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Food Chemistry 100 (2007) 972–976
Food Chemistry
www.elsevier.com/locate/foodchem

π-Complex formation of conjugated linoleic acid with iron

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Received 1 August 2005; received in revised form 3 November 2005; accepted 10 November 2005

Abstract

Conjugated linoleic acid (CLA) is known to have several beneficial biological effects in animal models, including anticarcinogenic and antiatherosclerotic effects, antiobesity, and antioxidant activity. However, reports of its antioxidant activities have been inconsistent. In this study, we investigated the possible occurrence of π-bonding between CLA and iron. CLA methyl ester was reacted with triiron dodecacarbonyl and confirmed to form π-complexes with iron tricarbonyl. This study may suggest the possible involvement of CLA in oxidation by way of interacting with iron.

Keywords: Conjugated linoleic acid; CLA; Iron

1. Introduction

Conjugated linoleic acid (CLA) is the acronym for a group of conjugated dienoic isomers of linoleic acid, primarily *cis*-9,*trans*-11 or *trans*-10,*cis*-12 octadecadienoic acid. Since its discovery as an anticancer principal from ground beef extract (Ha, Grimm, & Pariza, 1987), it has been studied extensively because of its wide range of beneficial biological activities, such as reducing the incidence of carcinogenesis in several animal models, reducing the severity of atherosclerosis, reducing adverse effects of immune stimulation, and reducing body fat while enhancing lean body mass in several animal species (Lee, Kritchevsky, & Pariza, 1994; Miller, Park, Pariza, & Cook, 1994; Pariza, Park, & Cook, 2001; Park et al., 1997).

A trace amount of transition metals is essential in many biological systems. π-Complexes with transition metals can be very stable and quite effective in certain reactions as catalysts. It has been postulated that the trace amount of transition metals in biological systems might be in the form of π-complex as intermediates in an enzymatic reaction involving a coenzyme (King, Treichel, & Stone, 1961; Manuel, Stafford, & Stone, 1961; Nakamura & Tsutsui, 1963, 1964). The possible use of organometallic complexes in the synthesis of interesting and important natural products has caught the attention of a number of organic chemists (Hwang, Liao, Horng, & Ong, 1989; Mandon & Astruc, 1989; Pearson & Shively, 1994; Pearson, 1981; Pearson, Gelormini, & Pinkerton, 1992). The objective of this research was to investigate the possible occurrence of π-bonding between CLA and the transition metal iron.

2. Materials and methods

2.1. Materials

Iron pentacarbonyl was purchased from Aldrich Chemical Company (Milwaukee, WI). Linoleic acid was purchased from Nu-Chek Corporation (Elysian, MN) and CLA (conjugated octadecadienoic acid) was prepared and methylated as described previously (Chin, Liu, Storkson, Ha, & Pariza, 1992). The purity of CLA was 97.8% (45.7% *cis*-9,*trans*-11, 47.6% *trans*-10,*cis*-12, 1.71% *trans*,

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doi:10.1016/j.foodchem.2005.11.006

trans, 3.04% other isomer). Potassium permanganate was purchased from Fisher Scientific (Fair Lawn, NJ) and ferrous sulfate, 8-hydroxyquinoline, kojic acid, silica gel, and alumina were from Sigma Chemical Company (St. Louis, MO). All solvents used were HPLC grade (EM Science, Gibbstown, NJ). Thin layer chromatography (TLC) was performed with Whatman pre-coated silica gel plates (60 Å, 250 μm thickness) with fluorescence at 254 nm (Whatman Ltd., Maidstone Kent, England).

High-performance liquid chromatography (HPLC) was performed with a Beckman 110B Solvent Delivery Module equipped with 163 Variable Wavelength Detector. A Beckman DU-50 Series Spectrophotometer was used for the ultraviolet spectra (Beckman, Fullerton, CA). Gas chromatography (GC) was conducted with a Hewlett–Packard 5890 Series II fitted with a flame ionization detector and 3396A integrator (Hewlett–Packard, Avondale, PA). Mass spectrometry (MS) was conducted with a Finnigan 4500 Mass Spectrometer (Finnigan Corp., San Jose, CA).

