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Incorporation of carotenoid esters into liposomes

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Abstract

Carotenoid esters are investigated for their interaction with liposomal membranes and compared with their corresponding free (non-esterified) carotenoids. A monoester (β-cryptoxanthin) and two diesters (zeaxanthin and lutein) were chosen. Egg yolk phosphatidylcholine liposomes served as the membrane model. We measured the sizes of the liposomes by photon correlation spectroscopy. The incorporation yields were determined spectrophotometrically. From liposomes simultaneously doped with the fluorescent dye Laurdan, fluidity changes of the liposomes were obtained.

In summary, the results indicate that the carotenoid esters: (i) get incorporated, but at a lower yield than their corresponding free carotenoids, (ii) also increase the membrane rigidity as do the free carotenoids, and (iii) increase the liposome sizes significantly, but after extrusion through an 0.1 μm filter the sizes resemble with the exception of the liposomes incorporated with lutein diesters, they remain bigger indicating an elastic property due to two different accessible locations in the membrane.

Keywords: Carotenoid esters; Incorporation yields; Liposomal sizes; Laurdan labelling

1. Introduction

Carotenoids are lipophilic pigments widespread in bacteria, plant and animal tissues [1]. Among 600 naturally occurring carotenoids, about 50 are found in the nutritional chain. Carotenoids are biosynthesized in plants and micro-organisms and are involved in photosynthesis and photo-protection. The human and animal diet provides a wide range of carotenoids, hydrocarbons, xanthophylls and other derivatives, but only around 20 of them are present in plasma [2]. The plant xanthophylls are present in free form or as acyl ester; usually the hydroxyl group being esterified with medium chain length saturated fatty acids [1,3]. The carotenoid profile in plasma is strongly influenced by the food composition, structural features of the carotenoids and also by membrane properties [4].

Biological functions of carotenoids in human and animals have been largely investigated and reviewed [5,6]. Experimental and epidemiological studies demonstrated the beneficial effect of carotenoids in preventing some types of cancer, cardiovascular and degenerative diseases (i.e. cataract, age related macular degeneration) [7–11]. Most of these effects are considered to be a consequence of antioxidant properties of carotenoids, which can act as quenchers of reactive oxygen species [6,12–14].

The involvement of carotenoids at cellular level is strictly related to their interaction with biological mem-

Abbreviations: AcCN, Acetonitrile; BCR, β-cryptoxanthin; EBCR, β-cryptoxanthin esters; BHT, Di-*t*-buthyl-*p*-cresol; CHL, Chloroform; DCM, Dichloromethane; DMF, Dimethylformamide; DPPC, 1,2-Dipalmitoyl-*sn*-glycero-3-phosphorylcholine; EA, Ethyl acetate; EC, Effective concentration; EE, diethyl ether; ELUT, Lutein esters; EP, Petroleum ether; EZEA, Zeaxanthin esters; EYPC, Egg yolk phosphatidylcholine; H, Hexane; HPLC, High Performance Liquid Chromatography; IC, Initial concentration; IY, Incorporation yield; Laurdan, 6-dodecanoyl-2-dimethylaminonaphtalene; LUT, Lutein; MeOH, Methanol; MLV, multilamellar vesicles; PL, Phospholipid; SUV, Small unilamellar vesicles; ZEA, Zeaxanthin.

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branes. A cohort of studies was done in order to elucidate the location and the distribution of carotenoids in biological membranes and their influence on membrane properties [17–21]. The most important dietary carotenoids were investigated hydrocarbons (β-carotene, lycopene) and xanthophylls (lutein, zeaxanthin, canthaxanthin, astaxanthin) in both natural and artificial membranes [22,23]. Some specific interactions which depend on specific carotenoid structures were noticed. Zeaxanthin, a planar dihydroxy xanthophyll, resides mainly perpendicular to the plane of membrane and has a rigidifying effect on a DPPC membrane [24] while lutein may adopt two different orientations, one perpendicular, as zeaxanthin, and one parallel to the membrane. Xanthophylls seem to interact by both hydroxyl end groups with the polar sides and the aqueous environment of the membrane [25]. Our previous studies showed that carotenoid incorporation into liposomes might be governed not only by carotenoid polarity but also by their ability to change membrane anisotropy [22,26].

