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Enzymatic and oxidative metabolites of lycopene☆

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Abstract

Using the post-mitochondrial fraction of rat intestinal mucosa, we have investigated lycopene metabolism. The incubation media was composed of NAD^+, KCl, and DTT with or without added lipoxygenase. The addition of lipoxygenase into the incubation significantly increased the production of lycopene metabolites. The enzymatic incubation products of $^2H_{10}$ lycopene were separated using high-performance liquid chromatography and analyzed by UV/Vis spectrophotometer and atmospheric pressure chemical ionization–mass spectroscopy. We have identified two types of products: cleavage products and oxidation products. The cleavage products are likely: (1) 3-keto-apo-13-lycopenone ($C_{18}H_{24}O_2$ or 6,10,14-trimethyl-12-one-3,5,7,9,13-pentadecapentaen-2-one) with λmax = 365 nm and m/z = 272 and (2) 3,4-dehydro-5,6-dihydro-15,15′-apo-lycopenal ($C_{20}H_{28}O$ or 3,7,11,15-tetramethyl-2,4,6,8,12,14-hexadecahexaen-1-al) with λmax = 380 nm and m/z = 284. The oxidative metabolites are likely: (3) 2-apo-5,8-lycopenal-furanoxide ($C_{37}H_{50}O$) with λmax = 415 nm, 435 nm, and 470 nm, and m/z = 510; (4) lycopene-5, 6, 5′, 6′-diepoxide ($C_{40}H_{56}O_2$) with λmax = 415 nm, 440 nm, and 470 nm, and m/z = 568; (5) lycopene-5,8-furanoxide isomer (I) ($C_{40}H_{56}O$) with λmax = 410 nm, 440nm, and 470 nm, and m/z = 552; (6) lycopene-5,8-epoxide isomer (II) ($C_{40}H_{56}O$) with λmax = 410, 440, 470 nm, and m/z = 552; and (7) 3-keto-lycopene-5′,8′-furanoxide ($C_{40}H_{54}O_2$) with λmax = 400 nm, 420 nm, and 450 nm, and m/z = 566. These results demonstrate that both central and excentric cleavage of lycopene occurs in the rat intestinal mucosa in the presence of soy lipoxygenase.

Keywords: Lycopene; Rat; Metabolites; Oxidation; Cleavage; Enzyme

1. Introduction

Lycopene has been implicated as a potential chemopreventive agent with respect to cancer. Reports from the epidemiological studies [1-5], studies in animals [6-9] and cell cultures [10-14] all suggest that lycopene has anticarcinogenic properties. These reports have given rise to several hypotheses that the inverse relation between lycopene intake and cancer risk might ascribe to 1) lycopene as an antioxidant, 2) increasing cell-cell communication, 3) reducing mutagenesis, 4) inhibiting tumor cell proliferation, and 5) improving antitumor immune responses [15]. However, the mechanism(s) by which this carotenoid might exert its biological activities and thereby modulate disease processes is still unknown. In 1996, Clinton et al. suggested the occurrence of *in vivo* isomerization of lycopene, since they detected higher amounts of *cis*-lycopene than *all-trans*-lycopene in human serum and in both benign and malignant prostate tissue [16]. In contrast to β-carotene, few studies have investigated the metabolism of lycopene in a biological system, and very little is known about oxidative breakdown products of lycopene in humans. The first report of a metabolite in human plasma was that of 5,6-dihydroxy-5′,6′-dihydrolycopene resulting from oxidation of lycopene

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Abbreviations: DTT, dithiothreitol; EDTA, ethylenediamine-tetraacetic acid; Hepes, N-(2-hydroxyethyl) piperazine-N′–(2-ethanesulfonic acid); HPLC, high-performance liquid chromatography; KCl, potassium chloride; LC/APCI-MS, liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry; LOX, lipoxygenase (soybean); NAD^+, nicotinamide adenine dinucleotide; RT, retention time; THF, tetrahydrofuran.

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[17,18]. It also reported that 2,6-cyclolycopene-1,5-diol A and B are *in vivo* oxidative metabolites of lycopene in humans [19,20].

Our laboratory has carried out extensive studies on the metabolism of β-carotene and we found that the enzymatic cleavage of β-carotene to retinoids can occur either by an excentric or central cleavage pathway depending on the absence or presence of antioxidants [21], and have identified various intermediates of the excentric cleavage of β-carotene [22-25]. Interestingly, it was found that β-carotene may react with either fatty acid hydroperoxides or their derivatives and quench alkoxyl and/or peroxyl radicals [26]. A similar approach was used in the present work to understand lycopene metabolism by intestinal enzyme with/without adding lipoxygenase. Since nothing is known about intestinal metabolism of lycopene, we investigated the metabolism of lycopene *in vitro* in the presence of intestinal mucosa and soy lipoxygenase.

2. Materials and methods

2.1. Chemical products

Deuterated all-trans lycopene (12, 12′, 14, 14′,19, 19, 19, 19′, 19′, 19′-$^{2}H_{10}$-lycopene, $^{2}H_{10}$ lycopene or D_{10} lycopene was provided by BASF (Ludwigshafen, Germany) (Fig. 1). The standards of lycopene 1,2-epoxide, 2,6-cyclolycopene-1,5-epoxide I, 2,6-cyclolycopene-1,5-epoxide II and 2,6-cyclolycopene-1,5-diol, were kindly provided by Dr. Frederick Khachik (University of Maryland). Hepes, soy lipoxygenase, NAD^{+}, DTT, Tween 40, and formaldehyde were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All HPLC solvents were obtained from J.T. Baker Chemical Co. (Philipsburg, NJ, USA) and were filtered through a 0.45 μm membrane filter before use. Lycopene solutions were prepared under red light immediately before use.

