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Original Contribution

CAROTENOIDS AS ANTIOXIDANTS: SPIN TRAPPING EPR AND OPTICAL STUDY

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Abstract—The role of several natural and synthetic carotenoids as scavengers of free radicals was studied in homogeneous solutions. A set of free radicals: $^{\bullet}$OH, $^{\bullet}$OOH, and $^{\bullet}CH_3$ were generated by using the Fenton reaction in dimethyl sulfoxide. It was shown that the spin trapping technique is more informative than optical methods for the experimental conditions under study. 5,5-Dimethyl-pyrroline-*N*-oxide (DMPO) and *N-tert*-butyl-α-phenylnitrone (PBN) were used as spin traps for the EPR studies. The results show that the scavenging ability of the carotenoids towards radical $^{\bullet}$OOH correlates with their redox properties. © 2001 Elsevier Science Inc.

Keywords—Carotenoid, Antioxidant, Fenton reaction, Absorption, Spin trapping, Free radicals

INTRODUCTION

Usually the role of carotenoids is discussed in relation to photosynthesis, quenching of singlet molecular oxygen, and provitamin A activity [1–3]. In the last decade several studies have indicated that carotenoids, as well as "antioxidant vitamins" C and E, may prevent or inhibit certain types of cancer, atherosclerosis, age-related muscular degeneration, and other diseases [4–6]. It was suggested that this function of carotenoids is related to their ability to scavenge free radicals. Although the fate of free radicals within cells remains a matter of debate, it has been suggested that they are capable of chemically altering all major classes of biomolecules: lipids, proteins, nucleic acids [7,8]. The primary radicals can undergo reactions with molecular oxygen to generate peroxyl radicals (ROO$^{\bullet}$), which can cause considerable biological damage [8,9]. A number of studies have indicated that carotenoids act as antioxidants in solution, micelles, and liposomes [10–14]. Usually three mechanisms are discussed for the reaction of free radicals ($^{\bullet}$R) with carotenoids (Car) [4,11]: (i) Radical addition to the carotenoid polyene chain:

$$\text{R}^{\bullet}\ (\text{or ROO}^{\bullet}) + \text{Car} \rightarrow (\text{Car–R})^{\bullet}\ \text{or}\ (\text{Car–OOR})^{\bullet} \qquad (1)$$

Different reactivities of carotenoids with ROO$^{\bullet}$ radicals were observed by using pulse radiolysis [10] and UV-vis absorption [11,12] techniques. (ii) Hydrogen abstraction from the carotenoid:

$$\text{R}^{\bullet} + \text{Car} \rightarrow (\text{Car–H})^{\bullet} + \text{RH} \qquad (2)$$

Usually hydrogen abstraction from an allylic position of the polyene chain (4-CH_2 and 5-CH_3) is discussed [4,11]. (iii) Electron transfer reaction:

$$\text{R}^{\bullet} + \text{Car} \rightarrow \text{R}^{-} + \text{Car}^{+\bullet} \qquad (3)$$

Manifestations of these processes are usually discussed as depending on the structure and redox properties of the carotenoids involved. In the present study we applied the spin trapping (ST) EPR technique to study the scavenging ability of a set of natural and synthetic carotenoids with different redox properties towards free radical species. It is known that the spin trapping technique can provide direct evidence of the presence of radicals in the

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reaction system, and allows observation of the individual reactions of carotenoids with the radicals involved in the process [15–18]. The main goal of this investigation is the determination of a correlation between the redox properties of the carotenoids and their scavenging ability of the free radicals.

EXPERIMENTAL PROCEDURES

Chemicals

β-Carotene was supplied by Sigma (St. Louis, MO, USA), 8′-apo-β-caroten-8′-al by Roche Vitamins and Fine Chemicals (Nutley, NJ, USA), canthaxanthin by Fluka (Milwaukee, WI, USA), and ethyl 8′-apo-β-caroten-8′-oate by Hoffmann-La Roche (Basel, Switzerland). 7′-Apo-7′,7′-dicyano-β-carotene and 7,7′-di-phenylcarotene were synthesized by Dr. Elli Hand. Synthesis methods are described elsewhere (see, for example [19, 20]). Purity of the carotenoids was checked by ^{1}H-NMR (360 MHz, $CDCl_3$) and TLC analyses. The compounds were stored at −16°C in the dark in a desiccator containing drierite or in ampules sealed in vacuo. The solvents CH_2Cl_2 (Aldrich, Milwaukee, WI, USA, anhydrous), dimethyl sulfoxide (DMSO) (99.5%, Aldrich, A.C.S.) were used as received. Hydrogen peroxide (H_2O_2, 30% aqueous solution) (Fisher, Pittsburgh, PA, USA, A.C.S.) and $FeCl_2$ (Aldrich) were used to prepare the Fenton reagent. The spin traps, purified 5,5-dimethyl-pyrroline-*N*-oxide (DMPO), was a gift from the National Institute of Environmental Health Science (NIEHS), and the *N-tert*-butyl-α-phenylnitrone (PBN) (98%) was obtained from Aldrich.

