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Mini review

INTERACTIONS BETWEEN CANTHAXANTHIN AND LIPID MEMBRANES – POSSIBLE MECHANISMS OF CANTHAXANTHIN TOXICITY

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Abstract: Canthaxanthin (β, β-carotene 4, 4' dione) is used widely as a drug or as a food and cosmetic colorant, but it may have some undesirable effects on human health, mainly caused by the formation of crystals in the *macula lutea* membranes of the retina. This condition is called canthaxanthin retinopathy. It has been shown that this type of dysfunction of the eye is strongly connected with damage to the blood vessels around the place of crystal deposition. This paper is a review of the experimental data supporting the hypothesis that the interactions of canthaxanthin with the lipid membranes and the aggregation of this pigment may be the factors enhancing canthaxanthin toxicity towards the *macula* vascular system. All the results of the experiments that have been done on model systems such as monolayers of pure canthaxanthin and mixtures of canthaxanthin and lipids, oriented bilayers or liposomes indicate a very strong effect of canthaxanthin on the physical properties of lipid membranes, which may explain its toxic action, which leads to the further development of canthaxanthin retinopathy.

Key words: Canthaxanthin, Retinopathy, *Macula lutea*, Model lipid membranes, Molecular interactions

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Abbreviations used: AMD – age-related macular degeneration; DHPC – 1,2 dihexanoyl-*sn*-glycero-3-phosphocholine; DMPC – dimyristoyl-phosphatidylcholine; DMPE – 1, 2-diacyl-*sn*-glycero-3-phosphoethanolamine; DPPC – dipalmitoyl-phosphatidylcholine; DSPC – distearoyl-phosphatidylcholine; EYPC – egg yolk phosphatidylcholine; FTIR – Fourier transform ultra red; GSTP – glutathione S-transferase, zeaxanthin-binding protein; NMR – nuclear magnetic resonance, qlLBP – lutein-binding protein; RPE – retinal pigment epithelial cell; XBP – xanthophyll-binding protein

INTRODUCTION

Retinopathy is a term that refers to forms of non-inflammatory retinal damage that are often progressive, and may result in blindness or severe vision loss. Some medicinal products can trigger drug-related retinopathy [1, 2]. Canthaxanthin intake is associated with golden yellow or red, birefringent crystalline deposits in the retina around the macula; this condition is called canthaxanthin retinopathy [3-8]. The formation of aggregates of canthaxanthin in the *macula lutea* membranes has been observed with an aid of ophthalmoscope [1, 3-7, 9]. There is an increasing number of publications on other undesirable effects on human health arising from the use of this carotenoid, conditions such as retinal dystrophy [9] or aplastic anaemia [10]. Canthaxanthin is frequently given to patients with tumours, as it can act as a strong antioxidant. Its anti-tumour and radical quenching action has been proved [11-14]. Canthaxanthin is also popular as a natural skin bronzer added to cosmetics [15, 16]. Experiments done on animals [8, 17] including humans [3-8, 18] show that even when used in small quantities such as in food colouring or cosmetics, canthaxanthin can form molecular aggregates that are deposited in the tissues, especially in the *macula lutea* of the eye. Although drug-related retinopathies generally affect patients taking large doses of a given drug, with canthaxanthin, the side effects were reported even without the use of high doses. From the great number and size of the crystals, on the one hand, and the relatively small amount of isolated canthaxanthin on the other, it was concluded that the crystals presumably represent a canthaxanthin-lipoprotein complex rather than pure canthaxanthin alone [4]. It has been shown that canthaxanthin retinopathy is strongly connected with damage to the blood vessels around the place of crystal deposition, and the growth of new, abnormal vessels [1, 4]. The analysis of the fatty acid composition of the retinal vasculature shows that the human retinal blood vessels are mainly composed of palmitate (25.1%), oleate (28.8) and stearate (16.8%) fatty acids [19]. Therefore, one can venture the hypothesis that this dysfunction may be based on specific interactions of canthaxanthin with the lipids containing these fatty acids.