2.2. Animals

Male rats (n = 20) of the Sprague-Dawley strain (300-500 g) were purchased from CAMM Research Animals (Wayne, NJ, USA). Animals were housed in the animal facility at the USDA Human Nutrition Research Center on Aging (HNRCA) at Tufts University and received Purina rat chow (Ralston Purina, St. Louis, MO, USA) and water ad libitum. Rats were fasted overnight before euthanasia under CO_2. The upper half of the intestines of the rats was collected. All animal procedures were reviewed and approved by the Animal Care and Use Committee of the USDA Human Nutrition Research Center on Aging at Tufts University.

2.3. Lycopene purification

D_{10} lycopene was purified by neutral aluminum oxide column chromatography [26]. Lycopene used for the incubation experiments is shown in Fig. 1 as it was analyzed using HPLC (Fig. 1A), UV-Visible spectruscopy (Fig. 1B) and LC/APCI-MS (Fig. 1C).

2.4. Sample preparation and incubation

The incubation of post-mitochondrial fraction and its incubation with lycopene were essentially as described previously [21,27]. Briefly, the fresh upper half of the intestine was washed with ice-cold isotonic saline (0.85% NaCl) and cut lengthwise. The mucosa was gently scraped off with a razor blade on ice and homogenized for 20 sec on ice using a Brinkmann (Westbury, NY, USA) Polytron homogenizer, with 50 mM Hepes buffer (weight:volume = 1:4), pH 7.4, containing 1 mM EDTA, 1.15% KCl, and 0.1 mM DTT. A post-nuclear fraction was prepared by centrifugation of the intestinal homogenate at 800*g* for 30 min in a Sorvall RT 6000 refrigerated centrifuge (Du Pont Co., Newtown, CT). The post-nuclear fraction was obtained by centrifugation in a L8-70MW ultracentrifuge (Beckman Inc., Palo Alto, CA, USA) at 10,000*g* at 4°C for 60 min in order to remove particulate matter and some organelles. To obtain the purified post-mitochondrial fraction, the resultant supernatant solution was applied to an Econo-Pac 10 DG Disposable Column (30 x 10 ml, BioRad Laboratories, Hercules, CA, USA) equilibrated with cold 10 mM Hepes buffer, pH 7.4, containing 0.1 mM EDTA, 0.05 M KCl, and 0.1 mM DTT. The protein fraction, eluted in the void volume (mol. wt. > 6 kDa) was then divided into aliquots of 1.5 ml and stored in liquid nitrogen until use. The post-mitochondrial fractions were used 12 hr to 2 weeks after collection. The protein concentration was determined using the BCA (bicinchoninic acid) Protein Assay (Pierce Co. Rockford, IL, USA). The standard incubation mixture contained 1.0-2.0 mg of protein fraction, 20 mM Hepes buffer, pH 8.0, 800 units LOX, 0.2 mM NAD^{+}, 2 mM DTT, 150 mM KCl, in a total volume of 1000 μl. After pre-incubation at 37°C in a shaking water bath for 5 min, the enzyme reaction was started by adding 80 μl of lycopene solubilized in aqueous Tween 40 to 920 μl of the incubation mixture with a final concentration of 2.7-4.9 μM of lycopene. After incubation for up to 90 min at 37°C, the incubation was terminated by adding 100 μl of formaldehyde (37%, w/w), and post-incubated for 10 min at 37°C. The vials were uncovered and the incubation mixture was exposed to room air as the gas phase. Two control vials were run lacking either lycopene or the protein fraction. We studied the effects of incubation period, protein fraction concentration, NAD^{+}, LOX, and formaldehyde. Crystalline D_{10} lycopene was solubilized in Tween 40/acetone (1:19, v/v) in a 1:10,000 ratio by weight (0.3 mg of D_{10} lycopene dissolved in 3.3 g of Tween 40/acetone) in a quartz tube since the lycopene tends to be adsorbed onto the surface of regular glass test tubes. To yield a clear mixture, the solution was vortexed and sonicated 5 x 3 min at 4°C, and the acetone was evaporated under N_2. Water (9 ml) was then added, and the solution

