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b-Carotene: The Reason Not to Feed Your Veggies to the Dog.

By

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Abbreviations:

AAPH, 2,2'-azo*bis*(2-amidinopropane)
CAR, Carotenoid
DNA, Deoxyribonucleic Acid
MDA, Malondialdehyde
$^1O_2$, Singlet Oxygen
ROS, Reactive Oxygen Species

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Abstract:

β-Carotene, an important component of fruits, vegetables and plants, is an important part of the human diet. As a precursor for vitamin A, as well as an antioxidant it is an invaluable part of the diet. β-Carotene's antioxidant functions are best carried out at low concentrations of oxygen. Therefore the biological relevance of this substance is easily understood. The scavenging mechanisms for quenching harmful agents in the body are also an important function. β-Carotene has been studied in order to discover the benefits to humans and other mammals. The visualization of this material was important in starting this research. While the importance of this carotenoid is understood, the mechanisms of all its functions are still being explored. This paper will focus on the structure, scavenging mechanisms of β-carotene and the ways in which β-carotene is detected.

Introduction:

We were always told by parents, teachers and other concerned adults to eat our vegetables when what we were trying to do was slip them under the table to the family dog. Whether they understood why they were telling us this or not, the fact remains that they were right. Vegetables contain many essential ingredients for the human diet one of these being β-carotene. Most recently it has been observed that increasing the levels of β-carotene in the blood plasma may help decrease the risk of some cancers [9]. β-Carotene also provides up to 50 percent of the daily requirement for vitamin A [9]. With this information it is evident why fruits and vegetables are an important addition to a diet.

b-**Carotene Background:**

In 1831 Dr. Wackenroder was the first to isolate carotene from carrots. This discovery marked the beginning of carotenoid research [10]. Carotenoids are natural pigments, generally oranges, reds and yellows, which are found in all plants. There are carotenoids found in green leaves and other foliage, however, the chlorophyll masks the visualization of these pigments [5]. Carotenoid pigment is what gives fruits, vegetables and flowers their bright and beautiful colors. β-Carotene is one member of this carotenoid family. Since the isolation of carotene there has been great interest in isolating and synthesizing β-carotene. Since 1954 β-carotene has been produced commercially and there are a total of six manufactured forms [10]. These manufactured forms are used as a color additive in processed foods. Naturally occurring β-carotene acts as a pro-vitamin becoming vitamin A. Carotenoids are also a biological antioxidant in radical processes [10]. A biological antioxidant is a compound that protects biological systems against the potentially harmful effects of oxidation reactions [10]. β-Carotene

and vitamin A are thought to be protective in the process of carcinogenesis and many studies have been done to try and demonstrate the beneficial aspects of ingesting β-carotene. Although carotenoids, especially β-carotene, are found in abundant quantities in plants, people and other mammals are unable to synthesize their own β-carotene. Thus eating plants with high levels of β-carotene is needed for the human diet. It has been demonstrated by numerous groups that diets rich in vegetables and fruits are associated with decreased incidences of certain cancers [6]. High β-carotene levels in blood plasma have been correlated to lower risk of cancer [6].

b-**Carotene Structure:**

The structure of β-carotene explains why it makes such a good antioxidant with strong tendencies to quench some very complex chemical reactions. β-Carotene is a long carbon chain isoprenoid, which generally is found in the *trans* configuration [9]. As seen in Figure 1 the two rings and multiple conjugated double bonds would seem to make β-carotene a good antioxidant. This antioxidant quality enables the molecule to quench $^1O_2$ by allowing singlet oxygen to add across these double bonds these chemical reaction will be discussed in more detail later [10].

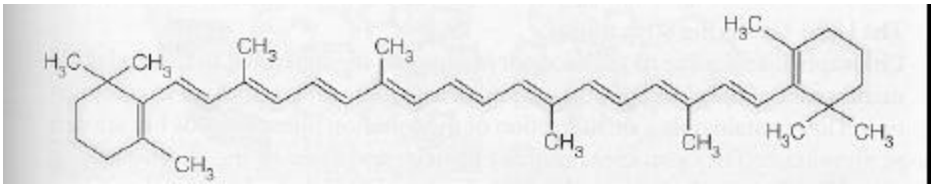

Figure 1: The structure of *trans* ß-carotene [8].

b-**Carotene Metabolism:**

Carotenoids are unstable species, which are sensitive to: light, variations in the level of oxygen, changes in the pH, and changes in temperature [10]. This is why studying β-carotene

has been so difficult. *In vitro* studies have shown that β-carotene inhibits peroxidation of simple lipids at a lower oxygen concentration but fails to perform at higher levels of oxygenation [6]. *In vivo* levels of oxygenation, the β-carotene causes antioxidant type chain breaks while the higher oxygen levels cause the β-carotene to react with O_2 causing the synthesis of carotenoid peroxyl radicals [9]. This explains the difficulty in researching the pro-vitamin but also explains why the antioxidant activity is beneficial to people. As humans, our cells function with a lower level of oxygen than is found outside the body.