MACULA LUTEA PIGMENTS

Lutein and zeaxanthin are the predominant carotenoids in the human *macula lutea*, although there is considerable evidence for the presence of other carotenoids (cis-isomers, 3' epilutein, 3-hydroxy β,ε-carotene-3'-one, echinenone, and a derivative of β-carotene, 11-cis-retinal) [20-24]. In humans, the levels of selected carotenoids in ng per g of macular tissue are: all-trans lutein – 32.93 ± 7.74; all-trans zeaxanthin – 12.70 ± 4.94; cis-isomers – 4.90 ± 2.70; 3' epilutein – 2.33 ± 0.50 [24]. Experiments have shown that zeaxanthin acts as an effective free radical quencher, while lutein is an effective filter of short wavelength radiation [25, 26]. Studies on humans show that lutein

supplementation results in increased macular pigment levels and improved vision in patients with age-related macular degeneration (AMD) and other ocular diseases [27, 28]. In healthy patients, under normal physiological conditions, canthaxanthin does not appear in the *macula lutea* membranes. In most cases, its presence is connected with its consumption in high doses, and very rarely when no canthaxanthin has been ingested [2, 29-33].

Experiments on monkeys receiving an excess of canthaxanthin-containing fodder showed the level of canthaxanthin in the *macula* as high as 250 ng/g of wet tissue (approx. 1.25 μg/g dry tissue), while the levels of zeaxanthin and lutein were respectively 180 and 50 ng/g (0.9 and 0.25 μg/g dry tissue). The highest level of canthaxanthin was found in the paracentral area of the retina [17].

It has been shown that lutein and zeaxanthin are located in the *macula* in an environment that is very similar to that of liposomes, with their axis perpendicular to the surface of the membrane [34].

Determining the canthaxanthin concentration in humans is quite difficult due to its correlation with the level of food consumption or use of cosmetics containing these carotenoids. The individual's metabolic ability to absorb xanthophylls is also of great importance. One has to consider the effect of cumulating this substance after long-term delivery. The canthaxanthin concentration can vary, depending on the tissue, and in the eye, on the distance from the *macula lutea*. No data on the canthaxanthin concentration in the *macula lutea* in humans is available.

Based on data obtained from a study on the human lens [35], an average of 2 mg of lipid is found in 1 g of *macula* tissue. A study on patients with retinopathy showed ca. 42 μg of canthaxanthin per 1 g of the whole *macula*, which accounts for the canthaxanthin concentration with respect to lipid in the range of a fraction to a few mol% [36].

Canthaxanthin localization and orientation in model unary lipid membranes as compared to other macular carotenoids

Polar carotenoid pigments usually span the lipid bilayer in such a way that their hydrophilic groups are anchored at the two opposite polar zones of the membrane [37-39]. The incorporation of carotenoids into membranes is governed by their polarity and stereometry [39, 53]. The localization and orientation of the xanthophylls within the lipid membrane are determined by the localization of the non-polar polyene chain, and by the localization of the hydrophilic groups, which remain in contact with the polar head groups of the lipid bilayer [40]. Of importance is the shape and orientation of the ionone rings and the stereometry of the functional groups that govern the carotenoid incorporation. The molecular structures of macular carotenoids are shown in Fig. 1. The latest published structures for lutein and zeaxanthin, based on crystallographic data, are given in [41], and for canthaxanthin in [42].

