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CAROTENOID PROTECTION AGAINST OXIDATION

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Abstract - Based on experimental observations made in photosynthetic organisms which lacked colored carotenoid pigments, a hypothesis was developed that these pigments could serve as protective agents against photosensitized oxidations. Over the last 20 years, many workers have confirmed this hypothesis and have extended the observations to non-photosynthetic bacteria, plants and animals. It has also been possible to demonstrate carotenoid protection in in vitro systems. This article reviews the major mechanisms whereby carotenoid pigments protect cells against harmful photosensitized oxidations. The mechanisms include:

(1) Carotenoid quenching of triplet sensitizers.
(2) Carotenoid quenching of O_2 ($^1\Delta_g$).
(3) Carotenoid inhibition of free radical reactions.

INTRODUCTION

Although it has been known for almost a century that sunlight was lethal to bacteria (1) an understanding of this phenomenon is still in progress. Buchbinder et al. (2) demonstrated that not only was sunlight lethal but that artificial light sources were also effective in killing various strains of streptococci. If an exogenous pigment or light-absorbing compound is added to the cellular system, the damaging effects of either natural or artificial light can be vastly increased. This was first reported by Raab (3) who, by using acridine orange added to an aerated culture of paramecia, was able to observe a light-induced death of the organism. This sensitization of living tissue by light, a suitable sensitizing pigment and air, has been referred to as the photodynamic effect. In 1941, Blum (4) published a monograph describing the history of the photodynamic effect and summarizing most of the observations made up to that time. More recent surveys have been published about this phenomenon (5-12) and as the understanding of photochemical oxidations has expanded, it has become possible to begin to understand the nature of the photodynamic damage including molecular mechanisms. The fact that some of the damage is not lethal but involves the induction of mutations is certainly a clear demonstration that there has been an effect, either direct of indirect, on the nucleic acids of the treated organisms (5, 10). Although this type of observation is not surprising when the light source consists of or contains far-ultraviolet radiation, it has also been observed using visible light. In some cases, different effects have been observed which are presumably due to different types of photochemistry occurring in the ultraviolet or the visible region of the spectrum. In addition, there is a region of near-ultraviolet light (320-400 nm) which also causes a photodynamic effect that can either lead to killing or mutations (13).

In this paper, I will discuss the damage done to biological systems by light, in particular the damage caused by visible light, and then discuss the photochemical basis for this damage. The role of carotenoid pigments in protecting cells against photosensitized oxidations will be described using observations based on both cellular and in vitro systems. Particular emphasis will be placed on the multiple reactions in which carotenoid pigments protect cells against harmful oxidations. The implications of these observations for other systems in which oxidants are generated non-photochemically will also be discussed.

BIOLOGICAL PHOTODAMAGE

Based on numerous experimental observations, investigators have concluded that the damage done by visible and near-ultraviolet light to cellular systems can be modified to a large extent by the presence of endogenous carotenoid pigments (14). There is an extensive literature on photodamage due to visible light in organisms which lack carotenoid pigments

(see Krinsky (7) for an extensive review of this material up to 1967) and this will be touched on very briefly in this article. The crucial observations were made by Sistrom et al. (15) who studied the physiology of Rhodopseudomonas spheroides, a purple sulphur bacterium and a blue-green mutant strain which they had isolated. Both strains displayed identical growth behavior under photosynthetic conditions, but in the presence of air and light the mutant strain stopped growing, its bacteriochlorophyll was destroyed and the organism was killed. Based on these observations, Sistrom et al. (15) suggested that the colored carotenoid pigments were acting as protective agents against a photodynamic effect initiated by the endogenous sensitizer, bacteriochlorophyll. Similar observations have also been made in non-photosynthetic bacteria (16-18). It should be pointed out that the blue-green mutant strain lacked colored carotenoid pigments but still retained the usual complement of bacteriochlorophyll.

Much of the recent work dealing with carotenoid protection has been reviewed recently (19) and only a few examples will be presented here of additional material. The effectiveness of carotenoid pigments in protecting organisms against the harmful effects of light were first believed to be limited to visible light. For example, both Kunisawa and Stanier (16) and Mathews and Krinsky (20) could find no protective effect of carotenoid pigments using bacteria that were exposed to ultraviolet radiation. Futhermore, Mathews and Krinsky (20) demonstrated that a radiation-resistant organism showed no differential effect to gamma-irradiation whether it contained colored carotenoid pigments or not. There have been several recent reports, however, that would indicate that colored carotenoid pigments may offer some protection against ultraviolet damage. David (21) observed that several different strains of Mycobacterium showed a negative correlation between carotenoid content and relative ultraviolet sensitivity when exposed to germicidal ultraviolet irradiation. It should be pointed out that the irradiation response of the mycobacteria also correlated well with the size of the bacterial genome as well as the degree of repair efficiency. Similarly, Morris and Subden (22) studied the effects of ultraviolet irradiation on carotenoid-containing and carotenoidless strains of Neurospora crassa and found that both albino strains and those rendered pigmentless by treatment with a carotenoid synthesis inhibitor, β-ionone, had a much greater sensitivity to ultraviolet irradiation than did the wild type strain. Another example of carotenoid protection was reported by Epstein (23). While treating hairless mice with either β-carotene or a placebo, the animals were exposed to ultraviolet-B (290-320 nm) and he reported that the tumors which normally form under this treatment grew more slowly in the carotenoid treated group.

