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Antioxidant activity of lycopene extracted from tomato paste towards trichloromethyl peroxyl radical $CCl_3O_2^{\cdot}$

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Abstract

The objectives of this paper were to evaluate the antioxidant activity of lycopene towards the trichloromethyl peroxyl radical ($CCl_3O_2^{\bullet}$) and to obtain a precise rate constant using a pulse radiolysis technique. Lycopene was extracted and purified from tomato paste and its purity reached 95.0% (HPLC analysis). $CCl_3O_2^{\bullet}$ was generated from a pulsed aqueous air-saturated solution of 2-propanol (2 M), acetone (1 M) and CCl_4 (10^{-2} M). The observed spectrum showed an intense bleaching of the lycopene ground-state absorption in the region 350–450 nm and the bleaching rate constant was determined to be 1.17×10^9 $M^{-1}s^{-1,}$ which indicated a rapid interaction between lycopene and $CCl_3O_2^{\bullet}$.

Keywords: Antioxidant; Lycopene; Trichloromethyl peroxyl radicals; Pulse radiolysis

1. Introduction

Lycopene (molecular structure as Fig. 1) is one dietary carotenoid found in fruits such as fresh ripe tomato, watermelon, papaya guava, grapefruit and one primary carotenoid component accumulated in human tissues and fluids such as the prostate, and the serum after absorption. Recent epidemiological studies show that supplementation of diets rich in lycopene reversely connects with the risk of many chronic diseases, such as cancers and heart diseases (Edward-Giovannucci, 1999; Rao & Agarwal, 1998, 1999), and the benefits of such diets are partly attributed to lycopene contents, and in nature to the antioxidant activity of lycopene, according to a free radical oxidative damage theory known in the field of pathology (Maix, 1987).

The antioxidant activity of lycopene has been extensively evaluated based on its ability to scavenge free radical in free radical models, or to protect cell components against oxidative damage in cell culture models, or in animal models. The accumulated experimental evidence suggests that lycopene can quench singlet oxygen (Di Mascio, Kaiser, & Sies, 1989), scavenge free radicals of nitrogen dioxide ($NO_2^{\bullet}$), thiyl ($RS^{\bullet}$) and sulphonyl ($RSO_2^{\bullet}$; Mortensen, Skibsted, Sampson, Rice-Evans, & Everett, 1997) and inhibit oxidative damage to lymphocytes DNA (Collins, Olmedilla, Southon, Granado, & Duthie, 1998) and to cell membrane (Woodall, Britton & Jackson, 1997). All the evidence is helpful for an understanding of the antioxidant role that lycopene can play.

Carbon tetrachloride (CCl_4) is a xenobiotic which produces hepatotoxicity in humans as well as in animals (Frederick, Ighofimoni, & Julie, 1998). The hepatotoxic effect of CCl_4 is thought to result from its reductive dehalogenation by cytochrome P-450 to the highly reactive trichloromethyl radical ($CCl_3^{\bullet}$; McGregor & Lang, 1996; Recknagel, Glende, Dolak, & Walter, 1989), and further to the trichloromethyl peroxyl radical ($CCl_3O_2^{\bullet}$) in the presence of oxygen (Slater, 1978). The interactions between some carotenoids and $CCl_3O_2^{\bullet}$ have been reported previously (Tessa, Edward, David, Wolfgang-Schalch, Jane, & George, 1995). However, the direct interaction of lycopene with $CCl_3O_2^{\bullet}$ has not, to date, been reported.

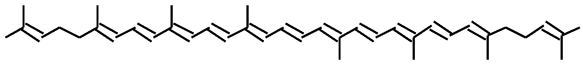

Fig. 1. Molecular structure of lycopene.

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The objectives of this paper are to study the direct interaction of lycopene with the $CCl_3O_2^{\bullet}$ radical and to obtain a precise rate constant using a pulse radiolysis technique.

2. Materials and methods

2.1. Chemicals

Tomato paste was purchased from Shanghai Tomato Paste Factory (Shanghai, China). Hexane, alcohol, KOH and 2-propanol were analytical grade and purchased from Shanghai Chemical Reagent Shop (Shanghai, China). Distilled water was made in our laboratory. Nitrogen, (Purity, 99.99%) was purchased from Shanghai Gas Shop (Shanghai, China)

2.2. Extraction and purification of lycopene

Lycopene was extracted from tomato paste. The extraction procedure was as follows: tomato paste was pretreated, using 95% alcohol at 50 °C. Then, the pretreated tomato paste was extracted, using hexane at 50 °C. After extraction, hexane was evaporated to dryness under a stream of nitrogen with a vacuum rotating evaporator (Shanghai No. 6 instrument Factory, Shanghai, China).

