The involvement of free radicals in the mechanism of β-Carotene Degradation

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***Abstract*:** β-Carotene, a pro-vitamin A carotenoid, was reacted with oxygen in benzene as solvent, in the dark. The products of oxidation were characterised by HPLC with a UV photodiode detector and LC-MS, EI mode. The products identified were oxygen-containing compounds of the type epoxides, aldehydes and ketones. ESR study of retinyl- and ionyl-derivatives revealed that the secondary sites C7 and C9 were the most reactive towards radical generation. Both the results of product analysis and the ESR study enabled us to propose that the products of β-carotene autoxidation were obtained by a free radical mechanism involving initial formation of a biradical during the *trans*-to-*cis* isomerisation at the 15,15' bond and attack by oxygen.

***Keywords*:** Carotene, Carotenoids, Apo-carotenoids, ESR, Degradation, Autoxidation, Free radicals.

Introduction

β-Carotene is a pro-vitamin A carotenoid possessing half the activity of vitamin A. Because of its structural resemblance to vitamin A, it was thought that two molecules of vitamin A could be obtained by central cleavage from β-carotene (Karrer et al. [1]). However, attempts to obtain two molecules of vitamin A directly from β-carotene have, so far, failed. In the absence of antioxidants, the carotenoids are unstable to heat, light and air/oxygen. Peto et al., [2] suggested that dietary β-carotene might function as an anti-carcinogenic agent. In both in vitro and in vivo studies β-carotene has been shown to act as both a pro-oxidant and an antioxidant, Packer et al. [3]; Krinsky and Deneke [4].

The question that has often arisen is whether it is β-carotene per se or its degradation products that is responsible for its antioxidant activity? In addition, the mode of action whereby β-carotene is degraded is largely unknown. It is the elucidation of this latter unknown aspect that is the objective of this paper through an examination of the products of β-carotene oxidation and an ESR study of reactive intermediates from retinyl- and

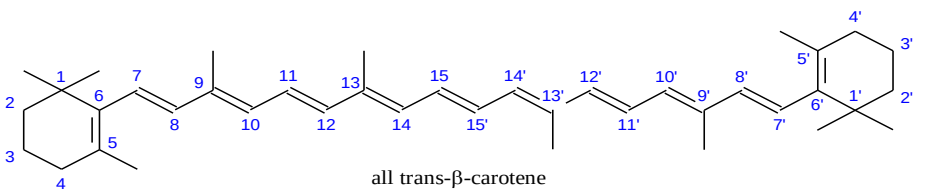
ionyl-derivatives. We propose the evidence as strongly favouring the involvement of free radicals in the degradation of β-carotene.

Materials and Method

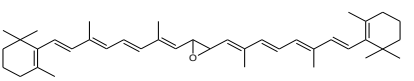
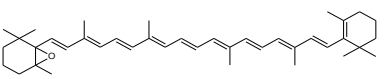
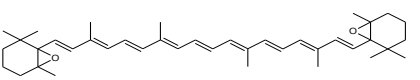
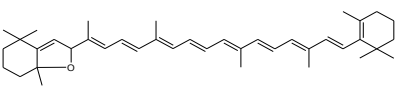
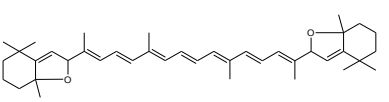
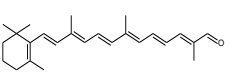
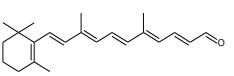
β-Carotene, β-ionone, β-cyclocitral, retinyl acetate, *cis*-9-retinal, *cis*-13-retinal and β-apo-8'-carotenal were obtained from Sigma-Aldrich. The epoxy compounds were prepared as described by Barber et al. [5], and the other relevant compounds were prepared according to the literature methods described in Haag et al. [6], Attenburrow et al. [7], Huisman et al. [8] and Haeck et al. [9]. The details of the experimental method for β-carotene oxidation can be found in Mordi et al. [10].

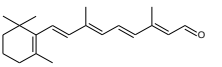
Results and Discussion

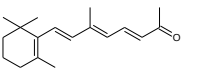
The products of β-carotene autoxidation identified by following the progress of reaction are listed in Figure 1. This list has been compiled from these references Mordi et al. [10]; Tang et al. [11]; Wang et al. [12], and Rodriguez and Rodriguez-Amaya [13].

