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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.

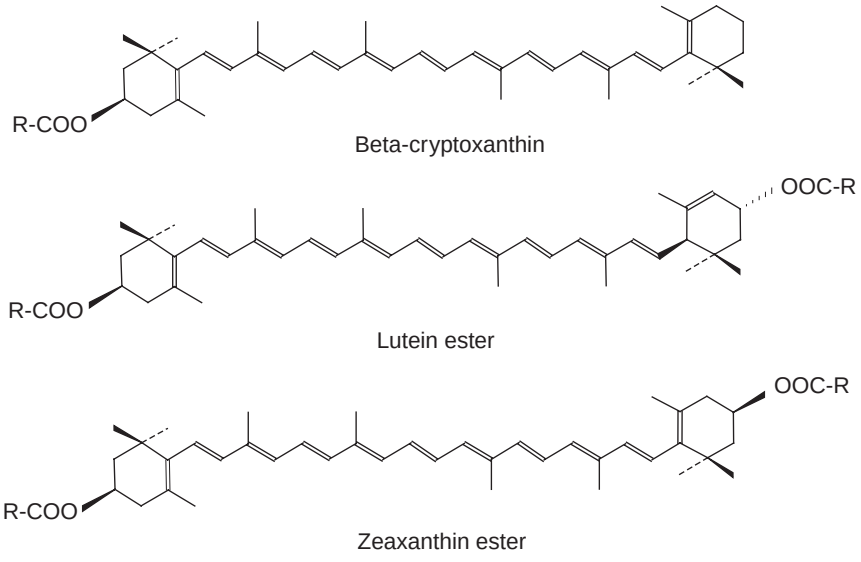

Fig. 1. Structures and abbreviations of xanthophyll esters used in this study: β-cryptoxanthin esters (EBCR), Zeaxanthin esters (EZEA), Lutein esters (ELUT).