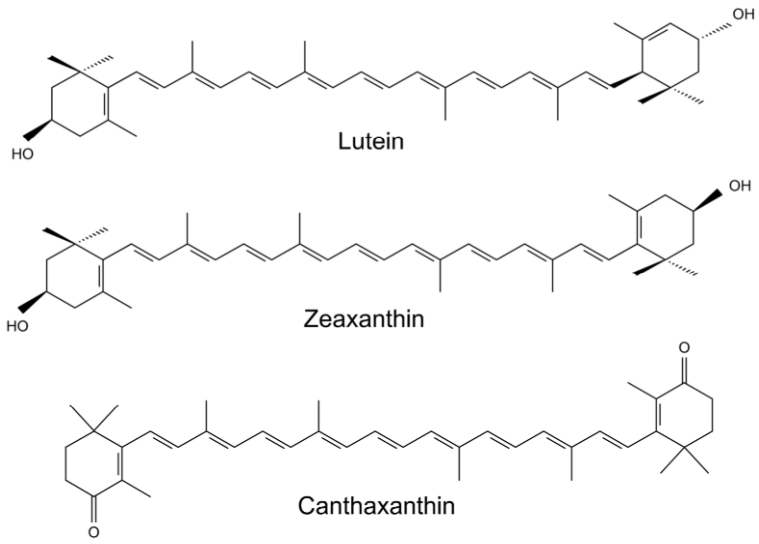

Fig. 1. The chemical structures of the macular carotenoids: lutein and zeaxanthin [41] (latest published) and canthaxanthin [42]. Note the differences in the end rings of lutein (β, ε) and zeaxanthin (β, β).

As can be seen in Fig. 1, lutein and zeaxanthin differ only by the presence of a double bond in the ionone ring in the position of C4'-C5' in the case of lutein and C5'-C6' in the case of zeaxanthin. The analysis of the position of the absorption spectra maximum of lutein in the lipid membrane clearly indicates that the lutein polyene chain (a chromophore) is localized within the hydrophobic core of the lipid membrane, similarly to zeaxanthin and canthaxanthin [39, 40, 43]. However, the results on the mean angle of the dipole transition moment of the macular pigment with respect to the axis normal to the plane of the membrane, received from linear dichroism measurements on oriented multibilayers, show that this angle is significantly bigger than in the case of zeaxanthin (for DPPC: zeaxanthin – 36 ± 4º, lutein – 57 ± 8º [40, 44]). Considering that the data received is the effect of averaging over the whole pool of the pigment, one possible explanation is based on the assumption that lutein is distributed among two differently oriented pools: one spanning the membrane similarly to zeaxanthin, and the other, albeit far smaller, oriented horizontally with respect to the surface of the lipid bilayer. The two hydroxyl groups in the lutein molecule are localized identically as in zeaxanthin, in positions C3 and C3'. On the other hand, these two molecules reveal the diverse construction of the end rings: both of the end rings in zeaxanthin are of the β-type, while in lutein, one is of the β-type and another of the ε-type. The natural consequence of this structure is the conjugation of the double bond of zeaxanthin both in the C5=C6 and C5'=C6' positions with the conjugated polyene chain, which results in the relative rigidifying of the end of the molecule with rings. In the case of

lutein, the double bond in the ε-ring in the position C4'=C5' is not conjugated with the polyene chain, which gives the potential ability to rotate the entire ε-ring around the C6'-C7' bond. Together with the data obtained on the different organization of lutein molecules in a monomolecular layer at the air-water interface (the lateral orientation of the lutein molecules even at relatively high surface pressures), this explanation allowed for a hypothesis about the horizontally oriented fraction of lutein molecules in lipid bilayers.

Another aspect to consider is the orientation of the dipole transition moment with respect to the molecular axis of lutein. This should differ significantly from zeaxanthin and canthaxanthin, which display a high symmetry. The difference with lutein should result from the asymmetry of the conjugated double bonds at the end of the molecule; much stronger effects would be expected (for a detailed discussion on the orientation of the dipole transition moment of polyenes, see [45]). This effect alone may determine the ostensible differences in the orientation of lutein and zeaxanthin with respect to the axis normal to the surface of the bilayer, without the necessity of introducing the two fractions oriented in quite a different way. Summing up, the large pool of lutein, which is of key significance for lipid membrane properties, is most probably localized and oriented similarly to the molecules of zeaxanthin, forming hydrogen bonds with the opposite polar zones of the bilayer.