2.2. Preparation of triiron dodecacarbonyl

Triiron dodecacarbonyl was prepared by oxidation of the anion $HFe(CO)_4^-$ with manganese dioxide as previously described (King & Stone, 1963). Briefly, iron pentacarbonyl (60 g, 0.3 mol) was treated with 12.5 M NaOH for 30 min, and then 125 ml saturated ammonium chloride was added. Separately, manganese dioxide was prepared by heating potassium permanganate until a brown precipitate of manganese oxide was formed. This MnO_2 was added to the buffered $HFe(CO)_4^-$ solution and incubated for 1 h. The excess MnO_2 was decomposed by addition of a solution of iron (II) sulfate heptahydrate dissolved in 2 N H_2SO_4. The black precipitate (triiron dodecacarbonyl) was filtered and washed successively with 2 N H_2SO_4, 95% ethanol, and petroleum ether. This precipitate was kept under nitrogen.

2.3. Preparation of CLA/Me–iron complex(es)

CLA methyl ester (CLA/Me)–iron complex(es) (CLA/Me–iron) were prepared as previously described (Nakamura & Tsutsui, 1964). Briefly, CLA/Me and triiron dodecacarbonyl in benzene were heated to 80–90 °C under nitrogen. The brown precipitate was removed by filtration and the yellow filtrate was evaporated to give a yellow semisolid. The semisolid was separated quickly by deactivated alumina. Elution with hexane and with hexane–benzene mixture (5:1 to 1:1, v/v) gave a yellow semisolid (reaction mixture A).

Reaction mixture A was separated with silica gel column chromatography by eluting with hexane:acetone (100:1, v/v). The suspected CLA/Me–iron complex fraction (reaction mixture B) was further purified with HPLC (column: Ultrasphere-ODS, C18 reversed phase, 5 μm, 20 × 4.5 mm, acetonitrile:water with mobile phase (85:15, v/v), 2 ml/min flow rate, and peaks were detected at 245 nm).

Thin layer chromatography was performed using a precoated silica gel plate and detected with UV absorbance (254 nm) and color development using 8-hydroxyquinoline-kojic acid (Zweig & Sherma, 1972). Reaction mixture A and purified reaction mixture B were analyzed by examining their ultraviolet spectra, TLC, HPLC, and gas chromatography. Gas chromatography was conducted using a Hewlett–Packard 5890 Series II fitted with a flame ionization detector and a 3396A integrator. A Supelcowax-10 fused silica capillary column (60 mm × 0.32 mm i.d., 0.25 μm film thickness, Supelco Inc., Bellefonte, PA) was used with split mode and helium as the carrier gas. Injector and detector temperatures were 250 °C. Oven temperature was programmed from 50 to 200 °C, increased 20 °C per min, held there for 50 min, increased 10 °C per min to 230 °C, and held for 20 min. The mass was measured from purified reaction mixture B with GC–MS, performed using the same GC conditions described above with a Finnigan INCOS 50 GC/MS (Finnigan Corp., San Jose, CA).

3. Results and discussion

CLA/Me had maximum absorbance at 232 nm, and iron pentacarbonyl did not have any absorbance at the same wavelength (Fig. 1). However, reaction mixture A had absorbance at 205 nm and 217 nm, the latter is the shift of maximum absorbance of CLA/Me. This suggests possible complex(es) formation of CLA/Me and iron tricarbonyl on the conjugated double bond. Fig. 2 shows the TLC diagram. Reaction mixture A (represented as #2 in TLC plate) had unknown compound(s) besides CLA/Me. This unknown compound(s) turned black after spraying with 8-hydroxyquinoline-kojic acid, which indicated the presence of iron in the compound(s) (Zweig & Sherma, 1972). From the R_f value, the unknown compound(s) had higher polarity than that of CLA/Me. Gas chromatograms (data not shown) and high-performance liquid