In several other organisms, carotenoid protection against visible light has been demonstrated. These include Micrococcus roseus (24) and Bdellovibrio bacteriovorus (25).

Not only do the presence of carotenoid pigments protect the cells against death, but it has now been demonstrated by Prebble and his associates (26,27) that individual enzymes and compounds can be protected in cell-free systems. Carotenoid pigments have continually been demonstrated to be useful in plants and animal systems. One manifestation of this protection is the fact that various herbicides are toxic because they interfere with the normal pathways of carotenoid biosynthesis, resulting in the accumulation of colorless polyenes and rendering the treated plant susceptible to photodestruction (28,29). A recent example of this kind of observation has been presented by Bartels and Watson (30) who not only demonstrated chlorophyll photodestruction in wheat seedling treated with two different herbicides, but also observed a destruction of chloroplast structure and lack of chloroplast ribosomes following illumination. The latter effect may be due to a direct photosensitizing effect of chlorophyll on chloroplast DNA, as originally suggested by Leff and Krinsky (31).

One of the most interesting aspects of carotenoid protection in photosynthetic systems was noted by Boucher et al. (32). These workers were investigating the photoreaction center from the photosynthetic bacterium, Rhodospirillum rubrum as well as a carotenoidless mutant strain (strain G9). When preparations of the photoreaction center from the mutant strain were exposed to intense near-infrared light in the presence of oxygen, the bacteriochlorophyll was irreversibly bleached whereas the effect was much smaller in similar preparations obtained from the wild type strain. Boucher et al. (32) have made the important observation that they can reconstitute carotenoid-containing photoreaction centers when the photoreaction centers from strain G9 were incubated with various purified bacterial carotenoids. Upon reconstitution, a measure of photoprotection against light-induced bacteriochlorophyll bleaching was obtained which in some cases was comparable to that observed in the wild type reaction center. Interestingly, they were unable to reconstitute a carotenoid-containg reaction center using the non-bacterial pigments, β-carotene or lutein. Some of the relevant data which they obtained can be seen in Table I. These authors conclude (32) that relative effectiveness as protective agents is not related to the ratio of the pigment to the bacteriochlorophyll but must be a function both of the structure of the carotenoid and the manner in which it binds to the reaction center.

There have been several recent reports on the ability of carotenoid pigments to protect animal systems against photosensitized damage. These include the observations of Hairston (33) who found a direct relationship between the concentration of carotenoids in the copepod Diaptomus nevadensis and their ability to survive in natural intensities of visible light. These observations indicate the evolutionary significance of carotenoid pigments for the enhanced ability of these organisms to survive and support the thesis first suggested by Mathews and Sistrom (17) that the possession of colored carotenoid pigments would enhance chances for survival when an organism is exposed to light in its environment.

TABLE 1. Carotenoid Effects in Reconstituted Photoreaction Centers (32)

Carotenoid	Carotenoid / Bacteriochlorophyll	Efficiency of Protection(%)
β-carotene	0	-
lutein	0	-
spirilloxanthin (wild type)	1.2	100
spirilloxanthin (reconstituted)	1.2	100
sphaeroidene	1.2	100
sphaeroidenone	1.0	20
chloroxanthin	0.4 - 1.5	40

In several in vitro systems carotenoids have been shown to offer some protection against photodynamic effects. Oxford et al. (34) utilized squid giant axons and measured either the irreversible block of sodium channels or the slowing of inactivation following the administration of photosensitizing dyes and light. They found that the addition of β-carotene could protect this preparation when methylene blue was used as a sensitizer but not when either Rose Bengal or a merocyanine dye was used as the sensitizer. One difficulty in interpreting these experiments is the fact that it is not precisely known where the sensitizing dye is initiating its actions and whether or not the carotenoid pigment can be localized in close enough proximity to exert a protective effect.

