Pure & Appl. Chem., Vol. 57, No. 5, pp. 723–728, 1985.
Printed in Great Britain.
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Carotenoids in photosynthesis

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Abstract - Wild-type photosynthetic organisms all contain carotenoids. These photosynthetic carotenoids are mainly packaged (together with the chlorophylls or bacteriochlorophylls) into specific pigment-protein complexes. This review lecture summarises the main types of photochemical reactions which carotenoids undergo *in vitro* and emphasises the organising role of the apoproteins, of these pigment-protein complexes, in controlling those reactions which are actually expressed *in vivo*.

INTRODUCTORY REMARKS

Carotenoids are essentially hydrophobic molecules and are typically found associated with photosynthetic membranes. However, they are not freely mobile within the liquid interior of these membranes, but are rather non-covalently bound to specific pigment-protein complexes. These complexes also usually contain chlorophylls or bacteriochlorophylls.

In general carotenoids are rather reactive molecules being able to undergo a wide range of photochemical reactions. *In vitro*, in organic solvents, their photochemistry has been, and continues to be, extensively studied. *In vivo*, however, because the carotenoids involved in photosynthesis are bound into specific pigment-protein complexes, which of the possible photochemical reactions are expressed is largely determined by the various pigment-pigment and pigment-protein interactions that exist within these complexes. A detailed description of the structure of these pigment-protein complexes is therefore required if the behaviour of carotenoids in photosynthesis is to be clearly understood. At present, unfortunately, this information is largely unavailable.

CAROTENO-CHLOROPHYLL(BACTERIOCHLOROPHYLL)-PROTEIN COMPLEXES

In purple photosynthetic bacteria the carotenoids are found in association with two distinct classes of pigment-protein complexes, the reaction centres and the light-harvesting or antenna complexes (1,2). Both these types of complexes are hydrophobic, integral membrane proteins, which can be readily isolated and purified following detergent solubilisation.

In *Rhodopseudomonas sphaeroides*, for example, two types of light-harvesting complexes are present (2-5) (the B875-complex and the B800-850-complex).

The B875-complex exists *in vivo* as an aggregate of a minimal unit that contains two molecules of bacteriochlorophyll *a* and two molecules of carotenoid, bound to two low molecular weight apoproteins (the α-apoprotein Mr = 6809 and the β-apoprotein Mr = 5441) (5,6). Both apoproteins have been sequenced (6). Similarly the B800-850-complex is an aggregate of three molecules of bacteriochlorophyll *a*, and 1 or 2 molecules of carotenoid, which are again bound to two low molecular weight apoproteins that have been sequenced (the α-apoprotein has an Mr = 5647, and the β-apoprotein has an Mr = 5850) (4-9).

By comparing the primary structures of a range of apoproteins from a variety of bacterial light harvesting complexes conserved histidine residues have been identified (one in the α-apoproteins and two in the β-apoproteins). It has been suggested that these conserved histidines may be ligands for the bacteriochlorophyll molecules (10). There is no clear evidence as yet as to where the carotenoids may be bound, however it has been suggested that this may occur near arginine,29, in the β-apoprotein of the B800-850-complex in *Rps. sphaeroides*, since the buried charge associated with this arginine residue could account for the red-shift in the carotenoid's absorption spectrum when it is bound to the complex (6).

In Rps. sphaeroides the antenna complexes show little if any specificity as to the type of carotenoid which is bound (7). The carotenoid composition of the B800-850-complex, for example, simply mirrors the carotenoid composition of the strain from which it was prepared.

When carotenoids bind to the antenna complexes their absorption spectra are red-shifted (by up to 30 nm) (11). Their binding also induces strong CD signals (7,11). Since, in Rps. sphaeroides the carotenoids present in the antenna complexes show no visible CD signals when they are extracted into organic solvents, these induced signals probably reflect both asymmetry induced by binding to the apoproteins and by any pigment-pigment interactions.