The DMPO spin adducts with oxygen-centered radicals give characteristic EPR spectra. The major advantage of mutual employment of PBN and DMPO is that the hyperfine coupling constants (hfc) of the corresponding spin adducts is very sensitive to the nature of the trapped radical.

Fenton reaction, experimental conditions

For the generation of both oxygen and carbon centered radicals we used the well-known Fenton reaction [18,21,22].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + HO^- \qquad (4)$$

Depending on the experimental conditions, different radical species are generated. In the present study $^{\bullet}$OH radicals can react with the solvent DMSO yielding the $^{\bullet}CH_3$ radicals [23]. Under neutral pH conditions this reaction has a near diffusion rate limit (for reference see Table 2). The $^{\bullet}$OH radical can also react with initial hydrogen peroxide to give $^{\bullet}$OOH radicals. To prove the presence of these radicals we applied the spin trapping technique [17,18,22]. We used two different spin traps (PBN and DMPO) in order to obtain more information about the nature of the trapped radicals and compare the rates of radical scavenging by carotenoids and spin traps.

Apparatus

EPR measurements were carried out with a X-band (9.5 GHz) Varian E-12 EPR spectrometer, equipped with a rectangular cavity. The magnetic field was measured with an ER 035M gaussmeter, and the microwave frequency was measured with a Hewlett Packard 5245M frequency counter. UV-visible absorption spectra were recorded with a Shimadzu UV-1610 spectrophotometer.

Sample preparation

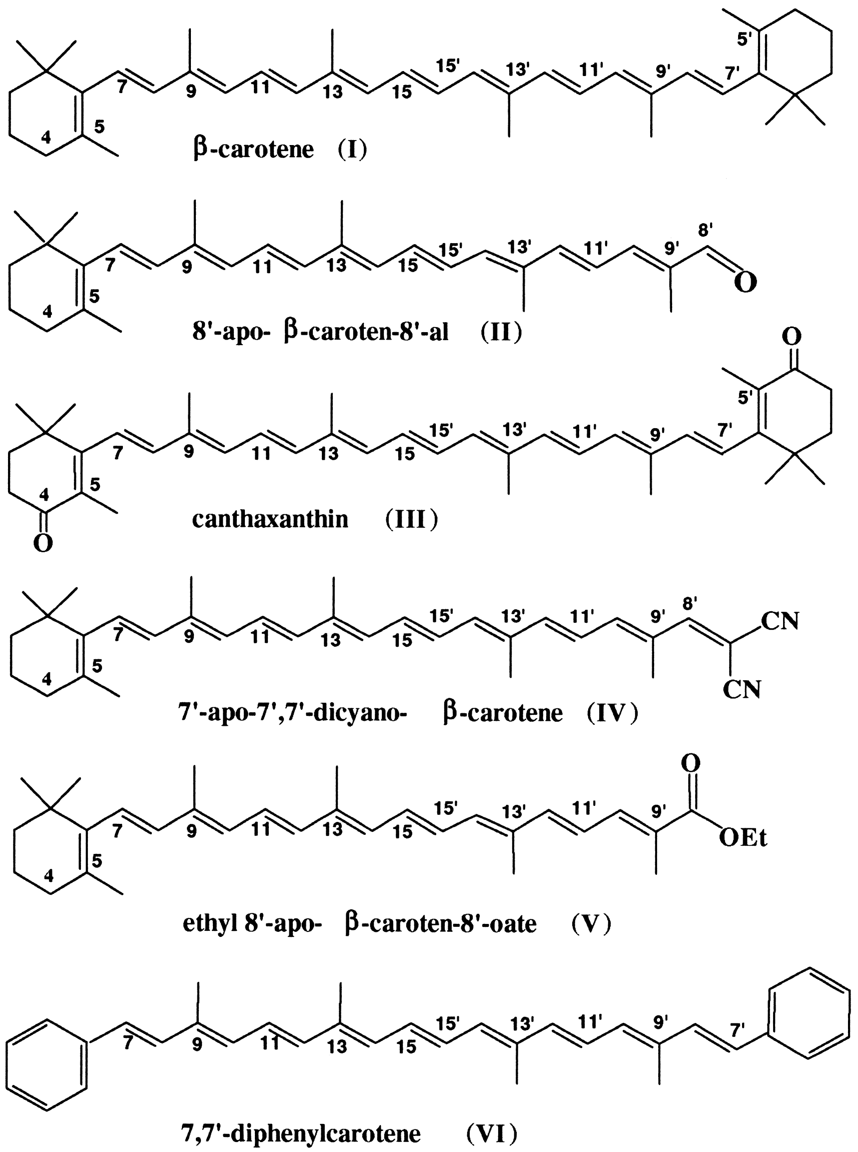
The solutions of all reagents were freshly prepared and purged with Ar. The concentration of carotenoids for optical analysis was on the order of 10 μM, and for EPR measurements about 1 mM, determined by optical absorption spectroscopy. Solutions of carotenoids in CH_2Cl_2 were added to DMSO solutions of spin traps (1–5 mM). The concentration of H_2O_2 varied from 0.17 to 500 mM. The reaction was started by addition of $FeCl_2$ dissolved in CH_2Cl_2. Then the solution was transferred to the stop-flow EPR tube by means of a Hamilton syringe, and the spectra were recorded within 1–5 min after mixing.

