This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2005)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2005 Term

Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

The Fine Print:

Because this is a paper written by a beginning student as an assignment, there are no guarantees that everything is absolutely correct and accurate.

In view of the possibility of human error or changes in our knowledge due to continued research, neither the author nor The University of Iowa nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such information. Readers are encouraged to confirm the information contained herein with other sources.

Reacting with β-carotene: A radical approach

by

Katrina S.M. Pedersen

Department of Radiation Oncology
Free Radical and Radiation Biology Program
The University of Iowa
Iowa City, IA 52242-1181

For 77:222, Spring 2005

9 February 2005

β-Car	β-carotene	$^1O_2$	singlet oxygen
Car$^\bullet$	β-carotene addition radical	**RO$^\bullet$**	alkoxyl radical
Car$^{\bullet+}$	β-carotene cation radical	**ROO$^\bullet$**	peroxyl radical

Abstract

Though plants produce over 600 carotenoid molecules, β-carotene is one of only several that are retained in human tissues and appear to be biologically relevant. β-carotene radicals are notable not only for their stability due to extensive conjugation of their carbon backbone, but also for their ability to undergo both antioxidative and prooxidative reactions. These reactions are dependent on environmental factors, such as oxygen tension and carotenoid concentration. Radicals may arise from β-Car by several reaction mechanisms with other radicals (*i.e.* RO$^{\bullet}$ and ROO$^{\bullet}$) resulting in localization of the unpaired electron over various areas of the molecule. This paper highlights such subtle structural differences between Car$^{\bullet}$ and Car$^{\bullet+}$ and discusses some of the most commonly used techniques for detecting and differentiating the various species.

Introduction

β-carotene is the most abundant and frequently studied of the plant-derived carotenoid molecules because of its presence within human tissues and its role as a vitamin A precursor [1, 2]. Carotenoids are accessory pigments to chlorophyll within photosystem II and have been shown to scavenge radicals and quench singlet oxygen ($^1O_2$) [3]. This scavenging ability is transferred to humans and other mammals through dietary routes and is at its greatest concentration in membranes and organelles with low oxygen tension [4, 5]. The lipophilic molecule is particularly proficient at forming relatively stable carbon-centered cation radicals ($Car^{\bullet+}$) upon oxidation with other radicals present in the cell [5, 11]. While the cation radical may undergo antioxidant activities in order to scavenge free radicals within the cell, recent studies of $Car^{\bullet+}$ physical chemistry have shown that it may actually have a prooxidant reaction mechanism as well. Much of the current research efforts are aimed at resolving the question of the efficacy of carotene supplementation in the diet.

The β-Car to $Car^{\bullet}$ and $Car^{\bullet+}$ transformation

β-Carotene ($C_{40}H_{56}$) consists of a long hydrocarbon backbone with extensive polyene conjugation. This chain is flanked on either end by cyclic groups (**Figure 1**).

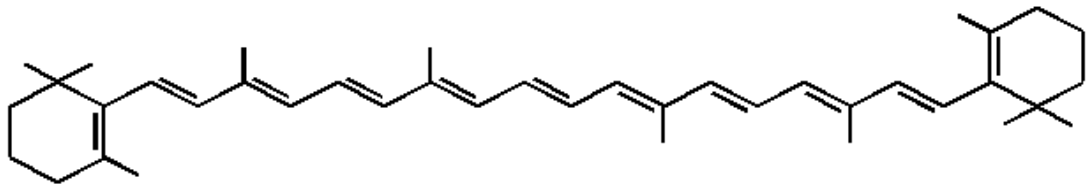

Figure 1. Structure of β-carotene

β-Car becomes a radical cation species when it is oxidized by peroxyl radicals present in *in vitro* and *in vivo* systems, specifically from ROO• formed by autooxidation of lipids [8]. This reaction may result in a resonance-stabilized carbon-centered cation radical with a relatively long half-life reported to be under 6 s (**Figure 2a**) [5, 7]. β-Car may become a radical through an addition reaction when the peroxyl radical forms a covalent bond to the first carbon of the carotenoid backbone (**Figure 2b**). The radical group takes an electron from the β-Car molecule and the newly unpaired electron is effectively delocalized across the structure of alternating double- and single-bonds.

