

Structure and properties of carotenoids in relation to function

GEORGE BRITTON¹

Department of Biochemistry, University of Liverpool, Liverpool, United Kingdom

Essentially all carotenoids, which are widespread in nature, possess certain common chemical features: a polyisoprenoid structure, a long conjugated chain of double bonds in the central portion of the molecule, and near symmetry around the central double bond. This basic structure can be modified in a variety of ways, most prominently by cyclization of the end groups and by the introduction of oxygen functions, to yield a large family of >600 compounds, exclusive of *cis/trans* isomers. They perform important functions in nature, including light-harvesting, photoprotection, protective and sex-related coloration patterns in many animal species, and as precursors of vitamin A in vertebrates. They may serve protective roles as well against age-related diseases in humans as part of a complex antioxidant network within cells. In this opening review, emphasis is placed on the close relationship between the physicochemical properties of carotenoids and their functions and actions in nature.

James A. Olson, Coordinating Editor

ABSTRACT The basic principles of structure, stereochemistry, and nomenclature of carotenoids are described and the relationships between structure and the chemical and physical properties on which all the varied biological functions and actions of carotenoids depend are discussed. The conjugated polyene chromophore determines not only the light absorption properties, and hence color, but also the photochemical properties of the molecule and consequent light-harvesting and photoprotective action. The polyene chain is also the feature mainly responsible for the chemical reactivity of carotenoids toward oxidizing agents and free radicals, and hence for any antioxidant role. *In vivo*, carotenoids are found in precise locations and orientations in subcellular structures, and their chemical and physical properties are strongly influenced by other molecules in their vicinity, especially proteins and membrane lipids. In turn, the carotenoids influence the properties of these subcellular structures. Structural features such as size, shape, and polarity are essential determinants of the ability of a carotenoid to fit correctly into its molecular environment to allow it to function. A role for carotenoids in modifying structure, properties, and stability of cell mem-

branes, and thus affecting molecular processes associated with these membranes, may be an important aspect of their possible beneficial effects on human health.—Britton, G. Structure and properties of carotenoids in relation to function. *FASEB J.* 9, 1551-1558 (1995)

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THE CAROTENOIDS ARE NOT JUST “another group of natural pigments.” They are substances with very special and remarkable properties that no other groups of substances possess and that form the basis of their many, varied functions and actions in all kinds of living organisms. Often traditionally thought of as plant pigments, the carotenoids have a much wider distribution and occur extensively also in animals and microorganisms. The striking natural colors that are due to carotenoids—e.g., in yellow, orange, and red flowers and fruit—are familiar to all of us, but other, less obvious roles make carotenoids essential components in oxygenic photosynthetic organisms. Without carotenoids, photosynthesis and all life in an oxygen atmosphere would be impossible. Carotenoids have recently been implicated as well in the prevention of or protection against serious human health disorders such as cancer and heart disease. Thus, these substances are clearly of major importance in biology.

The natural functions and actions of carotenoids obviously are determined by the physical and chemical properties of the molecules, and these properties are defined by the molecular structure. First, the overall molecular geometry (size, shape, presence of functional groups) is vital for ensuring that the carotenoid fits into cellular and subcellular structures in the correct location and orientation to allow it to function efficiently. Second, the conjugated double bond system determines the photochemical properties and chemical reactivity that form the basis of these functions. In addition, specific interactions with other molecules in the immediate vicinity are crucial for correct functioning.

¹Send correspondence and reprint requests to Dr. Britton, at: Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, United Kingdom.

²Abbreviations: AIBN, 2,2'-azo-bis-isobutyronitrile; AMVN, 2,2'-azobis (2,4-dimethylvaleronitrile).

The main objective of this article is to describe the structures and properties of carotenoids and to use this information as a basis for understanding how carotenoids may function and act in biological systems.

CAROTENOID STRUCTURES AND NOMENCLATURE

More than 600 different carotenoids have been isolated from natural sources and characterized (1, 2). For the newcomer to the field, the prospect of having to assimilate such a mass of detailed chemical information is daunting. Fortunately, although these are all different structures, for many purposes they can be considered as variations on a structural theme and, in terms of many of the basic chemical and physical properties, the carotenoids may be considered as a group.