Resonance-Raman studies on bacterial antenna complexes indicate that the carotenoids are in the all-*trans* configuration (12).

Reaction centres prepared from carotenoid-containing strains of Rps. sphaeroides contain ~1mole of carotenoid per mole of reaction centre (13). This carotenoid, together with the other reaction centre pigments (4 molecules of bacteriochlorophyll a, 2 molecules of bacteriopheophytin and 1-2 molecules of ubiquinone) is bound to the two lower molecular weight reaction centre subunits. Reaction centres show a mild degree of specificity with regard to carotenoid type, preferring methoxy-carotenoids (13).

Resonance-Raman and CD studies on the reaction centre carotenoid have indicated that it is in some kind of strained *cis*-isomeric form (14-16).

Various polarised light techniques have been used to investigate the angle between the transition dipole of the carotenoids and the other pigments in these pigment-protein complexes (17). However the results are really only clear cut in reaction centres. In reaction centres from wild-type Rps. sphaeroides the long axis of the carotenoid makes an angle of ~75° with the Qy axis of the 'special-pair' reaction centre bacteriochlorophylls (18). Recently reaction centres from Rps. viridis (19) and Rps. sphaeroides (20) have been crystalised. Hopefully determination of their complete three-dimensional structure by X-ray diffraction will unequivocally show how the reaction centre carotenoid is integrated into the overall structure of the reaction centre.

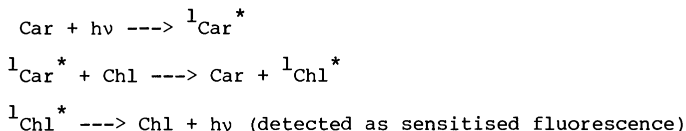
In oxygen evolving photosynthetic organisms the photosynthetic pigment-protein complexes also contain carotenoids. In the lower algae, such as dinoflagellates, a particularly well-studied example is the peridinin-chlorophyll a-complex (21,22). This light-harvesting complex contains four molecules of peridinin and one molecule of chlorophyll a bound to a single apoprotein with a molecular weight of ~35KD. Based on CD and energy transfer measurements it has been suggested that the four peridinin molecules are arranged in two parallel pairs around the centrally placed chlorophyll a molecule.

In higher plants, however, the situation is much more complex and correspondingly much less well understood (1-2). The photosynthetic unit of higher plants can be conveniently segregated into three macromolecular complexes. These are the major light-harvesting complex (the chlorophyll a/b proteins), and photosystems I and II. The two photosystems have a complicated composition, but seem to consist of an 'inner core', where the primary photochemical reactions are located, and an outer surrounding layer, of a variety of specific antenna complexes (1-2). In this way each photosystem has its own 'dedicated' antenna array as well as being able to accept photons absorbed by the chlorophyll a/b light-harvesting system. It is to be hoped in the near future that the procedures available for the isolation and preparation of these higher plant complexes will be improved, so that well-defined preparations can be routinely obtained. This should then allow much more reliable data upon their composition to be obtained.

However there are some generally agreed results in this area. Each type of complex, where it has been studied, does contain carotenoids (23). β-Carotene seems to be more abundant in the photosystem I and II complexes, while the xanthophylls are more abundant in the antenna complexes. There is very little data upon the conformation of the carotenoids in the higher plant complexes or indeed any structural data, but it is clear that the β-carotene in the photosystem I complex has a defined orientation relative to P700 (24).

THE LIGHT-HARVESTING ROLE OF CAROTENOIDS

It has been known for many years that carotenoids act as accessory light-harvesting pigments (25,26). This is a manifestation of singlet-singlet energy transfer from the carotenoid to the chlorophyll or bacteriochlorophyll.