RESULTS AND DISCUSSION

In the present work, the role of carotenoids as scavengers of free radicals was studied using β-carotene (**I**), 8′-apo-β-caroten-8′-al (**II**), canthaxanthin (**III**), 7′-apo-7′,7′-dicyano-β-carotene (**IV**), ethyl 8′-apo-β-caroten-8′-oate (**V**), and 7,7′-diapo-7,7′-diphenylcarotene (**VI**) (Scheme 1).

Let us consider which possible radical species are formed under our experimental conditions. As evident from Figs. 1 and 2 three types of spin adducts are observed during the Fenton reaction in DMSO depending on various H_2O_2 concentrations. For identification of the radicals trapped by DMPO and PBN we used data from the NIEHS Spin-Trap Database [24] (see Table 1).

Figure 1 demonstrates that only $^{\bullet}CH_3$ spin adducts were formed at low (1 mM) H_2O_2 concentration (spin adduct 3). With increasing H_2O_2 concentration (from 1 to 10 mM) the spin adducts of both $^{\bullet}$OH and $^{\bullet}CH_3$ radicals appear in the EPR spectrum (Fig. 1, spin adducts 1 and 3, and Fig. 2, top). At high H_2O_2 concentration



Scheme 1.

(500 mM) spin adducts of only $^{\bullet}OOH$ radicals were detected (Fig. 1, spin adduct 2, and Fig. 2, bottom). To understand the origin of the spin adducts we considered the possible reactions and estimated their kinetic parameters (Table 2). The data in Table 2 indicate that direct reaction of the primary $^{\bullet}OH$ radicals with spin traps is impossible because under our experimental conditions these radicals react with solvent molecules more rapidly than with spin traps. The $^{\bullet}CH_3$ spin adduct was most likely produced via reaction of primarily formed $^{\bullet}OH$ radical with solvent DMSO [23]:

$$\mathrm{DMSO} + {}^{\bullet}OH \rightarrow {}^{\bullet}CH_3 + CH_3SO(OH)$$

Since the ratio of the DMSO and spin trap concentrations is constant during changing the H_2O_2 concentration, the observation of $^{\bullet}OH$ spin adducts with increasing H_2O_2 is most likely due to secondary processes. Formation of the $^{\bullet}OOH$, which results in the spin adducts under high H_2O_2 concentrations, may be caused by two reactions: (i) direct reaction of $^{\bullet}OH$ radical with H_2O_2:

$$H_2O_2 + {}^{\bullet}OH \rightarrow HO_2^{\bullet} + H_2O$$

and (ii) reaction of the $^{\bullet}CH_3$ radical with H_2O_2:

$${}^{\bullet}CH_3 + H_2O_2 \rightarrow CH_4 + HO_2^{\bullet}$$

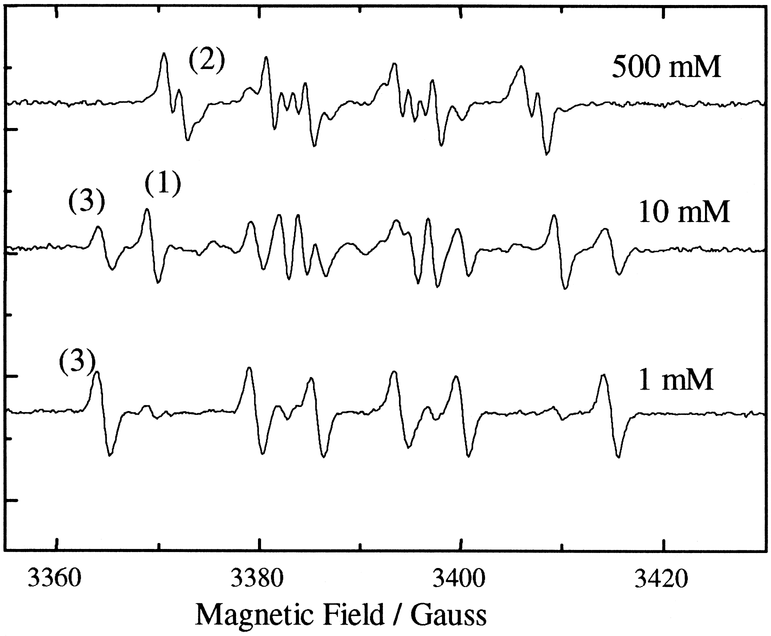


Fig. 1. EPR spectra of DMPO spin adducts recorded during the Fenton reaction in DMSO at different H_2O_2 concentrations ($[FeCl_2]$ = 1 mM), (1), (2), and (3) are $^{\bullet}OH$, $^{\bullet}OOH$, and $^{\bullet}CH_3$ radicals, respectively.