Basic structure

The carotenoids are isoprenoid compounds, biosynthesized by tail-to-tail linkage of two C₂₀ geranylgeranyl diphosphate molecules. This produces the parent C₄₀ carbon skeleton from which all the individual variations are derived. In Fig. 1A, this basic structure is illustrated by lycopene and β,β -carotene; the carotenoid numbering scheme is also shown. This skeleton can be modified 1) by cyclization at one end or both ends of the molecule to give the seven different end groups (illustrated in Fig. 1B), 2) by changes in hydrogenation level, and 3) by addition of oxygen-containing functional groups. Carotenoids that contain one or more oxygen functions are known as xanthophylls, the parent hydrocarbons as carotenes.

Nomenclature

Traditionally, carotenoids have been given trivial names derived usually from the biological source from which the carotenoid was first isolated. It is still common practice,

especially in biological work, to use the well-known trivial names even though generally they convey no information about the structure of the carotenoid. A semisystematic scheme has therefore been devised that allows any carotenoid to be named unambiguously and in a way that defines and describes its structure. In essence, all specific names are based on the stem name "carotene," preceded by the Greek-letter prefixes that designate the two end groups; the seven end groups are illustrated in Fig. 1B.

For clarity and to avoid confusion, the use of both end-group prefixes for a carotene is now recommended (3). Thus, for example, " β -carotene" is now more correctly referred to as β,β -carotene, α -carotene as β,ϵ -carotene, etc. Changes in hydrogenation level and the presence of oxygen-containing substituents are indicated by the standard prefixes and suffixes used in organic chemistry. Thus, the biosynthetic precursor phytoene becomes 7,8,11,12,7',8',11',12'-octahydro- ψ,ψ -carotene, and zeaxanthin is β,β -carotene-3,3'-diol. Many natural carotenoids are optically active, i.e., chiral compounds; chirality is indicated by the conventional *R,S* system.

The IUPAC-IUB rules are given in full in an IUPAC publication (4) and in volume 1A of the new series, *Carotenoids* (5).

Some carotenoids have a structure consisting of fewer than 40 carbon atoms and derived formally by loss of part of the C₄₀ skeleton. These compounds are referred to as apocarotenoids when carbon atoms have been lost from the ends of the molecule or as norcarotenoids when carbon atoms have been lost formally from within the chain.

The polyene chain

The most striking and characteristic feature of the carotenoid structure is the long system of alternating double and single bonds that forms the central part of the molecule. This constitutes a conjugated system in which the π -electrons are effectively delocalized over the entire

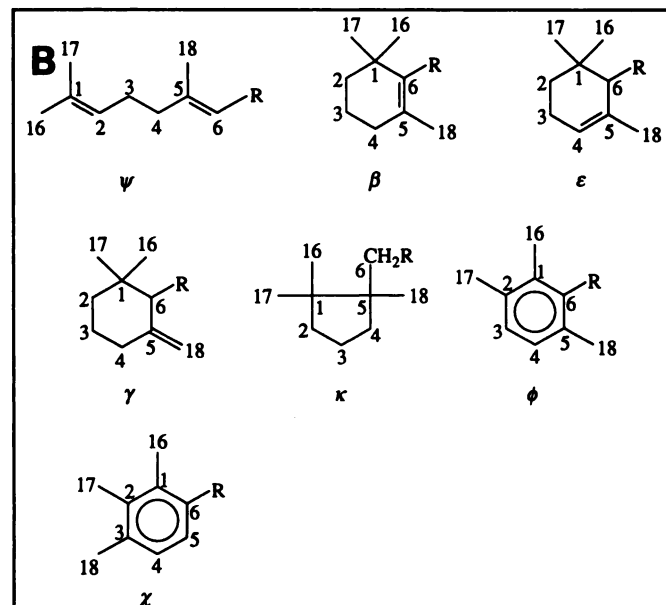
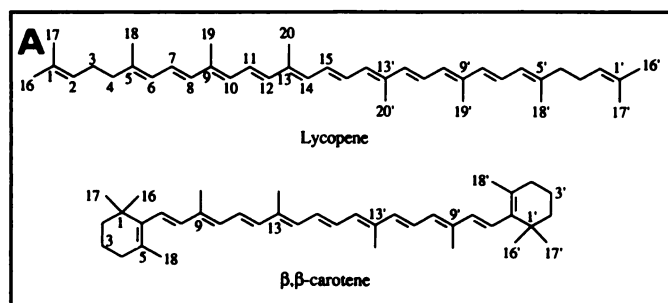


