Hidden Beauty of Carotenoids: From Brilliant Colors to Human Health

James Allen Olson Memorial Lecture 2008

Arun B. Barua
Department of Biochemistry, Biophysics and Molecular Biology,
Iowa State University, Ames, USA

Correspondence: Arun B. Barua, 4737 S Ellis Ave,
Chicago IL 60615, USA
Email: abarua@iastate.edu

First of all, I would like to thank Elizabeth Johnson, Chairperson, CARIG Symposium Committee, and the CARIG Steering Committee members for their kind invitation to deliver the 2008 James Allen Olson Memorial Lecture. It is a great privilege and honor to be asked to present this lecture. When Liz asked me if I would accept the invitation, I replied, "How can I refuse an invitation to speak in memory of James Olson, whom I was associated with for over twenty years?" Indeed, it was James Allen Olson who brought me to this country from India in 1980. During these twenty years, I learned a lot from this great scholar, an authority on retinoids and carotenoids. What impressed me most was his vast knowledge and incredible memory. I miss him very much.

The title of my talk is 'The Hidden Beauty of Carotenoids: From Brilliant Colors to Human Health.' You will understand why the beauty of carotenoids is often hidden as I go through my talk.

It is well known that carrot leaves are green. But hidden underneath the soil is the beautiful bright orange color of the carrot roots. In 1831, Wackenroder extracted the color from the roots and obtained a homogenous compound that he named 'Karotin' ('Carotin').[1] Tswett invented the technique of chromatography in 1906. Using chromatography, it was shown that the homogenous compound was actually a mixture of isomers, β-carotene being most predominant. In carrot extract, β-carotene crystallizes first leaving the β-isomer in the mother liquor. The vivid yellow or orange color of carotenoids in many fruits and vegetables is often hidden under the green color of chlorophyll. Just peel a green but ripe papaya, mango, watermelon, squash or grapefruit, and you see the beautiful yellow to orange color of carotenoids within the pulp inside. It is difficult to believe that no green plants can survive without

Table 1: Carotenoids identified in human plasma (excluding *cis* isomers)[7]

From diet	Presumably metabolites
α-Carotene	3'-Hydroxy-ε-ε-caroten-3-one
β-Carotene	3'-Hydroxy-ε-ε-caroten-3'-one
γ-Carotene	3'-Hydroxy-β-ε-caroten-3'-one
Lycopene	ε-ε-Caroten-3,3'-dione
Neurosporene	3-Hydroxy-3',4'-didehydro-β-γ-carotene
ζ-Carotene	3'-Hydroxy-2',3'-didehydro-β-ε-carotene
Phytofluene	3'-Epilutein
Phytoene	2,6-Cyclolycopene-1,5-diol
α-Cryptoxanthin	Anhydrolutein
β-Cryptoxanthin	
Lutein	
Zeaxanthin	

carotenoids. But where are the carotenoids in green leaves? In autumn, when the days are short and there is not enough sunlight, the chlorophylls say "goodbye" to the carotenoids and disappear from the leaves. Carotenoids are left exposed. They were hidden under the chlorophylls. The beauty of autumn leaves is spectacular, but short-lived. The leaves fall and with them the carotenoids. It seems there is an intimate relationship between carotenoids and chlorophylls.

Functions of carotenoids

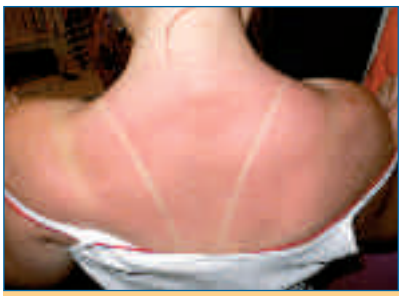
In non-photosynthetic tissues, carotenoids determine or contribute to the color of flowers and fruits. The color of carotenoids in fruits attracts birds and animals, and as the fruits are eaten their seeds get dispersed. The scales of certain fish turn brightly colored during spawning time. The brilliant colors of carotenoids in flowers attract insects and bees. As these creatures fly from flower to flower, pollination occurs. Bird feathers are often brightly colored with carotenoids to attract birds of the opposite sex.

