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THE INTRACELLULAR DISTRIBUTION OF TOCOPHEROLS IN *CALENDULA OFFICINALIS* LEAVES

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Abstract—From *Calendula officinalis* leaves, five cellular subfractions (chloroplasts, mitochondria, Golgi membranes, microsomes and cytosol) were obtained and their purity was checked. The contents of α-,γ- and δ-tocopherols were determined in these fractions. There were no tocopherols in Golgi membranes and cytosol. γ-Tocopherol and δ-tocopherol were found in the chloroplasts, mitochondria and microsomes, whereas α-tocopherol was present only in the chloroplasts.

INTRODUCTION

Previous studies have shown that plants contain four tocopherols, i.e. α-,β-,γ- and δ-tocopherols. Photosynthetizing tissue contains mostly α-tocopherol [1, 2], localized mainly in the chloroplasts [1–7] or plastids [6]. The remaining tocopherols occur in the green tissue in much smaller amounts than α-tocopherol, and thus data about the localization of these compounds within the cell are very limited. Some authors [3, 7, 8] have shown the presence of γ- and δ-tocopherols in the chloroplasts and postchloroplastic supernatant. Moreover, a certain amount of unidentified tocopherol has been detected in the mitochondrial fraction [9, 10]. So far only one paper has been published [5] concerning the distribution of the individual tocopherols in several cellular fractions obtained from the brown seaweed *Fucus spiralis*. However, the fractions which differed in their sedimentation constants were not further characterized.

The aim of the present study was to investigate the distribution and level of tocopherols in purified cellular subfractions from *Calendula officinalis* leaves. According to earlier studies [7], α-,γ- and δ-tocopherols occur in this plant.

RESULTS AND DISCUSSION

The different subfractions, obtained as reported in the Experimental, were characterized by the determination of their contents of chlorophyll and plastoquinon as markers for the chloroplasts; ubiquinine and succinic dehydrogenase activity [11] as markers for the mitochondria; activity of UDPG: sterol glucosyltransferase as a marker enzyme for Golgi membranes [12] and glucose-6-phosphatase activity as a marker for the microsomal fraction [13, 14]. The results are presented in Table 1 and they indicate that, in fact, the investigated subfractions were not contaminated with each other. The contents of the different tocopherols were determined in the purified subfractions. The mean results of three independent experiments are presented in Table 2. It can be seen that α-tocopherol occurs almost exclusively in the chloroplasts, although trace amounts were also present in the microsomal fraction. γ-Tocopherol occurred in three subfractions, its amount being the greatest in the chloroplasts, with less in the mitochondrial fraction and the smallest amount in the microsomal fraction. δ-Tocopherol occurred in the same subfractions in similar amounts. Moreover, trace amounts of this compound were found in the supernatant. No tocopherols were present in the Golgi membrane fraction.

This paper presents the first exact qualitative and quantitative determinations of α-, γ- and δ-tocopherols in homogeneous cellular subfractions obtained from a higher plant. The present results confirm the findings of other authors stating that α-tocopherol occurs only in the chloroplasts. Investigations proving that γ- and δ-tocopherols are present not only in the chloroplasts but also beyond them were extended in this study. They showed that these compounds are localized in the mitochondrial and microsomal fractions, whereas they are absent in Golgi membranes and probably in cytosol. The trace amount of δ-tocopherol detected in cytosol may originate from contamination with the microsomal fraction.

The present results can serve as a basis for further studies on the intracellular localization of the biosynthesis of tocopherols and on their possible transport between organellae, as suggested by other authors [15]. The problem of possible compartmentization of the tocopherol biosynthetic pathways seems of particular interest. The origin of these compounds is heterogeneous, because the aromatic ring is formed independently of the prenyl chain, and linking of these two moieties takes place at the last stages of their biosynthesis. The aromatic ring is formed in the

Table 1. Content of chlorophyll, plastoquinone and ubiquinone and succinic dehydrogenase, UDPG: sterol glucosyltransferase and glucose-6-phosphatase activities in subcellular fractions of *C. officinalis* leaves

Fraction	Protein (mg)	Chlorophyll (mg)	PQ* (μg)	UQ (μg)	Succinic dehydrogenase (μM/min/mg protein)	UDPG: sterol glucosyltransferase (μM/hr/mg protein)	Glucose-6-phosphatase (μM/hr/mg protein)
Chloroplasts	2.53	69.9	49.1	0.45	0.58	0	0
Mitochondria	1.01	2.3	1.3	38.8	12.76	81.4	0.01
Golgi membranes	0.27	0	0	0	0	520.3	0
Microsomes	26.01	0.4	0	+	0.01	1.8	67
Cytosol	8.03	0	0	0	0.003	0.2	1.58

* PQ = Plastoquinone; UQ = ubiquinone.

chloroplasts, mitochondria and microsomes. The phytyll chain is formed in the chloroplasts and nothing is known about its biosynthesis elsewhere in the cell. Its biosynthesis or transport from the chloroplasts, either as a free compound or possibly as a form bound with the aromatic system, calls for elucidation.

EXPERIMENTAL

Experiments were performed on leaves of 6-week-old *Calendula officinalis* L. var. Radio plants cultivated in a lumistat [16].

Preparation of cellular subfractions. Cut leaves were ground in a mortar 3× for 30 sec with an equal amount of Kieselgel (0.2–0.5 mm) in 0.3 M sucrose containing 0.01 M Tris–HCl buffer (5 ml of soln, 3 g of leaves). The suspension was filtered through 4 layers of cheesecloth and centrifuged successively at 200 *g*, 1 min; 3000 g, 10 min; 14 000 *g*, 20 min; 105 000 g, 6 min. The 14 000 g pellet was suspended in the homogenization soln and further fractionated by sucrose density gradient centrifugation at 95 000 g, exactly as described in ref. [17]. The two membrane layers localized at the 1.0/1.25 M sucrose and 1.25/1.5 M sucrose interfaces were collected as the Golgi fraction and pellet was the mitochondrial fraction.

Extraction. In each case the pellets and final supernatant were extracted as in ref. [5].

Preparative chromatography and quantitative determinations of quinones and tocopherols were performed as described in refs. [5, 18].

Table 2. Quantitative determination of the tocopherols in subcellular fractions of *C. officinalis* leaves (10 g)

Fraction	Tocopherols (μg) α-T	γ-T	δ-T
Whole tissue	16.8	6	4.5
Chloroplasts	13.1	2.56	1.17
Mitochondria	0	1.17	0.84
Golgi membranes	0	0	0
Microsomes	tr	0.60	0.96
Cytosol	0	0	tr

Chlorophyll content was determined according to Arnon [19].

Protein content of the cellular subfractions was determined by the method of ref. [20].

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