Apart from the functional keto- group in the ionone ring of canthaxanthin, the shapes of the polyene chains of all these xanthophylls differ to such an extent that this can affect their binding to the lipid membrane and the formation of molecular aggregates of xanthophyll molecules. The ends of the β-rings of canthaxanthin have dihedral angles of 43º [42], making it less probable that they will form a card-pack molecular aggregate, and increasing the likelihood of the formation of different types of aggregates. It also yields the possibility of contact of one polar surface, as in the case of lutein. The orientation angles of canthaxanthin in the lipid phase depend on the actual concentration of the pigment with respect to the lipid. The mean angle between the dipole transition moment and the axis normal to the plane of the DPPC membrane was determined as 20º- at 0.5 mol% and 47º- at 2 mol% of canthaxanthin [40]. The dimension of the hydrophobic core of DPPC is slightly larger than the length of the macular xanthophyll pigments, which may imply their vertical orientation with respect to the plane of the membrane [40]. The angle of 20º confirms the vertical orientation of the axis connecting the opposite keto- groups of the xanthophyll at the 4 and 4' positions. The result of 47º suggests that canthaxanthin forms molecular structures characterized by chromophores tilted with respect to the axis normal to the plane of the membrane. This angle, similar to that found for lutein, implies the possibility that canthaxanthin incorporated into lipid membranes can be distributed in such a way that a small fraction of this carotenoid can be oriented parallel to the plane of the membrane.

MECHANISMS OF CANTHAXANTHIN DELIVERY TO THE *MACULA LUTEA* MEMBRANES

The eye has a rich blood supply and a relatively small mass, making it susceptible to drugs in systemic circulation, such as canthaxanthin. Structures within the eye with high blood perfusion rates, such as the choroids, retina and optic disc, are particularly vulnerable to the toxic effects of drugs [46, 47].

The exact mechanism of delivering canthaxanthin to the retina and its operation is still unclear. Some theories indicate the role of the cellular retinol-binding protein, others assume that lipids play a role [8]. Generally, all the dietary carotenoids are solubilised in micellar form. They are usually absorbed as a part of chylomicra, and they become constituents of low density and high density lipoproteins [48]. The question of the transfer of canthaxanthin in its unchanged form arose from the observation that in the human intestine, about half of the dietary carotenoids are absorbed intact. Fifteen different dietary carotenoids are detectable in the human serum, but only lutein, zeaxanthin and their metabolites are found in the retina. They are spatially localized mainly in the Henle fiber layer [49]. This tissue exhibits highly selective uptake of xanthophylls. It requires specific membrane-associated xanthophyll-binding proteins (XBP) involved in the uptake of these carotenoids from the bloodstream. It was proposed that XBP might behave like enzymes mediating the inter-conversion of lutein, zeaxanthin and *meso*-zeaxanthin and their metabolites. It was speculated that the dysfunction of this protein can result in canthaxanthin uptake. Although studies showed specific binding of lutein and zeaxanthin to XBP, so far no binding of canthaxanthin has been reported [50-52]. Tubulin has been found in the human and bovine retina, but its relatively low specificity and affinity to binding ocular carotenoids has been observed *in vitro*. The paclitaxel (Taxol®) binding site of beta-tubulin has been found to be responsible for binding carotenoids. Further studies on XBP proteins showed glutathione S-transferase (GSTP) as having a high affinity to bind zeaxanthin and ocular *meso*-zeaxanthin. It has also been found in human lenses. The lutein-binding protein (qlLBP) was found and purified from quail liver, but has not yet been described in detail [53].

As both the macular pigments and canthaxanthin dissolve well in lipids and are frequently delivered unchanged to ocular tissues, it has been suggested that there is a high probability that they may follow the same absorptive pathways as other dietary lipids [54, 55]. However, this does not explain why no other carotenoids are found in this tissue. Wisniewska *et al.* [56] put forward the hypothesis that some specific properties of ocular carotenoids may be responsible for their presence in primate retinas.