Chlorophylls and carotenoids are integral components of the Light Harvesting Complex (LHC I and II) in photosystems. LHC II serves as the principal solar energy collector in photosynthesis. The structures of LHC I and II, in pea and spinach, have been determined by x-ray crystallography, and the binding sites of β-carotene, lutein, neoxanthin, violaxanthin and zeaxanthin have been identified.[2-5]

Carotenoids play an important role by absorbing blue-green light in this light harvesting process. In photosynthetic tissues, when there is excess light, photoprotection against harmful oxygen species is another important function of carotenoids. One mechanism for photoprotection by carotenoids is the quenching of chlorophyll triplets that would otherwise lead to the generation of oxygen singlets. These oxygen singlets can react with lipids, proteins, and other macromolecules, causing irreparable damage, even death, to the green plant. Another special mechanism, referred to as Non-Photochemical Quenching (NPQ), has evolved in plants to dissipate excess energy as heat.[2] The primary function of β-carotene is likely the quenching of singlet oxygen, produced after charge recombination, to the triplet state of chlorophyll.[3]

Carotenoids and human skin

When blood is analyzed for carotenoids, a number of dietary carotenoids, viz., α- and β-carotenes, lycopene, α- and β-cryptoxanthins, lutein and zeaxanthin, are seen.[6] There are a few other carotenoids such as anhydrolutein and keto-derivatives of lutein in the blood, however it is likely that these are metabolites of dietary carotenoids **(Table 1)**.[7] The presence of carotenoids in our circulating blood points towards some specific functions of the carotenoids in our body. Let us focus on four of the major carotenoids seen in our blood: β-carotene, lutein, zeaxanthin and lycopene. The color of our



Sunburn

skin is primarily due to melanin. The hemoglobin in red blood cells also affects the color of people with lighter skin. There may be a minor contribution determining skin color by fats that are present under the epidermis. Dietary carotenoids, such as β-carotene, lycopene and lutein, can contribute to normal human skin color but as in the case of green leaves, the carotenoids are often masked by hemoglobin and melanin. As in green plants, these carotenoids can participate in photoprotection.[8, 9] Too much exposure to sunlight may lead to sun-

burn (erythema) and other skin disorders, such as premature aging, photoallergic and phototoxic reactions, and skin cancer.

In plants, another role of carotenoids, besides photoprotection, is to protect against photosensitization by the plant's own chlorophyll. This same principle has been applied to the treatment of the human photosensitivity disease, Erythropoietic Protoporphyria (EPP), which has been studied extensively by Mathews-Roth.[10] EPP is an inborn error of heme biosynthesis resulting in high levels of protoporphyrin within red blood cells. Leakage of protoporphyrin from blood cells leads to a cascade of reactions

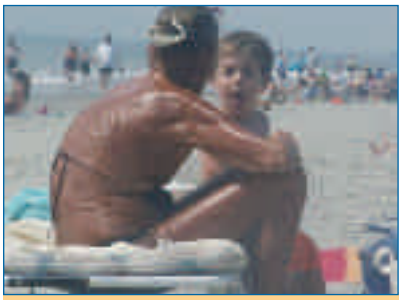
Goldfish

resulting in itching, burning, and ulceration of skin upon exposure to sunlight. Successful use of β-carotene (30–180 mg/day) for the treatment of EPP has been well documented. Canthaxanthin has also been used, but its application has been curtailed as long-term treatment results in deposits of crystalline canthaxanthin in the retina.

Carotenoids and the eye

The existence of a yellow spot in the macula of the human eye has been known for about two centuries. This yellow color in the macula has been found to be due to the presence of the carotenoids, lutein and zeaxanthin. These carotenoids must have a role in ocular health, and indeed, several studies have shown that these carotenoids are important contributors for maintaining good vision.[11]

Specifically, lutein and zeaxanthin have been associated with cataracts and age-related macular degeneration (AMD), which is regarded as the leading cause of vision loss, in the western world, for persons 55 years and older. In the USA, 200,000 new cases of AMD are diagnosed each year. Multiple health claims touting the benefits of lutein and zeaxanthin supplementation for maintaining good eye health have been made. In a report from November 2006, the Food and Drug Administration (FDA) concluded that, on the basis of evidence-based review, no credible evidence existed for a health claim about the intake of lutein and/or zeaxanthin and the risk of age-related macular degeneration or cataract.[12]



Photoaging

More recently, results from four large studies have been published showing that there may indeed be a positive association between lutein and zeaxanthin and cataracts and AMD. In one study involving 35,551 women, aged 45 years or older, who consumed diets higher in lutein/zeaxanthin or total vitamin E had lower risk of developing cataracts. The study did not claim that lutein/zeaxanthin could prevent cataracts, but reported that it might delay their formation.[13] A second study, known as Age-Related Eye Disease Study (AREDS), included 4,519 subjects aged 60–80 years. It was reported that higher dietary intake of lutein/zeaxanthin was independently associated with decreased likelihood of having neovascular age-related macular disease (AMD), geographic atrophy, and large or extensive intermediate drusen.[14]