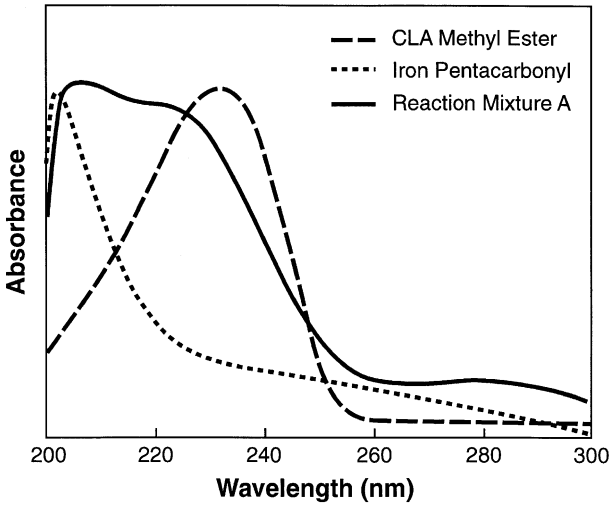


Fig. 1. Ultraviolet spectra of CLA methyl ester, iron pentacarbonyl, and reaction mixture A in methanol solution.

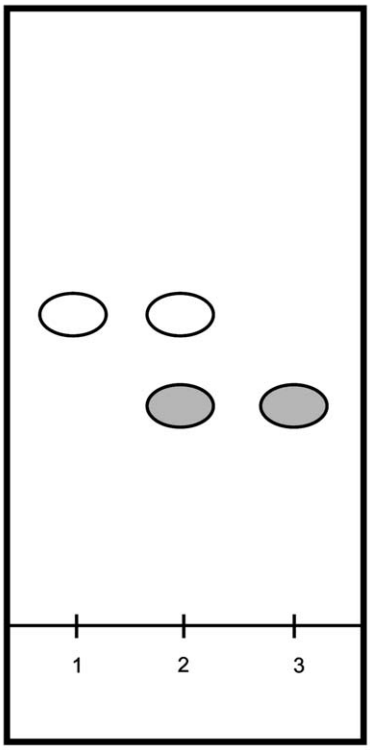


Fig. 2. Thin layer chromatogram of CLA methyl ester (1), reaction mixture A (2), and reaction mixture B (3). Developing solvent was hexane: acetone (20:1, v/v), and detection by ultraviolet absorbance at 254 nm (open circles) and by spraying with 8-hydroxyquinoline-kojic acid (filled circles).

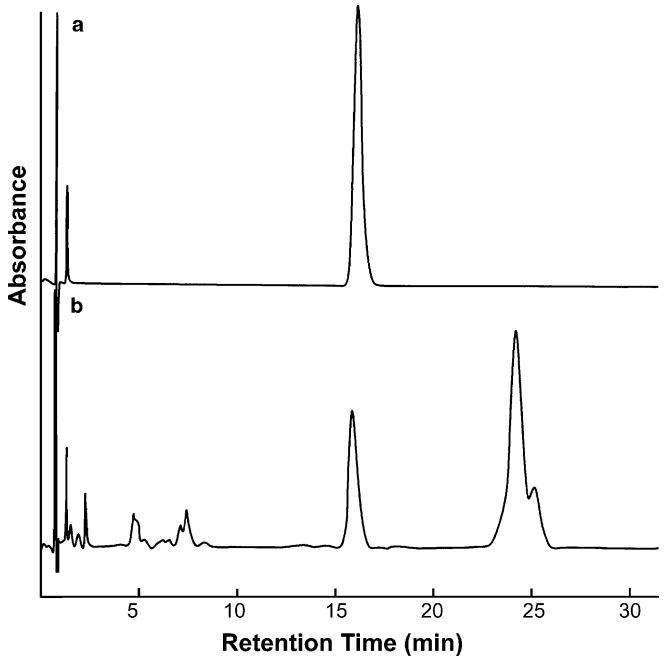


Fig. 3. High-performance liquid chromatograms of CLA methyl ester (a) and reaction mixture A (b). * indicates π-complex(es) formed with CLA methyl ester and iron. A reversed phase C18 column (20 × 4.5 mm) was used. Solvents were acetonitrile:water (85:15) with the flow rate of 2 ml/min. Peaks were detected at 245 nm.

chromatograms (Fig. 3) confirmed the presence of a new compound(s).