Experiments show that the solubility of canthaxanthin in lipids strongly depends on the length of the hydrophobic core of the lipid, the dimension of the polar head zone, and the presence of esther carbonyl groups [57]. It has been generally concluded that canthaxanthin demonstrates the highest solubility in lipids with

the dimension of the hydrophobic core comparable to the distance between the keto- groups of canthaxanthin.
On the other hand, macular carotenoids have poor aqueous solubility, so in the human *macula*, they are present either in the lipid membranes or are associated with proteins. Unlike lutein and zeaxanthin, canthaxanthin can easily dissolve in small concentrations in lipid/water or organic solvent/water mixtures. It can also easily form molecular aggregates under the same conditions.
Lipid bilayers have attracted much interest as idealized model systems for cellular membranes mainly because many of their physical properties are close to those of natural membranes [58]. Although it was shown that the incorporation efficiencies of lutein, zeaxanthin and canthaxanthin into different types of membranes are different and the conclusion drawn that neither liposomes nor microsomes may be considered model for studies of the incorporation of carotenoids into retinal pigment epithelial (RPE) cells [59], only model studies on defined membranes can give insight into the molecular interactions between these pigments and the membrane lipids.

CANTHAXANTHIN INTERACTION WITH MODEL LIPID MEMBRANES

It has been proposed that the toxicity of canthaxanthin towards lipid membranes is the result of the strong interaction between the pigment and lipid molecules, and of the formation of crystalline aggregates of canthaxanthin in the membranes even at very small concentrations of this pigment [40, 57]. This affects the physical properties of the retinal capillary walls, resulting in the destruction of the retinal vasculature and the further development of retinopathy.
Under experimental conditions, in the case of model DPPC lipid membranes, a canthaxanthin concentration below 1 mol% produces significant change in membrane properties. In some cases, the effects are seen at pigment concentrations as low as 0.05 mol% with respect to the lipid [57]. Based on the experimental data on phosphatidylocholines (mainly DPPC), several mechanisms of canthaxanthin interactions with the lipid membranes were listed.

The ordering effect of canthaxanthin on lipid acyl chains

The most pronounced effect of canthaxanthin with respect to lipids is the ordering of the lipid alkyl chains based, most probably, upon hydrophobic van der Waals interactions with the rigid carotenoid molecule containing a conjugated double-bond system (for carotenoids in general, see [37, 39]). The thickness of the hydrophobic core of the DPPC membranes containing canthaxanthin (calculated from the diffractometrically determined periodicity parameter of the lipid multibilayer) shows that it is slightly larger than the distance between the keto- groups of canthaxanthin. The reported distance between the canthaxanthin keto- groups is ~2.7 nm [42], and it has been experimentally determined that the hydrophobic core thickness of DPPC at 40ºC is 3.2 nm [40]. The observed increase in thickness of the hydrophobic core of

DPPC supplemented with canthaxanthin indicates that this could be the result of a strong interaction where the alkyl chains are forced to adopt an extended conformation [40, 44].

Additionally, the measurements of the isotherms of compression of canthaxanthin and DPPC show the effect of removing the *semi-plateau* in the registered isotherms even at relatively low surface pressures [60]. This represents the molecular interactions of alkyl lipid chains and the rigid polyene of canthaxanthin, leading to the ordering of the hydrocarbon lipid chains and the promotion of the vertical orientation of a certain fraction of canthaxanthin.

The analysis of the infra-red absorbance spectra of monolayers containing canthaxanthin indicated a condensing effect of canthaxanthin on the lipids. The elimination of the *end-gauche* and *double-gauche* conformations of lipid alkyl chains was observed [60].