β-Carotene has also been shown to possess antioxidant activity in liposomes, microsomal membranes, lipoproteins and corneal endothelial cells. As mentioned above, β-carotene, a pro-vitamin, is cleaved to make vitamin A. This is demonstrated in Figure 2. Cleavage of β-carotene involves a direct attack of a dioxygenase-like enzyme on the central 15,15'-double bond leading to the formation of two molecules of retinal [9].

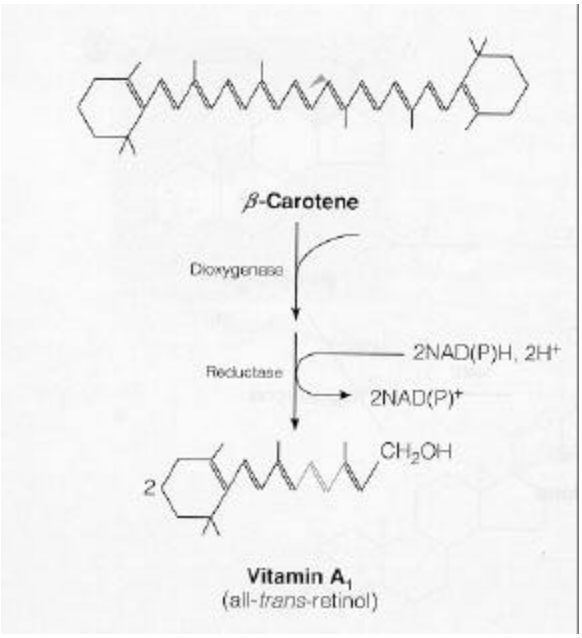

Figure 2: Cleavage of ß-carotene to vitamin A [8].

In vitro studies have suggested that β-carotene performs well as an antioxidant but performs better when other antioxidants are present. Having α-tocopherol and β-carotene in the system causes an additive beneficial effect in inhibiting radical initiated lipid peroxidation [11]. In Figure 3, lipid peroxidation was initiated in rat liver membranes by the addition of AAPH, which thermally decomposes to produce peroxyl radicals. The effects of individual use of β-carotene and α-tocopherol as well as the combined effect are shown in Figure 3. AAPH is added to control induction of peroxidation. β-Carotene by itself does not show much protective effect when added to the AAPH. α-Tocopherol seems to protect against the peroxyl radicals formed by the AAPH, but the combination of β-carotene and α-tocopherol exhibts an even greater protective effect against radical induced lipid peroxidation [11].

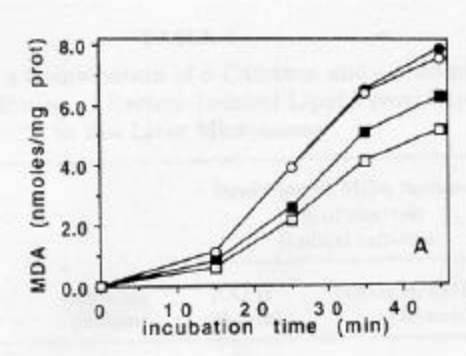

Figure 3: The induction of lipid peroxidation in rat liver by addition of AAPH. MDA formation was measured at 535 nm in the presence of AAPH (closed circle), ß-carotene (open circle), a-tocopherol (closed box) and a combination of both β-carotene and α-tocopherol (open box) [11].

b-Carotene Radical Scavenging Mechanisms:

The greatest benefit of β-carotene is the ability to scavenge radicals. In plants the antioxidant role of β-carotene occurs through the quenching of ROS (generally thought to be $^1O_2$ quenching) formed during photosynthesis. This is believed to be the same beneficial process in humans [6]. There are two mechanisms for β-carotene to scavenge radicals that will be

discussed in this paper. One of these mechanisms includes the deactivation of electronically active species such as $^1O_2$ and the deactivation of reactive chemical species such as peroxyl and alkoxyl radicals [10]. Increased oxidative damage *via* radicals occurs in many diseases and may play an active role in promoting lung damage in cystic fibrosis patients [7]. Lipid peroxidation in patients with cystic fibrosis is thought to be due to the increased generation of free radicals centered around the decreased availability of antioxidants such as β-carotene in the lung tissue [7].

