SOLVED AND UNSOLVED PROBLEMS OF CAROTENOID FORMATION

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Abstract - This review highlights some of the more recent developments in
the field of carotenoid biosynthesis and metabolism against the background
of our accumulated knowledge of the formation of these pigments. Plant,
microbial and animal carotenoids are all considered and attention is
focused on specific problems which have been solved, on those still
remaining unsolved and on some new problems.

INTRODUCTION

This review may appear unbalanced if it is read in isolation. The whole topic of carotenoid
biosynthesis was covered in detail in this series as recently as 1976 under the headings
"The early steps" and "Later reactions" by Davies and Taylor (1) and by Britton (2)
respectively. This is a progress report which is intended to update those earlier accounts,
and therefore it should preferably be read in conjunction with them. However, this paper is
not detailed in those areas which are considered in the accompanying reviews by other
authors. Furthermore, problems currently or recently under investigation in my own
laboratory are perhaps given more space than they might be allocated by other writers. My
intention is merely to underline those problems which have been solved, to reiterate those
questions which still remain unanswered, and to state new problems which have arisen since,
or were not considered in, the earlier reviews (1, 2).

EARLY STEPS OF CAROTENOID FORMATION IN CHLOROPLASTS

One problem we did not reconsider in 1976 but which has been discussed more recently by
Goodwin (3) is that relating to the role of mevalonic acid (MVA) as a carotenoid precursor
in chloroplasts. MVA is converted *in vitro* by chloroplasts into carotenoids (4, 5), but how
is the chloroplastidic MVA formed *in vivo*? In other systems capable of polyisoprenoid
biosynthesis, MVA is formed from acetyl-CoA with acetoacetyl-CoA and 3-hydroxy-3-methyl-
glutaryl-CoA (HMG-CoA) as intermediates. HMG-CoA reductase, the enzyme responsible for the
last step of this sequence, has been detected in chloroplasts (7) but, as chloroplasts are
not known to carry out glycolysis or fatty acid β-oxidation (those reaction sequences by
means of which pyruvate, and thence acetyl-CoA, is normally provided for biosynthetic
purposes), the origin of acetyl-CoA remains obscure. As CO_2 is such a good precursor of
chloroplastidic terpenoids (8), one possibility (9) is that pyruvate is formed from CO_2
using the glycollate-glyoxylate-glycine-serine pathway (10), and that it is converted into
acetyl-CoA by the pyruvate dehydrogenase which is bound to the lamellar membrane (11).
Alternatively, HMG-CoA could be formed directly from leucine and CO_2 (3), for the
chloroplast is permeable to leucine which is incorporated into carotenoids in green leaves
(12).

FORMATION OF PHYTOENE

Once MVA (C_6) is formed, and this is the case both in the chloroplast and in other
carotenogenic systems, it. is converted via its phosphate and pyrophosphate and with the loss
of CO_2 into isopentenyl pyrophosphate (IPP; C_5), the basic building unit of polyisoprenoid
biosynthesis. Isomerization to dimethylallyl pyrophosphate (DMAPP), the condensation of the
two C_5 molecules to yield geranyl pyrophosphate (C_{10}) and subsequent additions of C_5 units
(IPP) result in the formation of farnesyl pyrophosphate (FPP; C_{15}) and geranylgeranyl
pyrophosphate (GGPP; C_{20}). FPP undergoes a reductive dimerization via presqualene
pyrophosphate to form squalene (C_{30}) and there is evidence that FPP is also a precursor of
the C_{30} carotenoids in some non-photosynthetic bacteria (1). The first C_{40} hydrocarbon
normally formed in tetraterpenoid carotenoid biosynthesis, phytoene (7,8,11,12,7',8',11',12'-
octahydro-ψ,ψ-carotene) arises from two molecules of GGPP via prephytoene pyrophosphate.
Much of our detailed knowledge of the formation of the terpenyl pyrophosphates and their

role as carotenoid precursors has come from work in the laboratories of Rilling and of Porter and is reviewed in other papers in this Symposium.

While most organisms appear to form 15-cis phytoene (13, 14), some non-photosynthetic bacteria such as a Mycobacterium sp. (15), Flavobacterium dehydrogenans (14), Halobacterium cutirubrum (16) and Sarcina flava (17), form the all-trans isomer. The stereochemistry of hydrogen loss in the conversion of GGPP into phytoene is consistent in each case with the formation of all-trans phytoene in a Mycobacterium system (18) and of the 15-cis isomer in a fungal system (18) and in chloroplasts (5).