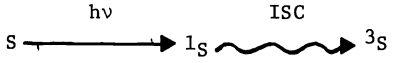
There have been several reports that carotenoid pigments can protect red blood cells against photohemolysis induced by exogenous dyes or endogenous porphyrin pigments since the original report by Krinsky et al. (35). Schothorst et al. (36), Swanbeck and Wennersten (37) and Nilsson et al. (38) have all shown that the addition of β-carotene to red blood cells exposed to light and containing either endogenous pigments such as protoporphyrin or exogenously added sensitizers can lead to an inhibition of photohemolysis.

These studies (36-38) involving photohemolysis of red blood cells that were either exposed to exogenous protoporphyrin or that have come from patients suffering from erythropoietic protoporphyria (EPP) were based on the observations made initially by Mathews-Roth et al.(39) that the oral administration of β-carotene could serve as a protective agent for patients suffering from EPP. There have now been many studies which have corroborated the efficacy of β-carotene as a treatment for this form of a human photosensitivity disease. Treatments of patients suffering either from EPP or other photosensitive diseases have been reviewed recently by Mathews-Roth (40,41).

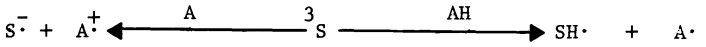
PHOTOCHEMICAL BASIS FOR PHOTODAMAGE

Before proceeding with the mechanisms whereby carotenoid pigments protect various biological systems from photosensitized damage, it is necessary to review the photochemical reactions which can induce such damage. These photochemical reactions are initiated by light ($h\nu$) which excites a sensitizer molecule (S) and forms the first electronically excited species

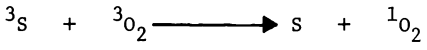
of the sensitizer, referred to as the singlet excited species (^1S). This singlet excited species has a very short lifetime ($\sim 10^{-11}$ sec) and dissipates its energy either by interacting with the solvent, emitting a photon in the form of fluorescence or by undergoing an inter-system crossing (ISC) to form a new excited species called the triplet sensitizer (^3S). These reactions, which are depicted below, can generate different proportions of the ^3S,



depending on other compounds in the environment and the chemical nature of the sensitizer. It is the ^3S species that has a sufficiently long lifetime to interact with other chemicals and initiate photochemical reaction. Among those reactions there are several that can lead to the harmful aspects of the photodynamic effect. The ^3S can now initiate a series or reactions, depending on the availability of O_2 and the nature of other potentially reactive species in the environment. Gollnick anc Schenck (42) have divided the subsequent photochemical reactions of ^3S into two general types. Type I reactions are redox reactions which frequently result in the formation of radical species that can then lead to further radical-catalyzed damage. The initial reactions frequently involves hydrogen or electron abstraction depending on the nature of both the sensitizer and the compound with which it reacts. The two forms of the type I reaction are seen below:



In the type II reaction, ^3S reacts directly with ground state O_2 . The reaction proceeds very efficiently since O_2 in its ground state is a paramagnetic molecule which exists as a triplet species, represented as $^3O_2$. The reaction then proceeds with conservation of spin forming the $^1\Delta_g$ species of O_2, hereafter referred to as singlet oxygen or $^1O_2$. This reaction is described below:



This latter reaction was originally postulated by Kautsky et al. (43) but was not widely accepted until the reports of Foote and Wexler (44) and Corey and Taylor (45) appeared in which they were able to demonstrate that the products of photosensitized oxidations using sensitizers such as methylene blue were identical to those that had been reported when $^1O_2$ was generated either chemically or by use of a radiofrequency discharge apparatus. Based on these results, Foote and Wexler (46) proposed that the Kautsky hypothesis of photo-sensitized production of $^1O_2$ had been confirmed. There have now been numerous confirmations of this observation (47-51). The production of photochemically active compounds is shown in Figure I.

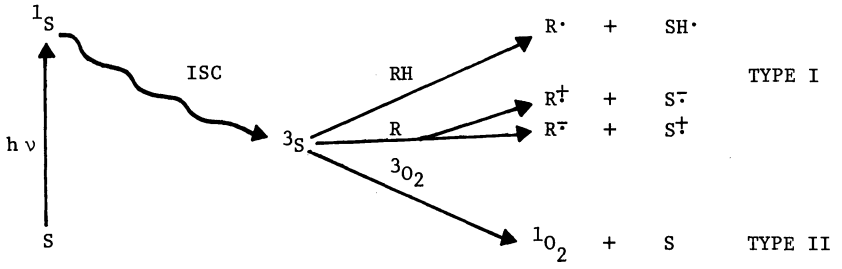


Figure I. Schematic representation of the reactions involved in both Type I and Type II photosensitized oxidations.

The damaging effects of free radical reactions in biological systems have been frequently described and the readers are referred to recent review by Pryor (52) dealing with this issue. The details of the chemical reactions of $^1O_2$ are described by Foote (53) and the biological role of $^1O_2$ by Krinsky (54,55). This species of oxygen has very different properties from $^3O_2$ and combines very rapidly with dienes such as those found in unsaturated fatty acids, aromatic and sulphur compounds such as those found in amino acids and various purines. All of these reactions have the capacity of initiating extensive cellular damage that can ultimately lead to the death of the organism (12).