According to the data in Table 2 the first reaction is totally suppressed by the reaction of $^{\bullet}OH$ with the organic solvent (DMSO). On the other hand, addition of ca. 1 mM H_2O_2 had no great effect on the second order decay kinetics of $^{\bullet}CH_3$ radical and only at high H_2O_2 concentrations does the reaction result in $^{\bullet}OOH$ radical [25]. Also, it is known that the $^{\bullet}OOH$ spin adduct is relatively unstable, especially in the presence of transition metal ions, which can reduce $^{\bullet}OOH$ radicals yielding the $^{\bullet}OH$ spin adduct [26,27]. With increasing H_2O_2 concentration the Fe^{2+} ions react primarily with the initial H_2O_2 and consequently, the stability of the $^{\bullet}OOH$ spin adduct will increase. The above indicates that under our experimental conditions only methyl and $^{\bullet}OOH$ radicals can be scavenged by small concentrations of the spin traps. Formation of the $^{\bullet}OH$ spin adducts is most likely a result of reduction of the $^{\bullet}OOH$ spin adducts. This provides a possibility to study the scavenging ability of carotenoids towards each radical. Control experiments were carried out to determine the ability of the carotenoids to scavenge stable radical adducts. Addition of the carotenoids to previously prepared reaction mixtures of

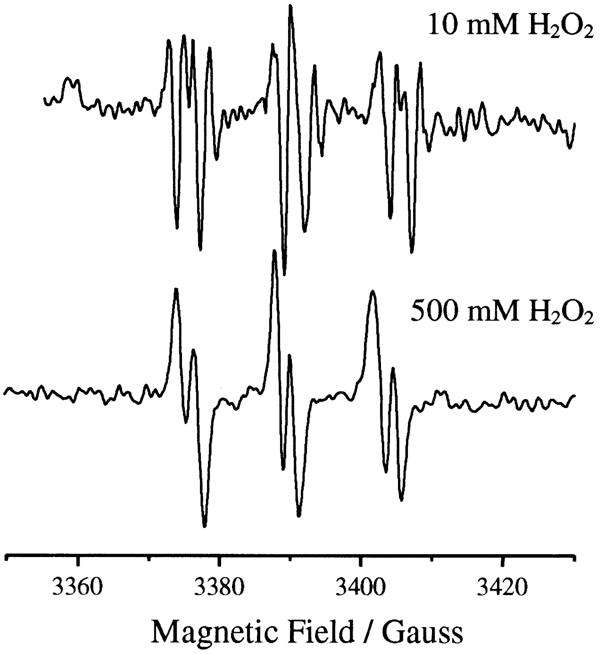


Fig. 2. EPR spectra of PBN spin adducts recorded during the Fenton reaction in DMSO at different H_2O_2 concentrations ($[FeCl_2]$ = 1 mM).

Table 1. Hyperfine Coupling Constants **a** (Gauss) of Spin-adducts in DMSO*

Spin-adduct	Radical	DMPO	PBN
1	OH	$\mathbf{a}_N = 13.2$ $\mathbf{a}_H = 14.5$	
2	OOH	$\mathbf{a}_N = 13.1$ $\mathbf{a}_{H\beta} = 10.3$ $\mathbf{a}_{H\gamma} = 1.2$	$\mathbf{a}_N = 13.9$ $\mathbf{a}_H = 2.3$
3	CH_3	$\mathbf{a}_N = 14.8$ $\mathbf{a}_H = 21.1$	$\mathbf{a}_N = 14.9$ $\mathbf{a}_H = 3.4$

* Identification of all spin-adducts was made by using the NIEHS Spin trap database [24].