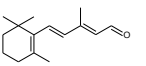


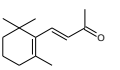
all trans-β-carotene

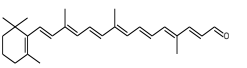
Product name	Structure
15,15'-epoxy-β-carotene	
5,6-epoxy-β-carotene	
5,6,5',6'-diepoxy-β-carotene	
5,8-epoxy-β-carotene	
5,8,5'8'-diepoxy-β-carotene	
β-apo-12'-carotenal	
β-apo-14'-carotenal	

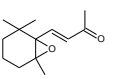
Retinal 

β-apo-13-carotenone 

β-ionylidene acetaldehyde 

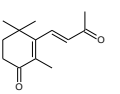
β-ionone 

β-apo-10'-carotenal 

5,6-epoxy-β-ionone 

β-cyclocitral 

Dihydroactinidiolide 

4-oxo-β-ionone 

2,2,6-trimethylcyclohexanone 

Fig 1 Products of β-Carotene oxidation

It is suggested that free radicals are involved in the oxidation, since the extended system of conjugated double bonds makes β-carotene susceptible to radical attack. Evidence for the involvement of free radicals in the oxidation of β-carotene is that the oxidation is accelerated by radical initiators (AIBN) and inhibited by radical chain-breaking antioxidants BHT and α-tocopherol. So how do these products arise? Zechmeister et al. [14] and El-Tinay and Chichester [15], suggested that the central double bond of β-carotene is less susceptible to oxidative attack than the terminal double bonds, which are the sites of greatest electron density. Glover [16], suggested that the degradation of β-carotene proceeded by initial attack at one end of the molecule, forming a series of apo-carotenoids by the removal of two carbon units. The initial evidence for these carotenoids was presented by Sharma et al. [17]. Mohamed et al. [18] and Doering and Sarma [19] recently confirmed that the terminal double bonds were the preferred sites of attack as there is a greater loss of stabilisation energy the radical formed as we move away from the centre of the polyene chain

compared to the loss towards the centre.

In our ESR study of retinyl- and ionyl-derivatives and spin trapping of the radicals generated from them, we were able to show that C7 and C9 for ionyl-derivatives and C7, C9 and C11 and probably C15 for retinyl-derivatives were the most reactive sites, Mordi and Walton [20]. Apart from C15, which is a primary site, these sites are secondary. The radical generated will, of course, be delocalised over several carbon atoms and the terminal carbons will be the most sterically exposed and so more susceptible to attack. By extension therefore, it is suggested that these sites would be vulnerable to attack on the conjugated system of β-carotene.

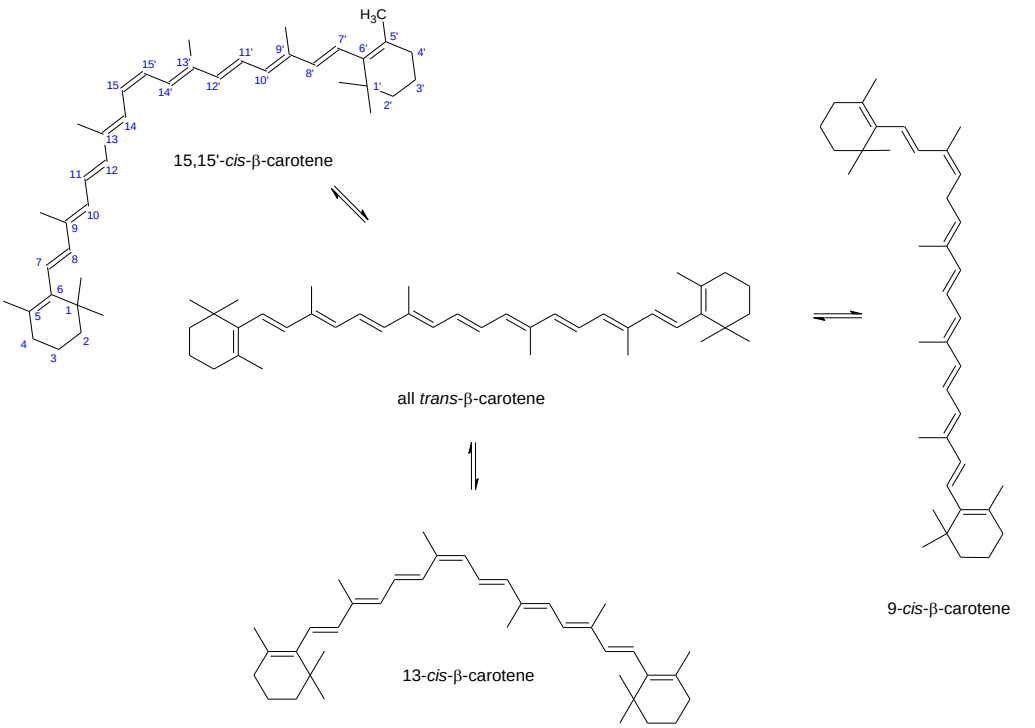
A study of self-initiated autoxidation of retinal showed that retinal is resistant to oxidation compared to β-carotene, Mordi et al. [10]. ESR study of retinal failed to give any ESR signal. Retinyl derivatives gave only a broad and weak ESR spectrum, Mordi and Walton [20]. Could this be an indication that there is a predetermined chain length to allow for isomerisation before biradical formation?