Carotenoid esters are major components of some vegetables and fruits, good sources of esters are red pepper (*Capsicum annuum*), papaya (*Carica papaya*) and loquat (*Eriobotrya japonica*) [27]. Lutein esters from *Tagetes erecta* are added to poultry feed, to improve skin and egg yolk pigmentation [28] and are used as eye-protecting medicine [29]. Factors which influence the bioavailability of xanthophylls were recently reviewed [30,31]. Four major events are modulating the absorption of xanthophylls: release from food matrix, transfer to lipid micelles, and uptake by intestinal mucosal cells by passive diffusion across membranes, and transport to the lymph system. Lutein esters seem to be hydrolyzed before absorption but small amounts of lutein esters were found in blood [32] and in skin [33] after a long term dietary supplementation with a mixture of lutein esters. There are few data regarding carotenoid ester metabolism and the specific enzymes involved in their hydrolysis are not yet known. Recent investigations [34] showed that porcine pancreatic lipase and cholesterol esterase can hydrolyze carotenoid esters, but human pancreatic lipase accepts only retinol esters as substrates. It was demonstrated that lutein esters are absorbed in the blood stream and the esterification of lutein does not impair lutein bioavailability [35]. It was shown that lutein myristate esters were more stable than free lutein against heat and UV-light [15] and the esterification of lutein with fatty acids does not affect their antioxidant properties [16].

Few investigations on incorporations of xanthophyll derivatives into liposomes were done. Studying the effects of thermozeaxanthin (a zeaxanthin–glucoside–ester) Hara et al. [36] showed that the incorporation of thermozeaxanthin (up to 1 mol%) into EYPC LUV (large unilamellar vesicles) stabilized the liposomes. The effect of thermozeaxanthin incorporation on membrane properties was proved to be influenced by the length of the fatty acid chains of phosphatidylcholine. LUV composed of dimirystoylphosphatidylcholine were not stabilized and lost the fluorescent dye. The solubility of lutein and lutein esters was investigated in food grade non-ionic microemulsions. Both free and esterified lutein showed a better solubility in reverse micellar and water/oil composition with a maximum within the bicontinuous phase [37].

The aim of the present study was to extend our previous researches on carotenoid acyl esters, to evaluate their ability to be incorporated and to investigate how they modulate physical liposome properties. Three types of naturally occurring carotenoid esters were used: β-cryptoxanthin esters, zeaxanthin esters and lutein esters — Fig. 1. These compounds are widely found in human and animal food.

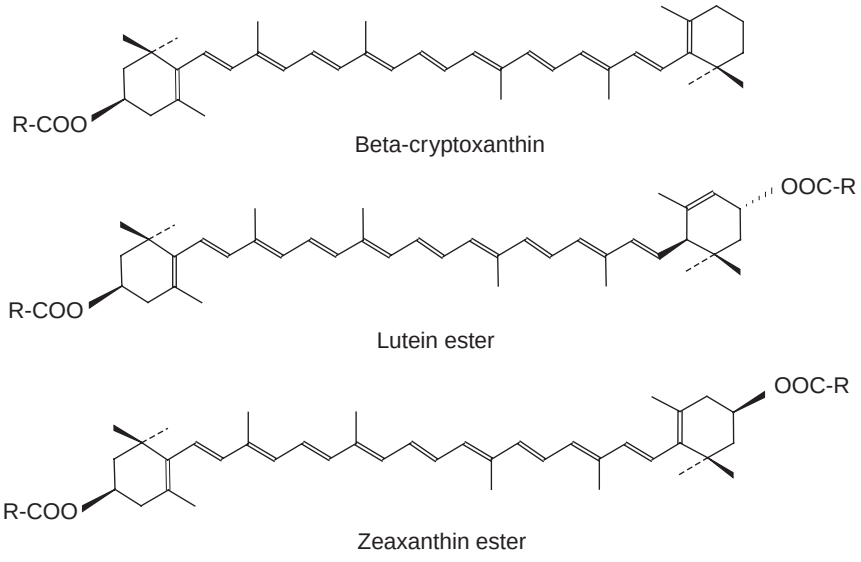

Fig. 1. Structures and abbreviations of xanthophyll esters used in this study: β-cryptoxanthin esters (EBCR), Zeaxanthin esters (EZEA), Lutein esters (ELUT).