The explanation for carotenoid protection against photosensitized oxidations must therefore reside in the ability of carotenoid pigments to intercept the reactive species described in Figure I.

The first clearly defined active species in this figure consists of ^3S. Depending on the nature of the photochemical system under investigation, varying porportions of ^1S will be converted to ^3S. This depends to a large extent on the intersystem crossing rate for various sensitizers. In photosynthetic systems, this rate is known for chlorophyll (56) and amounts to .04% of all the ^1S molecules being converted to the ^3S of chlorophyll. In 1957, Fujimori and Livingston (57) demonstrated that carotenoid pigments had the capacity to quench the triplet state of chlorophyll (^3Chl) in vitro and there are now many examples of this triplet-triplet energy transfer from chlorophyll to carotenoids, with examples being observed both in vitro and in vivo.

The reaction which occurs is apparently a direct energy transfer between ^3Chl and the carotenoid as shown below:

$$^3Chl + Car \longrightarrow Chl + {}^3Car$$

This reaction was first demonstrated by Chessin et al. (58) and has now been reported by numerous investigators working with photosynthetic systems (59-66). This form of triplet-triplet energy transfer in photosynthetic bacteria has been reviewed recently by Cogdell (67). The high efficiency of the quenching reaction between β-carotene and ^3Chl has permitted Foote (53) to calculate that only 10% of ^3Chl would survive carotenoid quenching within intact chloroplasts where the local concentration of β-carotene might be as high as 2×10^{-2} M. Carotenoid quenching of other ^3S species has been demonstrated in vitro both by Mathis and his associates (59, 68, 69) and Land and his colleagues (70-73). In particular, Bensasson et al. (72) have demonstrated that the singlet → triplet intersystem crossing efficiency for carotenoid pigments is extremely low and would therefore preclude the ^3Car arising by any other means than direct energy transfer from ^3S, with the exception of the reaction with $^1O_2$, to be discussed below. One of the compounds studied by Land and his associates (73) is the dimethyl ester of protoporphyrin IX, the pigment which accumulates in EPP. These workers have demonstrated, using a series of carotenoid pigments of varying polyene lengths, that β-carotene gives the maximum quenching of ^{3}protoporphyrin IX.

In looking at the other potentially damaging species depicted in Figure I, we are left with the radical intermediates formed in the Type I reactions and $^1O_2$ formed in the Type II reaction. A great deal of emphasis has been placed on the interaction of carotenoid pigments with $^1O_2$, sometimes to the detriment of the possible relationship of carotenoid pigments to the radical intermediates generated by Type I photoreactions. The emphasis on $^1O_2$ is based on the very elegant work of Foote and his collaborators who have been instrumental in explaining this important protective function of carotenoid pigments. In 1968, Foote and Denny (74) first demonstrated that carotenoid pigments can quench $^1O_2$ in an in vitro system. This significant observation has been verified in many different systems so that it now is clear that carotenoid pigments are excellent quenchers of $^1O_2$ and can therefore confer protection on either chemical or biological reactions. The quenching constant for this reaction approaches a diffusion-controlled limit and has now been determined to be 1.3×10^{10} M^{-1} sec^{-1} in benzene solutions (75). This value seems to be similar for many carotenoid pigments which contain at least nine conjugated double bonds (76).

The relationship between the chromophore length and the ability to protect cells has been described in some detail by Krinsky (14). What seems to be quite clear is that carotenoid pigments containing nine or more conjugated double bonds are effective quenchers of $^1O_2$ as well as capable of offering good protection against photosensitized damage in vivo. Carotenoid pigments which contain seven or less conjugated double bonds are not as effective $^1O_2$ quenchers and are not as effective in in vivo systems. The relationship between the length of the conjugated double bond system of the carotenoids and either protection against photobleaching of chlorophyll, as measured by Claes (77) and Claes and Nakayama (78) and the $^1O_2$ quenching rate as determined by Foote et al. (76) is depicted in Figure II.