A third study in Australia, involving 2,454 people, showed that whereas lutein and zeaxanthin reduced the risk of long-term incidence of AMD, β-carotene intake was associated with increased risk of AMD in both smokers and non-smokers.[15] A report from March 2008 found in 1,802 women, 50–79 years of age, that diets rich in lutein and zeaxanthin were moderately associated with decreased prevalence of nuclear cataracts. However, the report stated that other protective aspects of such diets might in part explain these relationships.[16]

Lycopene and human health

Lycopene is another carotenoid that has been of significant interest to carotenoid researchers and the tomato industry. Lycopene is a potent antioxidant and has been shown to be protective against several forms of cancers – prostate cancer in particular.[17] A recent lycopene forum discussed the health benefits of the consumption of lycopene through tomato-based products. According to Giovannucci, a moderate inverse association was observed between tomato products and prostate cancer in most prospective and plasma-based studies of lycopene.[18] Relative to other cancers, the association was suggestive but not always consistent.

Health claims had also been made of the benefits of lycopene. The FDA reviewed the health claims of lycopene and stated in 2007: "On the basis of evidence-based review of claims, the FDA found no credible evidence to support an association between lycopene intake and a reduced risk of prostate, lung, colorectal, gastric, breast, ovarian, endometrial, or pancreatic cancer. The FDA also found no credible evidence for an association between tomato consumption and a reduced risk of lung, colorectal, breast, cervical, or endometrial cancer. The FDA found very limited evidence to support an association between tomato consumption and reduced risks of prostate, ovarian, gastric, and pancreatic cancers."[19]

Recently, the excentric cleavage products of lycopene, the lycopenoids, have gained interest.[20, 21] We look forward to learning more about the health benefits of lycopene and its cleavage products and tomato products in the near future.

Bioavailability of carotenoids

What happens to the carotenoids ingested in food? Some but not all dietary carotenoids are absorbed. Information about the bioavailability of carotenoids is important from a nutritional viewpoint. Knowledge about the SLAMENGHI factors was learnt from the late Clive West.[22] SLAMENGHI is a mnemonic used to describe the factors that can affect the bioavailability of β-carotene and other carotenoids. It stands for Species of carotenoids, molecular Linkage, Amount of carotenoids consumed in a meal, Matrix in which the carotenoid is incorporated, Effectors of absorption and bioconversion, Nutrient status of the host, Genetic factors, Host-related factors, and Interactions.[22] However, there is still more to be learned about these factors that control the absorption of carotenoids.

A considerable section of the world population is vegetarian, and carotenoids play a vital nutritional role in this group of people. These vegetarians depend on provitamin A carotenoids as their only source of vitamin A. In the United States, about 26% of vitamin A consumed by men and 34% of vitamin A consumed by women is in the form of provitamin A carotenoids.[23]

Nora O'Brien's laboratory, in Cork, Ireland, is involved in a project on the bioavailability of carotenoids. Her study on human intestinal Caco-2 cells showed that the xanthophyll carotenoids were more bioavailable than hydrocarbon carotenoids.[24] These results may have a relation with a finding that will be discussed later in this paper.

Conversion of carotenoids to vitamin A

After β-carotene is absorbed in the mucosal cells of the small intestine, it is cleaved to vitamin A aldehyde (retinaldehyde, retinal). There are two pathways for the conversion of β-carotene to vitamin A: the central cleavage by cleavage of the 15,15'-double bond to give rise to two molecules of vitamin A, and the excentric cleavage by cleavage of a terminal double bond to give rise to only one molecule of vitamin A.

James Olson became well known to carotenoid and retinoid investigators when he, in association with Hayaishi, described an enzyme, 15,15'-β-carotene dioxygenase, that they were able to isolate from rat liver and intestine and characterize.[25] The enzyme cleaved β-carotene molecule to two molecules of vitamin A supporting the central cleavage mechanism. This same enzyme was also reported independently and simultaneously by Goodman and Huang.[26] During

the 2007 Olson Memorial Lecture, von Lintig gave an update on cloning and expression of the enzymes, CMO1 and CMO2. These enzymes are capable of cleaving the carotenoid molecule at 15,15'-double bond and at 9',10'-double bond to give rise to retinal by central cleavage and to apocarotenal by excentric cleavage, respectively.[27–31] Further support for excentric cleavage was published by Andrew Clifford's group, describing the identification of apo-β-8'-carotenal as a metabolite of β-carotene in the plasma of a human volunteer three days after an oral dose.[32] Assuredly, more about cleavage of other carotenoids by these enzymes will be reported in the near future.