2. Experimental

2.1. Chemicals

Lutein (LUT) and lutein esters (ELUT) were purified from *T. erecta* flowers. Free β-Cryptoxanthin (BCR) and Zeaxanthin (ZEA) and their esters (EBCR, EZEA) were purified from *Physalis alkekengi*. The β-cryptoxanthin ester fraction contains 95% β-cryptoxanthin monopalmitate and other esters (myristate and stearate). The EZEA fraction contains 95% dipalmitate and other esters (myristoyl–palmitoyl, palmitoyl–stearoyl and distearoyl). The ELUT fraction is a more complex mixture of lutein diesters with saturated fatty acids of middle chain length. The main esters were: dimyristoyl lutein — 17.9%, myristoyl–palmitoyl lutein — 29.4%, dipalmitoyl lutein — 31.3%, palmitoyl–stearoyl lutein — 14%. Other esters were present up to 100%. The identification of all samples was made by LC-MS and the purity of all compounds was checked by HPLC as described before [44].

Egg yolk phosphatidylcholine (more than 99% purity) were purchased from Lipoid KG (Ludwigshafen, Germany).

The fluorescent probe used in the experiments was 6-dodecanoyl-2-dimethylamino-naphtalene (Laurdan), purchased from Molecular probes (Eugene, OR, USA).

Di-*t*-buthyl-*p*-cresol (BHT), dichloromethane (DCM), methanol (MeOH), chloroform (CHL), ethanol (EtOH), and ammonium ferrothiocyanate were from Sigma (Deisenhofen, Germany). Hexanes (H), petroleum ether (EP), diethyl ether (EE), ethyl acetate (EA) were from Fluka (Deisenhofen, Germany). Dimethylformamide (DMF) was obtained from ACROS (Geel, Belgium) and acetonitrile (AcCN) from Riedel (Seelze, Germany). All chemicals were of research grade or HPLC grade. The solutions were prepared in deionized ultra pure water.

2.2. Preparation of liposomes

Control liposomes: The liposomes were prepared following classical methods [38,39]. The EYPC (50 mg) was dissolved in 15 ml DCM/MeOH (2:1, v/v) and evaporated in the Rotavapor (vacuum system). The film was dried under vacuum (Vacutherm, Heraeus Instr., Hannover, Germany) for 24 h at 45 °C.

Sample liposomes were prepared from EYPC as before, but dissolved together with a corresponding amount of each carotenoid: 0.374 mg LUT, 0.374 mg ZEA, 0.364 mg BCR, 0.682 mg ELUT, 0.689 mg EZEA, 0.521 mg EBCR, previously dissolved in 5 ml DCM. The amounts of carotenoids were calculated using their specific absorption coefficient, in order to obtain a final concentration ratio of 1 mol% carotenoid to lipid. In the case of free carotenoids, tabulated absorption coefficients $A_{1\ cm}^{1\%}$ were used [1]. To obtain the specific absorption coefficients for carotenoid esters the specific absorption of the parent carotenoid was multiplied by the ratio of the molecular mass of the parent carotenoid to the weighted average molecular mass of the corresponding carotenoid ester. The specific ester absorption coefficients appear to be 1719 for EBCR, 1277 for EZEA and 1451 for ELUT. The solutions were evaporated and the films were dried under the same conditions.

In all cases, the dried film was vortexed for 10 min at 40 °C with 10 ml phosphate buffer (0.1 mM, pH 7.4), giving a heterogeneous suspension of multilamellar vesicles (MLVs). Then the MLVs were sonicated using the Bandelin sonoplus HD70 sonicator (Bandelin Electronics, Germany) at maximal power of 70–80% (cycle 30%) at 40 °C, under nitrogen, for 15 min.

The suspensions of unilamellar vesicles obtained after sonication were centrifuged at 45 000 rot/min (105 000 $\times g$) in an ultracentrifuge. Aggregated lipids and carotenoids, forming an upper thin layer, were removed. The suspensions containing mainly small unilamellar vesicles (SUVs) were extruded using the Thermostat Membrane Extrusion Equipment of Lipex Biomembranes Inc. (Vancouver, Canada) through polycarbonate filters of 0.4, 0.2, and 0.1 μm.

The carotenoid concentration into SUV, before (IC) and after (EC) incorporation, was determined from the absorption spectra recorded on a Shimadzu UV-2102 PC Scanning Spectrophotometer. The incorporation yield (IY) was calculated as the ratio between EC and IC. The phospholipid concentration of each type of liposomes was determined by the Steward method [40].

2.3. Determination of vesicle sizes

The size distribution of particles usually is determined by light scattering methods. For optically turbid solutions like liposomes, photon correlation spectroscopy is the method of choice. To avoid the disturbing effects of multiply scattered light the three-dimensional cross-correlation technique has been developed theoretically and experimentally and has been applied here [41]. This way we investigated the liposomal solutions in their original state.

