA mixture had a greater inhibitory activity on cyclooxygenase than either c9,t11-CLA or t10,c12-CLA under the same experimental conditions (*19*).

It was observed in our previous study that a mixture of CLA isomers could directly react and quench the stable 2,2-diphenyl-1-picryhydrazyl radical (DPPH•) as measured by electron spin resonance (ESR) spectrometry and spectrophotometric methods (*8*). In view of the growing evidence that individual CLA isomers differ in their physiological and biological activities, it becomes interesting whether CLA isomers act differently in their reactions with free radicals. In the present study, ESR and spectrophotometric methods were used to compare high-purity c9,t11-CLA and t10,c12-CLA for their actions in CLA−DPPH• reactions. The effects of simulated gastric fluid on isomer distribution were also investigated in this study because isomerization of ingested CLA may take place in the acidic environment of the stomach.

MATERIALS AND METHODS

High-purity c9,t11-CLA (>98%) and t10,c12-CLA (>98%) were purchased from Matreya, Inc. (Pleasant Gap, PA). DPPH• was a product from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents were of the highest commercial grade and used without further purification.

ESR Analysis. The ESR analysis was conducted according to a previously described procedure (*8*). Briefly, solutions of CLA and DPPH• in benzene were mixed and incubated at 38−40 °C for 15 h. The ESR analysis was performed at ambient temperature before and after incubation. The ESR was conducted using a Bruker EMX ESR spectrometer (Bruker Instruments, Inc., Billerica, MA) with a modulation frequency of 100 kHz, a sweep width of 100.00 G, a 3458 G center field, and a 3.17×10^4 receiver gain. The final concentration of DPPH• was 2.0 mM, and CLA concentrations were 0 and 10 mg/mL in the reaction mixtures.

Characteristics of Individual CLA Isomer—DPPH Radical Reactions. The kinetics of CLA isomer−DPPH• reactions was investigated by measuring the disappearances of DPPH radicals in the reaction mixtures at room temperature (*8*). Eight levels of each CLA isomer were used in the kinetic study. One milliliter of CLA solution was mixed into 1 mL of 200 μM DPPH• ethanol solution. Absorbance of each reaction mixture at 517 nm was measured against an ethanol blank at 0, 5, 10, 20, 40, 80, 160, 320, 1000, and 2000 min. The DPPH• level remaining in each reaction vial at each tested time point was calculated using a DPPH• standard curve (*8*, *20*). The initial velocity of the CLA−radical reaction was obtained by plotting the absolute amount of DPPH• remaining against time at each level of CLA. The initial velocities were then plotted against the initial concentrations of individual CLA isomer to demonstrate the kinetic properties of CLA−radical reactions. The initial concentrations of individual CLA stock solutions were determined by gas chromatographic (GC) analysis. The ED_{50} of CLA was obtained by plotting the percent DPPH• remaining at steady state of the reaction against the corresponding CLA concentration. The ED_{50} is the level of individual CLA isomer required to quench 50% DPPH• under the experimental conditions. Triplicate reactions were carried out for each level of individual CLA isomers.

Synergistic Effects of c9,t11-CLA and t10,c12-CLA in Quenching DPPH Radicals. Total free radical scavenging capacities of selected combinations of c9,t11-CLA and t10,c12-CLA were estimated and compared according to the procedure described above using DPPH•. The final concentrations were 100 μM for DPPH• and 80 mg/mL for total CLA in each sealed reaction vial except for the control, which contained no CLA. The selected isomer combinations include (1) 100% c9,t11-CLA, (2) 75% c9,t11-CLA and 25% t10,c12-CLA, (3) 50% c9,t11-CLA and 50% t10,c12-CLA, (4) 25% c9,t11-CLA and 75% t10,c12-CLA, and (5) 100% t10,c12-CLA. The absorbance at 517 nm was measured against a blank of pure ethanol at 0, 15, 30, and 50 min of the reaction. Initial reaction velocity was obtained according to the procedure described above. All tests were conducted in triplicate.

Effect of Gastric Fluid on CLA Isomer Distribution. Eighty milligrams of c9,t11-CLA or t10,c12-CLA was added into and mixed with 20 mL of simulated gastric fluid prepared according to the *United States Pharmacopeia* (*21*). The resulting mixture was stirred at 37−39 °C. Aliquots (2 mL) were taken at 0, 15, 30, and 60 min. The aliquots were extracted immediately with 5 mL of nitrogen-saturated hexane at ambient temperature. The hexane was not removed from each extract until methylation. The tests were done in duplicates.