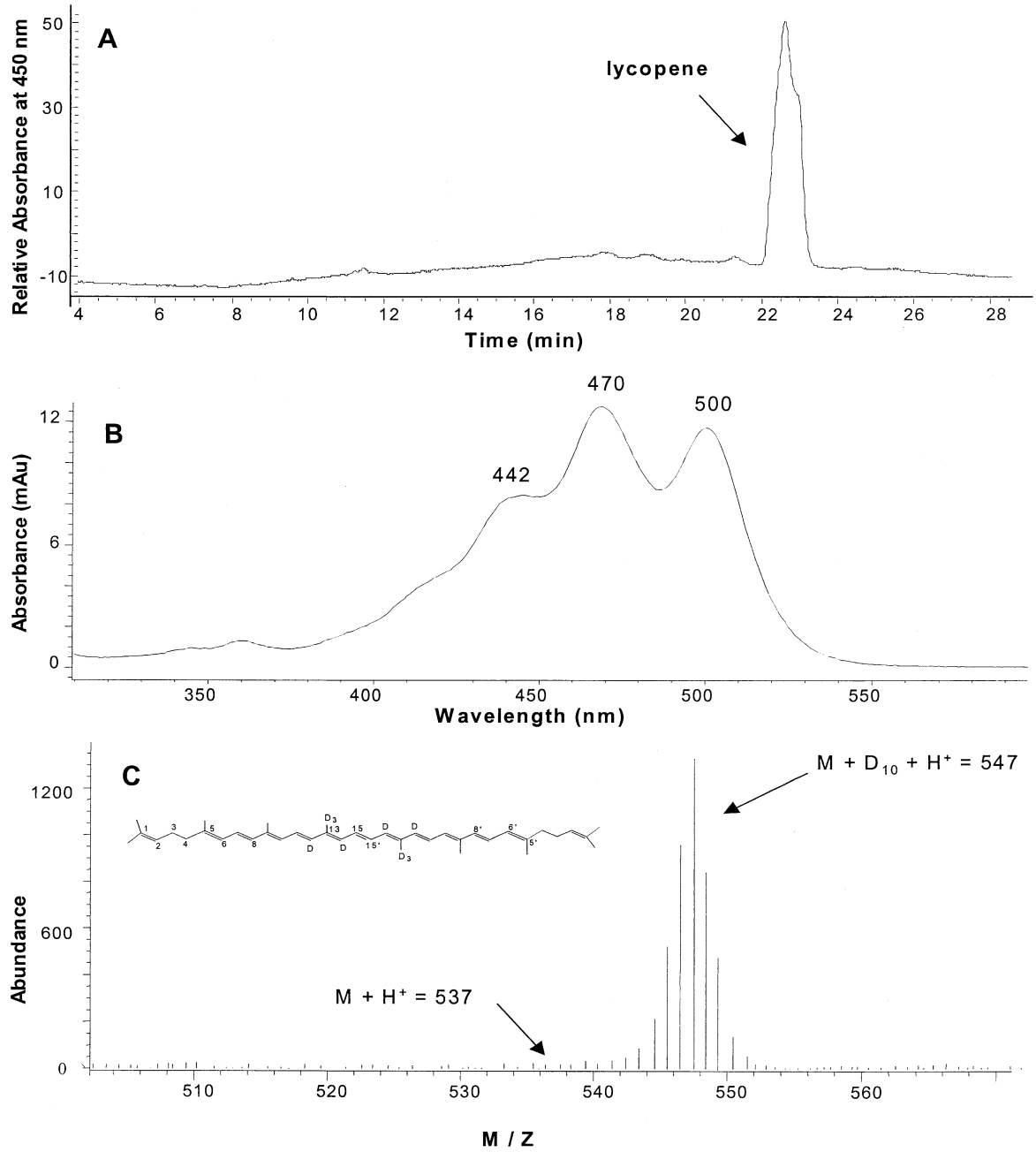

Fig. 1. Synthetic lycopene used for the incubation procedures. (A), HPLC profile of purified deuterated lycopene using a C18 column with the UV detector set at 450 nm. (B), Representative UV-Visible absorption spectrum of deuterated lycopene. (C), MS profile of D_{10} lycopene from LC/MS analysis.

was again vortexed and sonicated 5 x 3 min at 4°C. Lycopene solution was added to the incubation mixture as soon as it was prepared. All experimental procedures were repeated five times and carried out under red light.

2.5. HPLC analyses

Tissue extraction was done using 1.5 ml of $CHCL_3$: CH_3OH (2:1, v/v) plus 0.5 ml of hexane, followed by 1.5 ml of hexane. The mixture was centrifuged for 20 min at 800*g* at 4°C. After centrifugation, the chloroform and hexane layers were combined and evaporated to dryness under N_2. The residue was re-dissolved in 150 μl ethanol, sonicated twice for 30 sec each time and vortexed for 30 sec. A 50 μl aliquot of the final extract was injected into the HPLC system after centrifugation for 2 min at 800*g* at 4°C. The HPLC system consisted of Series 410LC pumps (Perkin-Elmer, Inc. Norwalk, CT, USA), Waters 717 plus autosampler (Waters, Milford, MA, USA), a Pecosphere-3 C18 0.46 x 8.3 cm cartridge column (Perkin-Elmer, Inc. Nor-

walk, CT, USA), 994 programmable photodiode array detector and Waters Millennium 32 software. The HPLC mobile phase was CH_3CN : THF : H_2O (50:20:30, v/v/v, 1% ammonium acetate in H_2O, solvent A) and CH_3CN : THF : H_2O (50:44:6, v/v/v, 1% ammonium acetate in H_2O, solvent B). The gradient procedure at a flow rate of 1 ml/min was as follows: 90% solvent A and 10% solvent B were used for 2 min followed by a 12 min linear gradient to 100% solvent B, a 15 min hold at 100% solvent B, then a 2 min gradient back to 90% solvent A and 10% of solvent B. The Waters 490E multi-wavelength spectrophotometer detector was set at 340, 375 and 450 nm, respectively. Unknown peaks were quantified by determining peak areas in the HPLC chromatograms. Furthermore, absorption spectra were recorded by the diode array detector for the unknown peaks and compared with standards.