The tomato paste extract was further purified by saponification, referring to a patent (No. WO 98/43620). The saponification was carried out in a 40 wt% KOH solution. Tomato paste (10 g) extract was first dispersed into 2-propanol (1:5, wt./wt.) at 60 °C for 1 h under a stream of nitrogen. Then 37 wt% KOH solution (1:4, v/v) was added and the mixture was stirred at 50 °C for 2 h. Finally, the mixture was washed with distilled water to neutrality and then filtered to obtain 0.50 g lycopene crystals. The purity of lycopene crystals reached 95.0% as assessed by high performance liquid chromatography (HPLC).

2.3. HPLC analysis

The HPLC system (HP, Hewlet Packard, USA) consisted of a 5 μm particle size Zorbax ODS column (USA; 250×4.6 mm), and a UV detector. The flow phase was methanol; the flow rate was 1.0 ml/min; the injection volume was 10 μl; the peaks were detected at 473 nm (maximum absorbance for lycopene in the flow phase).

2.4. Pulse radiolysis

The pulse radiolysis experiment was conducted using a linear accelerator, providing a 10 MeV electron pulse with a duration of 8 ns at room temperature. The dosimetry of the electron pulse was determined by a thiocyanate dosimeter containing 10 mM KSCN solution, by taking $\epsilon\ (SCN)^- = 7600\ mol^{-1}l\ cm^{-1}$ at 480 nm. In the present work, the average pulse dose was 20 Gy. The reaction was monitored by a 500 W xenon lamp. When pulse electron and analytical light vertically passed the 20 mm quartz sample cell, light signal of reaction in the solution would enter the 44 W grating through reflection and was transformed into a digital signed and recorded in a computer.

2.5. Generation of the $CCl_3O_2^{\bullet}$ radical

$CCl_3O_2^{\bullet}$ was generated in aqueous air-saturated 2-propanol (2 M) and acetone (1 M) solution containing CCl_4 (10^{-2} M) by pulse radiolysis. The reactions were described as follows (Packer, Slater & Wilson, 1998).

$$H_2O \rightarrow OH - + H^{\bullet} + \text{e-aq}$$

$$OH^- + (CH_3)_2CHOH \rightarrow H_2O + (CH_3)_2C^{\bullet}OH$$

$$H^{\bullet} + (CH_3)_2CHOH \rightarrow H_2 + (CH_3)_2C^{\bullet}OH$$

$$\text{e-aq} + (CH_3)_2CO \rightarrow (CH_3)_2CO^{\bullet} \rightarrow (CH_3)_2C^{\bullet}OH$$

$$CCl_4 + (CH_3)_2C^{\bullet}OH \rightarrow (CH_3)_2CO + CCl_3^{\bullet} + H^+ + Cl^-$$

$$CCl_3^{\bullet} + O_2 CCl_3O_2^{\bullet}$$

All these reactions were completed within 1 μs, and the primary products after radiolysis was $CCl_3O_2^{\bullet}$.

3. Results and discussion

3.1. Identification of lycopene extracted from tomato paste

The HPLC spectrum (473 nm) of lycopene extracted from tomato paste is shown in Fig. 2, where there are two peaks at retention times of 17.02 and 22.31 min, respectively. The peak at 17.02 min represented beta-carotene, as suggested from its corresponding UV spectrum, which displayed the maximum characteristic peaks of beta-carotene (454 and 482 nm in the inset), while the peak at 22.31 min represented lycopene, as shown by its corresponding UV spectrum, which contained four characteristic absorption peaks of lycopene (296, 444, 473 and 501 nm in inset). Compared with the areas of the 22.31 min peak and that of 17.02 min peak, it could be found that lycopene was the primary occurring carotenoid while beta-carotene was only in a trace. The purity of lycopene reached 95.0%, as assessed by HPLC. It is known that tomato paste contains many other carotenoid such as phytoene, phytofluene, zeta-