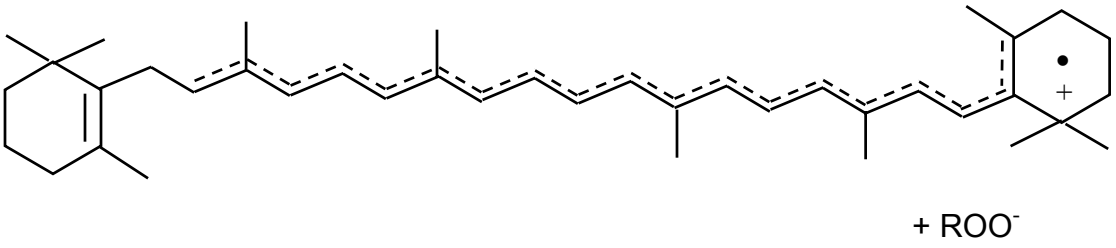

Figure 2a. Carotene cation radical formed by ROO• induced oxidation of β-Car.

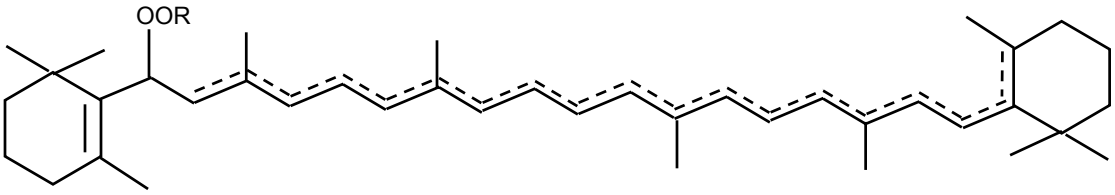

Figure 2b. Structure of β-Carotene radical adduct formed by ROO•

Researchers have noted that myeloperoxidase must be present within cells and *in vitro* systems during the transformation of β-Car to $Car^{\bullet}$ and $Car^{\bullet+}$ with phenoxyl and hydroxyl radicals, suggesting that the enzyme is necessary to catalyze oxidation with certain radicals [6].

Radical chemistry

As mentioned above, $Car^{\bullet}$ and $Car^{\bullet+}$ are resonance stabilized due to the planar double bond nature of its carbon skeleton. The stability conferred by this structure is an important determining factor in the reactions that $Car^{\bullet}$ and $Car^{\bullet+}$ can later undergo [5]. While β-Car may react with many different radical species, the following sections will discuss only peroxyl radical reactions reflecting the emphasis of the research being performed on such reactions.

Antioxidant reactions

The $Car^{\bullet+}$ arises from β-Car during antioxidant processes that prevent lipid peroxidation by free radicals (i.e. $ROO^{\bullet}$) in the cell [4, 5]. $ROO^{\bullet}$ species are strongly oxidizing and may, therefore, react directly with β-Car to form $Car^{\bullet+}$ and peroxyl anion. Though less oxidizing than the peroxyl radical, $Car^{\bullet+}$ may still undergo chemistry and will subsequently accept electrons from other donors; however, these radicals are not able to propagate lipid peroxidation [2, 5, 8]. Additionally, $ROO^{\bullet}$ may bind to β-Car through an organic addition reaction (**Equation 1**) [5, 8]. The resultant peroxyl carotenoid radical may react with a second incident $ROO^{\bullet}$ to finally create the net polar, non-radical products (**Equation 2**) [5, 8]. Though the reactions are oxidative, the overall reaction

serves to trap the radicals that are most likely to damage lipids and other cellular structures [5].

$$ROO^{\bullet} + \beta\text{-Car} \rightarrow ROO\text{-}Car^{\bullet} \quad (1)$$

$$ROO\text{-}Car^{\bullet} \rightarrow \text{polar products of epoxide oxidation} \quad (2)$$

Prooxidant reactions

The most notable prooxidative process involving a β-carotene radical is that involving the ROO-Car$^{\bullet}$ (**Equation 1**). When O_2 is present, it may react with the relatively stable addition radical to create a now unstable diperoxyl radical (**Equation 3**). This highly reactive radical may then further react with bimolecular oxygen and propagate more radicals and polar molecules (**Equation 4**) [5]. When such processes occur, the oxidative cycle will likely continue unchecked.

$$ROO\text{-}Car^{\bullet} + O_2 \rightarrow ROO\text{-}Car\text{-}OO^{\bullet} \quad (3)$$

$$ROO\text{-}Car\text{-}OO^{\bullet} + O_2 \rightarrow \text{radicals } (ROOCARO_2^{\bullet}) \quad (4)$$