Once retinaldehyde is formed, a series of other enzymatic reactions occur in the small intestine to give rise to retinol, retinoic acid, glucuronides and retinyl esters. A number of enzymes belonging to the reductase, hydrolase and esterase groups are involved in this process. James Olson described the possible pathways of the conversion of β-carotene to retinoids.[33] An update on absorption and transport of vitamin A has been published by Wongsiriroj and Blaner.[34]

Flamingo colored by canthaxanthin

Vitamin A deficiency

Why are carotenoids so vital to people, especially vegetarians? The fact that β-carotene and a number of related provitamin A carotenoids are converted to vitamin A in our body is well known. Vitamin A is required for growth, reproduction, cell division and differentiation, and vision. Vitamin A aldehyde, retinal or retinaldehyde binds with the protein opsin to form rhodopsin, which plays an important role in vision. Lack of vitamin A affects rhodopsin formation in the eye, consequently resulting in a visual defect. The serum/plasma retinol concentration in a healthy person should be above 0.7 µmol/L. A retinol concentration of 0.35–0.7 µmol/L is regarded marginal, and levels below 0.35 µmol/l are regarded as deficient.[35] The first sign of deficiency of vitamin A is night blindness. Xerophthalmia is a disease of the eye resulting from vitamin A deficiency.

Vitamin A deficiency is regarded as a public health problem in more than half of all countries in this world. It is estimated that 250 million preschool children are vitamin A deficient. It is also estimated that 250,000–500,000 of those children will become blind every year, with half of them dying within 12 months of losing their sight.[36, 37] It is well known that vitamin A deficiency can lead to blindness, but this is a preventable disease. However, people are often unaware that even before blindness occurs, vitamin A deficient children face a 23% greater risk of dying from ailments such as measles, diarrhea or malaria.[36, 37]

Moreover, it is also likely that in vitamin A-deficient regions a substantial proportion of pregnant women are deficient in vitamin A. Occurrence of night blindness is common in such mothers. A baby born to a vitamin A-deficient mother is almost certain to be vitamin A deficient as the breast milk will be lacking vitamin A. If the vitamin A supply of the pregnant woman is inadequate, the supply to the fetus will also be diminished, as well as later in her milk. Usually, such mothers depend on carotenoids as the source of vitamin A and a low dietary intake of provitamin A carotenoids would result in vitamin A deficiency. Hence, vitamin A deficiency is not only a problem for children, but also for pregnant and lactating women.

Retinoyl glucuronide hydrolysis test

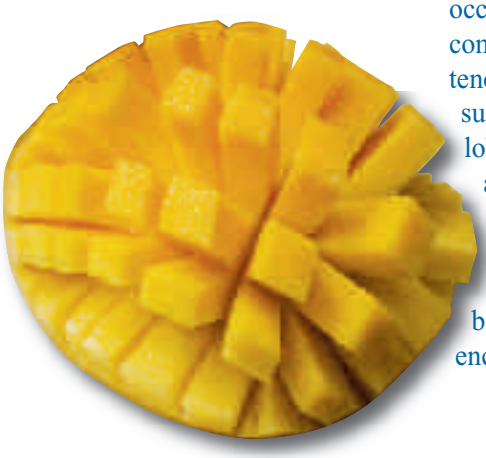
There are several methods of determining vitamin A deficiency. Analysis of serum retinol is a very good biochemical method to assess vitamin A status. However, it has some drawbacks. The blood level of vitamin A is homeostatically controlled. Only when stored vita-

min A in the liver is almost exhausted, is there a fall in the level of serum vitamin A. During infection and diarrhea, serum levels of retinol fall, making serum retinol reading an unreliable indicator of vitamin A status. Researchers have developed various other methods to determine the status of vitamin A, including the Relative Dose Response (RDR)[38] and Modified RDR (MRDR)[39] methods.

In this James Olson Memorial lecture, it would be appropriate to mention some of Olson's own work. Jim Olson was the first to report, in the 1960s, the existence of water-soluble metabolites of retinol and retinoic acid, known as retinyl glucuronide (ROG) and retinoyl glucuronide (RAG).[40, 41] After the successful chemical synthesis of the retinoid glucuronides in the mid-1980s, the biological properties of these metabolites have been determined. RAG is biologically active, less toxic and less teratogenic than retinoic acid (RA).[42] In vitamin A-adequate mice, RAG administered by injection was only partially hydrolyzed to RA.[43] RA was not detected in the serum of orally administered RAG in vitamin A adequate rats, although a small quantity of intact RAG could be detected in the liver. On the other hand, in vitamin A-deficient rats, RAG was converted to RA that appeared in serum as well as in the liver.[44, 45] Following an oral dose of RAG to healthy American adults, neither RAG nor RA could be detected in serum.[45] The conclusion was that, when the vitamin A status was normal, orally-dosed RAG was not converted significantly to RA.