Figure 1. A) Basic structure and numbering scheme of an acyclic carotenoid (lycopene) and a dicyclic carotenoid (β,β -carotene). B) The seven different end groups found in natural carotenoids.

length of the polyene chain. It is this feature that gives the carotenoids as a group their distinctive molecular shape, chemical reactivity, and light-absorbing properties.

Stereochemistry

Because of isomerism around C=C double bonds, different configurations are possible. These are distinctly different molecular structures that can be isolated as separate compounds. In addition, rotation is theoretically possible about any C-C single bond in the polyene chain, so the carotenoids can, in principle, adopt an enormous number of shapes or conformations.

Configuration: geometrical isomers

In principle, each double bond in the polyene chain of a carotenoid can exist in two forms (configurations), designated *trans* or *cis*, depending on the disposition of substituent groups, specifically those that constitute a continuation of the polyene chain, about that double bond (5) (Fig. 2A). [In current chemical usage, the configuration about a double bond is designated *E* or *Z*. In carotenoids *E* and *Z* are usually equivalent to *trans* and *cis*, respectively, although there are some exceptions.] This gives rise to a large number of theoretically possible *monocis* and *polycis* isomers. In reality, only a few of these geometrical isomers are encountered in nature. The presence of a *cis* double bond creates greater steric hindrance between nearby hydrogen atoms and/or methyl groups, so that *cis* isomers are generally less stable thermodynamically than the *trans* form. Most carotenoids, therefore, occur in nature predominantly or entirely in the *all-trans* form. With some double bonds, i.e., those that bear three substituents and also the disubstituted C-15,15' double bond, the steric hindrance is small so that isomers with *cis* double bonds in these positions are relatively easily formed and relatively stable (e.g., 9-*cis*-, 13-*cis*-, and 15-*cis*- β , β -carotenes). With other disubstituted

double bonds, however, a *cis* configuration results in major steric interference between a methyl group and a hydrogen atom (5) (Fig. 2B). Isomers containing such sterically hindered double bonds are hard to form and are rarely encountered in nature, e.g., 7-*cis*- and 11-*cis*- β , β -carotene.

Conformation

Although, in principle, a carotenoid with a defined *cis/trans* configuration can adopt a very large number of shapes, in practice the carotenoid will exist in a particular preferred, low-energy conformation.

The polyene chain. By far the most stable form of the conjugated polyene chain is a linear, extended conformation. Two major factors are responsible for this. First, a conjugated system is greatly stabilized when the double bonds are coplanar. Second, steric hindrance is smallest when each C-C single bond is in the *s-trans* conformation (equivalent to a *trans* double-bond configuration, Fig. 2C). X-Ray crystallographic data reveal that carotenoids are essentially extended linear molecules with only a slight S-shaped distortion to relieve steric tension (6).