2.4. Steady-state fluorescent measurements

A stock solution of 0.1 mM Laurdan in DMF was prepared and 68.4 μl of this solution was added to 60.4 μl SUV suspension containing 0.05 mg phospholipid/ml, in order to obtain a lipid/probe ratio of 500, and diluted to 5 ml with phosphate buffer. The mixture was incubated for 1 h at 25 °C, under magnetic stirring. The emission spectra were recorded between 375 and 600 nm at λ_{exc}=350 nm. The excitation spectra were recorded between 300 and 420 nm at λ_{obs}=440 nm. For β-cryptoxanthin ester the fluorescence measurements were recorded also at various temperatures.

All fluorescence measurements were performed with a computer controlled Perkin Elmer LS-50 Luminescence Spectrometer equipped with a thermostatic cuvette (Julabo Labortechnik, Seelbach, Germany). The scan speed was 400 nm/min and the slits were fixed at 5 nm.

3. Results and discussion

3.1. Determination of liposome sizes

We determined the size of the liposomes prepared from EYPC, and of those incorporated with free or esterified xanthophylls. The measurements were made both, on sonicated liposomes and on ultra-centrifuged extruded vesicles. Table 1 presents the mean size of the liposomes. The sonicated vesicles are larger than the corresponding control vesicles. The difference is highly significant for the liposomes containing esters or free lutein.

It is observed that always the extruded liposomes are of lower dimension than the sonicated ones. The dimension of the carotenoid doped liposomes after extrusion is close to that of the corresponding control liposomes without carotenoids, except the lutein diesters sample, which has a larger diameter. It is evident that the extrusion procedure uniformed the vesicle sizes.

Among the extruded liposomes those containing lutein diesters and lutein have the largest diameter of the esterified and the non-esterified carotenoids, respectively. An explanation may be that lutein esters and lutein can adopt two different locations in the membrane, one perpendicular and one more parallel to the membrane plane. The simultaneous existence of the two locations of lutein in the membrane has been postulated [24,46]. It has been shown that also DPH is localized in two populations in lipid vesicles, mainly parallel to the membrane plane [47]. β-carotene is only located in the core of the membrane parallel to its plane while Zeaxanthin because of its stereometry and its polar headgroups intercalates well into the membrane structure being only oriented normal to the membrane plane [48]. Lutein is discriminated from zeaxanthin by a different position of the double bond in one of its rings. This lets the ring rotate and tilts it out of the molecule axis. For those reasons lutein fits less well into the membrane perpendicular location and it has been proposed that simultaneously a second location is possible where the polar headgroups of lutein attach to headgroups of the same membrane-bilayer while the polyenic chain of lutein is oriented to the membrane core [24]. The equilibrium between both locations will be subjected to external forces. The extrusion stress can transfer lutein molecules from the membrane perpendicular to the parallel location because the density in the membrane core is lower than in the head group region. After the extrusion, lutein molecules relax into the perpendicular position and the vesicle increases again its size. The other carotenoids not having two equilibrated membrane locations will not be able to undergo such an elastic effect. This can explain why the incorporation yield of the zeaxanthin diesters in the extruded liposomes is much lower than that of the lutein diesters (Table 2). Zeaxanthin diesters will be squeezed out because only this way the liposome can pass the extrusion stress. After the extrusion the liposome cannot re-incorporate the ester. Formerly we have shown already that liposomes have a low ability to incorporate carotenoids from a solution [26]. Therefore the liposomal sizes and the incorporation yields of the esters of lutein and zeaxanthin are correlated.

Table 1
Sizes of EYPC liposomes containing free and esterified xanthophylls (1 mol%)

Sample	Sonicated liposomes (nm)	0.1 μm extruded liposomes (nm)
β-cryptoxanthin	197	98
Lutein	292	108
Zeaxanthin	195	101
β-cryptoxanthin esters	257	111
Lutein diesters	340	144
Zeaxanthin diesters	343	105
Control	187	105

The measurements have been made after sonication — column 2, and then after centrifugation and extrusion — column 3; S.D. = ±10 nm.