Gas Chromatographic Analysis. The levels of pure CLA isomer (*cis*-9,*trans*-11 CLA or *trans*-10,*cis*-12 CLA) in the stock solutions and in gastric fluid treated samples were analyzed using a GC method after free CLAs were converted to their fatty acid methyl esters according to a previously described methylation procedure (*22*). Briefly, 1 mg of CLA was vortex-mixed with 0.2 mL of 4% HCl−MeOH, and the reaction was carried out for 5 min at ambient temperature. Distilled water (3 mL) was added to stop the reaction, and the reaction products were extracted with hexane (5 mL). The hexane extract was dried using anhydrous sodium sulfate, concentrated under a stream of N_2, and subjected to GC analysis.

GC analysis of individual CLA isomer contents was performed using an HP 6890 gas chromatograph equipped with an autosampler, Chemstation, and FID (Hewlett-Packard Co., Avondale, PA). A fused silica capillary column SP-2380 (30 m × 0.25 mm with a 0.25 μm film thickness) from Supelco (Bellefonte, PA) was used with helium as the carrier gas and the following temperature program: 165 °C for 6 min, then 0.4 °C/min to 175 °C, held for 4 min, followed by 10 °C/min to 195°C, and held for 1 min. The quantification of individual CLA isomers was performed using heptadecanoic acid (17:0) as an internal standard.

Statistic Analysis. Data are reported as mean ± SD for triplicate measurements, except ESR analysis. An independent samples *t* test (SPSS for Windows, version release 10.0.5, 1999, SPSS Inc., Chicago, IL) was conducted to identify differences among means ($n = 3$). A $p < 0.05$ was considered statistically significant.

RESULTS

ESR Results. ESR measurements showed that both c9,t11-CLA and t10,c12-CLA could directly react with and quench DPPH radicals under the experimental conditions (**Figure 1**). No differences in ESR absorbance were observed among samples containing 0 CLA, 10 mg/mL c9,t11-CLA, and 10 mg/mL t10,c12-CLA at the beginning of the reactions (**Figure 1A1−C1**), whereas suppressed ESR absorbance was detected

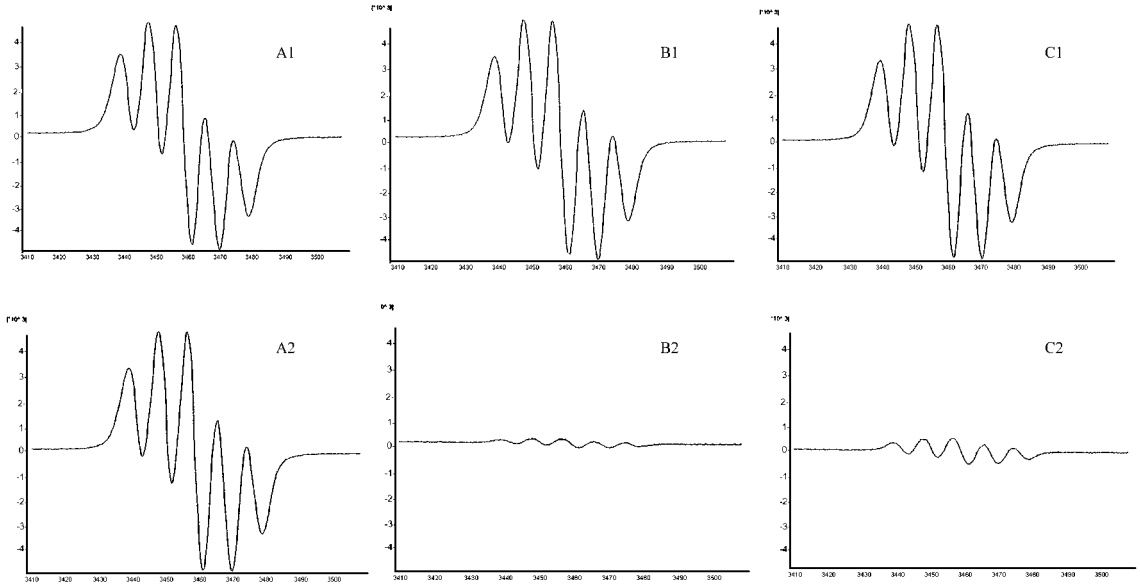

Figure 1. Free radical scavenging activity of CLA isomers determined by ESR. The final concentration of DPPH• was 2.0 mM in all tested samples. A1, B1, and C1 are the ESR spectra measured at the beginning of CLA–DPPH radical reactions, whereas A2, B2, and C2 represent the ESR absorbance determined in the samples after 15 h of incubation at 38–40 °C, respectively. The final CLA concentrations were 0 for A1 and A2, 10 mg/mL of c9,t11-isomer for B1 and B2, and 10 mg/mL of t10,c12- isomer for samples C1 and C2.