DESATURATION OF ACYCLIC CAROTENES

In higher plants, phytoene is desaturated to lycopene (ψ,ψ-carotene) by a series of four didehydrogenations occurring alternately at either side of the central chromophore (19). The intermediates are phytofluene (7,8,11,12,7',8'-hexahydro-ψ,ψ-carotene), ζ-carotene (7,8,7',8'-tetrahydrolycopene) and neurosporene (7,8-dihydro-ψ,ψ-carotene). Each didehydrogenation is a trans elimination of the 2-pro-S and 5-pro-R hydrogens originating from MVA (20, 21).

An isomer with an unsymmetrically placed chromophore, 7,8,11,12-tetrahydrolycopene(7,8,11,12-tetrahydro-ψ,ψ-carotene), replaces ζ-carotene entirely at the conjugated heptaene level in Rhodospirillum rubrum (19) and the occurrence of both isomers in some fungi (22) implies the operation of alternative routes from phytofluene to neurosporene. Lycopene is usually the end product of the desaturation of the acyclic carotenes but desaturation can proceed further in certain organisms for 3,4-dehydrolycopene (3,4-didehydro-ψ,ψ-carotene) is a minor component of some fungi (23) while bisdehydrolycopene (3,4,3',4',-tetradehydro-ψ,ψ-carotene), the ultimate product of desaturation, has been detected in Valencia oranges (24).

In organisms which form all-trans rather than 15-cis phytoene from GGPP, the carotenes retain the all-trans configuration throughout desaturation (25), although some cis as well as all-trans lycopene appears in S. flava when C_{50} carotenoid formation is blocked by nicotine (17). Since neurosporene and lycopene are usually predominantly in the all-trans configuration (except in some tomato mutants which form the poly-cis proneurosporene and prolycopene) it is clear that a cis to trans isomerization must occur early in the desaturation sequence in those organisms which initially form 15-cis phytoene. This occurs at the phytoene level in Rhodospirillum rubrum (26, 27), in Phycomyces blakesleeanus (26, 27) and in a Flavobacterium sp. (28) and at the phytofluene level in higher plants (29, 30) while there is evidence that the cis configuration is retained as late as ζ-carotene in the PG1 mutant of Scenedesmus obliquus (31).

FORMATION OF ALICYCLIC CAROTENES

Much has been written about the route of cyclic carotene (e.g. β,β-carotene) formation and there has been much discussion about which acyclic carotene (lycopene or neurosporene) is the actual substrate of the β-cyclase enzyme in higher plants, algae and fungi. The specificities of the dehydrogenases and cyclases may be wide enough to allow alternative routes to operate in the same organism (32). Such alternative routes to β-carotene have been demonstrated in Phycomyces blakesleeanus (33).

New information has recently been obtained, however, about the mechanism of carotene cyclization. Studies with stereospecifically-labelled MVA showed, some years ago, that β- and ϵ-rings are formed independently but possibly from a common carbonium ion or other intermediate (34-36). A cyclized carbonium ion could be stabilized by proton loss either from C-6 (to yield a β-ring) or from C-4 (to yield an ϵ-ring); loss of a proton from C-18 would give a γ-ring (2). Such a mechanism would require an initial proton attack at C-2 and this proton would be retained in the cyclized product.

The PG1 strain of Scenedesmus obliquus has been cultured heterotrophically in the dark, when it accumulated ζ-carotene. Cells were harvested, resuspended in D_2O and their starch reserves depleted, and then the suspension was illuminated in an air-CO_2 atmosphere. Bicyclic carotenoids were formed and α-carotene (β,ϵ-carotene), β-carotene (β,β-carotene), lutein (β,ϵ-carotene-3,3'-diol) and zeaxanthin (β,β-carotene-3,3'-diol) were purified and submitted to mass spectrometric analysis. Each bicyclic molecule formed from the unlabelled acyclic precursor contained 2 atoms of deuterium (37).

The same approach was used with a Flavobacterium sp., R1519 (38) in which nicotine causes the accumulation of lycopene since cyclization is blocked; removal of the inhibitor and aerobic reincubation results in the formation of zeaxanthin (39). After removal of the nicotine, aerobic incubation was in a medium prepared using D_2O. The resulting dideuterio-zeaxanthin was examined by [1]H n.m.r. spectroscopy which revealed the location of the

deuteriums at C-2 and C-2' and showed that the sample had the (2S,2'S) configuration. It was concluded that the initial proton attack in the cyclization of lycopene is on the re,re face of the C-1,2 double bond (38).