Mathews-Roth and Krinsky (18) found an interesting opportunity to extend these observations by studying a mutant strain of Micrococcus luteus (Sarcina lutea) whose major carotenoid pigment contained only eight conjugated double bonds. In this mutant strain, the carotenoid pigment did not protect the organism from the harmful effects of oxygen and an exogenous photosensitizer. These studies were then extended by Mathews-Roth et al. (79) who

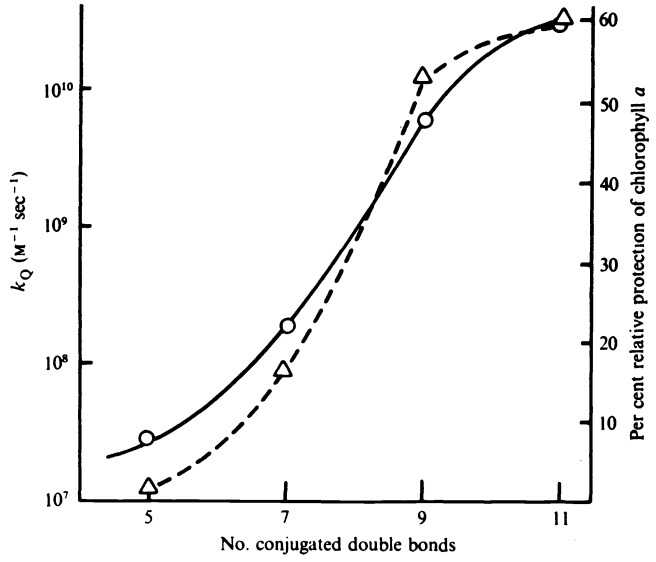


Figure II. The relationship between the number of conjugated double bonds and either $^1O_2$ quenching rates (76) or protection against photobleaching of chlorophyll a (77,78).

investigated the ability of a large number of naturally occurring carotenoid pigments to quench $^1O_2$ as well as effect free radical reactions. These authors found that the major pigment of the wild-type M. luteus contained nine conjugated double bonds and was as effective a quencher $^1O_2$ as either β-carotene, lutein or isozeaxanthin. The carotenoid pigment from the mutant strain which contained only eight conjugated double bonds was two to three times less efficient than the wild-type pigment, whereas phytofluene (5 conjugated double bonds) and phytoene (3 conjugated double bonds) were 100 and 1,000 times less efficient than β-carotene at quenching $^1O_2$. What then is the basis for these differences among carotenoid pigments of different polyene lengths in their ability to quench $^1O_2$ and offer protection in vivo?

MECHANISMS OF CAROTENOID PROTECTION AGAINST $^1O_2$

Although the rate of $^1O_2$ quenching by β-carotene estimated by Foote et al. (76) at 3×10^{10} M^{-1} sec^{-1} may be somewhat high, it is not very far above the value of 1.3×10^{10} M^{-1} sec^{-1} reported more recently (75). The data reported in Figure II by Foote et al. (76), Claes (77) and Claes and Nakayama (78) as well as that reported by Mathews-Roth et al. (79) would indicate that some limiting factor prevents all of these pigments from efficiently quenching $^1O_2$. The limiting factor may in fact be the triplet energy level (E_T) of the carotenoid pigments in comparison to the energy difference between $^1O_2$ and $^3O_2$. In the latter case, the energy difference is 94 kJ mol^{-1} for O_2 ($^1\Delta_g$). It has been very difficult to obtain direct measurements of the triplet energies of carotenoids. However, there have been several excellent estimates of these energies and a number have been brought together in Figure III. The theoretical calculations of Salem (80) would indicate that an inverse relationship should exist between the number of conjugated double bonds in polyenes and the triplet energy levels. Data for a number of polyenes and carotenoid pigments, shown in Figure III, have been taken from the publications of Mathis and Kleo (69) and Bensasson et al. (72) which clearly display this linear relationship between the inverse of the triplet energy level ($1/E_T$) and the length of the conjugated polyene chain. The dashed line in Figure III represents the energy level of $O_2(^1\Delta_g)$. These data clearly demonstrate that only carotenoid pigments containing nine or more conjugated double bonds could be involved in an exothermic energy transfer relationship with $^1O_2$, and those of shorter polyene chains would be much less efficient in this process. This is precisely the observation that has been demonstrated by several workers in the past (76-79).

As mentioned earlier, carotenoid pigments serve as excellent quenchers for various ^3S, including ^3Chl. In Figure III, the $1/E_T$ of chlorophyll is also plotted. From this relationship it can be seen that carotenoid pigments containing seven or more conjugated double bonds should be effective quenchers of ^3Chl. Again, this is precisely the

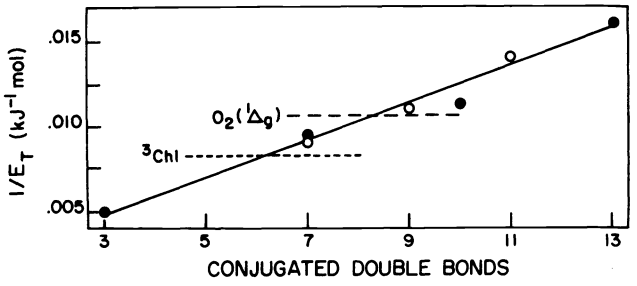


Figure III. The relationship between the reciprocal of carotenoid triplet energy levels ($1/E_T$) and the number of conjugated double bonds in the pigment. Data are taken from Bensasson et al. (72) (●) and from Mathis and Kleo (69) (○). The energy difference between $^1O_2$ and $^3O_2$ (– – – – –) and the triplet energy level of chlorophyll (-----) are also depicted.