Table 2. Possible Reactions and Rate Constants During the Fenton Reaction in the Process Under Study

	Reaction	Rate constant, $M^{-1}s^{-1}$	pH	Ref.
Radical generation				
4	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$	76		[28]
5	$HO_2^{\bullet} \leftrightarrow H^+ + O_2^{\bullet -}$, pK = 4.45			[28]
6	$H_2O_2 + {}^{\bullet}OH \rightarrow HO_2^{\bullet} + H_2O$	$2.7 \cdot 10^7$	7	[29]
7	$HO_2^{\bullet} + {}^{\bullet}OH \rightarrow H_2O + O_2$	$6.6 \cdot 10^9$	1 to7	[30]
8	$HO_2^{\bullet} + Fe^{2+} \rightarrow H_2O_2 + Fe^{3+}$	$1.2 \cdot 10^6$	1 to7	[31]
9	$HO_2^{\bullet} + Fe^{3+} \rightarrow H^+ + Fe^{2+} + O_2$	$2.1 \cdot 10^5$	1.51	[32]
		$1.0 \cdot 10^6$	2.0	
10	$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow H_2O_2 + O_2$	$8.3 \cdot 10^5$	>2	[33]
		$1 \cdot 10^6$	≤2	
11	$HO_2^{\bullet} + H_2O_2 \rightarrow {}^{\bullet}OH + H_2O + O_2$	0.50	0.5 to3.5	[34]
Reactions in the presence of DMSO				
12	$DMSO + {}^{\bullet}OH \rightarrow {}^{\bullet}CH_3 + CH_3SO(OH)$	$7.0 \cdot 10^9$	7	[23]
13	${}^{\bullet}CH_3 + H_2O_2 \rightarrow CH_4 + HO_2^{\bullet}$	$1 \cdot 10^6$		[25]
14	${}^{\bullet}CH_3 + {}^{\bullet}CH_3 \rightarrow CH_3CH_3$	$9 \cdot 10^8$	7	[25]
Reactions of PBN				
15	$PBN + HO_2^{\bullet} \rightarrow {}^{\bullet}PBN(OOH)$	$1 \cdot 10^6$	7	[35]
16	$PBN + {}^{\bullet}OH \rightarrow {}^{\bullet}PBN(OH)$	$6.1 \cdot 10^9$	7	[36]
17	$PBN + {}^{\bullet}CH_3 \rightarrow {}^{\bullet}PBN(CH3)$	$1–5 \cdot 10^7$	7	a
Other reactions of carotenoids				
18	$Car + {}^{\bullet}OH \rightarrow {}^{\bullet}Car(OH)$	$\sim 10^9$		b
19	$Car + {}^{\bullet}CH_3 \rightarrow {}^{\bullet}Car(CH_3)$	$<1 \cdot 10^6$		b
20	$Car + Fe^{3+} \rightarrow Car^{+\bullet} + Fe^{2+}$	Not determined		[37]

[a] Estimated from the scavenging rates of CH_2OH and CH_3CHOH radicals [38].

[b] Reactions rates were estimated from reactions of other polyene compounds (piperylene).

the Fenton reagent and spin traps (DMPO, PBN) had no effect on the intensity of the EPR signal of the ${}^{\bullet}OOH$ spin adduct. We conclude that carotenoids did not react with the spin adducts of DMPO or PBN.

As noted above, several reaction types of radicals with carotenoids (1–3) are possible. These reactions should have different spectroscopic features. Nonselective addition of radicals to the polyene chain leads to disruption of the carotenoid chromophore in the products. The result of this addition is a sizable blue shift in the absorption spectra. This process can be clearly observed during UV-vis measurements (see

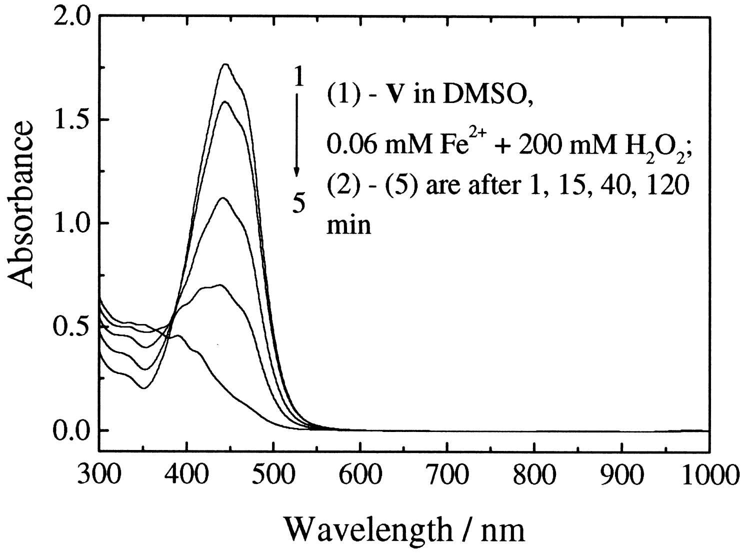


Fig. 3. Optical absorption spectra of carotenoid V in MeOH before reaction (1) and during the Fenton reaction in 1 min (2), 15 min (3), 40 min (4), and 120 min (5) after starting.