2.6. LC/APCI-MS analysis of metabolites

The final extract from incubation re-dissolved in 100 μl ethanol was evaporated to dryness under N_2. The residue was re-suspended in 300 μl $CHCl_3$ and applied to Sep-Pak Vac NH_2 cartridges (3 ml) (Waters, Milford, MA, USA) rinsed with hexane. The Sep-Pak column was then eluted with 8 ml hexane. The eluent was evaporated under N_2, and the residue was re-suspended in 200 μl ethanol for LC/MS analysis using atmospheric pressure chemical ionization (APCI). The same mobile phase described for HPLC, (except the absence of ammonium acetate), was pumped through a 0.005 in. ID PEEK by an Agilent 1100 LC pump at a flow rate of 1.0 ml/min. The Agilent 1100 HPLC was equipped with an autosampler, UV/VIS diode array detector, and a quaternary pump. An Esquire LC (Wilmington, DE, USA) mass spectrometer (Bruker Daltonic, Inc., Bremen, Germany) with APCI source and ion trap was used for analyses. APCI was performed in the positive mode [28]. The vaporizer temperature, drying gas temperature, and corona current were all optimized with regard to maximum signal intensity of molecular ions. The following optimal conditions were used: vaporizer temperature, 300°C; nebulizer pressure, 50 psi of N_2; drying gas temperature 350°C. The counter-current drying gas was supplied from a liquid N_2 cylinder, and was optimized at a flow rate of 4.0 L/min. The APCI source was a corona discharge from a needle generated by the following potentials: corona needle = + 2200 V, endplate offset = −500 V, and capillary = −4000 V. The mass scan range was at 200 to 700 daltons in 0.1 dalton steps with an accumulation time at 50 ms and a half maximum peak width of 0.6 daltons. From the LC/APCI-MS analysis, the mass spectra for unknown metabolites with a characteristic isotopic profile similar to D_{10} lycopene were identified to determine their mass.

2.7. Statistical analysis

Results are expressed as mean ± SEM and the significance of differences were calculated by Student's t test and ANOVA using SigmaStat version 2.0 for Windows 95, NT & 3.1 (Jandel Scientific Software, San Rafael, CA, USA). Differences were considered significant when $P < 0.05$.

3. Results

3.1. Peak identification by HPLC

After incubation of lycopene in the absence of tissue and LOX, there were only minor peaks observed at 340 nm (Fig. 2A) and some distinct peaks at 450 nm (Fig. 2C), suggesting that there was some auto-oxidation of lycopene during the 90 min incubation. After incubation with tissue and LOX, many new peaks were observed by HPLC at both 340 (Fig. 2B) and 450 nm (Fig. 2D). We were able to identify seven major peaks (peaks 1-7) based on their well-defined UV-visible spectra, and these were analyzed further by LC/APCI-MS to determine possible mass of each peak. As shown in Fig. 2, peak 1 had a retention time of 6.6 min and was seen at a wavelength setting of 340 nm (Fig. 2B). Peaks monitored at 450 nm were at retention times 11.3, 15.5, 16.5, 18, 22, and 27.5 min, and are labeled peaks 2, 3, 4, 5, 6, and **7,** respectively (Fig. 2D). Relatively minor peaks in the HPLC profile were not studied for identification because these peaks consisted of a mixture of products without well-defined spectral properties, or were the same as the peaks from the control incubations.

When tissue was added to the incubation, the quantitative analysis of peak areas for the seven peaks (Table 1) indicated significant increases in the production of these compounds, and the increases of these compounds were 2 to 128 fold over baseline. When LOX was added to the incubations without tissue, the increases in the production of the 7 peaks were 2 to 31 fold over baseline, in which, the production of peaks 1, 6, and 7 were significantly lower than those produced from the incubation with tissue alone. When both tissue and LOX were added to the incubation together, the production of peaks 1, 3, 4, and 5 showed an added effect as compared to incubations with either tissue or LOX alone. Although only peak 1 was significantly greater using incubations with the combination of tissue and LOX vs the incubations with tissue alone or LOX alone (synergistic effect), peaks 2, 3, 4, 5, 6, and 7 were of the same magnitude when using tissue alone as when using the combination of tissue and LOX (no effect) (Table 1). To obtain maximum production of these compounds, we carried out incubations with both tissue and LOX to identify these compounds.