Table 2
Incorporation yields (IY) of carotenoids (1 mol%) in extruded SUV liposomes

Sample	IC (nmol/ml)	EC (nmol/ml)	IY
β-cryptoxanthin	65.8	25.1 ± 1.2	0.38 ± 0.02
Lutein	66.0	24.0 ± 1.2	0.36 ± 0.02
Zeaxanthin	65.9	23.1 ± 1.2	0.35 ± 0.02
β-cryptoxanthin esters	66.0	5.3 ± 0.3	0.08 ± 0.01
Lutein diesters	65.9	7.2 ± 0.4	0.11 ± 0.01
Zeaxanthin diesters	65.9	1.3 ± 0.1	0.02 ± 0.01

3.2. Incorporation yields of carotenoids into liposomes

Fig. 2a, b, c present the absorption spectra of the liposomes containing carotenoid esters and the spectra of carotenoid esters dissolved in hexane. In all cases the absorption spectra of the suspensions show a bathocromic shift of 4 nm compared to the ester spectra in hexane. Moreover, the fine structure of the spectra of the carotenoid esters in liposomes is less evident than of those in hexane. The more polar environment of carotenoid esters in liposomes causes both these effects. Previously we observed the same effect for free zeaxanthin and lutein and for β-carotene in liposomes as well. The esterified ZEA and LUT show the same absorption spectra as the non-esterified ones, and the bathochromic shifts are observed as well [26].

The absorption spectra were used for the calculation of the IC, EC and IY values of the carotenoids into SUV. The carotenoids were extracted in hexane from a defined volume of liposomal suspension and evaluated spectrophotometrically.

Table 2 contains the mean values of IC, EC and IY for free and esterified xanthophylls in SUV. The IC for all was 66 nmol/ml suspension, which corresponds to 1 mol% carotenoids/PL. The determination was repeated on samples

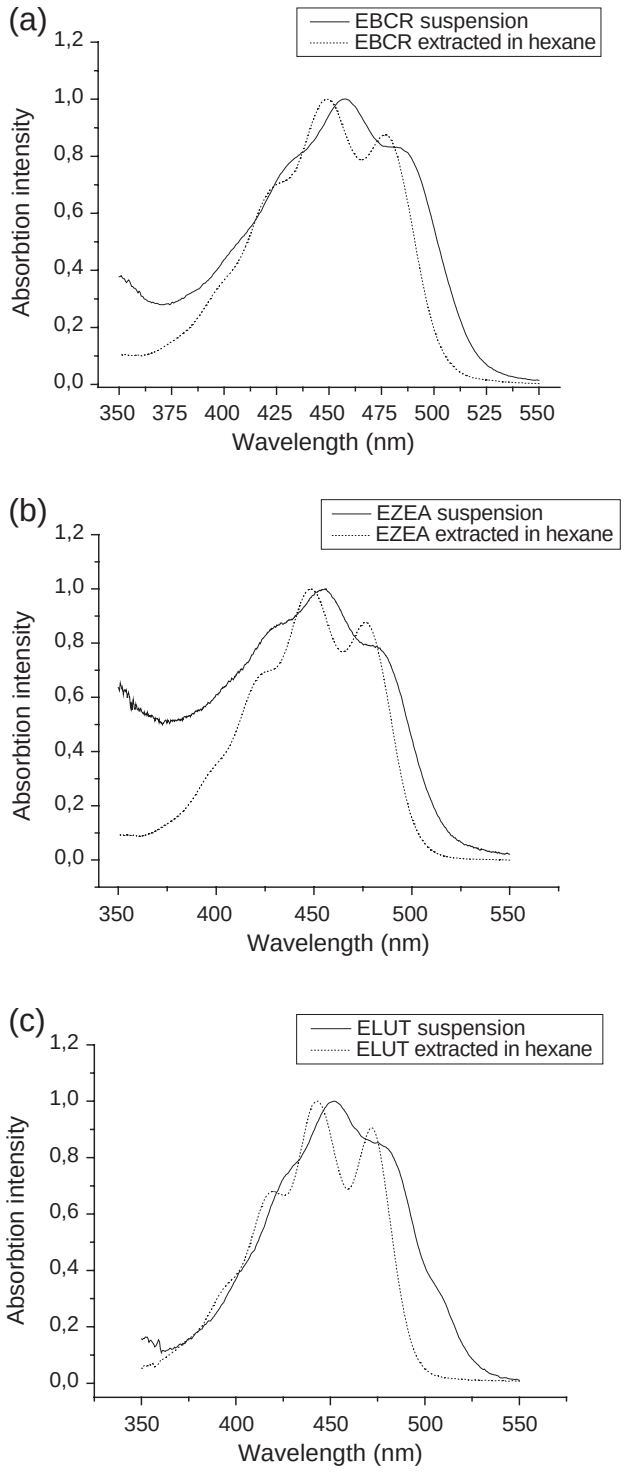

Fig. 2. Normalized absorption spectra of xanthophyll esters in hexane and in liposome suspension (1 mol%), (a) β-cryptoxanthin esters, (b) Zeaxanthin esters, (c) Lutein esters.

containing 2 mol% esters, which did not change significantly the IY. It indicates that we are still below the saturation limit. The incorporation yield of phospholipids into control liposomes was about 82%.