EFFECTS OF NICOTINE ON CAROTENOID FORMATION

A number of characteristic reactions of acyclic carotenes can be visualized (2) as involving an attack at C-2 by a positively charged species. These include the formation of (a) β-, (b) ε- or (c) γ-rings in C_{40} carotenoids when a proton is incorporated in each case at C-2 and then a proton is lost from C-6, -4 or -18 respectively, (d) the 1,2-hydration reaction (in e.g. Rhodospirillaceae) when a proton is gained at C-2 and, instead of cyclization and proton loss, OH^- is added at C-1 to form e.g. rhodopin (1,2-dihydro-ψ,ψ-caroten-1-ol) and (e) the hydrogenation reaction which forms 1,2-dihydrocarotenes in Rhodopseudomonas viridis. Also included are reactions which may form C_{45} and C_{50} homocarotenoids in non-photosynthetic bacteria where the initial attack at C-2 is by an electrophilic C_5 species (derived from IPP or DMAPP by loss of pyrophosphate?) and is followed by (f) addition of OH^- at C-1 to form e.g. bacterioruberin [2,2'-bis(3-hydroxy-3-methylbutyl)-3,4,3',4'-tetrahydro-ψ,ψ-carotene-1,1'-diol] or by cyclization and proton loss (g) from C-6 to form e.g. decaprenoxanthin [2,2'-bis(4-hydroxy-3-methyl-2-butenyl)ε,ε-carotene] or (h) from C-18 to form e.g. sarcinaxanthin [2,2'-bis(4-hydroxy-3-methyl-2-butenyl)γ,γ-carotene].

Since the formation of β-carotene in many organisms is blocked at lycopene by nicotine (e.g. 40), studying the effect of this cyclization inhibitor on the other reaction types (b - h) is a logical first approach to determining whether they are as similar in vivo as mechanistic theory predicts. Recent observations that have been made are as follows. The cyclase forming ε-rings in banana leaves is less sensitive than the β-cyclase to nicotine (41). Such a difference in sensitivity to nicotine by different enzymes in the same organism is also apparent in Rhodomicrobium vannielii where a higher concentration is required to block 1,2-hydration than is required to prevent β-cyclization (42). When nicotine-inhibited cultures of Rm. vannielii are resuspended in the absence of nicotine, 50% of the accumulated lycopene is converted into rhodopin as long as diphenylamine (DPA) is present to prevent de novo lycopene formation (42). The effect of nicotine in preventing the 1',2'-hydration of neurosporene and hence the formation of spheroidene (1-methoxy-3,4-didehydro-1,2,7',8'-tetrahydro-ψ,ψ-carotene) in Rhodopseudomonas sphaeroides is particularly useful in the preparation of $[^{14}C]$neurosporene from $[2,3-^{14}C]$succinate (33). The 1,2-dihydrogenation of acyclic carotenes in Rhodopseudomonas sphaeroides is also nicotine-sensitive (2).

C_{45} AND C_{50} HOMOCAROTENOIDS

The effect of nicotine on the formation of C_{50} carotenoids has now been tested in two different organisms, Sarcina flava (17) which forms carotenoids of the sarcinaxanthin series (γ-rings) and Halobacterium cutirubrum (43) which forms the acyclic bacterioruberin series.

The experiment with S. flava was part of a study (17) carried out by Dr. H.K. Al-Windawi in my laboratory in order to determine whether the alleged C_{50} carotene, sarcinene [2,2'-bis(3-methyl-2-butenyl)-ε,ε-carotene or its γ,γ-analogue], described in Sarcina spp. (44, 45) has any role in the biosynthesis of C_{50} xanthophylls. Both nicotine and DPA were used, separately and together, as inhibitors of carotenoid biosynthesis and in no instance was any C_{50} carotene found. DPA used alone (25μM) completely inhibited homocarotenoid formation and all-trans phytoene and phytofluene accumulated. On removal of the DPA and reincubation of cells, other carotenes were formed and C_{45} and C_{50} carotenoids appeared. When nicotine was used as inhibitor (7mM), there was a stimulation of carotenoid formation compared with a no-substrate control. Over 40% of the total carotenoid was C_{40} carotenes and the rest was homocarotenoids. The carotene fraction contained all the acyclic carotenes from phytoene to lycopene, including both isomeric conjugated heptaenes. When the cells were washed and reincubated, the carotenes, with the exception of lycopene, disappeared and there was a concomitant synthesis of sarcinaxanthin and other xanthophylls. Thus, in S. flava, nicotine is not as good an inhibitor of homocarotenoid formation as it is of e.g. β-cyclization or 1,2-hydration in other organisms. It seems certain that neurosporene is a better precursor than lycopene of C_{45} and C_{50} carotenoids in S. flava; possible intermediates in such a pathway [e.g. 2-(4-hydroxy-3-methyl-2-butenyl)-7',8'-dihydro-γ,ψ-carotene] were detected during the study (17). The fact that lycopene is the only carotene detectable in normal cultures may mean that its further metabolism is sluggish.