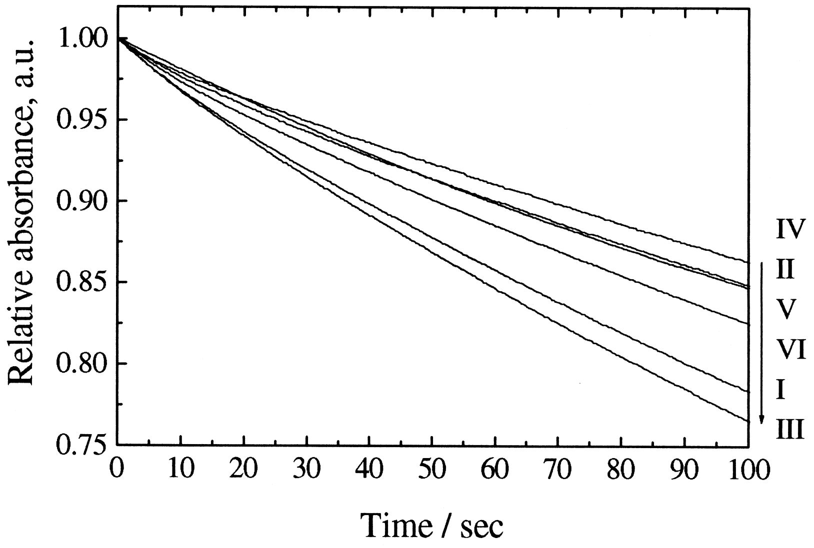


Fig. 4. Time course of absorption band of carotenoids I–IV recorded during the Fenton reaction in DMSO at high H_2O_2 concentration. The concentrations are 0.01 mM for carotenoids, 1 mM Fe^{2+}, and 500 mM H_2O_2 (λ_{obs} = 460 nm).

Fig. 3). The absence of an isobestic point in UV-vis spectrum most likely indicates that radical addition to the carotenoid during the Fenton reaction is nonselective. In contrast, abstraction of a hydrogen atom from the 4-CH_2 (or 5-CH_3) groups [11] should not lead to considerable shifts of the absorption band of the products compared to the initial carotenoid. This process cannot be clearly detected in an absorption spectrum. Figure 3 shows both the disappearance and transformation of the absorption band in the region of the initial carotenoid spectrum.

Figure 4 shows the kinetics of the carotenoid disappearance under the conditions of $^{\bullet}OOH$ radical generation (high H_2O_2 concentration). Because there are many possible reactions in these processes, complete kinetics analysis is very complicated. Most of the kinetic data are available only for aqueous solutions. Figure 4 shows that the carotenoid I–VI decay rates do not differ by more than 1.5 times for the same carotenoid concentrations. Similar decay behavior of different carotenoids indicates the presence of reactions that do not depend on the carotenoid properties and their ability to trap oxygen radical species. Our attempts to simulate the kinetics of the carotenoid disappearance (with known rate constants from Table 2) by using the *IBM Chemical Kinetic Simulator* show that the most appropriate candidate for this role is the slow reduction of Fe(3+) to Fe(2+). If other fast reactions (for example, cleavage of the radicals or oxidation of the carotenoids by the radicals) play a limiting role in this process, then the time of the carotenoid half-decay must be in the millisecond range, at least with the employed concentrations of the initial compounds. For these reasons we used the more informative spin trapping technique to obtain kinetic data.

As shown above, different spin traps (DMPO and PBN) show the same behavior (Figs. 1 and 2). With high concentration of H_2O_2 (500 mM) only the DMPO-OOH or PBN-OOH spin adducts were detected. Addition of the carotenoid in these cases results in a decrease of the spin adduct EPR signal. Figure 5 shows the EPR spectra of the PBN/OOH spin adduct at different concentrations of carotenoid.

It is reasonable to assume that the decrease of spin-adduct (SA) yield is due to competing reactions of rad-

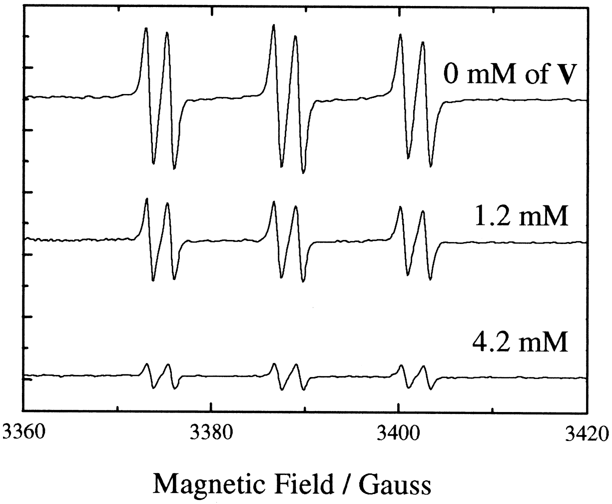
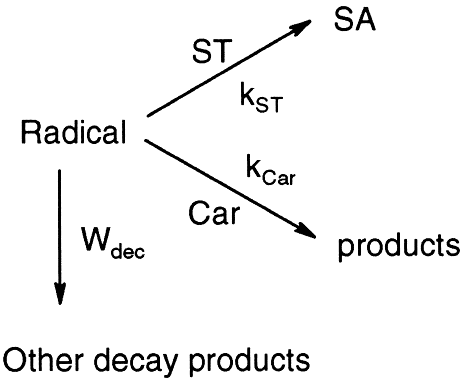