$^{1}Chl^{*}$ ---> Chl + hν (detected as sensitised fluorescence)

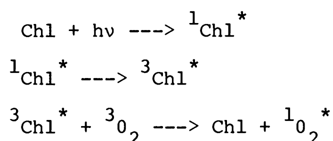
The overall efficiency of this process varies, being ~30% in the B890-complex from Rhodospirillum rubrum (27) (spirilloxanthin ->bacteriochlorophyll a), ~50% in higher plants (28) (mainly β-carotene ->chlorophyll a), ~90% in the B800-850-complex from Rps. sphaeroides (29) (spheroidene ->bacteriochlorophyll a) and ~100% in the peridinin-chlorophyll a complex from dinoflagellates (22). The reasons for these large variations in efficiency remain to be determined. They may depend upon either the type of carotenoid present or upon how the carotenoids and the chlorophylls (bacteriochlorophylls) are packaged within the pigment-protein complexes.

Although the accessory light-harvesting role of carotenoids is easy to demonstrate experimentally by sensitised fluorescence (26) the details of the mechanism of this singlet-singlet energy transfer process are still unclear. In vitro carotenoids are essentially non-fluorescent (30) and time-resolved resonance-Raman studies have estimated the fluorescence lifetimes of carotenoids to be less than one psec (31). This makes conventional mechanisms of singlet-singlet energy transfer such as the dipole-dipole exchange mechanism of Förster seem rather unlikely. There have been two proposals for ways out of this problem. Firstly it has been suggested that energy transfer may be occurring from a lower lying singlet state (32), or secondly if the carotenoid-chlorophyll distance was small enough (say 4-8 Å) an electron exchange mechanism could operate (33).

Recent attempts to resolve this problem have focused upon experiments with model compounds. Moore et al. (34,35) have synthesised a range of covalently linked caroteno-porphyrin dimers. In these model compounds singlet-singlet energy transfer from the carotenoid to the porphyrin only occurs when the two molecules are held in very close contact i.e. when the carotenoid is caused to fold back over the porphyrin plane so that the two π systems interact directly. In vitro with non-covalently linked mixtures of carotenoids and chlorophylls no singlet-singlet energy transfer from the carotenoid to the chlorophyll occurs. It is very clear from these studies that a major role of the antenna apoproteins in photosynthesis is to hold the carotenoid and chlorophyll molecules in close proximity to each other so that singlet-singlet energy transfer from the carotenoid to the chlorophyll can be an efficient process.

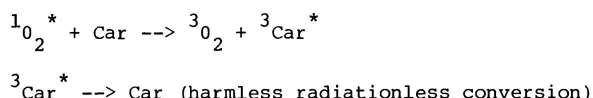
THE PHOTOPROTECTIVE ROLE OF CAROTENOIDS

Photosynthesis probably only occurs in an aerobic environment because of the presence of carotenoids (36). When chlorophylls, or bacteriochlorophylls, are irradiated in the presence of oxygen the harmful photodynamic reaction occurs. In this reaction light generates chlorophyll triplets. These triplet states can then interact with molecular oxygen to produce singlet oxygen, which is a strong enough oxidant to be able to degrade both chlorophyll itself as well as proteins, nucleic acids and lipids. Carotenoids prevent these harmful reactions.



The protective role of carotenoids is mainly due to two reactions (36). Firstly carotenoids can interact directly with singlet oxygen to quench it (37). The requirement for this reaction is that the carotenoids have a triplet state whose

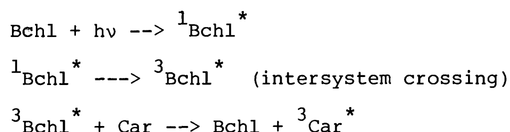
energy level is below that of singlet oxygen i.e. below 94 KJ $mole^{-1}$. In practice this means carotenoids with more than seven conjugated double bonds.