After further purification with column chromatography and HPLC, we collected reaction mixture B (represented as #3 in Fig. 2). Reaction mixture B showed the same characteristics in GC and HPLC as the unknown compound(s) of reaction mixture A, which confirms that this is the newly formed product. Since CLA is a mixture of mainly two isomers, the reactivity to iron between CLA isomers may differ. However, GC analysis showed no preferential disappearance of specific CLA isomers (data not shown), which indicates both major isomers may have reacted with iron in a similar manner. From previous reports (Nakamura & Tsutsui, 1964; von Gustorf, Pfajfer, & Grevels, 1971), we inferred that the reaction mixture B may contain mainly π-complexes with iron tricarbonyl and CLA/Me (I, Fig. 5). This was further confirmed by mass spectrometry (Fig. 4). The molecular weight peak [M^+, CLA/Me–$Fe(CO)_3$, 434.2] was not detected, however, this may be due to the liable nature of carbonyls as suggested by Nakamura and Tsutsui (1964) and Pearson (1981). The peaks at *m/e* 350.2 and 378.2 indicated $M^+ - 3(CO)$ and $M^+ - 2(CO)$, respectively. Reacting CLA/Me with $FeCl_3$ did not form any CLA-iron complex (data not shown) suggesting that it is likely that carbonyls were eliminated during the GC–MS process rather than formation of CLA/Me complex with Fe alone. Even though the molecular weight peak was not detected, the possibility of formation of dimers [2(CLA/Me)–Fe(CO), II in Fig. 5] was eliminated because of the peak at *m/e* 378.2 [$M^+ - 2(CO)$] and the weak extent of *m/e* 294 (CLA/Me molecular weight) (von Gustorf et al., 1971). This study suggests π-complex formation between CLA methyl ester, particularly conjugated dienes, and iron tricarbonyl (I, Fig. 5) (Nakamura & Tsutsui, 1964; Pearson, 1981). Although this was an in vitro study between CLA and iron, it implies the possible involvement of CLA in oxidative reactions by interactions with iron molecules.

Effects of CLA as an oxidation are not consistent. In earlier studies, it was suggested that CLA has antioxidant activities in vitro (Ha, Storkson, & Pariza, 1990). In addition, Yurawecz, Hood, Mossoba, Roach, and Ku (1995) reported the formation of an antioxidative furan fatty acid from CLA. This is also supported by others suggesting that CLA can be effective in protecting against oxidative damage under both in vitro and in vivo conditions (Kim et al., 2005; Palacios, Piergiacomi, & Catala, 2003; Yu, 2001). Contrarily, others have reported that the antioxidant effects of CLA were not different from arachidonic or linoleic acid, and were much less than the effects of butylated hydroxytoluene (BHT) and vitamin E in their systems, in both in vitro and in vivo models (Banni et al., 1998; Cantwell, Devery, O'Shea, & Stanton, 1999; Chen, Chan, Kwan, & Zhang, 1997; Seo, Endo, & Fujimoto, 1999; van den Berg, Cook, & Tribble, 1995; Yamasaki et al., 2000; Yang, Leung, Huang, & Chen, 2000).