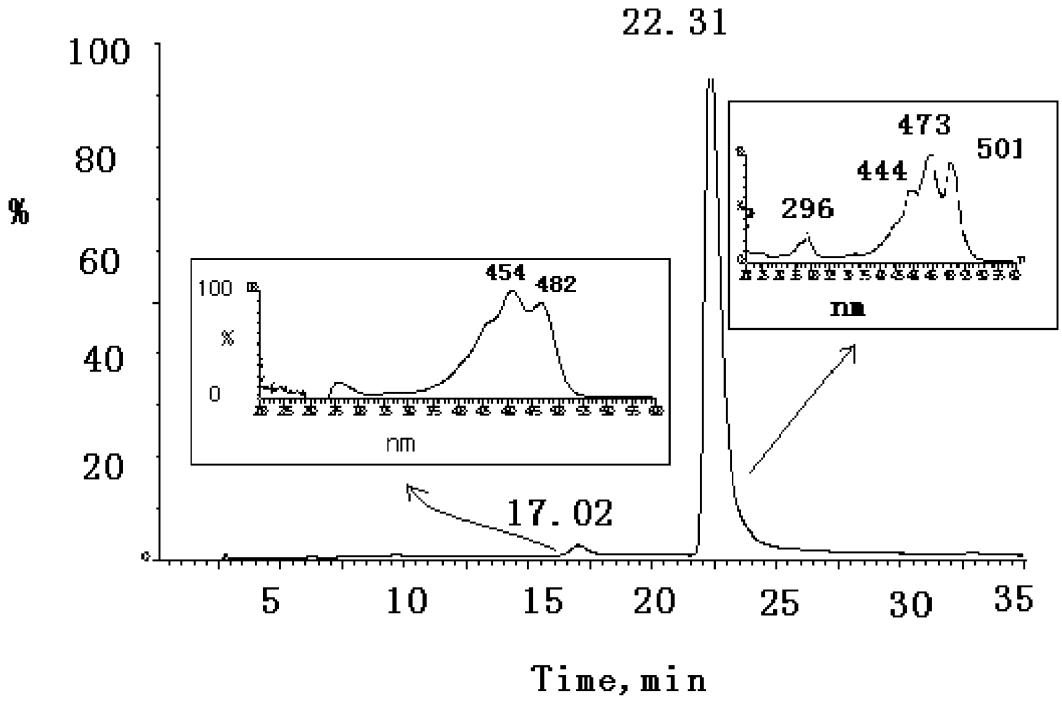

Fig. 2. HPLC spectrum (473 nm) of lycopene extracted from tomato paste. Inset: the UV spectra of corresponding peaks at 17.02 and 22.31 min.

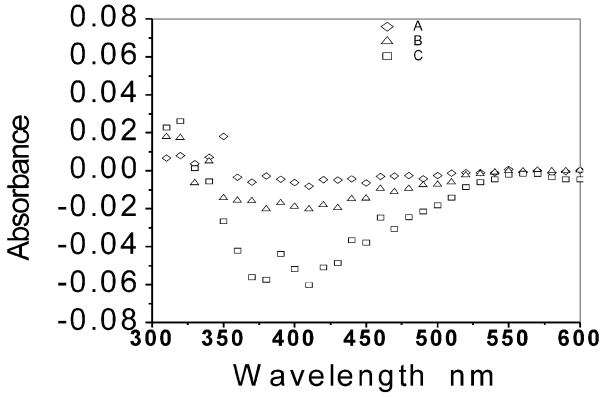

Fig. 3. Time-resolved absorption spectrum after pulse radiolysis of 0.5 mM lycopene in aqueous air-saturated 2-propanol (2 M) and acetone (1 M) solution containing CCl_4 (10^{-2} M). A: 3 μs; B:10 μs; C:50 μs.

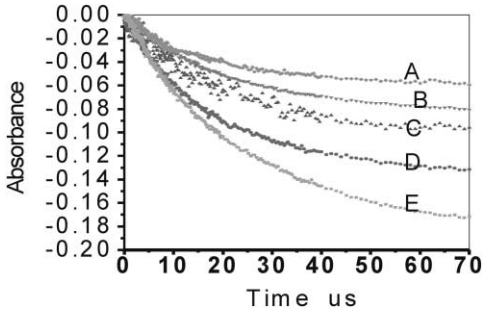

Fig.4. Kinetic absorption traces observed at 430 m following pulse radiolysis of of (A) 0.02 mM, (B) 0.1 mM, (C) 0.2 mM, (D) 0.3 mM, and (E) 0.5 mM lycopene in aqueous air-saturated 2-propanol (2 M) and acetone (1 M) solution containing CCl_4 (10^{-2} M).

carotene, gamma-carotene and neurosparene, but they were unseen in this spectrum. They may be removed during pretreatment or saponification treatment.