in the samples containing either c9,t11-CLA or t10,c12-CLA (**Figure 1A2**−**C2**) after incubation. However, ESR data did not demonstrate the different capacities of c9,t11- and t10,c12-CLA isomers in their free radical scavenging capacity because the experiments were not conducted in a quantitative manner, although the ESR absorbances in **Figures 1B2** and **1C2** might appear to be different.

Characteristics of Individual CLA Isomer—DPPH Radical Reactions. The dose and time effects of c9,t11-CLA and t10,c12-CLA are shown in parts A and B of **Figure 2**, respectively. With both CLA isomers, free radical scavenging activity was detected at all tested concentrations, and the lowest concentration was 2.5 mg of CLA/mL in ethanol (**Figure 2**). Dose dependence was observed for both c9,t11-CLA and t10,-c12-CLA under the same testing conditions. Higher concentrations of either CLA isomer were more effective in quenching free radicals in the system. The reaction kinetic curves of the two CLA isomers against DPPH radicals appear to be similar (**Figure 2**). The percent DPPH• remaining at steady state was plotted against the corresponding CLA concentration to obtain the ED_{50} values for the individual isomers. ED_{50} values of CLA to quench DPPH radical are approximately 56 mg CLA/mL for c9,t11-CLA and 60 mg/mL for t10,c12-CLA. Both CLA isomers were observed to immediately react and quench DPPH radicals at all tested levels, and no lag phase was observed in CLA−DPPH• reactions.

Initial velocities of CLA−DPPH reactions were determined for c9,t11-CLA and t10,c12-CLA at six selected concentrations for each isomer. t10,c12-CLA had greater initial velocity than c9,t11-CLA at all tested concentrations (**Figure 3**). However, c9,t11-CLA quenched more DPPH radicals in the reaction mixtures than t10,c12-CLA when the CLA−DPPH• reaction reached the steady state (1400 min) at all tested concentrations (**Figure 4**), although significant differences between these two isomers were observed only at 2.5, 5, 10, and 80 mg/mL levels ($p < 0.05$).

Synergistic Effects of c9,t11-CLA and t10,c12-CLA in Quenching DPPH Radicals. The synergistic effects between c9,t11-CLA and t10,c12-CLA in quenching DPPH radicals are illustrated in **Figure 5**. Mixtures of c9,t11-CLA and t10,c12-CLA, at ratios of 25:75, 50:50, and 75:25, reacted with DPPH radicals at greater initial velocities than either pure isomer on

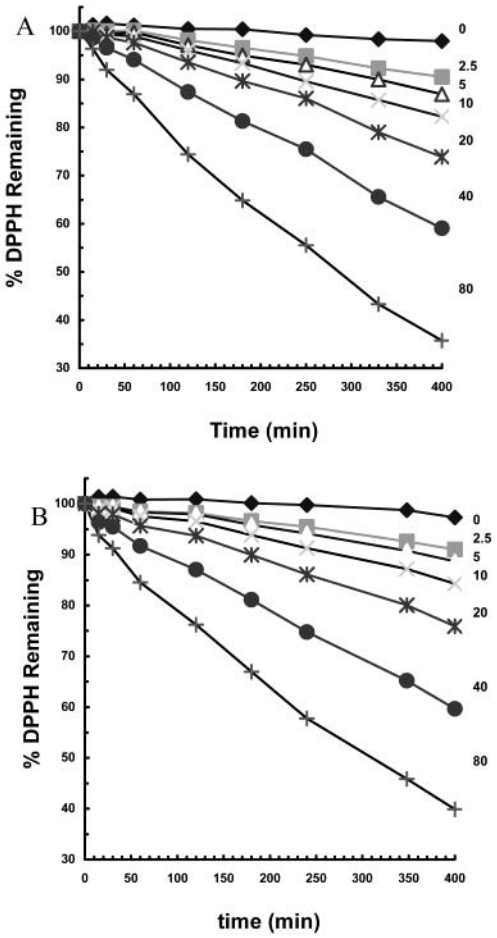

Figure 2. Dose and time effects of radical DPPH–CLA isomers: (A) reactions of c9,t11-CLA; (B) reactions of t10,c12-CLA; 0, 2.5, 5, 10, 20, 40, and 80 represent the final CLA concentrations (mg/mL) in the reaction mixtures. DPPH• concentration was 100 μM in all reaction mixtures.