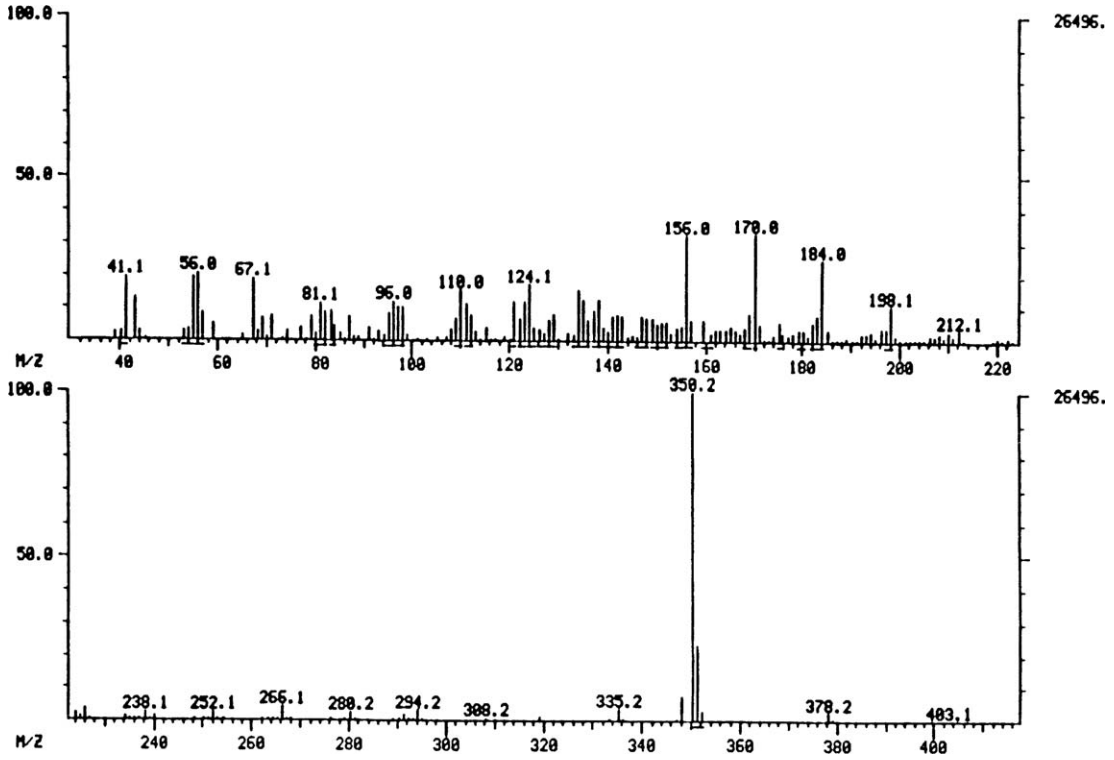
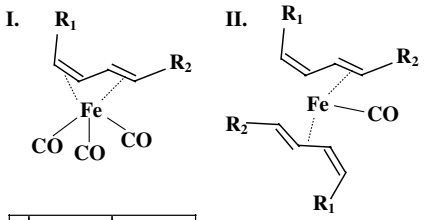


Fig. 4. Mass spectrum of CLA methyl ester–iron complex(es) or reaction mixture B.



	c9,t11 CLA	t10,c12 CLA
R1	$(CH_2)_7COOH$	$(CH_2)_4CH_3$
R2	$(CH_2)_5CH_3$	$(CH_2)_8COOH$

Fig. 5. Suggested structure of CLA methyl ester–iron tricarbonyl (I) and a possible structure for CLA methyl ester dimmer with iron carbonyl (II).

Although van den Berg et al. (1995) suggested that oxidative effects of CLA were independent of metal ions, this inconsistency regarding CLA's oxidative effects may need further evaluation based on our data of π-complex formation between CLA and iron.

In summary, π-complex formation between CLA methyl ester and iron tricarbonyl was investigated and confirmed. Further studies are required to determine the possible occurrence and significance of complex formation between CLA and iron in biological systems.

Acknowledgements

We thank Ms. Jayne M. Storkson for assistance with manuscript preparation. This research was supported in part by gift funds administered through the University of Wisconsin-Madison, Food Research Institute, and by the Department of Food Science at the University of Massachusetts.

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