$$^{1}O_2^{*} + Car \rightarrow {}^{3}O_2 + {}^{3}Car^{*}$$

$$^{3}Car^{*} \rightarrow Car \text{ (harmless radiationless conversion)}$$

One of the interesting features of carotenoids is the large energy gap between their first excited singlet state and their first excited triplet state. The energetic first excited singlet state is important for the light-harvesting role of carotenoids, while the low lying first excited triplet state is important for their photoprotective function. This low lying triplet state makes carotenoids good acceptor molecules in triplet-triplet energy transfer reactions. The second way in which carotenoids prevent the photodynamic reaction is to quench the lifetime of the chlorophyll and bacteriochlorophyll triplets which are responsible for singlet oxygen production (36).



$$Bchl + h\nu \rightarrow {}^{1}Bchl^{*}$$

$$^{1}Bchl^{*} \rightarrow {}^{3}Bchl^{*} \text{ (intersystem crossing)}$$

$$^{3}Bchl^{*} + Car \rightarrow Bchl + {}^{3}Car^{*}$$

In bacterial reaction centres, for example from the carotenoidless mutant of _Rps. sphaeroides_ strain R26, triplet bacteriochlorophyll lasts for a few microseconds (38). However in reaction centres containing carotenoid, this bacteriochlorophyll triplet state is 'effectively' replaced by a carotenoid triplet (39,40). The carotenoid triplet arises from a triplet-triplet energy transfer with the reaction centre bacteriochlorophyll triplet (as depicted above). Carotenoid triplet formation takes place in a few nanoseconds, and therefore the lifetime of the potentially dangerous bacteriochlorophyll triplet state is reduced by about three orders of magnitude. This provides a very efficient block upon the formation of singlet oxygen.

Carotenoids will quench triplet chlorophyll _in vitro_ (41), but again to achieve the very high rates of triplet-triplet exchange required to block the formation of singlet oxygen, the apoproteins play a vital structural role, holding the pigments in close proximity to one another.

In vitro direct excitation of monomeric carotenoid to give a triplet state has yet to be reported. This probably reflects both the very short lifetime of the first excited singlet state (31), which allows almost no time for intersystem crossing, and the very large energy gap between the singlet and triplet states. However recently _in vivo_ in some photosynthetic bacteria carotenoid triplets have been formed by direct excitation (42-44).

In chromatophores from _R. rubrum_ for example the yield of carotenoid triplets was higher when the carotenoid (in this case mainly spirilloxanthin) was directly excited, than when the bacteriochlorophyll was excited (43). This is the reverse of what might be predicted if the carotenoid triplets were solely arising by a triplet-triplet exchange reaction with bacteriochlorophyll triplets. The key to understanding this process was the discovery that this carotenoid triplet formation was sensitive to external magnetic fields. The easiest way in which the magnetic field dependence could be sensibly explained was to assume that singlet fission was taking place. This type of process has been well documented in aromatic crystals (45).

REDOX REACTIONS OF CAROTENOIDS

In vitro carotenoid radical cations and anions have been generated, both photochemically (46) and radiochemically by pulse radiolysis (47). Carotenoid radical ions produced from carotenoids with 7 or more conjugated double bands have typical strong absorption bands in the near infa red. The radical cation of β-carotene in hexane absorbs maximally at 1040 nm and its radical anion at 880 nm (47).

So far there have been no reports of the production of carotenoid radical anions in photosynthesis. However it has now become clear that under some experimental conditions the radical cation of β-carotene can be generated, photochemically, in

photosystem II (48). The largest Car^{+} signal is generated when normal oxygen evolution is inhibited, but it can still be detected in untreated chloroplasts. Further work will be required to see whether β-carotene is indeed a physiologically important electron donor for photosystem II or only on a side-path that becomes important under non-physiological, inhibited conditions.

CAROTENOIDS AND FLUORESCENCE QUENCHING

In vitro carotenoids have been shown to quench the fluorescence of chlorophyll and porphyrins (34,49), though the mechanism by which this quenching occurs is not yet clear.

In vivo carotenoids will also quench the fluorescence of chlorophyll or bacteriochlorophyll, but only when the carotenoid is in its triplet state (50-53). This quenching by carotenoid triplets is an example of an excited state annihilation process (in this case a singlet-triplet fusion).

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