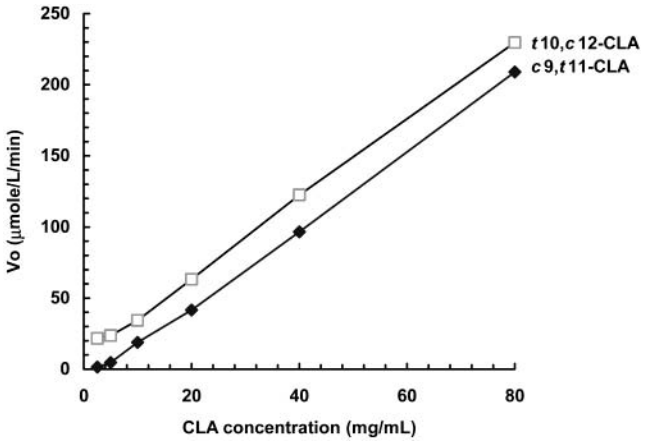

Figure 3. Initial velocity of CLA isomer–DPPH radical reactions. DPPH• concentration was 100 μM in all velocity measurements.

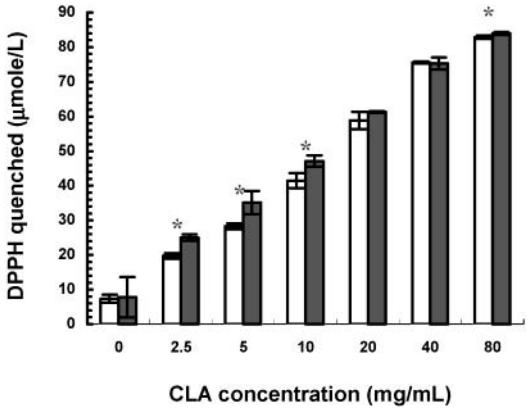

Figure 4. Thermodynamic properties of CLA isomer–DPPH radical reactions. Total DPPH radical scavenging capacities of c9,t11-CLA and c10,t12-CLA were measured and compared when the reactions reached equilibrium. DPPH• concentration was 100 μM in all reaction mixtures. The white columns represent t10,c12-CLA, whereas the shaded columns represent c9,t11-CLA. An asterisk (∗) at the top of a column indicates that a significant difference was detected between the two CLA isomers at that concentration. Vertical bars represent the standard deviation of each data point ($n = 3$).

the same concentration basis. The initial velocity is the micromoles of DPPH radicals quenched by the CLA at a final concentration of 80 mg/mL in 1 L of reaction mixture per minute. The initial velocity is a measurement of the radical scavenging capacity of CLA with an emphasis on the CLA−DPPH• reaction kinetics. A higher percentage of t10,c12-CLA in the mixture resulted in a greater initial velocity of the CLA−DPPH• reaction. In addition, it is further demonstrated in **Figure 5** that pure t10,c12-CLA resulted in a greater initial velocity than did c9,t11-CLA.

Effects of Gastric Fluid on CLA Isomer Distribution. To determine the effects of acidic gastric condition on the isomer distribution of CLA preparation, pure c9,t11-CLA and t10,c12-CLA were treated separately with the simulated gastric fluid for different time periods and analyzed by GC. The recovery rate of the CLA was >85%. The results showed that no t10,c12-CLA was converted to c9,t11-CLA, and no c9,t11-CLA was detected in treated t10,c12-CLA samples, at any of the tested time points. The coefficient of variation (CV) value for the GC analyses was <10%. Furthermore, no significant changes in total t,t-CLA were observed in the CLA samples treated with simulated gastric fluid for 0, 15, 30, and 60 min.