The ring-chain junction. Because of theoretical free rotation about the C-6,7 single bond in carotenoids with cyclic end groups, in principle there can be an infinite number of possible angles of twisting between the ring and the main polyene chain. In the β -ring carotenoids, the C-5,6 double bond is formally conjugated with the polyene chain, so that coplanarity of the ring and chain double bonds should be favored. There are two extreme structures, 6-*s-cis* and 6-*s-trans* (Fig. 2D), that allow such coplanarity, but these would generate considerable steric crowding (5). It is now known that the preferred conformation is 6-*s-cis*, but this is distorted approximately 40° from planarity to relieve steric interference between the C-5 methyl substituent on the ring and the C-8 hydrogen atom of the chain (7). With the ϵ -ring, the cyclic double bond (C-4,5) is not in conju-

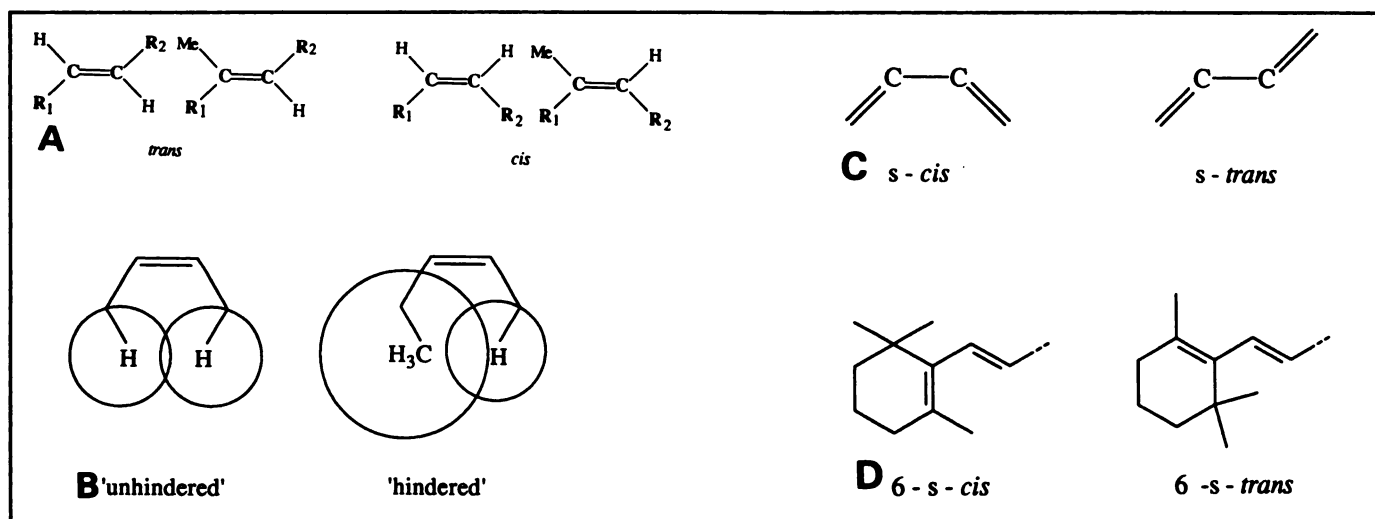


Figure 2. A) *cis* and *trans* double bonds in carotenoids. B) Sterically "hindered" and "unhindered" *cis* double bonds in the polyene chain of carotenoids.

gation, so that relief of steric crowding becomes the only major determinant of the preferred ring-chain conformation.

The ring-chain conformation, together with the shape of the ring itself—normally a chair or half-chair conformation determined by the presence of functional groups—establish the effective bulk of the end group.

PROPERTIES

General properties: size, shape, solubility

The carotenoids as a group are extremely hydrophobic molecules with little or no solubility in water. They are thus expected to be restricted to hydrophobic areas in the cell, such as the inner core of membranes, except when association with protein allows them access to an aqueous environment. (See section on Properties and Molecular Interactions of Carotenoids in vivo.) Polar functional groups obviously alter the polarity of carotenoids and affect their interactions with other molecules.

The overall size and shape of the molecule are extremely important in relation to the properties of a carotenoid and, hence, to function. All colored carotenoids in the all-*trans* configuration have an extended conjugated double-bond system and are linear, rigid molecules. The *cis*-isomers, however, are no longer simple linear molecules. Their overall shape differs substantially from that of the all-*trans* form, so their ability to fit into subcellular structures may be greatly altered. The tendency of *cis*-isomers to crystallize or aggregate is usually much less; therefore *cis*-isomers may be more readily solubilized, absorbed, and transported than their all-*trans* counterparts.