relationship that has been observed by Claes (81) for carotenoid protection against the anaerobic photobleaching of chlorophyll, which one must assume proceeds through ^{3}Chl. In this study, the major change in the ability of carotenoid pigments to protect against chlorophyll photobleaching occurred in the pigments containing between five and seven double bonds and indicated that the carotenoid with seven double bonds was effective in this process (81).

Another possibility for carotenoid quenching of $^1O_2$ has been raised by Foote et al. (82). Their suggestion involved reversible electron transfer from carotene to $^1O_2$ which could also be dependent on the polyene chain length. The product of this reaction would then be the carotenoid radical cation and the superoxide radical anion, as shown below:

$$^1O_2 + \text{Car} \longrightarrow O_2^{\cdot-} + \text{Car}^{\cdot+}$$

There is some evidence that the carotenoid radical cation can be formed by an electron transfer process. Mathis and Vermeglio (68) studied the reaction between irradiated toluidine blue and carotenoids. They observed the triplet-triplet energy transfer from ^{3}toluidine blue to form the ^{3}Car. In addition, they observed an electron transfer reaction from the carotenoid to both the mono- and bi-protonated ^{3}toluidine blue. Dawe and Land (83) have also reported the formation of the radical cation of carotenoids as well as the radical anions, but these are formed by direct pulse radiolysis techniques, as opposed to the energy transfer system described by Mathis and Vermeglio (68). The reactions of the radical cations and anions of carotenoid pigments described earlier (83) have now been studied by Lafferty et al. (84,85). In the first of these papers, Lafferty et al. (84) demonstrated that electron transfer reactions could occur readily between carotenoid radicals and chlorophyll generating the respective radical anion or radical cation of chlorophyll as seen below:

$$\text{Car}^{\cdot+} + \text{Chl a} \longrightarrow \text{Car} + \text{Chl a}^{\cdot+}$$

$$\text{Car}^{\cdot-} + \text{Chl a} \longrightarrow \text{Car} + \text{Chl a}^{\cdot-}$$

However, they were unable to detect the reverse reaction which would involve either Chl$^{\cdot+}$ or Chl$^{\cdot-}$ reacting with β-carotene to generate radical carotenoid species. In their most recent publication, Lafferty et al. (85) have demonstrated that the rate of electron transfer from the radical cations and anions of several carotenoid pigments, varying in double bond length from eight to fifteen conjugated double bonds, could occur readily with chlorophyll a but they could find no reaction of chlorophyll b with the radical cation of β-carotene. Beddard et al. (86) have attempted to draw a relationship between these reactions of the radical species of carotenoids with chlorophyll and the mechanism of photosynthesis. These workers reported that β-carotene was able to quench chlorophyll fluorescence. Their explanation of this phenomenon is based on the fact that the energy of the excited singlet state of chlorophyll lies too far below that of β-carotene to permit an efficient energy transfer process. Since β-carotene also has a low ionization potential, Beddard et al. (86) proposed that the quenching of chlorophyll fluorescence by carotenoids

occurs by means of an electron transfer process, shown below:

$$^1\text{Chl } \underline{a} + \text{Car} \longrightarrow \text{Chl } \underline{a}^{-\cdot} + \text{Car}^{+\cdot}$$

This radical cation species of carotene could then react with another molecule of chlorophyll a, as described earlier (84) to generate a radical cation species of chlorophyll as seen below:

$$\text{Car}^{+\cdot} + \text{Chl } \underline{a} \longrightarrow \text{Car} + \text{Chl } \underline{a}^{+\cdot}$$

The overall effect of these electron transfer reactions would be to produce the charge separated pair, Chl $\underline{a}^{+\cdot}$/Chl $\underline{a}^{-\cdot}$ which could be involved in the reaction center of photosystem II. Whether these reactions occur in vivo and whether they have any relationship to the protective function of carotenoid pigments remains to be demonstrated.

