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SIGNIFICANCE OF THE *CIS–TRANS* ISOMERIZATION OF EARLY INTERMEDIATES IN THE CAROTENE BIOSYNTHETIC PATHWAY

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Using semi-empirical self-consistent field molecular orbital (SCF-MO) quantum chemical calculations, structures of putative intermediates of the desaturation pathway for both *cis*- and *trans*- carotene biosynthesis have been optimized. It was observed that the *cis* isomers of the early biosynthetic intermediates are more stable than corresponding *trans* isomers. Both desaturation and cyclization steps confer increased stability on these carotenes. The results also argue for phytofluene, rather than the earlier suggested phytoene or ζ-carotene, as the energetically favored branch point for poly-*cis*-carotene biosynthesis.

INTRODUCTION

Two major classes of pigments that occur in the photosynthetic membranes of plants are chlorophylls and carotenoids. The main function of carotenoids in photosynthetic organisms is to provide protection against photodynamic destruction and to assist in light harvesting.[1] Carotenoids are also present at high concentrations in some flowers and fruits. β-Carotene, the principal carotene in higher plants, also serves as the precursor for vitamin A in mammals.[2]

Most carotenoids found in nature show an all-*trans* configuration about the conjugated polyene chromophore. The natural occurrence of *cis*-poly-*cis*-carotenes in plants, although observed, is not very common.[3] Treatment of plant tissues with substituted secondary or tertiary amines can also induce the synthesis of poly-*cis*-carotenes in an otherwise normal *trans*-carotene-producing system.[4] Daffodil chromoplasts form poly-*cis*-prolycopene (7,7′,9,9′-tetra-*cis*) under aerobic conditions *in vitro*.[5]

The first step in the biosynthesis of carotenoids is the head-to-head condensation of two molecules of geranylgeranyl pyrophosphate to form phytoene, via the intermediate prephytoene pyrophosphate.[6] 15-*cis*-Phytoene is the most predominant phytoene formed in eukaryotic plants and most bacteria; the all-*trans* isomer is found only in a few organisms.[7] The formation of all-*trans*-lycopene in higher plants appears to involve five steps: one isomerization and four dehydrogenations. 15-*cis*-Phytoene is first dehydrogenated to *cis*-phytofluene, which is then isomerized to *trans*-phytofluene and converted by a series of three dehydrogenations into ζ-carotene, neurosporene and lycopene[5] (Figure 1). Lycopene is then cyclized to form either α- or β-carotene.

The pathway for the biosynthesis of poly-*cis*-carotenes (Fig. 1) in tissues where they occur is still less understood. Clough and Pattenden[8] established the stereochemistry of the poly-*cis*-carotenes of the tangerine tomato as C-15-mono-*cis*-phytoene, C-15, C-9′-di-*cis*-phytofluene, C-9,C-9′-di-*cis*-ζ-carotene, C-9,C-9′,C-7′-tri-*cis*-proneurosporene and C-7,C-9, C-9′,C-7′-tetra-*cis*-prolycopene. The remaining double bonds in these carotenes have *trans* geometry. Clough and Pattenden[8] also proposed that the branch point for the synthesis of *cis*-carotenes was phytoene, rather than *cis*-ζ-carotene. Studies done on cell-free extracts of tangerine tomato fruit plastids,[9] however, indicated that the branch point in the conversion of poly-*cis* compounds is *trans*-ζ-carotene.[10] Raymundo and Simpson[11] suggested that proneurosporene and prolycopene could be formed from poly-*cis*-phytoene, poly-*cis*-phytofluene and poly-*cis*-ζ-carotene, which in turn can be formed from phytoene, phytofluene and ζ-carotene, respectively.