Fig. 5. EPR spectra of PBN/OOH spin adduct recorded during the Fenton reaction in DMSO at different concentrations of V; (PBN 5 mM, H_2O_2 500 mM, Fe^{2+} 1 mM).



Scheme 2.

icals with carotenoids (Car) and spin traps (ST) (Scheme 2). Note that k_{Car} reflects all possible ways of the radical interaction with the carotenoids and can include (i) addition to carotenoid, (ii) proton abstraction from carotenoid, and (iii) oxidation of the carotenoid by single electron transfer. All above processes prevent free radicals to be scavenged by spin traps.

Kinetic analysis of this scheme shows that the ratio of spin adduct concentrations in the absence and presence of carotenoid is:

$$\frac{A_0}{A} = \frac{k_{ST}[ST] + W_{dec} + k_{Car}[Car]}{k_{ST}[ST] + W_{dec}}$$

where A_0 is the concentration of the spin adduct in the absence of the carotenoid and A is that in the presence of the carotenoid. W_{dec} includes all other pathways of the radical decay. If our experimental conditions satisfy this equation, then the experimental data for constant ST concentration should be linear in coordinates $(I_0/I - 1)$ and [Car], where I_0 and I are the EPR signal intensities in the absence and presence of the carotenoid, respectively. The slope will be $k_{Car}k_{ST}[ST] + W_{dec}$. The results of such a plot are presented in Fig. 6 and Table 3. The experimental data are in good agreement with the competing processes in Scheme 2. Although experimental data linearity for carotenoid V seems poor compared to the other carotenoids, at low carotenoid concentrations the correlation coefficient of linear fitting is 0.988. This permits us to estimate the slope with good reliability.

Table 3. Kinetic Data for the Reaction of Carotenoids with $^{\bullet}$OOH Radical

Carotenoid	$k_{Car}/k_{ST}[ST] + W_{dec}$	Correlation coefficient
I	0.13 ± 0.02	0.939
II	0.64 ± 0.02	0.993
III	0.39 ± 0.01	0.997
IV	4.80 ± 0.01	0.999
V	2.48 ± 0.12	0.988
VI	1.65 ± 0.02	0.998

As mentioned above, possible reactions of the carotenoid with the radicals include three processes: radical addition, hydrogen abstraction, and carotenoid oxidation. In our experiments the carotenoids I–II and V–VI have identical cyclohexene terminal fragments. III has carbonyl groups in the 4 and 4′ positions of the terminal rings, and in VI the cyclohexene fragment was changed to phenyl. If, as previously discussed in the literature [4,11], hydrogen abstraction from the 4 position of the cyclohexene ring plays a major role in the radical scavenging processes, then carotenoids III and VI should be

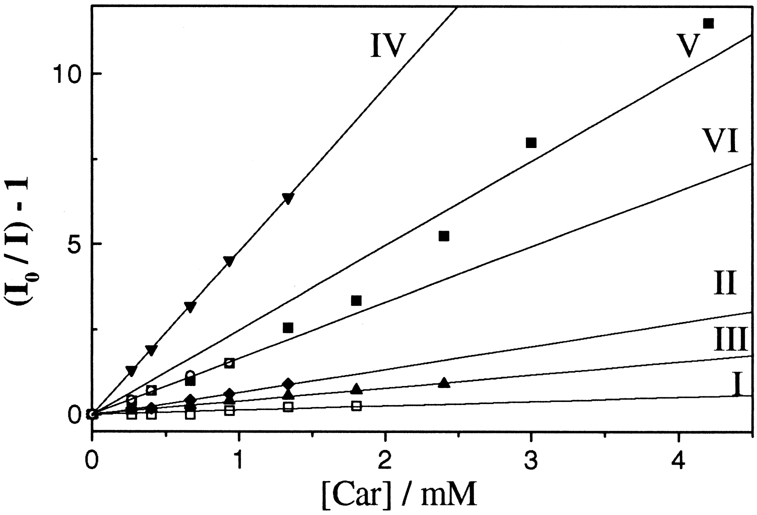


Fig. 6. Dependence of the ratio of the PBN-OOH EPR intensities on the concentration of different carotenoids. [ST] = 5 mM in all experiments.