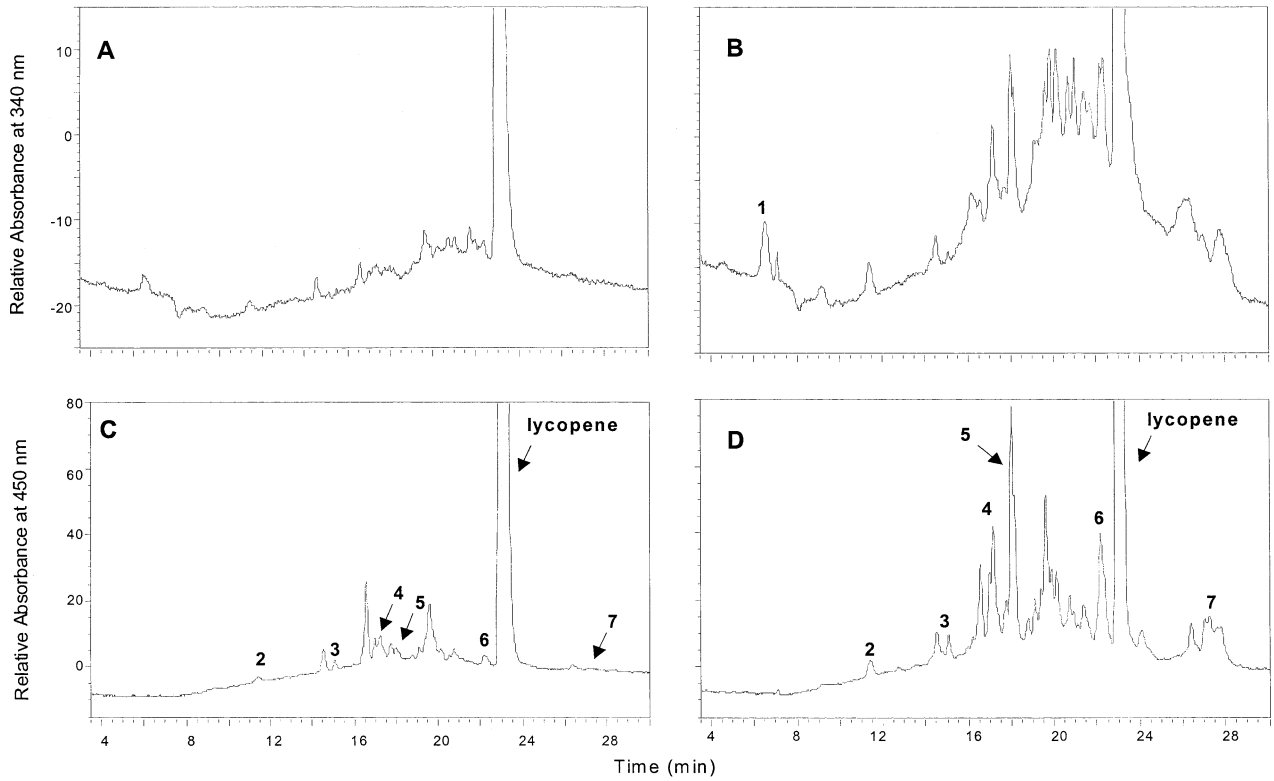

Fig. 2. Oxidative and enzyme-catalyzed cleavage products of lycopene. Purified D_{10} lycopene 2.7 μM (solubilized in aqueous Tween 40) was incubated in absence (A, C) or presence (B, D) of purified post-mitochondrial fraction of rat intestine (2 mg protein) plus LOX (800 units), and cofactors (NAD^+, DTT) for 90 min at 37°C in a total volume of 1 ml. The HPLC profiles of the extracts are displayed when monitored at both at 340 nm (A, B) and 450 nm (C, D). The peak at 22.8 min is lycopene.

3.2. Peak identification using UV/VIS and LC/APCI

The *in vitro* metabolites which were collected from the HPLC system were applied to LC-APCI-MS (positive ion mode) to determine the molecular masses. Metabolites were identified by the presence of a semi-symmetrical pattern of molecules, each differing by a single mass unit attributable to the deuterium from the parent D_{10} lycopene (Fig. 1). The incubation of deuterated lycopene with both post-mitochondrial fraction of rat intestinal mucosa and lipoxygenase produced two groups of products: cleavage products (peaks 1 and 2) and oxidation products (peaks 3-7).

Table 1
Effect of tissue and/or lipoxygenase (LOX) on the formation of lycopene products[b]y the post−mitochondrial fraction of rat[1,2,3]

Condition	1	2	3	4
Tissue	−	+	−	+
LOX	−	−	+	+
Peaks				
1	33.1 ± 6.4[a]	135.5 ± 12.9[b]	69.8 ± 9.1[c]	181.3 ± 14.1[d]
2	26.3 ± 1.1[a]	84.7 ± 12.3[bd]	55.5 ± 4.8[b]	77.8 ± 5.6[d]
3	27.8 ± 3.1[a]	66.6 ± 5.9[b]	60.9 ± 7.5[b]	81.1 ± 7.4[b]
4	47.4 ± 3.7[a]	412.3 ± 79.4[b]	375.7 ± 69.9[b]	539.4 ± 134.3[b]
5	16.5 ± 8.5[a]	396.2 ± 72.4[bd]	302.7 ± 47.2[bc]	642.7 ± 120.4[d]
6	60.5 ± 2.5[a]	337.2 ± 49.7[b]	136.6 ± 35.9[a]	336.3 ± 58.8[b]
7	6.4 ± 1.1[a]	819.3 ± 171.9[b]	201.7 ± 80.5[ac]	797.6 ± 97.4[bd]

[1] Values are mean of areas ($\times 10^3$) ± SEM of five separate experiments. Values in the same row with different superscript letters are significantly different, $p < 0.05$ (Student's test).

[2] Cofactors (NAD^+, DTT) and lycopene (4.9 μM) solubilized in aqueous Tween 40 were incubated at 37°C in presence (+) or absence (−) of purified post-mitochondrial fraction (less than 10 days since it was collected and stored under liquid nitrogen) of rat intestine (1 mg protein), and (+) or (−) LOX, for 90 min.

[3] Peak 1 was monitored at 340 nm. Peaks 2-7 were monitored at 450 nm.