The strong van der Waals interactions of canthaxanthin and the lipid alkyl chains were also concluded on the basis of the infra-red spectra (IR) of DPPC multibilayers containing 2 mol% of canthaxanthin [40]. Upon incorporation of the canthaxanthin into the membranes, the position of the band corresponding to the scissoring vibrations of the CH_2 groups of alkyl chains was shifted towards a lower wavenumber, and became narrower, which indicated an ordering effect of canthaxanthin with respect to the hydrocarbon core of the membrane. The IR measurements on a single DPPC monomolecular layer containing canthaxanthin (0.5 and 5 mol%) revealed the existence of an ordered lipid phase [60].

Modifying the properties of the lipid surface

Another effect of canthaxanthin on lipid membranes is the modification of their surface properties. An analysis of the small DPPC and EYPC (egg yolk phosphatidylcholine) liposome size distribution profiles showed that canthaxanthin affects the physical properties of the liposomes, resulting in vesicle aggregation. Canthaxanthin caused the immobilization of the C-O-P-O-C and PO_2^- groups, resulting in the aggregation of the liposomes [40].

^{1}H-NMR resonance experiments showed that canthaxanthin influenced the segmental molecular motion of DPPC lipid molecules both in the head-group region (the $N^+(CH_3)_3$ choline polar head-groups) and in the hydrophobic core of the bilayer (the CH_2 and CH_3 groups of the alkyl chains). The strongest immobilization of this part of the lipid molecules was observed at pigment concentrations between 1 and 1.5 mol% [40]. Such an effect may be an indication of pigment aggregation. Unfortunately, the change in the molecular organization of canthaxanthin cannot be seen directly via electronic absorption spectra [40] as with other macular xanthophylls [43]. In the case of canthaxanthin, the band representing the electronic transition between the ground energy level ($^1Ag^-$) and the Bu^+ state can be broadened upon the aggregation of this pigment [40].

The FTIR measurements on the DPPC multibilayers containing canthaxanthin showed a very strong interaction of canthaxanthin with the C-O-P-O-C region of

lipids that are close to the surface of the membrane. This region stays in close contact with the canthaxanthin ionone rings, or a certain fraction of the pigment may be located in its vicinity. In the case of the monolayer, the band representing the vibrations of the $-N^{+}(CH_3)_3$ choline group was insensitive to the presence of canthaxanthin. The spectral analysis indicated the possibility of hydrogen bonding to the phosphate groups. The immobilization of the PO_2^- group has also been observed in canthaxanthin multibilayers [40] and in a single monolayer [60].

Changes in the lipid membrane fluidity

Usually, carotenoids fluidise the membrane in its gel L_c and crystal $L_{\beta'}$ phases and rigidify it in its liquid crystalline L_α phase [37, 61-67]. The effects from polar carotenoids were found to be much greater than those from non-polar pigments [37, 68, 69]. The effect of canthaxanthin on the thermotropic properties of lipid membranes formed with different lipids was discussed in detail in [57]. Generally, with the addition of canthaxanthin, the DSC (Differential Scanning Calorimetry) peaks were broadened and less intensive, and the main phase transition temperature tended to shift towards lower values.

Like other macular xanthophylls, canthaxanthin changed the membrane thermotropic properties, but compared to lutein and zeaxanthin, the effect was much stronger. The 50% decrease in the maximal value of the membrane molar heat capacity of DPPC multilammellar vesicles required 1-2 mol% of lutein or zeaxanthin, while only 0.5 mol% of canthaxanthin produced the same effect [57].

The strongest influence of canthaxanthin on the main transition and pre-transition phases was observed on phosphocholines with the thinnest hydrophobic region: DMPC as compared with DPPC or DSPC [57]. In the case of DMPC, a strong decrease in the enthalpy of the main phase transition was observed at a canthaxanthin concentration as low as 0.05 mol%. For DPPC and DSPC, the effect was much weaker, but a distinct change in the enthalpy was observed at pigment concentrations between 0.1 and 0.2 mol%. The observed disappearance of the pre-transition peak of DMPC (and DPPC) indicated fluidisation of the $L_{\beta'}$ phase, as reported previously for other xanthophylls [68, 70].