The above results with nicotine are remarkably similar to those obtained in a study on _Halobacterium cutirubrum_ (43). Here again, nicotine (3mM) stimulated carotenoid formation. The formation of monoanhydrobacterioruberin [2-(3-hydroxy-3-methylbutyl)-2'-(3-methyl-2-butenyl)-3,4,3',4'-tetradehydro-1,2,1',2'-tetrahydro-ψ,ψ-carotene] and bacterioruberin was blocked completely and virtually the only carotenoids present were lycopene and bisanhydro-bacterioruberin [2,2'-bis(3-methyl-2-butenyl)-3,4,3',4'-tetradehydro-1,2,1',2'-tetrahydro-ψ,ψ-carotene], comprising 79 and 19% respectively, of the total carotenoid. The accumulation of lycopene shows that nicotine will inhibit the C_5 addition reaction, but the presence of a good proportion of bisanhydrobacterioruberin means that this inhibition is by no means complete. On the other hand, the total absence of both bacterioruberin and monoanhydro-bacterioruberin from the inhibited culture shows that nicotine has as powerful an effect on 1,2-hydration as it does in the photosynthetic bacteria.

Clearly, the addition of the C_5 unit at C-2 of an acyclic C_{40} carotene shows a quite different sensitivity to nicotine compared with reactions involving proton addition at C-2. Indeed, the stereochemistry of the attacks are different, for while β-rings are formed as a result of a proton attacking the re,re face of the 1,2- double bond (38), the (2R,2'R) chiralities determined for decaprenoxanthin (46, 47) and sarcinaxanthin (48) result from a C_5 electrophile attacking the si,si face.

CAROTENOID METABOLISM IN ANIMALS

Although carotenoids are not formed de novo by animals (49), some recent work on animals has raised problems which are of a biosynthetic nature. One of the most remarkable of these stems from a detailed analysis of the carotenoids of the ladybird beetle, _Coccinella septempunctata_ (50, 51). The mixture of 18 carotenoids included all the acyclic carotenes from phytoene to 3,4-dehydrolycopene (including both ζ-carotene isomers), both the mono-β-cyclic and mono-γ-cyclic isomers of each of the last 4 acyclic carotenes of the desaturation sequence, and β-carotene, β,γ-carotene and γ,γ-carotene. While the β-cyclic and acyclic carotenes are normally regarded as red yeast carotenoids (27), some of the γ-cyclic carotenes are formed by the discomycete fungus _Caloscypha fulgens_ (52). The key to this metabolic enigma probably lies in an earlier observation (53) that a pink variety of the aphid _Macrosiphum liriodendri_ contains acyclic and β-cyclic carotenes while a green variant contains both β-cyclic and γ-cyclic pigments. Other aphids in the diet of the ladybird may have a similar pigment composition. The conclusion reached (50, 51) is that, since aphids are known to be rich in microbial symbionts, the carotenoids are formed within the aphid and/or ladybird and the actual biosynthesis must be carried out by symbiotic microorganisms.