Olson was planning to carry out a study to prove this theory in a developing country where VAD existed. Unfortunately, before such a study could be done, James Olson passed away suddenly in September 2000. After his demise, I became aware about the deaths of thirty children and sickness involving thousands of children following supplementation of vitamin A under the WHO-UNICEF Global Vitamin A program in Assam, India in 2001. There were protests against the supplementation program alleging that VAD did not exist in that region. There was conflicting support and criticism regarding the supplementation program.[46-49]

I developed a keen personal interest in determining if VAD existed in Assam, since it is where I spent my college and university life. In collaboration with Gauhati University and Sri Sankardeva Nethralaya (a prominent eye institute) in Guwahati, Assam, the retinoyl glucuronide hydrolysis test was performed on Assamese children.[50] A total of 61 children were included in the study. An oral dose of 25–75 mg of RAG was given to 42 children. Blood was collected from all 61 children 4 hours after the oral dose was given to the group of children receiving the RAG dose. The serum of all 61 children was analyzed simultaneously for retinol, RA and carotenoids. It was found that the majority of the children were vitamin A-deficient or marginally deficient (serum retinol <0.7 µmol/L). The children with serum retinol concentration below 0.85 µmol/L showed RA in their serum resulting from hydrolysis of RAG, whereas children with serum retinol concentration >0.85 µmol/L did not show any RA. The conclusion was that detection of RA following an oral dose of retinoyl glucuronide was an indicator of marginal or deficient level of serum vitamin A. In this study, we were able to identify marginal deficiency in children without any clinical or sub-clinical signs of VAD simply from hydrolysis of RAG to RA.



The concentrations of lutein and β-carotene were also determined in these children. Most of the children showed low but satisfactory levels of lutein in serum, but the level of β-carotene was surprisingly very low in all the children. The reason for such low level of β-carotene was not determined. As mentioned before, the University of Cork study showed that lutein was more efficiently absorbed than β-carotene. Olson showed that lutein affected the absorption of β-carotene when present together.[51] Perhaps these are the reasons for the observed difference of lutein and β-carotene levels in the children in our study. It is possible that these children, being deficient in vitamin A, had an increased demand for vitamin A and any dietary β-carotene available in food was converted to vitamin A resulting in less storage of β-carotene.

Future direction

There are more than 600 naturally occurring carotenoids. Besides the commonly identified serum carotenoids, other dietary carotenoids such as astaxanthin (from shrimp, lobster and salmon),[52] bixin (from annatto seeds),[53] crocetin and crocin (from saffron or crocus flower)[54] have been shown to appear in human and mouse blood. We also know of the presence of phytofluene, phytoene, and

metabolites like anhydrolutein and keto-carotenoids, in circulation in our blood. Although some limited information about astaxanthin, bixin and crocetin is available, there is still much to be discovered about the role of these carotenoids in our health.

Conclusion

Only a few carotenoids, out of hundreds, are absorbed and found circulating in human blood. These carotenoids, such as α- and β-carotenes, lycopene, α- and β-cryptoxanthins, lutein and zeaxanthin, must have an important and necessary role in our bodies. The role of provitamin A carotenoids in humans as the source of vitamin A has been known for a century. There is no doubt that, without provitamin A carotenoids in the diet, a large section of the human population would be blind and the mortality rate significantly increased. The photoprotective action of carotenoids against sunburn and other skin conditions, such as photoaging, is likely similar to photoprotection of chlorophylls by carotenoids in green plants. Why are only lutein and zeaxanthin found in the macula of the eye? There is likely an association of lutein/zeaxanthin with cataracts and AMD, but the exact role is yet to be discovered. Similarly, there is probably an association of lycopene with cancer but its exact role in fighting cancer is still unknown and this area of research remains promising. The exact role of other carotenoids, such as phytoene, phytofluene, astaxanthin, bixin, and crocetin, is still unknown but we are on the verge of discovery. The important and necessary roles that these carotenoids play in normal bodily function, fighting diseases, and maintaining health remain a hidden beauty waiting to be unveiled through our continued research.

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