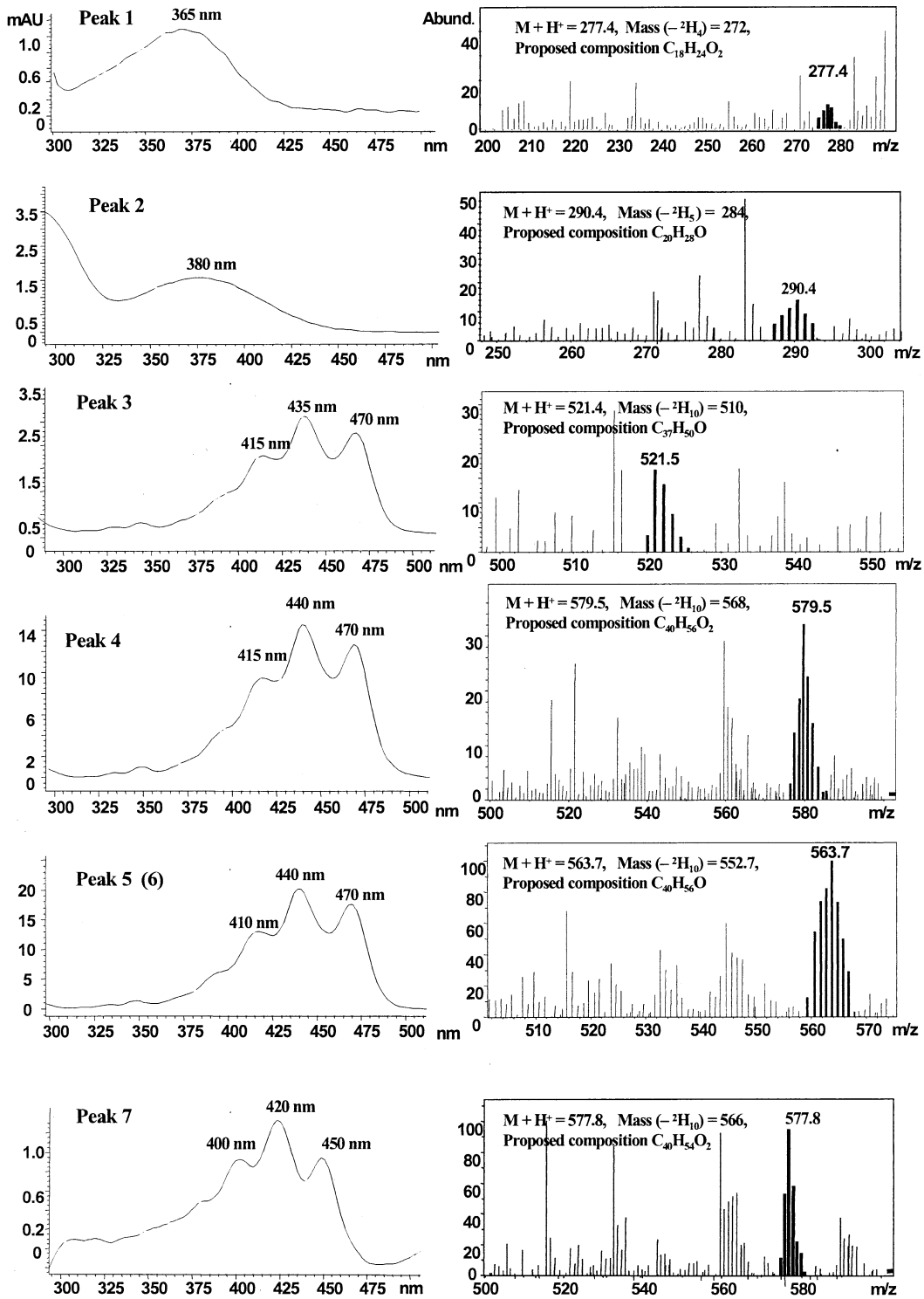

Fig. 3. UV/Vis diode array detection and LC/APCI-MS analysis of lycopene metabolites when purified post-mitochondrial fraction of rat intestine (1 mg protein) was incubated at 37°C for 90 min with LOX (800 units), cofactors (NAD^+, DTT) and 4.9 μM lycopene solubilized in aqueous Tween 40.

3.3. Cleavage products

As shown in Fig. 3, peak 1 (retention time = 6.6 min) had a single absorption peak at 365 nm and m/z = 277 for the protonated molecular mass. When corrected for H^+ and for 4 deuterium atoms, the molecular mass was 272.4 and the proposed composition is $C_{18}H_{24}O_2$. The absorption maximum (365 nm) of this peak is similar to a compound with a carbonyl conjugated to 4 double bonds (365 nm) reported as a product of autoxidation of lycopene [29].

Since peak 1 has an additional atom of oxygen, we are proposing the structure for peak 1 as 3-keto-apo-13-lycopenone or 6,10,14-trimethyl-12-one 3,5,7,9,13-pentadecapentaen-2-one. Peak 2 (retention time = 11.2 min) has a λmax = 380 nm and m/z = 290 for a protonated molecular ion. Correcting for five deuterium atoms would give a m/z = 284 and a composition of $C_{20}H_{28}O$. This composition corresponds to 15,15′-apo-lycopenal (acycloretinal), but the maximum absorbance at 380 nm suggests that peak 2 has a carbonyl conjugated to only 5 double bonds. We propose the structure for peak 2 as 3,4-dehydro, 5,6-dihydro-15,15′-apo-lycopenal (Fig. 3).