The lack of reactivity of retinol could be attributed to the compound's inability to undergo the initial *cis-trans* isomerisation at the C15 carbon atom and the presence of the hydroxyl group preventing the initial epoxide formation at C15. The observed products after prolonged reaction time could be due to end-chain peroxyl attack on retinal. Although retinal is known to undergo *cis-trans* isomerisation at C11 for vision, if this happened in the oxidation reaction system, through the suggested biradical mode, the biradical would not be as strongly stabilised (delocalised) as the biradical derived from β-carotene and therefore more energy would be needed to bring about its formation.

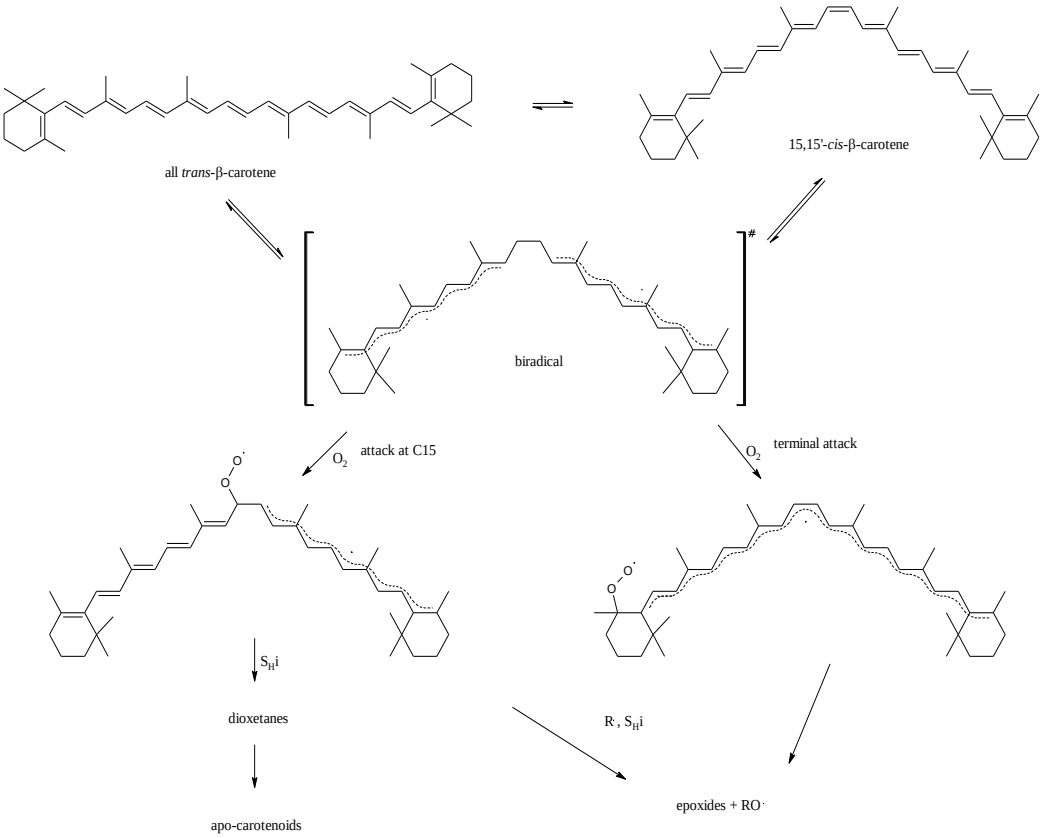
It is thought that the mechanism of autoxidation begins by an initial *trans-cis* isomerisation at 15,15' central double bond, Mordi et al. [10] and Doering and Sarma [19]. The original idea of the involvement of *cis* isomer was put forward by Glover [16] and recent studies have shown that *cis* isomers are truly involved in β-carotene autoxidation and degradation. We showed that there were other isomers of β-carotene in the product mixture during oxidation but were unable to properly identify which isomers. Other workers have been able to identify these isomers as 9-cis and 13-cis-β-carotene, Mohamed et al. [18], Penicaud et al. [21], Achir et al. [22], Marx et al. [23] and Henry et al. [24]. This trans-cis isomerisation implies the formation of a biradical intermediate, Mordi et al. [10].