CAROTENOIDS AND FREE RADICAL REACTIONS

Since the demonstration that carotenoid pigments could quench $^1O_2$ (74), many investigators have concluded that all reactions in which it is possible to demonstrate carotenoid inhibition involve $^1O_2$. Anderson and Krinsky (87) used this phenomenon to demonstrate that carotenoid pigments could protect artificial membranes, called liposomes, from photodynamic damage and went on to show a similar effect when the $^1O_2$ was produced by radiofrequency discharge (88). In the report of Anderson et al. (88) some destruction of the β-carotene was observed at the same time that the pigment was protecting the liposomes against $^1O_2$. Both of these reports (87,88) involved the use of unsaturated fatty acids in egg phosphatidylcholine (PC) and the possibility always existed that radical reactions could also be involved in the carotenoid destruction. There has certainly been much evidence in the literature that lipid peroxidation can result in carotenoid destruction, although very little is known of the mechanism of this process. There have been a number of suggestions that carotenoids might in fact interact directly with hydroperoxide intermediates in fatty acid oxidation. Yamane and Lamola (89) reported that β-carotene could inhibit red blood cell hemolysis induced by cholesterol hydroperoxide, and Kellogg and Fridovich (90) reported that β-carotene inhibited lipid peroxidation in a system in which the peroxidation was initiated by xanthine oxidase.

We have also reported briefly (91) that carotenoids may inhibit lipid peroxidation reactions under conditions in which one would not expect $^1O_2$ to have been formed. Susan Deneke and I have extended our earlier observations on carotenoid protection of liposomes and have looked at this phenomenon under other experimental conditions (92). In one experiment the type of fatty acids necessary for demonstrating the carotenoid destruction was investigated. As can be seen in Figure IV, when liposomes are made with either the highly unsaturated

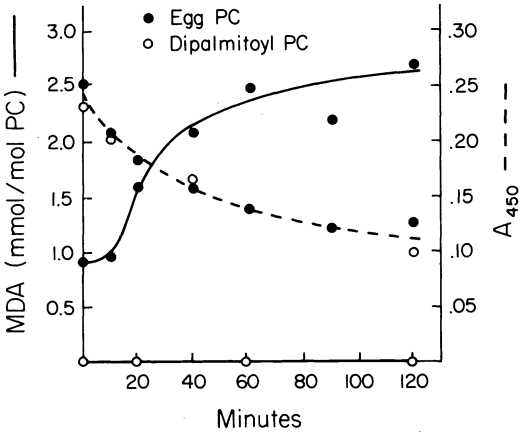


Figure IV. Photosensitized bleaching of β-carotene and malondialdehyde (MDA) formation in egg PC and dipalmitoyl PC liposomes. The liposomes were irradiated with white light at 0.45 J/cm^{2}sec at pH 8 in the presence of 8 x 10^{-5} M toluidine blue O (92).

fatty acids present in egg PC or the saturated dipalmitoyl PC, β-carotene destruction still occurs upon irradiation in the presence of toluidine blue O. As measured by the production of MDA, there is no lipid peroxidation occurring in the presence of the saturated fatty acids. This would seem to indicate that there can be a direct relationship between the excited dye or one of its products and the carotene leading to carotenoid destruction. In an attempt to avoid the introduction of $^1O_2$ by photochemical processes, Deneke and Krinsky (92) resorted to using chemical treatment to induce lipid peroxidation. In Figure V, the data on lipid peroxidation, as measured by the appearance of MDA induced by

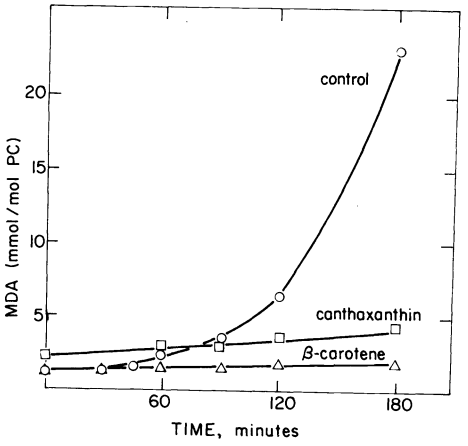


Figure V. Fe^{2+} generated radical oxidation of liposomes. Egg PC liposomes containing either β-carotene, canthaxanthin or no pigment were incubated with 0.1 M $FeCl_2$ for varying times, as indicated.

Fe^{2+} are shown. As can be seen, either β-carotene or canthaxanthin incorporated into the liposome membrane prevents the formation of MDA normally initiated by the addition of Fe^{2+}. Another system that has been used for generating lipid peroxidation is the irradiation of unsaturated fatty acids with ultraviolet light. Deneke and Krinsky (52) looked at this process in liposomes containing canthaxanthin and as seen in Figure VI the presence of