The lack of ester carbonyl groups in DHPC resulted in a narrowing of the pre-transition component, especially for canthaxanthin concentrations as low as 0.05 mol%, which suggested an ordering effect of canthaxanthin. Adding canthaxanthin produced the shift of the main transition peak position towards higher temperatures (~3ºC at 0.1 mol% of canthaxanthin), which additionally accounted for the ordering effect of canthaxanthin on the lipid [57].

The decrease in the dimension of the polar head size in DMPE was less than that in DMPC, probably due to the tight packing of DMPE making it impossible for canthaxanthin molecules to penetrate the lipid hydrophobic core individually. A component analysis indicated a distinct cooperativity change, which colligated with the formation of new thermotropic phases with lower and higher phase transition temperatures compared to a single compound in the case of pure

lipids. The effect of canthaxanthin was almost negligible in the case of lipids without the ester carbonyl group, especially at low canthaxanthin concentrations. The phase separation of the membrane components may result from exposure to extreme environmental conditions [71]. One can come to the conclusion that it can also be the result of the existence of a membrane addition that interacts strongly with the lipid. It is of high probability that the presence of canthaxanthin within the membrane can determine the membrane behavior, including the membrane stability. Interactions leading to a destabilization of the membrane and the formation of additional phases can result in the loss of membrane compactness. In addition, in the case of the retinal vasculature, it can lead to an increase in the permeability of the retinal capillary walls and the development of retinopathy.

Interactions by means of hydrogen bonds

Based on experimental data on canthaxanthin dissolved in organic solvents, it was shown that the behaviour of this pigment strongly depends on the ability of the pigment molecules to form hydrogen bonds with the mediation of the canthaxanthin keto- groups located at the 4 and 4' positions either directly or indirectly via water molecules [40]. FTIR spectrum analysis of DPPC membranes containing 2 mol% of canthaxanthin confirmed that these bonds can be created directly with the lipid ester carbonyl group or indirectly via water bridges using the keto- groups of canthaxanthin [40, 60].

The experiments on β-carotene and on zeaxanthin show that the hydrophobic polyene of a carotenoid is able to carry water molecules bound by weak hydrogen bonds in which the water oxygen atom acts as a proton acceptor. It is also possible that water molecules can be bound to the polyene by weak hydrogen bonds with the π-conjugated double-bond system [72, 73]. The FTIR absorption spectrum analysis indicated that water molecules bound to the polyene chain may serve as a channel to the hydronium ion carrying an extra proton, which can migrate along the carotenoid molecule. In small concentrations, canthaxanthin spans the lipid membrane, which can be important for facilitating transfer of the proton across the membrane. Such a role has not yet been proven for canthaxanthin. Under physiological conditions, carotenoid pigments do not usually exist directly in the lipid phase, but considering the strong interaction of canthaxanthin with the lipid membranes, one can postulate their strict appearance in the lipid phase.

CONCLUSIONS

Canthaxanthin toxicity towards the *macula lutea* lipid membranes could be due to its very strong molecular interactions with the lipid molecules. The effects of canthaxanthin at a molecular level are observed at much lower concentrations of the pigment in the lipid phase (below 1 mol%; in some cases as low as 0.05 mol%) than those of other xanthophylls such as lutein or zeaxanthin. The action of

canthaxanthin in the membranes is accomplished by the following molecular mechanisms:

1. Aggregation of canthaxanthin molecules in the lipid phase;
2. Strong van der Waals interactions between the polyene chain of canthaxanthin and the lipid acyl chains;
3. Modifications of the lipid properties in the polar head zone;
4. The introduction of new thermotropic phases in the lipids upon the incorporation of canthaxanthin;
5. The formation of hydrogen bonds between the canthaxanthin keto-groups and the C=O group of the lipid or hydrogen bonds between the polyene chain and water.

This last mechanism may have a crucial significance in the formation of the molecular aggregates of canthaxanthin that lead to further development of canthaxanthin retinopathy.

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