The shape and size of the end groups are also important factors. Acyclic carotenoids such as lycopene are essentially long, linear molecules with flexible end groups. Cyclization shortens the overall length of the molecule and increases the effective bulk of the end groups and the space they occupy. The effective bulk depends on the preferred conformation, as determined by steric factors and the presence of substituent groups, and can be altered significantly by apparently small structural changes such as epoxidation-deepoxidation.

Light absorption and photochemical properties

The absorption of light energy by an organic molecule produces a higher-energy excited state of that molecule. In the case of carotenoids, the relevant transition is a $\pi \rightarrow \pi^*$ transition, in which one of the bonding π -electrons of the conjugated double-bond system is promoted to a previously unoccupied π^* antibonding orbital. The π -electrons are highly delocalized and the excited state is of comparatively low energy, so the energy required to bring about the transition is relatively small and corresponds to light in the visible region in the wavelength range of 400–500 nm. Carotenoids are therefore intensely colored yellow, orange, or red. The relationship between

in the identification of carotenoids, is developed more fully elsewhere (8).

But coloration is not the sole function of carotenoids. They have distinctive photochemical properties that form the basis of other, even more important functions (9). The origin of these photochemical properties lies in the disposition of the low-lying excited energy states (both singlet and triplet) of the carotenoids. The characteristic strong absorption of light in the visible region is now attributed to a strongly allowed transition from the ground state S_0 to the second singlet excited state, S_2 (1^1Bu). The energy levels of this excited state, and of the somewhat lower first excited state S_1 (1^1Ag) that can be formed from it by internal conversion, lie close to but above those of chlorophyll, so that singlet-singlet energy transfer can take place from excited carotenoid to generate the excited singlet state S_1 of chlorophyll, which is active in photosynthesis (9).

Direct formation of the carotenoid triplet state (T_1) from the excited singlet states S_2 or S_1 via singlet-triplet intersystem crossing is not favored. Its formation by energy transfer from other triplet-state molecules acting as photosensitizers can be very efficient, however, provided the carotenoid contains more than seven conjugated double bonds, because the carotenoid triplet-state energy is low (9). Transfer of energy from triplet-state chlorophyll or other porphyrins to carotenoids occurs much more readily than the alternative energy transfer to oxygen to form the highly reactive and destructive singlet oxygen $^1\text{O}_2$. Carotenoids can also accept excitation energy from $^1\text{O}_2$ if any should be formed. The triplet-state carotenoid is of such low energy that it is unable to generate other species by energy transfer and it dissipates its excitation energy harmlessly to its surroundings. This allows β, β -carotene to protect photosynthetic reaction-center complexes against damage that would be caused by a combination of light and oxygen (9) and to provide an effective treatment for human patients suffering from erythropoietic protoporphyria, a condition in which free porphyrins accumulate in the skin and sensitize the formation of $^1\text{O}_2$ (10).

For efficient energy transfer involving either singlet or triplet states, the molecules concerned must be held in close proximity and in the correct orientation by specific interactions with other molecules such as, for example, proteins (9) (see section on Properties and Molecular Interactions of Carotenoids in vivo).

Chemical properties

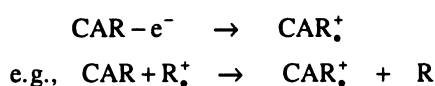
The fundamental chemistry of carotenoid radicals and of the reactions of carotenoids with oxidizing agents, peroxy radicals, etc., is important for evaluating the proposed actions of carotenoids as antioxidants, but it is not well understood. The strategy adopted here is not to survey the literature that deals with carotenoids as antioxidants, but to consider the properties of the carotenoids and their

ing agents as a basis for assessing whether an antioxidant or other chemical role in vivo is feasible.

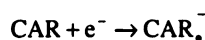
Carotenoid radicals

Many of the important oxidations are free-radical reactions, so a consideration of the generation and properties of carotenoid radicals and of carbon-centred radicals derived from carotenoids by addition of other species is relevant (11). The carotenoid radicals are very short-lived species. Some information has been obtained about them by the application of radiation techniques, particularly pulse radiolysis. In principle, carotenoid radicals can be generated in different ways.