The oxygen effect

While any reaction between cellular O_2 and Car$^{\bullet}$ is normally very slow, the progression is more favorable when the O_2 tension (partial pressure) of the system is high [3]. This effect is further enhanced with increasing concentrations of carotenoids [2, 3]. Researchers have found that Car$^{\bullet}$ reacts most often in the antioxidant capacity in tissues under physiological oxygen tensions; however, there are instances in which a higher pressure may exist, such as in the lungs [9]. The addition of both a lipid peroxidation initiator and varying concentrations of β-Car at low and high O_2 tension showed an increase of oxidative events within the system (**Figure 3**) [4]. This finding indicates that environmental factors may play a role in determining whether carotene radicals play a

prooxidant or antioxidant role. Though this gain of prooxidative activity at the expense of antioxidative processes is not yet understood, it could well be the result of carotene chemistry involving either the cation or the addition radical [2]. The physical features of bimolecular oxygen and its greater reactivity with organic molecules when pressure is increased could play a causative role in this functional reversal; however, recent data suggests that carotene radicals do not directly interact with O_2 to halt chain propagation and likely some other mechanism is involved [14]. In light of this data, smokers are particularly advised to avoid β-car supplementation in their diets because of increased radicals and subsequent damage in the lungs.

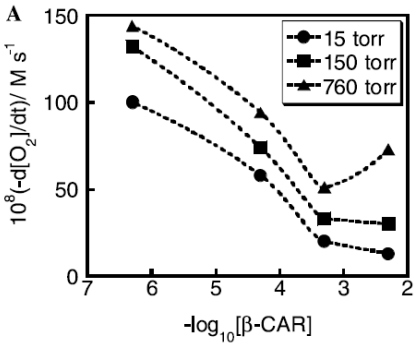

Figure 3. [β-Car] and oxygen partial pressure affects initial oxidation rates of methyl linoleate. As both pressure and concentration increase, oxidation also increases. (From [4].)

Detecting Car$^{\bullet/+}$

Many techniques are utilized to detect free radicals; however, due to the unique chemistry and stability of the carotenoids, some methods prove to be more informative than others. Electron paramagnetic resonance (EPR) and the closely related electron-nuclear double resonance (ENDOR) methods are among the most powerful tools in detecting the presence and structure of radicals, respectively [3,10]. EPR spectra obtained have shown not only the prevalence of the carotene radicals but have been able to differentiate the radical species due to their characteristic peaks.

UV-vis spectrophotometry has been an extremely important tool in carotene radical detection and characterization because the main function of carotene and its radicals is in light-harvesting complexes. When the wavelength of light from the spectrophotometer matches that needed to excite the electrons, the light is absorbed by the molecule and recorded[1]. Because of this property, the wavelength at which they absorb light allows species to be identified and differentiated [3, 14]. Spectrophotometry has also been employed in the literature to measure the half-life of some radicals [3, 11].

Photobleaching through photolysis and pulse radiolysis is a much more sensitive technique for detecting radical lifetime, kinetics, and the reaction mechanism [12, 13]. The radicals are optically detected by bleaching carotenoids with a photomultiplying xenon lamp in brief pulses of light to deliver electrons to the molecules [12]. The transient spectra measuring the amount of electrons absorbed at varying wavelengths may then be used to determine the radical's interactions and the time period required for such reactions to occur (**figure 4**) [12]. The radical cation has an exclusive wavelength (~942 nm maximum [15]) from other products

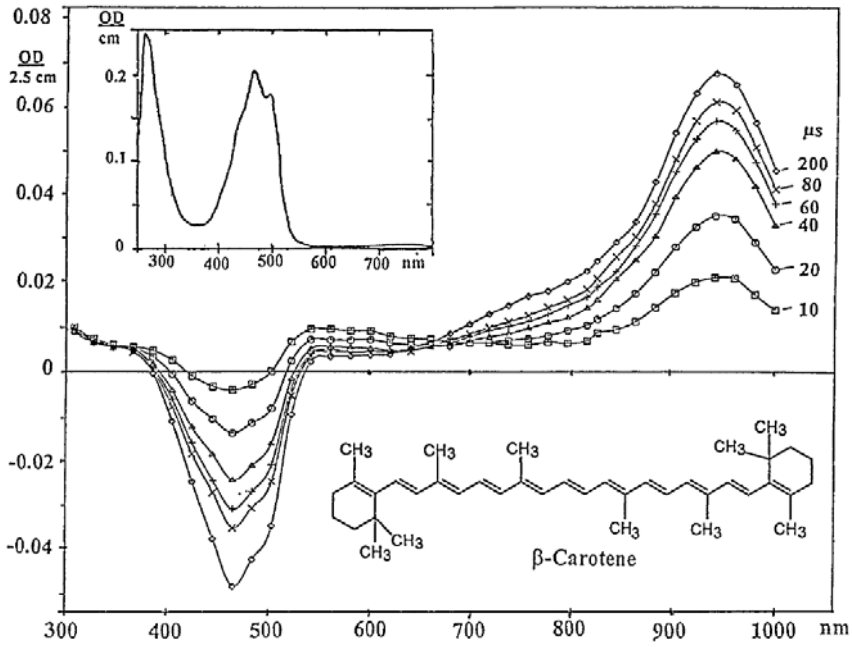

Figure 4. Pulse radiolysis spectra of Car•+ and triplet β-car performed with various pulse lengths (as labeled). The spectra show how various oxidation products may be differentiated (From [15].)