It is observed that the IYs of the three free xanthophylls have about the same value and that they are comparable with previous data obtained for SUV [26]. The IY of the esters are much lower than those of their corresponding free carotenoids (Table 2). They are close to the IY of β-carotene (0.1), as previously determined in SUV [26]. This cannot be explained by the high lipophilicity of carotenoid esters, which is more similar to hydrocarbons than to xanthophylls, because high lipophilicity should cause just a high and not a low incorporation yield.

The incorporation results correlate with the size measurements of liposomes. Low incorporation yields correspond to large molecules. The large size of the ester molecules hinders them to enter well into SUVs. Obviously the low incorporation yield of the carotenoid esters is ester-bound and the inter-ester specificity may not only be determined sterically but also by the type of fatty acids of which the esters consist mainly. Zeaxanthin diesters consist mainly of dipalmitate, β-cryptoxanthin ester of monopalmitate and lutein diesters of shorter fatty acids. According to this sequence, the fatty acid fluidity increases and the corresponding carotenoid esters fit easier into the membrane.

3.3. Fluorescence spectra of the liposomes

Fig. 3 shows the emission spectra of Laurdan incorporated into liposomes which contain free xanthophylls (a) and xanthophyll esters (b). The quenching effect plotted has been normalized to identical virtual concentrations. β-cryptoxanthin and β-cryptoxanthin esters were plotted as measured. According to the effective concentrations given in Table 2, for zeaxanthin diesters the measured difference to control was multiplied by the factor of 4.02 and for lutein diesters by 0.73 — Fig. 3b. This normalization implies a linear relation between carotenoid concentration and Laurdan fluorescence quenching and may not be fulfilled exactly. But for a qualitative evaluation it will be appropriate. In Fig. 3a a corresponding processing was performed, the factors for the plotting of zeaxanthin and lutein were 1.04 and 1.09, respectively.

The incorporation of carotenoids causes a strong quenching of the Laurdan emission which is most effective in liposomes containing free xanthophylls and less in liposomes which contain esters. The Laurdan emission quenching in the liposomes roughly corresponds with their carotenoid concentration. The reason for the quenching very probably is due to a resonance energy transfer from the excited Laurdan (donor) to the carotenoid (acceptor). The strong overlap of the Laurdan emission spectra (Fig. 3) and the absorption spectra of the carotenoids (Fig. 2) indicate a resonance energy transfer process.

Another effect onto the Laurdan fluorescence emission spectra, independent from the quenching effect, is the spectral shift within the double-peaked Laurdan emission (Fig. 3). The dual fluorescence spectra of Laurdan indicate the existence of two emissive states. Molecular physicists design them the locally excited (LE) state and the charge