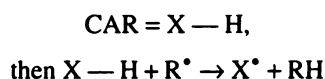
Oxidation. Oxidizing radicals with high redox potential can remove one electron from the carotenoid molecule to give the radical cation.



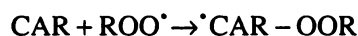
Reduction. The addition of one electron to the carotenoid molecule would give the radical anion.



Hydrogen abstraction. The abstraction of a hydrogen atom H[•] from a saturated carbon atom in a position allylic to the polyene chain can generate a resonance-stabilized neutral radical by homolytic cleavage of a C-H bond. For instance, if



Addition. The addition of a radical species such as a peroxy radical ROO[•] or the hydroxyl radical HO[•] to the polyene chain could generate a carotenoid-adduct radical, e.g.,



In the carotenoid radicals, the unpaired electron is highly delocalized over the conjugated polyene chromophore. This has a stabilizing effect and also allows subsequent reactions, e.g., additions, to take place at many parts of the molecule. The cation and anion radicals can be detected by their characteristic spectral properties, with intense absorption in the near infrared region (11, 12).

Chemical reactions: oxidation

The reactions of carotenoid end groups are important in classical chemistry, for example, in characterization, derivatization, and synthesis. Such reactions, however, are generally not of great significance in relation to function. Here the most important part of the molecule is the

system that is susceptible to attack by electrophilic reagents, is responsible for the instability of carotenoids toward oxidation, and is the important feature of the molecule in relation to free-radical chemistry.

Pure carotenoids, even in the crystalline state, are susceptible after isolation to oxidation and may be broken down rapidly if samples are stored in the presence of even traces of oxygen. In vivo, the carotenoids are usually stabilized to a considerable degree by proteins and other molecules in their immediate vicinity. Even in vivo, however, the carotenoids are still susceptible to oxidative damage if they become exposed to oxidizing species or free radicals that may be generated. The usual indication of carotenoid breakdown is bleaching, i.e., loss of color due to breaking of the chromophore.

Oxidative degradation was used for many years in the classical chemistry that led to the elucidation of carotenoid structures (13). Controlled chemical reactions were used to break various carbon-carbon double bonds in the polyene chain to generate apocarotenals and apocarotenones as well as small fragments arising from complete breakdown.

Oxidative breakdown is also caused by free-radical reactions; indeed, rapid bleaching by hydroxyl radicals is well known (14). Recent work in this area has concentrated on the reactions with peroxy radicals generated in some cases by pulse radiolysis, e.g., by the CCl₃OO[•] radical (15), but mostly by use of so-called azo-initiators such as 2,2'-azo-bis-isobutyronitrile (AIBN) and 2,2'-azo-bis(2,4-dimethylvaleronitrile) (AMVN). Controlled thermal decomposition of these substances generates free radicals that, in the presence of oxygen, give rise to the corresponding peroxy radicals (16). These in turn react with the carotenoids. A wide variety of products have been detected in these studies. Most seem to be apocarotenals or apocarotenones of various chain lengths produced by cleavage of any double bond in the polyene chain; epoxides are also commonly found (17, 18).

Similar chemical reactions also occur between carotenoids and singlet oxygen, and again generate apocarotenals and apocarotenones as the major products. The mechanism may be different, however, and may involve the addition of ¹O₂ to a double bond to form a labile dioxetane, which is then cleaved to give the corresponding carbonyl products (18).

In all the work on the reactions of carotenoids with oxidizing and free-radical reagents, however, the products that are seen are usually detectable only in much smaller amounts than the amount of carotenoid that is destroyed. Also, the substances that can be isolated are not the primary products of the reactions. The primary products will be generated extremely rapidly and have only a very short lifetime before they undergo further reactions that eventually lead to the formation of stable products that can be isolated. Nevertheless, attempts have been made to infer what the primary reactions may be and to deduce the subsequent course of the reactions from the identities

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