3.4. Oxidation products

Peak 3 (retention time = 15.2 min) has λmax at 415 nm, 440 nm, and 470 nm and m/z = 521 for $(M + H)^+$. Correcting for 10 deuterium atoms yields a mass of 510, corresponding to a $C_{40}H_{56}O_2$ composition. The spectrum indicates that there are no conjugated carbonyls, and is very similar to ε,ε-carotene with 9 conjugated double bonds [30]. The structure proposed is 2-apo-5,8- lycopenal-furanoxide (Fig. 3). There are no previous reports on this compound.

Peak 4 (retention time = 16.2 min) showed λmax = 415 nm, 440 nm, and 465 nm and m/z = 579 for $(M + H)^+$. Correcting for 10 deuterium atoms yields a mass of 568, corresponding to a $C_{40}H_{56}O_2$ composition. It was assigned as lycopene-5,6,5′,6′-diepoxide (Fig. 3). This assignment is in agreement with the structure with an identical mass and absorption maximum described earlier [31].

Peak 5 (retention time = 18.0 min) has a λmax = 410 nm, 440 nm, and 465 nm and m/z = 563 for $(M + H)^+$. Correcting for 10 deuterium atoms yields a mass of 552, corresponding to a $C_{40}H_{56}O$ composition. The spectrum indicates a chromophore of 9 conjugated double bonds, and it was assigned as lycopene-5,8-furanoxide. This structure has not been identified before.

Peak 6 had similar UV/Vis absorbance peaks and an identical mass as peak 5, but with a different retention time (22.0 min). Therefore, Peaks 5 & 6 were assigned as lycopene-5,8-epoxide isomers.

Peak 7 (retention time = 27.5 min) had λmax = 400 nm, 420 nm, 450 nm and m/z = 577 for $(M + H)^+$. Correcting for 10 deuterium atoms yields a mass of 566, corresponding to a $C_{40}H_{56}O_2$ composition. The absorption peak is reminiscent of carotenoids with 8 conjugated double bonds [27], and on the bases of the chromophore and mass, it was assigned as 3-keto-lycopene-5′,8′-furanoxide.

The proposed structures of these peaks are presented in Fig. 4. All these metabolites showed different UV/Vis profiles and masses from the standards: 1) 1,2-epoxide, 2,6-cyclolycopene-1,5-epoxide I, 2) 2,6-cyclolycopene-1,5-epoxide II, 3) and 2.6-cyclolycopene-1,5-diol.

4. Discussion

It has been shown that under certain conditions provitamin A carotenoids, such as β-carotene [32] and non-provitamin A carotenoids, such as canthaxanthin [33], astaxanthin [34] and lycopene [35-38] can inhibit neoplastic transformation. In addition, a recent report [34] demonstrated that lycopene and/or its oxidative metabolites can reduce proliferation of cultured human prostate cancer cells. In contrast, it has also been reported that lycopene or its metabolites may induce oxidative DNA damage in the human foreskin fibroblast Hs68 cell [39]. It is not clear whether those effects are due to the intact carotenoid molecules or its metabolites.

In the present study, we used deuterated lycopene with a characteristic enrichment profile that helped to identify the incubation products. Oxidative and cleavage products of lycopene appeared when lycopene was incubated with a post-mitochondrial fraction of rat intestine and cofactors with/without LOX at 37°C for up to 90 min. All lycopene metabolites were formed after 15 min incubation with the post-mitochondrial fraction of rat intestine with LOX at 37°C, and the amount was not significantly changed between 60 to 90 min of incubation. Our observation is not like the auto-oxidation of lycopene reported by Kim et al., where the lycopene was incubated in 37°C for 24 to 60 hr to obtain incubation products [29].

Our assignment for peak 2 is based on the maximum UV/Vis absorbance at 380 nm. The maximum absorbance of standard acyclolycopenal was reported as 399 nm [29,40]. Therefore, structure of 3,4-dehydro-5,6-dihydro-15,15′-apo-lycopenal is a reasonable assignment.

Our results demonstrate that both central and excentric cleavage of lycopene occur in rat intestinal mucosa. In addition, the products of lycopene were both oxidative products as well as enzyme-catalyzed cleavage products. β-carotene produced many different apo-carotenals under similar incubation conditions as previously reported [27], whereas lycopene produced only a few of them. Rather more epoxides and furanoxides were formed, suggesting that lycopene can be readily oxidized during incubation as compared with β-carotene. Under our experimental incubation conditions, which was not an acid catalyzed condition as previously reported [20], we did not observe any 1,2-epoxide, 2,6-cyclolycopene-1,5-epoxide or related compounds, and 2.6-cyclolycopene-1,5-diol. In addition, our incubation procedure produced significant amount of *cis* isomers (Peaks 5 & 6) from the original *all-trans* lycopene.

We used the APCI-MS to identify lycopene metabolites, because APCI-MS has been successfully used before to analyze the intact β-carotene molecule with the highest sensitivity [28]. Due to limited production of the metabolites of lycopene and current NMR detection limits, the proposed metabolites from the lycopene incubation in this study have not been confirmed by an on-line LC-NMR analysis.