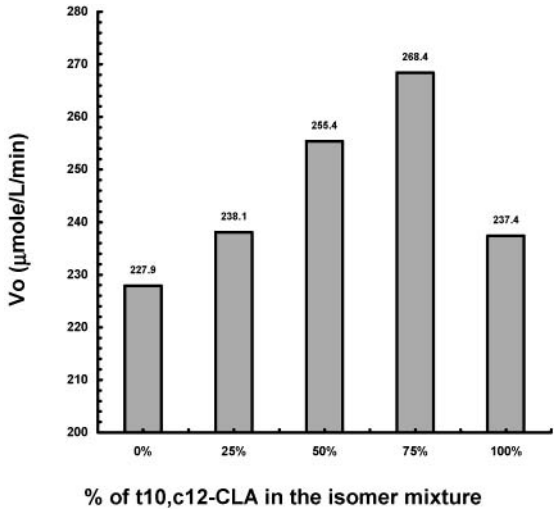

Figure 5. Synergistic effects of c9,t11-CLA and t10,c12-CLA in quenching DPPH radicals. Total DPPH• scavenging capacities of selected c9,t11-CLA and c10,t12-CLA combinations were measured and compared using initial velocities of CLA–radical reactions. Final concentrations were 80 mg/mL and 100 μM for total CLA and DPPH•, respectively. 0%, 25%, 50%, 75%, and 100% represent samples containing 100% c9,t11-CLA; 75% c9,t11-CLA and 25% t10,c12-CLA; 50% t11-CLA and 50% t10,c12-CLA; 25% c9,t11-CLA and 75% t10,c12-CLA; and 100% t10,c12-CLA, respectively, in the total CLA.

DISCUSSION

It has been accepted that free radicals and radical-mediated oxidation play roles in many aging-related health problems including cancers and heart diseases. Mixtures of CLA isomers have been investigated and demonstrated for their beneficial effects against carcinogenesis and atherosclerosis. Free radical scavenging capacities of CLA have been detected (*8*, *9*) and might contribute to their beneficial activities against these aging-related health problems. Recently, more evidence is indicating that c9,t11-CLA and t10,c12-CLA may act differently in biological systems and respond to the different biological and physiological effects (*4*). To better understand the beneficial effects of individual CLA isomers, it is important to investigate whether these isomers differ in their reactions with free radicals because free radical scavenging activity is a potential mechanism of their biological actions. This study was conducted to address (i) whether c9,t11-CLA and t10,c12-CLA act differently in the presence of radicals, (ii) whether and how the isomers influence each other in their radical scavenging properties, and (iii) whether the acidic condition of gastric fluid causes isomerization of individual CLA isomers.

Characteristics of the CLA−DPPH• reaction were evaluated using c9,t11- and t10,c12-CLA isomers in the present study by a spectrophotometric method. These characteristics included initial velocities of the reactions, the dose and time effects, and the total radical quenching capacity at steady state, which represents the kinetic and thermodynamic properties of the CLA−DPPH• reaction. The initial velocity of the t10,c12-CLA−DPPH• reaction is greater than that of the c9,t11-CLA−DPPH• reaction at all tested CLA levels, indicating that t10,c12-CLA differs from c9,t11-CLA in the kinetics of their reactions with DPPH radicals. Furthermore, t10,c12-CLA may be a kinetically preferred substrate for free radicals. This result is supported by the previous finding that t10,c12-CLA exhibited stronger oxyradical scavenging capacity than c9,t11-CLA in 70 min (*9*).

Similar to the CLA isomer mixture, both c9,t11- and t10,c12-isomers showed dose and time dependence in their reactions with DPPH radicals (*8*, *9*). Interestingly, both c9,t11-CLA and t10,c12-CLA had linear relationships between percent of DPPH• remaining in the system and reaction time (**Figure 2**), which is different from what was observed for the CLA isomer mix or pure linoleic acid (*8*). Furthermore, the ED_{50} values of c9,t11- and t10,c12-CLA isomers were 56 and 60 mg/mL, respectively. These ED_{50} values are much higher than that of the isomer mixture, 18 mg CLA/mL (*8*), suggesting that the isomer mixture may have a greater free radical scavenging capacity than any single isomer. In other words, individual CLA isomers may have synergistic effects in their radical scavenging activities. In the present study, a series of combinations of c9,-t11-CLA and t10,c12-CLA resulted in a more rapid initial velocity of CLA−DPPH• reaction (**Figure 5**). Changing the ratios of c9,t11-CLA/t10,c12-CLA from 100:0 to 75:25, 50:50, and 25:75 corresponded to a continuous increase in the initial reaction velocity. The mixtures at all testing ratios of the two CLA isomers had greater initial velocity than either pure isomer, on the same basis of final total CLA concentration. These data indicate that individual CLA isomers may have synergistic effects in their reactions with free radicals.