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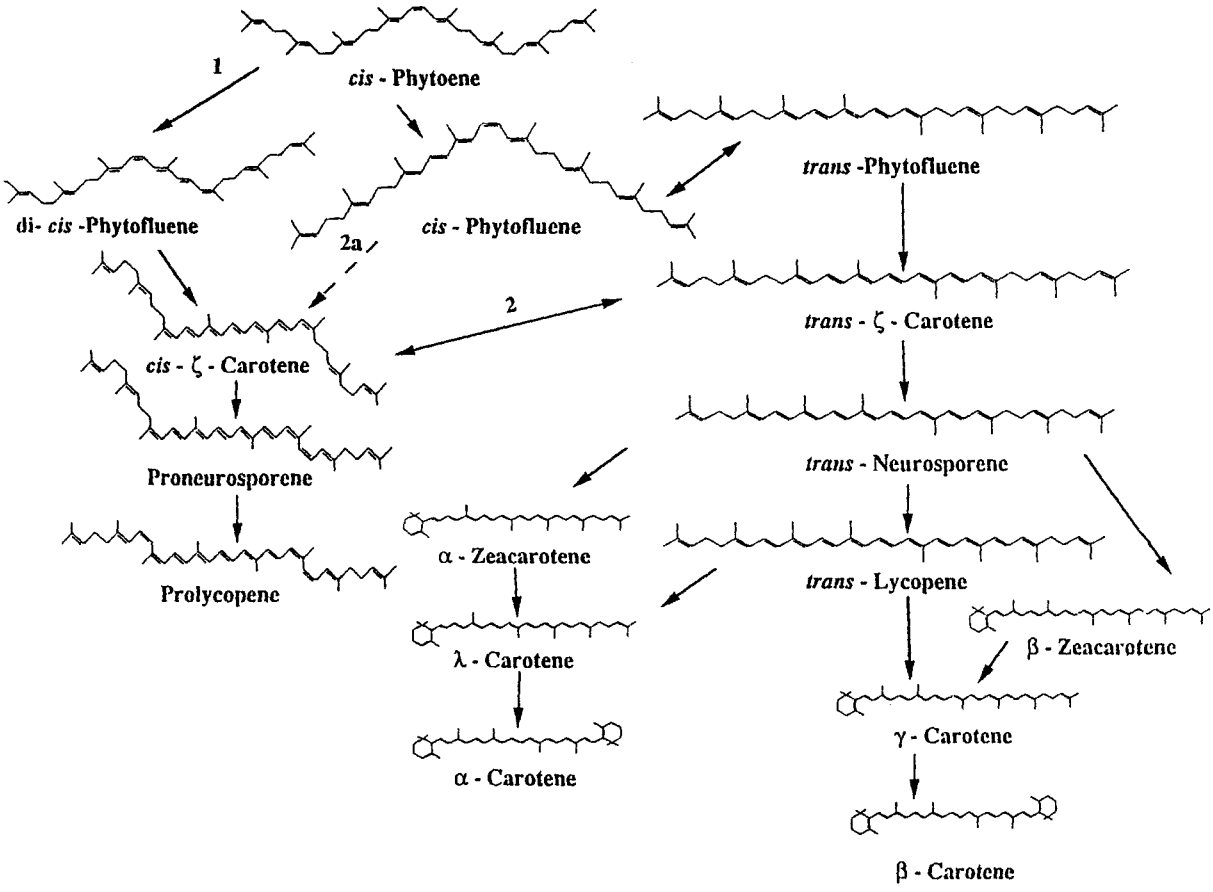

Figure 1. Pathway for the biosynthesis of carotenes in plants; 1, 2, and 2a (Refs. 8, 9 and 10, respectively) indicate the proposed branch points for the synthesis of poly-*cis*-carotenes

It is obvious from earlier studies that there is still uncertainty regarding the branch point of the biosynthetic pathway leading to the formation of poly-*cis*-carotenes. The aim of this study was (a) to optimize structures of putative intermediates of the desaturation pathway of both *cis*- and *trans*-carotene biosynthesis at a semi-empirical self-consistent field molecular orbital (SCF-MO) level and (b) to find the most energetically favorable intermediates and the biosynthetic step at which the *cis-trans* isomerization could possibly occur and serve as the branch point for the formation of poly-*cis*-carotenes. Semi-empirical SCF-MO methods have been proved to be successful tools in finding energetically favored pathways and their mechanistic details in other systems.[12,13]