The likelihood of involvement of free radicals is enhanced by the fact that the products of β-carotene oxidation by molecular oxygen are similar to those obtained by singlet oxygen oxidation. The similarity of products in these reactions has led to the suggestion that a singlet biradical

of β-carotene is formed in the *cis* configuration, which is stabilised by extensive delocalisation of the two unpaired electrons. The biradical is then attacked by oxygen on either side of the *cis* bond leading to the formation of carotenyl peroxyl triplet biradical, Scheme 1 and Scheme 2.



Scheme 1. Cis-trans isomerisation of all-trans-β-carotene prior to formation of the biradical, (Mordi et al. [10]; Mordi [25], and Penicaud et al. [21].)



Scheme 2. Steps in the formation of oxidation products, (Mordi et al. [10], Mordi [25] and Penicaud et al. [21].)

These peroxyl triplet radicals are sufficiently long-lived to act as initiators of autoxidation.

Following homolytic intramolecular substitution, S_Hi, (Mayo [26]; Mayo and Miller [27]; Porter et al. [28], and Porter and Zuraw, [29]), the peroxyl biradicals will collapse most probably by hydrogen abstraction to give hydroperoxides, thereby allowing the epoxides to appear as products whereas attack by oxygen at any position of the biradical would give the apo-carotenoids. By monitoring the progress of reaction, it was shown that the apo-carotenoids begin to appear at the same time, Mordi et al. [10].

By following the progress of reaction we have shown that all the double bond positions are vulnerable to attack by oxygen, but the initial site of attack appears to be at the terminal and central double bonds, as 5,6-epoxy, 5,6,5',6'-diepoxy and 15,15'-epoxy carotenes were identified at the initial stages of reaction. The attack at 15,15' double bond would probably lead to formation of dioxetane, which eventually collapses to the apo-carotenoids detected, whereas attack

at the terminal double bonds would lead to the epoxides. The apo-carotenoids were also identified, again suggesting the vulnerability of all the double bond positions (Mordi, [25]). These cleavage products have been identified by other workers and the formation of in-chain epoxides has been suggested as precursors to apo-carotenoids (Rodriguez and Rodriguez-Amaya [13]; Hiranvarachat et al. [30]; Caris-Veyrat et al. [31]). The formation of 5,8-epoxy- and 5,8,5',8'-diepoxy-β-carotene has been linked to the acid catalysed isomerisation of the 5,6-epoxy equivalents in food ripening (Rodriguez and Rodrigues-Amaya [13]) . We also suggested that these furanoids are formed directly from the peroxyl radicals.

Conclusion

We can conclude that β-carotene is degraded by initial *cis-trans* isomerisation, followed by the formation of a singlet biradical. The biradical is then attacked by oxygen to produce peroxyl radicals at the double bonds. The peroxyl radicals at the terminal double bonds are transformed to epoxides and furanoids and along the chain are transformed to apo-carotenoids.

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