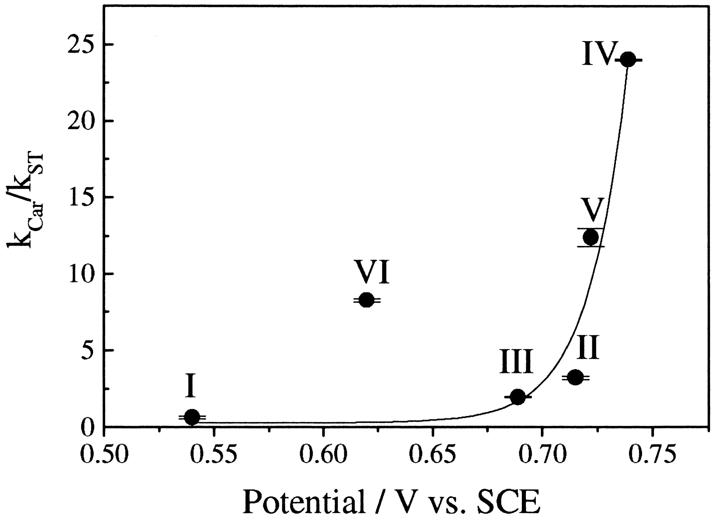


Fig. 7. Dependence of scavenging ability of carotenoids (I–VI) on their oxidation potentials.

less effective towards the radicals compared to other carotenoids. Nevertheless, their ability to scavenge free radicals is comparable to that of the other carotenoids, which have unsubstituted 4 positions of the cyclohexene fragment. Therefore we suggest that an alternative pathway is a radical addition. In this case efficiency should correlate with the charge distribution in the initial carotenoids. Another possible pathway is the redox process. In this case the effectiveness of the carotenoids should correlate with the oxidation potentials of the different carotenoids [39,40]. The last two processes may be independent, simultaneous, or consecutive reactions. Note that both radical adducts and radical cations of carotenoids were observed as primary products of the reactions of $CCl_3OO^{\bullet}$ radical with carotenoids [10].

W_{dec} includes mainly reactions with ferrous ions and also bimolecular recombination, but referring to Table 2 one can conclude that for initial concentrations of ST (5 mM) and $FeCl_2$ (1 mM) W_{dec} is slower then k_{ST}[ST]. With this assumption it is possible to obtain the relative scavenging ability insert equation from msp 14 from data of Table 3. For spin trap concentrations (5 mM) the dependence of relative scavenging ability of carotenoids towards the $^{\bullet}$OOH on their redox properties is presented at Fig. 7, which shows that the scavenging ability of carotenoids increases with the increase of their oxidative potential with the exception of VI. The main difference of carotenoid VI from the other carotenoids is the replacement of the cyclohexene terminal fragment by phenyl groups that can increase the probability of radical adduct formation. It is known that a phenyl group can react with $^{\bullet}$OH and $^{\bullet}$OOH radicals resulting in cyclohexadienyl-type radical adducts [41]. It is interesting to note that the scavenging ability of the carotenoids increases with their oxidation potentials. The presence of such a correlation indicates formation of the charged form of the carotenoid in the reaction process. As mentioned above, formation of the carotenoid radical cation is possible via oxidation by $^{\bullet}$OOH [10]. Nevertheless, radical cation formation does not exclude the formation of radical adducts. In this case, reaction of the carotenoid radical cation with HO_2^- can result in radical adduct formation. Comparison of the variance of the scavenging rates from spin trap experiments (about 40 times, see Table 3 and Fig. 7) with the data of UV-vis experiments (not more then 1.5 times) clearly confirms the above assumption that slow disappearance of the carotenoid optical absorbance (Fig. 4) under our experimental conditions did not originate from the fast scavenging processes of free radicals.

Another reason for the correlation between carotenoid redox properties and their relative radical scavenging ability may be connected with the equilibrium between $^{\bullet}$OOH and superoxide ion, $O_2^{\bullet-}$ [28]. It is known from electrochemical experiments that $O_2^{\bullet-}$ is stable in aprotic solvents or in basic media; for example, in pure DMSO its half-lifetime is more than 5 h [42]. On the other hand, most redox reactions of superoxide are faster than those of the hydroperoxyl radical [43].

CONCLUSIONS

Spin trapping EPR and UV-vis spectroscopy techniques were employed to determine the relative rates of the reaction of carotenoids with $^{\bullet}$OOH radicals generated by the Fenton reaction in DMSO. In all cases a decrease of the spin adduct yield was observed when the reaction was carried out in the presence of carotenoids. This process was also monitored optically by the decrease of the carotenoid absorption band. Three possible mechanisms are discussed: addition of radicals to the polyene chain, hydrogen abstraction from the C(4) position of the cyclohexene ring, and the redox process. It was found that the scavenging ability of the carotenoids increases with their oxidation potentials.

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