A synergistic effect between c9,t11- and t10,c12-CLA isomers was observed in a previous study (*19*). In that study, c9,t11-CLA, t10,c12-CLA, and a mixture of CLA isomers showed their inhibitory activities on human platelet thromboxane B2 formation from exogenously added [^{14}C]arachidonic acid. The I_{50} values were 15.6, 13.3, and 9.1 μM for c9,t11-CLA, t10,c12-CLA, and the isomer mixture of CLA, respectively. The I_{50} value is the inhibitor concentration required to reduce the cyclooxygenase activity to half of its original activity under the same experimental conditions. A stronger inhibitor has a lower I_{50} value. Therefore, the I_{50} values from this study indicate that t10,c12-CLA may be a stronger inhibitor for the human platelet cyclooxygenase than c9,t11-CLA and that the isomer mixture of CLA may be a stronger inhibitor of cyclooxygenase than any single CLA isomer including c9,t11-CLA and t10,c12-CLA. The finding from Tuitte and co-workers' study (*19*) is in agreement with our observation in the present study and supports the conclusion that individual CLA isomers may act and contribute differently to the biological effects of CLA and that there may be synergistic effects among individual isomers.

In contrast to the synergistic effects, the mixture of CLA isomers was observed to be a poorer substrate for the mitochondrial carnitine palmitoyl transferase I (CPT-I) compared to either c9,t11- or t10,c12-CLA isomer (*15*), suggesting that there may be inhibitory effects between CLA isomers. It was also reported that the isomer mix was a poorer substrate for the acyl-CoA oxidase (ACO) than t10,c12-CLA, but more favored than c9,t11-CLA. This observation indicated that interactions between individual CLA isomers might be more complex than synergistic or inhibitory effects. In addition, it was demonstrated in the same study (*15*) that both the CLA isomer mixture and t10,c12-CLA were better substrates for ACO than for CPT-I. Earlier in 1999, Moya-Camarena and co-workers (*14*) investigated the activities of individual CLA isomers and the isomer mixture of CLA on the activation of PPARα using a rat hepatoma cell line. They found that a mixture of CLA isomers, containing 41% of c9,t11-/t9,c11-CLA and 44.1% of t10,c12-CLA, was a significantly weaker activator for PPARα than c9,t11-CLA, t10,c12-CLA, or t9,c11-CLA. These results support the conclusion that individual CLA isomers may act and interact differently in contributing to each physiological activity observed for CLA preparations.

The present study also demonstrated that experimental design and testing conditions might alter the outcomes of individual CLA isomers in a particular bioactivity. c9,t11-CLA had a lower ED_{50} and removed more radicals when the CLA−DPPH• reaction reached the steady state (**Figure 4**), whereas t10,c12-CLA showed a greater initial velocity in reacting and quenching DPPH radicals at all testing concentrations (**Figure 3**). Determining the kinetics of the CLA−DPPH• reaction might have led to a conclusion that t10,c12-CLA is a stronger scavenger against DPPH• in this study, whereas measuring the remaining DPPH• at steady state might have resulted in a conclusion that c9,t11-CLA is a more powerful isomer to directly react and quench free radicals. This makes the comparison and interpretation of the results from different laboratories or different studies of CLA more challenging.

ESR spectrometry is a widely accepted method for free radical detections and has been successfully used to study radical scavenging capacities of antioxidants (*23*, *24*). The unpaired electron in a free radical results in a special ESR absorbance. This absorbance disappears when the free radical obtains an electron from antioxidants. ESR has been successfully employed to demonstrate the radical scavenging capacity of CLA isomer mixtures (*8*). In this study, ESR measurements showed that 10 mg/mL of either c9,t11- or t10,c12-isomer of CLA directly reacted with and quenched DPPH radicals in benzene, a nonpolar organic solvent, indicating that both c9,t11-CLA and t10,c12-CLA have radical scavenging capacities.

In conclusion, the present study demonstrated that both c9,t11- and t10,c12-CLA could directly react and quench DPPH radicals as measured by ESR and spectrophotometric methods. These two isomers differed in their kinetic and thermodynamic properties in reacting with DPPH radicals. t10,c12-CLA had a greater initial velocity in quenching DPPH radicals, whereas c9,t11-CLA scavenged more DPPH• at the steady state of the reaction. Synergistic effects were observed between c9,t11- and t10,c12-CLA isomers. In addition, no effect of acidic gastric fluid on CLA isomer distribution was detected under the experimental conditions. Additional studies are necessary to evaluate the radical scavenging capacity of individual CLA isomers in different free radical systems and under physiological conditions, the mechanism(s) of the CLA isomer−radical reactions, and the link between their radical scavenging properties and health beneficial effects.

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