PROCEDURE

The semi-empirical quantum chemical calculations were performed on a IBM 3090 600J computer with use of the PM3[14] Hamiltonian as found in MOPAC 5·0 and 6·0 (QCPE 831).[15] Geometries were optimized from the appropriate idealized starting geometries using the Broyden–Fletcher–Goldfarb–Shanno method. The optimization limits were set by using the keyword PRECISE, which is a routine procedure in optimizing geometry. Symmetry functions were not used in the calculations. The rotational barriers were followed by a systematic increase of the corresponding torsional angle along the C-15—C-15′ bond. All stable geometries were verified by the absence of negative eigenvalues of the force constant matrix (using analytical first derivatives). SCF calculations were optimized to $< 10^{-8}$ kcal mol^{-1} (1 kcal = 4·184 kJ) and geometries were optimized to $< 10^{-4}$ Å.

RESULTS AND DISCUSSION

The heats of formation (ΔH_f) of all carotenes included in the calculations are given in Table 1. Heats of for-

Table 1. Calculated heats of formation of carotenes

Carotene	ΔH_f (kcal mol^{-1})	E_{corr} (kcal mol^{-1})[a]
trans-Phytoene	−14·7	0
cis-Phytoene	−17·2	−2·5
trans-Phytofluene	15·0	0·7
Di-*cis*-Phytofluene	9·3	−5·0
cis-Phytofluene	8·1	−6·2
trans-ζ-Carotene	31·1	−12·2
cis-ζ-Carotene	30·6	−12·7
Unsym.-ζ-Carotene	29·6	−13·7
trans-Neurosporene	53·9	−18·4
α-Zeocarotene	49·3	−23·0
β-Zeocarotene	45·2	−27·1
trans-Lycopene	78·6	−22·7
λ-Carotene	74·2	−27·1
γ-Carotene	70·4	−30·9
ε-Carotene	63·3	−38·0
α-Carotene	57·5	−43·8
β-Carotene	56·8	−44·5

[a] $E_{corr} = \Delta H_f - n \times 29{\cdot}0$ (kcal mol^{-1}), where n is the number of desaturation steps the carotene underwent compared with phytoene.

mation resulting from the calculations can be directly compared for the molecule stability only for isomeric molecules. In order to compare the stabilities of molecules which differ in saturation (and the number of hydrogen atoms) we have introduced a corrected energy (E_{corr}). The correction is based on the energy difference between butane and butene (29·0 kcal mol^{-1}), calculated under same approximations as all other calculations:

$$E_{corr} = \Delta H_f - n \times 29{\cdot}0 \text{ (kcal mol}^{-1}\text{)} \quad (1)$$

where n corresponds to the number of desaturation steps the molecule underwent compared with phytoene. *trans*-Phytoene was arbitrarily assigned the value of 0 kcal mol^{-1}. The carotenes listed in Table 1 are given in order of increasing stability within groups of isomers. Relative stabilities of different carotenes are presented qualitatively in Table 1 by vertical placement of the corresponding molecules.

These calculations indicate that *trans* isomers of carotenes are always less stable than the corresponding *cis* isomers (Table 1). This is consistent with the fact that the phytoene found in eukaryotic plants and most bacteria is predominantly 15-*cis*.[7] Desaturation steps in the carotene biosynthetic pathway lead to an increase in stability of these carotenes, due to conjugation of the systems. Comparison of energies between the acyclic lycopene and all other isomeric cyclic carotenes support the fact that with cyclization there is a further increase in stability of these carotenes (Table 1). The cyclic carotenes, especially β-carotene and to a lesser extent α-carotene, are known for their functional role in light