[1] http://www.chemistry.mtu.edu/pages/courses/ch2400-dbates/Chapter%2011-Extended%20Summary.pdf

formed by β-car oxidation, such as triplet β-car (~552 nm maximum), allowing species to be distinguished.

Finally, chemicals are also utilized to give an indirect measure of carotene radical presence by measuring the lipid peroxidation due to radical insult after a manipulation in the system. One such fluorescent indicator is PnA (*cis*-parinaric acid), a fatty acid with four conjugated double bonds [11]. The pi-bonds make PnA an attractive candidate for oxidation, at which point the molecule will cease fluorescing [11]. Thus, when PnA is incorporated into lipids, the loss of fluorescence is a sign of not only the process of lipid peroxidation but also the extent to which it is occurring.

Altogether, the techniques mentioned above have been crucial in helping determine not only the presence of carotene radicals, but also the mechanisms by which β-Car reacts to either help protect or damage the cell. Though no consensus has been reached concerning the efficacy of β-Car supplementation in the diet, current research continues to drive at an answer.

References

[1] Yamauchi R, Miyake N, Inoue H, Kato K. (1993) Products formed by peroxyl radical oxidation of β-carotene. *J Agri Food Chem.* **41**: 708-713.

[2] El-Agamey A, Lowe GM, McGarvey DJ, Mortensen A, Phillip DM, Truscott TG, Young AJ. (2004) Carotenoid radical chemistry and antioxidant/pro-oxidant properties. *ABB* **430**: 37-48.

[3] Grant JL, Kramer V, Ding R, Kispert LD. (1988) Carotenoid cation radicals: Electrochemical, optical, and EPR study. *J Am Chem Soc.* **110**: 2151-2157.

[4] Burton GW, Ingold KU. (1984) β-Carotene, an unusual type of lipid antioxidant. *Science* **224**: 569-573.

[5] Jorgensen K, Skibsted LH. (1993) Carotenoid scavenging of radicals: Effect of carotenoid structure and oxygen partial pressure on antioxidative activity. *Z Lebensm Unters Forsch* **196**: 423-429.

[6] Day BW, Bergamini S, Tyurina YY, Carta G, Tyurin VA, Kagan VE. (1998) β-Carotene: An antioxidant or a target of oxidative stress in cells? In: Quinn and Kagan, ed. *Subcellular Biochemistry*. Vol. 30. New York: Plenum Press.

[7] Mortensen A, Skibsted LH. (1997) Importance of carotenoid structure in radical-scavenging reactions. *J Agri Food Chem.* **45**: 2970-2977.

[8] Liebler DC. (1993) Antioxidant reactions of carotenoids. *Annals of the NY Academy of Sciences* **691**: 20-31.

[9] Palozza P, Calviello G, Bartoli GM. (1995) Prooxidant activity of β-carotene under 100% oxygen pressure in rat liver microsomes. *Free Rad Biol & Med* **19**: 887-892.

[10] Piekara-Sady L, Kispert LD. (1994) Carotenoid cation radical: An EPR and ENDOR study. *Molecular Physics Reports* **6**: 220-223.

[11] Omaye ST, Krinsky NI, Kagan VE, Mayne ST, Liebler DC, Bidlack WR. (1997) β-Carotene: Friend or foe? *Fundamental and Applied Toxicology* **40**: 163-174.

[12] Mortensen A, Skibsted LH, Sampson J, Rice-Evans C, Everett SA. (1997) Comparative mechanisms and rates of free radical scavenging by carotenoid antioxidants. *FEBS Letters* **418**: 91-97.

[13] Mortensen A, Skibsted LH. (1996) Kinetics of photobleaching of β-carotene in chloroform and formation of transient carotenoid species absorbing in the near infrared. *Free Rad Res.* **25**: 355-368.

[14] El-Agamey A, McGarvey DJ. (2003) Evidence for a lack of reactivity of carotenoid addition radicals towards oxygen: A laser flash photolysis study of the reactions of carotenoids with acylperoxyl radicals in polar and non-polar solvents. *J Am Chem Soc.* **125**: 3330-3340.

[15] Getoff N. (2000) Pulse radiolysis studies of β-carotene in oxygenated DMSO solution. Formation of β-carotene radical cation. *Rad Res* **154**: 692-696.