3.2. Interaction of lycopene with $CCl_3OO^{\bullet}$

Fig. 3 shows the transient spectrum of the interaction of lycopene and $CCl_3OO^{\bullet}$. The observed spectrum showed an intense bleaching of lycopene ground-state absorption in the region 350–450nm after 50 μs. The bleaching degree of lycopene at 430 nm varied with its concentration and is displayed in Fig. 4. The bleaching rate constant of lycopene was determined to be 1.17×10^9 $M^{-1}s^{-1}$ from the slope of linear plot of the observed bleaching rate (K_{obs}) versus lycopene concentration in Fig. 5. The bleaching is a common phenomenon, occurring in the interaction between carotenoid pigments and free radicals (Mortensen et al., 1997), which directly suggests a loss of the carotenoids and indirectly reflects an ongoing interaction. In this experiment, the bleaching of the lycopene ground state absorption reflected the interaction between lycopene and $CCl_3O_2^{\bullet}$.

Our result showed that lycopene could react with $CCl_3O_2^{\bullet}$ at a rate constant of 1.17×10^9 $M^{-1}s^{-1}$. This rate constant value was among those of 1.9×10^7, 4.8×10^7, 1.6×10^9, and 1.26×10^9 M^{-1} s^{-1} at which lycopene reacted with $NO_2^{\bullet}$, $GS^{\bullet}$, $HO(CH_2)_2S^{\bullet}$ and

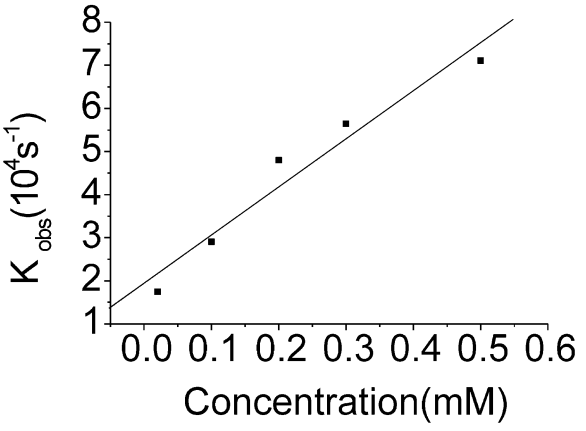

Fig. 5. The dependence of K_{obs} on the concentration of lycopene.

$CH_3SO_2^{\bullet}$, respectively (Mortensen et al., 1997). In addition, beta-carotene, a similar carotenoid to lycopene, has previously been intensively studied. (Packer, Mahood, Mora-Arellano, Slater, Willson, & Wolfenden, 1981). The rate constant of beta-carotene reacting with $CCl_3O_2^{\bullet}$ was determined to be 1.5×10^9 $M^{-1}s^{-1}$, which is similar to the value for lycopene measured by us. It was also found that the bleaching of beta-carotene ground-state absorption was accompanied by an increase in positive absorption in the near infrared region (950–1000 nm), indicating that beta-carotene radical cations were produced. According to this, the reaction that carotenoids (Car) and $CCl_3O_2^{\bullet}$ underwent was determined, via electron transfer to be as follows:

$$CCl_3O_2^{\bullet} + Car \text{—} CCl_3O_2^{\bullet -} + Car^{\bullet +} \quad (1)$$

Since lycopene, reacting with $CCl_3O_2^{\bullet}$, produced a similar bleaching of ground state absorption to beta-carotene, it was deduced that lycopene reacting with $CCl_3O_2^{\bullet}$ also underwent a reaction as described in Eq. (1) in our systems. This needs to be verified in the future.

In conclusion, the bleaching of lycopene ground-state absorption with a fast rate constant of 1.17×10^9 $M^{-1}s^{-1}$ indicated that lycopene could quickly react with $CCl_3O_2^{\bullet}$. This result may be an explanation for the benefit of lycopene in increasing survival of CCl_4-damaged rats, as reported by Kapitanov, Pimenov, and Nesterova (1995).

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