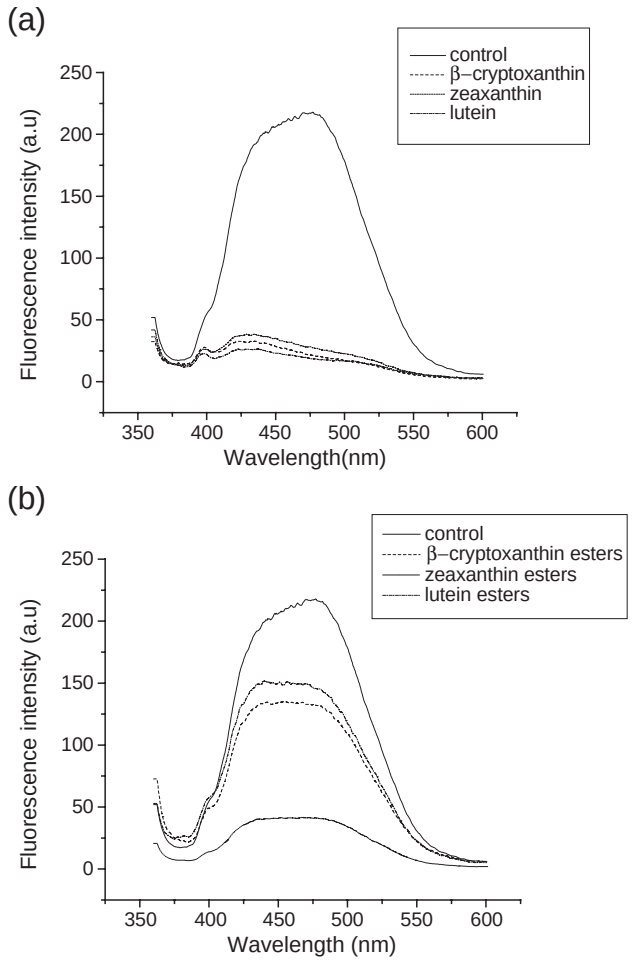

Fig. 3. Normalized emission spectra of Laurdan incorporated into liposomes (1 mol%) (λ_{exc}=350 nm, 25 °C). (a) Liposomes with free carotenoids, (b) liposomes with xanthophyll esters.

transfer (CT) state, respectively, [43]. The S_1(CT) state is created during the lifetime of the excited S_1(LE) state due to an intermolecular charge transfer between the twisted dimethylamino functional group and the naphthalene moiety. Conjugated molecules, which possess electron-releasing and electron-withdrawing functional groups, on excitation undergo a large change of their dipole moment. Therefore the fluorescence properties of such molecules are very sensitive to the polarity of the solvent and Laurdan provides a suitable probe to study its adjacent membrane polarity. The two emission peaks at 436 and 478 nm represent the emissions from the S_1(LE) state and the S_1(CT) state, respectively. Any viscosity decreases, be it by temperature enhancement in a glycerol solution of Laurdan [43], or by extrinsic effects on Laurdan in membranes, allow a higher rate of charge transfer and thus an enhancement of the long wavelength emission intensity on expenditure of the short wavelength emission intensity. (It is a shift of the emission ratio within a bimodular fluorescence emission and not a peak wavelength shift.)

The temperature effect onto Laurdan in liposomes we demonstrated by plotting the Laurdan emission spectra from control liposomes (Fig. 4a) and from liposomes incorporated with 2 mol% of β-cryptoxanthin ester (Fig. 4b), each at four temperatures from 10 to 50 °C. In all preparations the long wavelength emission intensity enhances with temperature. At identical temperatures the intensity ratio of the Laurdan short wavelength/long wavelength emission is always higher from the ester containing liposomes than from the control liposomes indicating a membrane rigidisation by the incorporation of the esters into the liposome.

According to Parasassi and Gratton [42] the bimodal spectral emission of Laurdan is empirically quantified as the "Emission Generalized Polarization" $GP_{em}=(I_{436}-I_{478})/(I_{436}+I_{478})$, where I_{436} and I_{478} are the Laurdan emission intensities at 436 and 478 nm, respectively, and the excitation wavelength is 350 nm. The GP_{em} values have been calculated from Fig. 3 and plotted in Table 3. The lower GP_{em} values correspond to a more fluid membrane state which allows intruded water molecules to have a dipolar relaxation rate in the order of magnitude of the Laurdan reciprocal fluorescence life time leading to the

