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GALACTOLIPIDS AND PHOSPHOLIPIDS OF ORANGE PEEL AND JUICE CHROMOPLASTS

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Key Word Index—*Citrus sinensis*; Rutaceae; orange; chromoplasts; chloroplasts; galactolipids; phospholipids.

Abstract—Chromoplasts from yellow orange (*Citrus sinensis*) fruit peel contain monogalactosyl diglycerides, (MGDG), digalactosyl diglycerides (DGDG) and phosphatidyl glycerol (PG) in amounts similar to those found in chloroplasts from green fruit peel. Juice chromoplasts contain relatively little MGDG and no DGDG with high levels of phosphatidyl choline and phosphatidyl ethanolamine but no PG.

INTRODUCTION

Chromoplasts are found within mature citrus fruits in the flavedo (the outer, colored layer of the peel) and in the juice. Although both chromoplast types are rich in carotenoid pigments, their history is different. Flavedo chromoplasts originate from green photosynthetically active chloroplasts, whereas juice chromoplasts develop from the pigment-less plastids of juice vesicles. The purpose of the present study was to determine whether the different developmental origin of the two kinds of chromplasts is reflected in their lipid composition.

RESULTS AND DISCUSSION

Table 1 shows the important differences between flavedo and juice chromoplasts and, on the other hand, the great similarity between flavedo chromoplasts and chloroplasts. Flavedo chromoplasts contain monogalactosyl diglycerides, (MGDG), digalactosyl diglycerides (DGDG) and phosphatidyl glycerol (PG) in amounts similar to those found in mature green flavedo chloroplasts. Juice chromoplasts contain some MGDG, little or no DGDG and no PG. Juice chromoplasts extracts revealed an additional major spot which moved about 0.1 R_f unit behind MGDG (in the GL detection TLC system) and reacted in addition to iodine vapour and with the sugar reagents anthrone (green) and *p*-anisidine phosphate (purple-gray) but not with the periodate–Schiff reagent. Juice chromoplasts contained phosphatidyl choline (PC), phosphatidyl ethanolamine (PE) as their only major phospholipid (PL) components.

Chromoplasts are known to evolve either directly from non-differentiated pigment-less plastids or from chloroplasts which are transformed in senescing tissues into chromoplasts [1]. Juice chromoplasts apparently belong to the first, while flavedo chromoplasts belong to the latter type.

Flavedo chromoplasts which have lost all their chlorophyll and photosynthetic membranes [2, 3] nevertheless retain considerable amounts of chloroplast membrane lipids. Chromoplasts of daffodil petals which have recently been found to be rich in DGDG, MGDG and PG may also be developmentally linked to chloroplasts [4]. However, juice chromoplasts also contain some MGDG, suggesting a more complex relationship between the origin of chromoplasts and their lipid composition.

Table 1. Quantitative estimation of galactolipid (GL) and phospholipid (PL) components in chromoplasts of orange peel and juice and in chloroplasts of green orange peel

Type of plastid and tissue	GL (μM/g ft. wt)		PL in plastids (μM P/g ft. wt)	PL components (% of plastid PL)		
	MGDG	DGDG		PC	PE	PG
Chromoplasts—yellow flavedo	0.26	0.10	0.52	tr	tr	>90
Chromoplasts—juice	0.16	tr?	1.41	48	42	—
Chloroplasts—green flavedo	0.37	0.09	0.39	tr	tr	<90

tr = trace.

EXPERIMENTAL

Yellow, or mature-dark green orange (*Citrus sinensis* CV Valencia) fruits were used. Flavedo layers were carefully removed and homogenized with an 'UltraTurrax' homogenizer (24 000 rpm) in ice cold 0.1 M, pH 7.4 Pi buffer containing 0.4 M sucrose. Upon centrifugation of flavedo extracts (6000 *g*, 10 min) the plastid material which is probably composed mainly of broken plastids separates as a dense, floating pellet which contains essentially all the pigmented material [5]. The plastid pellet was washed in the same buffer and reisolated by centrifugation. Pericarp segments from yellow fruit were homogenized as above and juice chromoplasts were recovered as a pellet between two centrifugations (1000 *g*, 3 min and 6000 *g*, 10 min). The pellet was washed in the same buffer and reconcentrated by centrifugation. Alternatively, freshly expressed juice was squeezed through 4 layers of gauze and filtered through Whatman No. 1 filter paper. Chromoplasts could be recovered from the filter paper [6]. Both methods yielded preparations with relatively little contamination as judged by the light microscope, and gave identical results in lipid analyses.

Lipids were extracted from whole tissues or from plastid fractions with $CHCl_3$–MeOH–0.1 N NaCl (25:25:2) to which butylated hydroxy anisole had been added [7]. One vol. of 0.1 M NaCl was added toward the end of the extraction. The $CHCl_3$ phase was separated and used for lipid analysis. TLC was performed on preactivated Si gel HR plates [8]. Galactolipids were tentatively identified by sugar spray reagents, mainly with *p*-anisidine phosphate in EtOH [9] and by cochromatography with authentic substances. The Vaskovsky and Kostetsky reagent [10] served for tentative identification of specific PL in combination with reference substances. A system of Me_2CO–toluene–HOAc–H_2O (60:40:2:1) was found to give good separation between PL which remain at the origin and the 2 major galactolipids (DGDG R_f 0.2, MGDG R_f 0.7) whereas pigments and neutral lipids move close to the front. PL were separated by developing the plates first in Me_2CO–HOAc–H_2O (100:2:1) [11] and then, again in the same direction up to the pigment zone with $CHCl_3$–Me_2CO–MeOH–HOAc–H_2O (10:22:12:12:1) [12]. For quantitative determinations lipid bands were visualized by short exposure to I_2 vapour and outlined. After the I_2 had evaporated from the chromatogram each band was removed, eluted and analysed. Galactolipids (MGDG and DGDG) were determined by the direct method of ref. [8]. Total PL, scraped from the origin zone of plates developed for galactolipid separation, or separated PL components were determined after digestion in 72% perchloric acid according to ref. [13].

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