β-Carotene is a $^1O_2$ scavenger and the ability to quench $^1O_2$ is related to the number of conjugated double bonds present [6,10]. This system quenches $^1O_2$ back to ground state oxygen ($^3O_2$). This drop in energy back to ground state is shown in reactions (1) and (2).

$$^1O_2 + CAR \rightarrow {}^3O_2 + {}^3CAR \text{ [10]} \qquad k = 4x10^9 \text{ L mol}^{-1} \text{ s}^{-1} \text{ [4]} \qquad (1)$$

$$^3CAR \rightarrow CAR + \text{heat energy [10]} \qquad k = 3x10^4 \text{ s}^{-1} \text{ [4]} \qquad (2)$$

These are important reactions because $^1O_2$ has been shown to inactivate proteins, cause peroxidation of lipids and cause DNA damage. In Figure 4 the quenching of singlet oxygen by β-carotene and other carotenoids is shown. The two other carotenoids that are studied in Figure 4 are lycopene and lutein, which are considered to be members of the β-carotene family. Lycopene is also a long carbon chain isoprenoid with multiple conjugated double bonds like β-carotene but lacks the ring structures on the ends. Lutein is also a long carbon chain isoprenoid with conjugated double bonds and the ring structures like β-carotene, however this species has OH groups attached to the rings, unlike β-carotene. In Figure 4 the quenching ability of $^1O_2$ is measured and shows that β-carotene in these conditions does not quench as well as lycopene but is a better quencher than lutein. The quenching rates (k_q) of these three carotenoids were calculated and measured with the assumption that $^1O_2$ lifetime is 10 μs. The quenching rates are

as follows: β-carotene $k_q = 4x10^9\ M^{-1}s^{-1}$, Lycopene $k_q = 9x10^9\ M^{-1}s^{-1}$ and Lutein $k_q = 2x10^9\ M^{-1}s^{-1}$ [4].

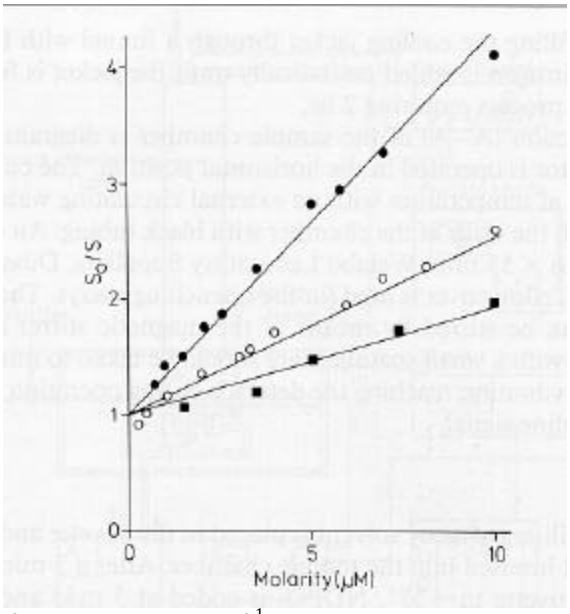

Figure 4: Stern-Volmer plots of data on quenching of $^1O_2$ by lycopene (closed circle), ß-carotene (open circle) and lutein (closed box) [3].

β-Carotene is an effective quencher of peroxyl and alkoxyl radicals as well, but the mechanisms are not very well defined. These peroxyl type radicals are created during lipid peroxidation. When dealing with O_2 and peroxyl radicals there is an equilibrium that is set up according to reaction (3).

$$\beta\text{-carotene}^{\bullet} + O_2 \leftrightarrow \beta\text{-carotene-}OO^{\bullet}\ [10] \qquad (3)$$

At physiological (low) oxygen concentration the hypothesis of reactions is (4) [10]. Implying that the β-carotene will react directly with a peroxyl radical to form a more stable carbon centered radical. At these O_2 concentrations reaction (3) is shifted to the left thus reducing the number of peroxyl radicals available.

$$\beta\text{-Carotene} + ROO^{\bullet} \rightarrow ROO\text{-}\beta\text{-Carotene}^{\bullet}\ [10] \qquad (4)$$

At these physiological conditions the β-carotene will compete with fatty acids for peroxyl radical reactions thereby preserving the fatty acid structure [2]. This competition occurs because the β-

carotene traps the chain propagating peroxyl radical before it can react with the fatty acid. These reactions only happen when there is a low concentration of O_2.

At higher concentrations of O_2 β-carotene becomes a pro-oxidant leading to more oxidative cellular damage. This damage is because the equilibrium of reaction (3) is shifted to the right allowing the formation of more peroxyl radicals and causing chain damage [2, 10]

b-Carotene Detection Methods:

There are many ways to detect β-carotene. β-Carotene can be visualized *via* visible light absorption (VIS), infrared spectra (IR), mass spectrometry (MS), NMR and high-pressure liquid chromatography (HPLC) [10]. β-Carotene is seen with VIS at 425 nm and by MS at 536 M^+ [10]. The most efficient way to visualize β-carotene is using reversed phase HPLC to separate β-carotene from its oxidation products and then resolving to the individual components by cyano column HPLC [10].

Summary:

As demonstrated above the benefits of β-carotene as a provider of vitamin A and an antioxidant are well understood. β-Carotene not only quenches physiological radicals to prevent peroxidation of lipids but may also be beneficial for preventing cancers. Understanding how β-carotene works as an antioxidant will help us to further understand how other naturally occurring species will help in the betterment of human lives. This is why you should eat your vegetables and not try to give them to the dog.

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