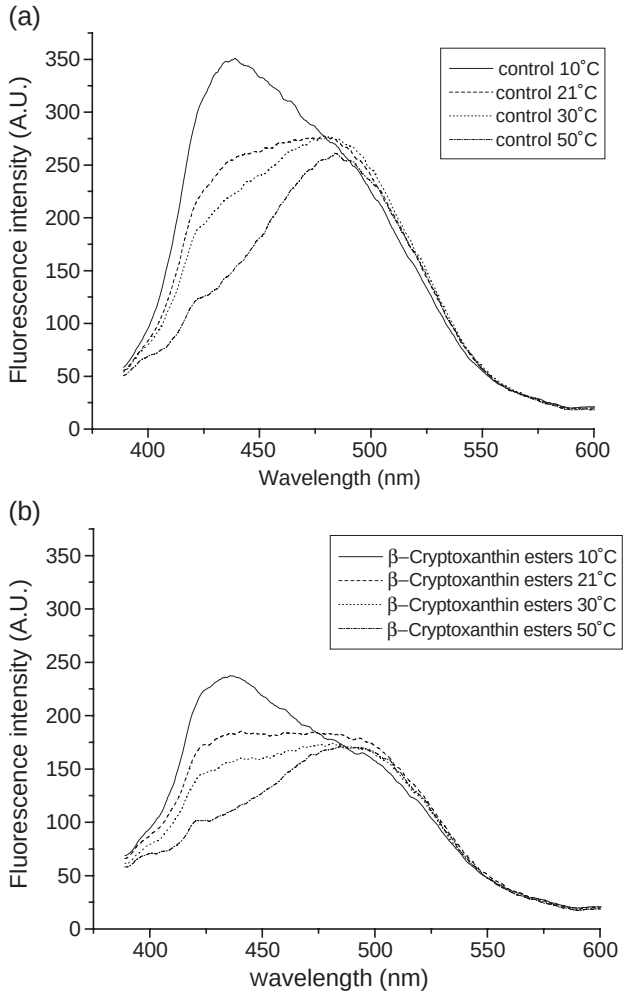

Fig. 4. Emission spectra at different temperatures of Laurdan incorporated into liposomes (a), and (b) at co-incubation with β-cryptoxanthin esters (2 mole %).

Table 3
The GP(em) values for liposomes containing free and esterified carotenoids (1 mol%) and Laurdan, S.D.=±0.010

Sample	$GP_{em}(I_{436}-I_{478}/I_{436}+I_{478})$
β-cryptoxanthin	+0.220
Lutein	+0.176
Zeaxanthin	+0.163
β-cryptoxanthin esters	+0.003
Lutein esters	+0.013
Zeaxanthin esters	−0.011
Control	−0.007

increase of the long wavelength emission at 478 nm, while higher GP_{em} values indicate a more rigid membrane state where the water dipolar relaxation rate is restricted and does not match with the Laurdan reciprocal fluorescence lifetime. Following the interpretation that water intrusion into the membrane, enhancing the Laurdan long wavelength emission, is correlated with increasing membrane fluidity, and, vice versa, the short wavelength emission to be correlated with more rigid membranes, we conclude from Table 3 that the carotenoid esters at the incorporation level of only 1% cause a very low or no membrane rigidisation, whereas the free carotenoids cause a strong one as is known already [21,24,26]. The GP_{em} values from the liposomes containing the carotenoid esters in a concentration of only 1% are not significantly different from their control values. This effect can be expected from the incorporation rates (Table 2). At the level of 2 mol% β-cryptoxanthin ester incorporation (Fig. 3), a tendency to membrane rigidisation can be seen.

4. Conclusions

We have shown that xanthophyll esters can be incorporated into liposomes. Efficiencies are lower than those of the corresponding free xanthophylls. But the quality of physical effects onto liposomes is the same.

Xanthophyll esters increase the liposomal sizes considerably. The biggest size enhancers, by nearly 100%, are the biggest esters used: zeaxanthin and lutein. β-cryptoxanthin monoester increases the vesicle size by only about 50%. Among the free carotenoids the sterically most bulky one, lutein, increases the liposome size by about 60% and the other free carotenoids, β-cryptoxanthin and zeaxanthin, not significantly at all. The pattern of incorporation yields is nearly a mirror image of the sizes. The exceptional features of free and esterified lutein to show a size elastic effect against the extrusion procedure confirm their ability to adopt two different locations in the membrane. Lutein esters seem to be more adaptable to membranes than the other esters. Because of their mainly short chain fatty acids and the ability of lutein to locate parallel as well as perpendicular to the membrane plane, it provides a particular elasticity to the positioning of lutein esters.

The xanthophyll esters have the same absorption characteristics like the corresponding free carotenoids. The fluorescence emission of the membrane label Laurdan is quenched by the xanthophylls and their esters. The quenching efficiency correlates with the concentration of the incorporated pigment and can even be used to determine it. We find this feature relevant in cases where a carotenoid concentration is to be determined from the membranes of living cells. We are applying this method on the quantification of some carotenoids from the membranes of retinal pigment epithelial cells [45]. Alternatively the cell membranes must be dissolved and the carotenoids must be extracted.

The Laurdan specific bimodal fluorescence emission reveals that the incorporation of xanthophylls at concentrations of about 24 nmol/ml clearly rigidisizes the liposomes. The esters cannot be incorporated to such an extent because of their low incorporation yields. A β-cryptoxanthin ester incorporation of about 10 nmol/ml reveals a membrane rigidisation effect against control as does a temperature decrease.

In summary we conclude that xanthophyll esters can enter and pass membranes, especially lutein ester which among the esters investigated is incorporated with the highest yield. To whatever degree xanthophylls in the blood stream may be esterified one can expect that they also in a direct manner can substitute free xanthophylls.

Acknowledgements

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