Carotenoids with hydroxyl groups at C-2 of the β-ring have been isolated from the green alga _Trentepohlia iolithus_ (54), the blue-green bacterium _Anacystis nidulans_ (55), the red yeast _Rhodotorula aurantiaca_ (56) and from crustaceans, _Idotea_ spp. (57). Although suggestions have been made that such structures may be formed from an acyclic 1,2-epoxide (54) or by an OH^+ initiated cyclization (2), it is just as likely that they are the products of a direct hydroxylation. Such carotenoids have now been found in insects, β,β-caroten-2-ol in the moth _Cerura vinula_ (58) and both β,β-caroten-2-ol and the 2,2'-diol in the stick insect _Carausius morosus_ (59). The main red pigment of _Carausius_ is a novel dione, 3,4,3',4'-tetradehydro-β,β-carotene-2,2'-dione, and other pigments which could be intermediates between β-carotene and the dione are minor components (60). That β-carotene is the precursor of β,β-caroten-2-ol in _Cerura_ spp. has been demonstrated directly by using the labelled carotene (61). The suggestion has been made that the 2-one structure is formed first and then reduced to the 2-ol (60); such a situation would be unusual and inconsistent with the mechanism of similar changes at C-4 where the 4-ol is formed first and then oxidized to the 4-one (62, 63).

The cone cells of the retinas of diurnal birds (and of reptiles etc.) are characterized by the inclusion of coloured oil droplets (64, 65). Different types of cone have droplets of different colours, which may be red, orange, yellow or yellow-green. One study on the chicken (_Gallus domesticus_) showed that single cones and the chief and accessory members of the double cones contain red, yellow and yellow-green droplets, respectively (66) but a more recent microspectrophotometric analysis (67) showed six oil droplet types. The position and size of the droplets means that incoming light destined for the retinal-based photoreceptors in the cone outer segments has to pass through the droplets, which are assumed to be colour discriminators or bandpass filters to improve visual acuity (65). In contrast to man, diurnal birds can discriminate colours over the whole of their visual field (68). The colours of the droplets are due to a number of carotenoids (69, 70), which can occur at very high concentrations. Absorbance data for the droplets in the turtle (71) show that the carotenoid concentration may be as high as molar.

The first systematic study of these carotenoids was commenced in the late 1930's by Wald (69, 72) who isolated, from saponified extracts of chicken retinas, the greenish yellow galloxanthin, the red astacene (3,3'-dihydroxy-2,3,2',3'-tetradehydro-β,β-carotene-4,4'-dione), the golden-yellow lutein and a yellow hydrocarbon fraction. Carotenoids with similar properties to these have been observed by other workers in chicken retinas (73, 74) homogenates (75), isolated oil droplets (70) and in pigeon retinas (76). Astacene, of course, is an artifact, formed on saponification from astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione), since shown to occur in the chicken retina (75). The yellow hydrocarbon fraction was correctly described (69) as having properties "almost identical with those of the hydrocarbon sarcinene" from Sarcina lutea (44); unfortunately, this designation for the carotene has persisted in the literature.

Studies in my own laboratory (by Rosemary Apps and Susan Ashworth) of the unsaponifiable fraction of turkey retinas have confirmed the presence of a mixture of carotenoids including galloxanthin, astacene, lutein, zeaxanthin and a carotene with a conjugated nonaene chromophore. All the properties of galloxanthin, including the mass spectrum of its diacetate, are consistent with its being the C_{27} diol, 10'-apo-β-carotene-3,10'-diol. A slight chromatographic difference between the natural compound and the authentic diol (prepared from zeaxanthin) may reflect a stereochemical difference.

The carotene was identified first by t.l.c. comparison with α- and β-carotenes as ε-carotene (ε,ε-carotene) and this was confirmed by its mass spectrum and comparison with synthetic ε-carotene (provided by Prof. C.H. Eugster). A recent study of chick retinas failed to detect ε-carotene although it was present in blood (74).

One can but speculate on the origin of these various retinal carotenoids. Both astaxanthin and galloxanthin could be formed from zeaxanthin, by insertion of 4-oxo groups in one case and by partial oxidative degradation in the other; neither transformation would be regarded as unusual in animals (77). In the long term, the ultimate source of carotenoids in birds must be their food and, indeed, Japanese quail with colourless oil droplets have been obtained by maintaining a colony for more than one generation on a diet free from carotenoids but supplemented with vitamin A (78). In the short-term, however, the carotenoids must be obtained by young birds from the egg for the pigmented oil droplets are present even in embryo (69, 73, 79).

It is difficult to rationalize the presence of ε-carotene in the turkey (as the sole retinal carotene constituting 15% of the total carotenoid) against this background, especially since neither feed pellets nor egg yolks contained any detectable ε-carotene. It may be that β- and ε-rings are not as metabolically immutable in animals as they are in plants and that some interconversion is possible. This is not a unique case of a carotenoid with two ε-rings in an animal for ε,ε-carotene-3,3'-diols occur, as the epimeric chiriquixanthins A and B in a Costa Rican frog (80) and as tunaxanthin in fish (81). In such animals, however, the possibility of a dietary source of ε-carotene cannot be ruled out although it is unlikely to be in the diet in any quantity. There is evidence from the Californian sheephead fish, Pimelometopon pulchrum that tunaxanthin is converted into astaxanthin, possibly via zeaxanthin (82).