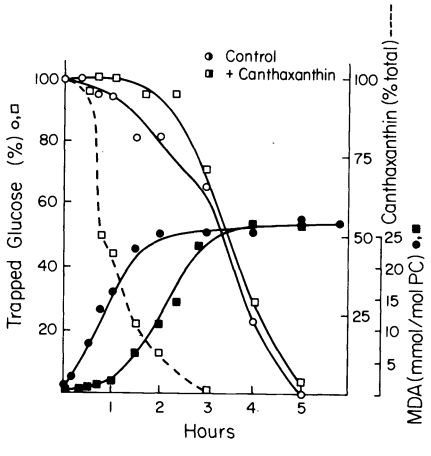


Figure VI. The effects of canthaxanthin on ultraviolet-induced lipid damage Egg PC liposomes, prepared with or without canthaxanthin, were irradiated with germicidal ultraviolet lights (254 nm) at 0.4 J/cm^2 sec.

canthaxanthin retards the formation of MDA during ultraviolet irradiation. Liposome integrity was measured by the rate of release of trapped glucose, and this was also prolonged. The protective action of canthaxanthin against ultraviolet light-induced radical reactions was apparently a function of the canthaxanthin concentration, and ceased when 50% of the pigment had been bleached. Deneke and Krinsky also studied the effect of carotenoid pigments on protecting liposomes against air oxidation. In Figure VII, a

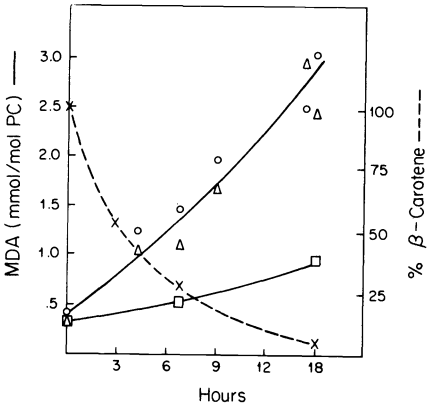


Figure VII. Egg PC liposomes containing either β-carotene (o), vitamin E (□) or no antioxidant (Δ), were incubated at 40° under air and either MDA or β-carotene concentrations followed with time.

comparison between β-carotene and Vitamin E as protective agents is presented. As can be seen, the liposomes, when incubated at 40° and exposed to air, underwent lipid oxidation with concomitant destruction of the β-carotene. During this time, Vitamin E was quite effective in inhibiting the production of MDA, whereas there appeared to be no difference between the liposomes containing β-carotene and those containing no protective agent whatsoever. It would seem therefore, that carotenoids can protect only certain radical-induced reactions from proceeding and these pigments are also oxidized during this process. These observations would seem to demonstrate that an inhibition of a chemical or biological reaction in the presence of carotenoids cannot be used as conclusive evidence that a $^1O_2$ reaction has occurred. All too many reports have appeared in the literature during the last ten years in which this argument was made to conclusively demonstrate that a reaction was occurring via $^1O_2$. Unless other supporting evidence is available, it would appear that a $^1O_2$ mechanism is not obligatory for the carotenoid pigments can have many effects on biological systems.

CONCLUSIONS

As reviewed in this article, carotenoid pigments are very effective agents for protecting systems against the harmful effects of oxidants. In the case of photochemically induced oxidations, carotenoid pigments have the capacity to quench the first potentially harmful intermediate, ^3S, at a very significant rate. In the case of chlorophyll, 90% of ^3Chl would be quenched by carotenoids, thus leading to the inhibition of any potential Type I or Type II reactions. The remaining ^3Chl could then continue to initiate Type I or Type II reactions. Under aerobic conditions, $^1O_2$ could be formed. As calculated by Foote (53) 10^5 molecules of $^1O_2$ would be quenched by carotenoids for each one that would react with a typical cell $^1O_2$ acceptor such as histidine. Furthermore, as demonstrated in the preceding section, carotenoid pigments can also quench the lipid peroxidation reactions which one would anticipate would be initiated by Type I reactions with any of the ^3Chl that had escaped quenching or reaction with oxygen. These aspects of the protective action of carotenoids against oxidation are summarized schematically in Figure VIII.

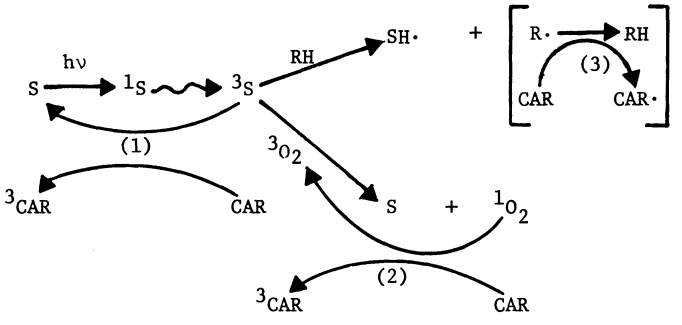


Figure VIII. Mechanisms of carotenoid protection against oxidations. Carotenoids can (1) quench triplet sensitizers, (2) quench $^1O_2$ and (3) quench free radical intermediates by mechanisms not yet adequately described.

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