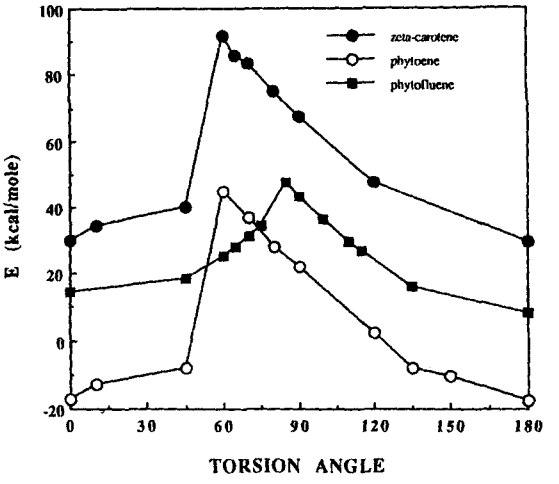

Figure 2. *Cis–trans* rotational pattern for (○) phytoene, (■) phytofluene and (●) ζ-carotene

and for the assembly of photosystems, of the photosynthetic apparatus of all algae and higher plants.

We also attempted to evaluate the significance of *cis–trans* rotation of the early intermediates phytoene, phytofluene and ζ-carotene in the carotene biosynthetic pathway. The rotational patterns for all three carotenes are illustrated in Fig. 2. The torsion angle, θ, is defined for the C-15—C-15′- bond. The 0° angle corresponds to *cis* isomers and 180° to *trans* isomers. We were unable to optimize transition states along these barriers. A striking observation from these results is that the result of *cis–trans* isomerization for phytofluene is different from those for both phytoene and ζ-carotene. The maximum point corresponds to 90° in phytofluene compared with 60° in both phytoene and ζ-carotene. The barrier is more symmetrical on both sides of the maximum and it is much smaller than for the other two carotenes. These observations are summarized in Table 2. In addition to the torsion angle, the height of the barrier (ΔE) and the difference between the corresponding *cis* and *trans* isomers ($\Delta E_{cis-trans}$) are listed in Table 2.

Extensive information on carotene biosynthesis in several prokaryotes and eukaryotes exists at the

Table 2. Parameters of *cis-trans* rotational barriers for early intermediates

Molecule	ΔE (kcal mol^{-1})	θ(°)	$\Delta E_{cis-trans}$ (kcal mol^{-1})
Phytoene	54	60	2.5
Phytofluene	28	90	6.9
ζ-Carotene	61	60	0.5

biochemical level.[6] However, very little information is available on the enzyme(s) that mediate(s) the isomerization and dehydrogenation reactions in both *trans*- and *cis*-carotene biosynthetic pathways, with the exception of the enzymes phytoene synthetase[16] and phytoene desaturase.[17] There is still no clear understanding on the branch point from which the biosynthesis of poly-*cis*-carotenes occurs. Phytoene[8] and *trans*-ζ-carotene[9] have been proposed as those points from which the poly-*cis*-carotenes were synthesized. Data obtained from our calculations indicate that *cis*-phytofluene may be a strong candidate for this position rather than both phytoene and *trans*-ζ-carotene. *cis*-Phytofluene is much more stable than *trans*-phytofluene (Tables 1 and 2). Porter and Spurgeon[10] also indicated the possibility of the occurrence of direct conversion of *cis*-phytofluene into *cis*-ζ-carotene in the poly-*cis*-carotene biosynthetic pathway of tangerine tomato fruit plastids. A comparatively lower conformational barrier of phytofluene, as indicated from our calculations, strongly suggests it to be an additional candidate for the *cis–trans* isomerization along with phytoene and ζ-carotene in the carotene biosynthetic pathway. It will be interesting to identify the enzyme(s) that catalyze this step in the carotene biosynthetic pathway to support our findings.

CONCLUSIONS

Our results argue for phytofluene rather than the earlier suggested phytoene or ζ-carotene as the energetically favored branch point for poly-*cis*-carotene biosynthesis in tissues where they occur. Results from our calculations also suggest that (a) *cis* isomers of the early intermediates of the biosynthetic pathway are more stable than corresponding *trans* isomers and (b) saturation and cyclization steps in the carotene biosynthetic pathway confer increased stability on these carotenes.

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