SOME STEREOCHEMICAL CONSIDERATIONS

One characteristic of ε-rings is their chirality at C-6. A recent comparison of the chirality at C-6 of both γ- and ε-rings (83) in both C_{40} and C_{50} carotenoids has singled out what may be an ambiguity. The configuration of the γ-ring in β,γ-carotene from Caloscypha fulgens is 6'S (83). In γ- or ε-rings which are substituted at C-2 (as in C_{50} carotenoids) exactly the same stereochemistry at C-6 has to be designated 6R (or 6'R) because the C_5 ligand at C-2 now imposes a different sequence of priorities at C-6. Decaprenoxanthin (ε-rings) and sarcinaxanthin (γ-rings) are both 6R,6'R (46 - 48) and therefore have the same configuration as (6'S)-β,γ-carotene. The ambiguity results from the fact that all C_{40} carotenoids with ε-rings are reported to have the opposite configuration (i.e. 6R or 6'R); thus such rings in α-carotene, δ-carotene (ε,ψ-carotene) and ε-carotene are described as 6R (84) and the chiriquixanthins A and B are also 6R,6'R, so designated on the basis of their ^{1}H n.m.r. spectra and because their CD spectra show positive Cotton effects at 268 nm (80).

Returning now to the ε-carotene isolated from avian (turkey) retinas, a CD comparison was made (by Dr. P.M. Scopes) of the retinal carotene with authentic (6S,6'S)-ε,ε-carotene. The CD spectra were virtually identical and their features included a negative Cotton effect at 268 nm. Thus the avian ε-carotene can be designated 6S,6'S. A further CD comparison with ε-carotene isolated from the marine green alga Ulva lactuca showed this, too, to be 6S,6'S.

If ε-carotenes with different configurations occur naturally, this situation must reflect their biosynthesis by different pathways or mechanisms. Other examples have recently been brought to light of which one involves the two chiriquixanthins A and B, (3R,6R,3'S,6'R)-ε,ε-carotene-3,3'-diol and (3S,6R,3'S,6'R)-ε,ε-carotene-3,3'-diol respectively. These are epimeric at C-3 and in the case of chiriquixanthin A the end groups are constitutionally identical but stereochemically different (80).

The other example concerns astaxanthin. Following the determination of the (3S,3'S) absolute configuration for astaxanthin from the carapace of the lobster Homarus gammarus and from lobster eggs and for the monoester from the alga Haematococcus pluvialis (85) a comparison was made with an astaxanthin diester from the spider mite Schizonobia sycophanta; again the chirality was shown to be 3S,3'S (86). The results of these comparisons supported the view then held that individual carotenoids exhibit identical optical properties irrespective of their source.

That this view is now untenable was demonstrated when astaxanthin from the novel fermenting red yeast Phaffia rhodozyma (87, 88) was shown, by comparison with (3S,3'S)-astaxanthin using CD and ^{1}H n.m.r. with chiral shift reagents, to be 3R,3'R (89). A possible explanation for the different chiralities of the yeast and lobster astaxanthin is as follows (89). The crustacean pathway to astaxanthin probably involves the formation of keto groups at C-4 and C-4' of zeaxanthin. Thus 3-hydroxylation precedes ketone formation at C-4. In P. rhodozyma, other carotenoids present are echinenone (β,β-caroten-4-one) and canthaxanthin (β,β-carotene-4,4'-dione) but not zeaxanthin or isozeaxanthin (β,β-carotene-4,4'-diol). Thus, in this organism, ketone formation probably precedes 3-hydroxylation. The stereospecificity of hydroxylation may depend on whether the β-ring is already substituted or not. This can only be investigated using a more biochemical approach.

CONCLUSION

As this account shows, a number of the problems of carotenoid formation have been solved since 1976. One of the earlier reviews upon which this paper is based (2) suggests that "it is possible that as yet undetected differences in the stereochemistry of biosynthesis may be revealed". Such differences are now apparent; these and the other unresolved problems of carotenoid formation must be the subject of further study.

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