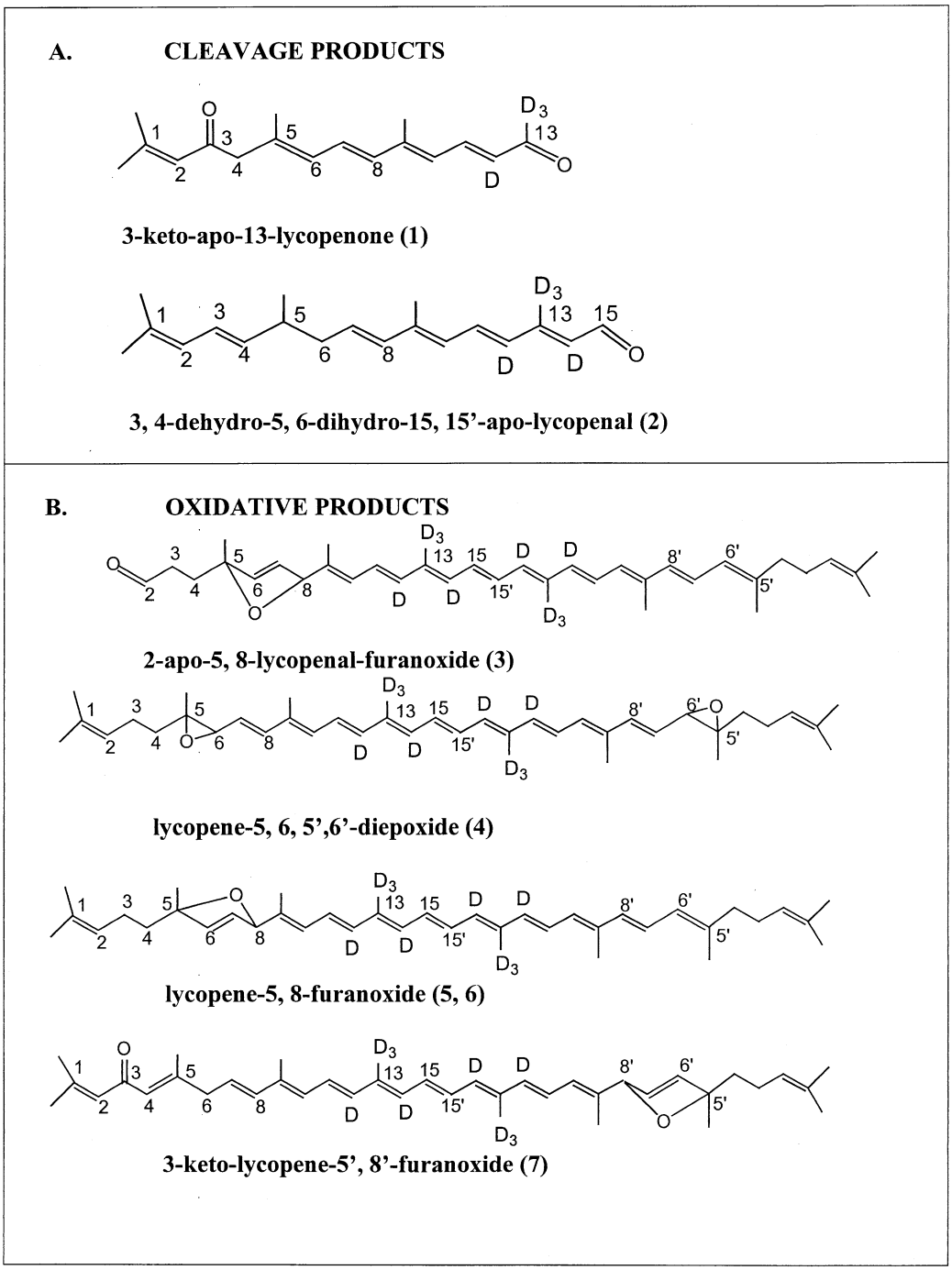

Fig. 4. Proposed structures of the metabolites detected through the incubation of D_{10}-lycopene with rat intestinal mucosa post-mitochondrial fraction and soy lipoxygenase. The purified post-mitochondrial fraction of rat intestine (1 mg protein) was incubated at 37°C for 90 min with LOX (800 units), cofactors (NAD^+, DTT) and 4.9 μM lycopene solubilized in aqueous Tween 40.

It is well known that retinoic acid is involved in the regulation of gene expression through the nuclear receptors RAR and RXR [33,41]. Under current incubation conditions, we found 3,4-dehydro-5,6-dihydro-15,15′-apo-lycopenal but not the corresponding acid. This may be due to low production of 3,4-dehydro-5,6-dihydro-15,15′-apo-lycopenal (peak 2) as shown in Table 1 and Fig. 2 under the incubation conditions used. In addition, it may be due to concentrations of cofactor, NAD^+ (0.2 mM), protein (2 mg), and lycopene (4.9 μM) used in the incubation, which could be insufficient to produce the corresponding acid. In the autoxidation of lycopene experiment [29], 2 mM NAD and 9.1 mg protein were used to convert acycloretinal to acycloretinoic acid. It has been reported that the addition of 1 mM NAD^+ to the β-carotene incubation media produced retinoic acid as well as retinal and β-apo-carotenal [21].

Although the biological importance of these lycopene metabolites, including their *cis-trans* isomers, is still unknown, the health effect attributed to lycopene in humans may be due to the activity of some of these oxidation products.

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