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POLYPRENYL QUINONES AND α-TOCOPHEROL IN *CALENDULA OFFICINALIS*

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Key Word Index—*Calendula officinalis*; Compositae; polyprenyl quinones; α-tocopherol; intracellular localization and dynamics of labelling.

Abstract—In various cellular subfractions of *Calendula officinalis* leaves a study was made of the distribution of polyprenyl quinones and α-tocopherol and the dynamics of their labelling with $^{14}CO_2$ and acetate-[1-^{14}C] and incorporation of mevalonate-[2-^{14}C] after 3 hr. It was confirmed that plastoquinone occurs only in the chloroplasts, ubiquinone only in the mitochondria and α-tocopherol in both these subfractions. Phylloquinone was found in the chloroplast and mitochondrial fractions as well as in the post-mitochondrial supernatant. Studies of the dynamics of radioactive precursor incorporation indicated that α-tocopherol is metabolized more rapidly than the polyprenyl quinones studied; the incorporation of mevalonate-[2-^{14}C] suggests that the side chain of plastoquinone can be synthesized in the cytoplasm and transported to the chloroplasts.

INTRODUCTION

Previous work on the distribution of polyprenyl quinones in plant cells has shown that ubiquinone occurs in the mitochondria, whereas plastoquinone and phylloquinone are present in the chloroplasts [1]. Tocopherols are found in all subfractions of plant cells [2].

In earlier unpublished work on the incorporation of radioactive precursors into some chloroplastic quinones and α-tocopherol we demonstrated that acetate and CO_2 are utilized to the same degree for the biosynthesis of α-tocopherol and phylloquinone. This finding pointed to the possibility that the biosynthesis of both these compounds may take place not only in the chloroplasts but also in the exochloroplastic region.

RESULTS AND DISCUSSION

In studies on the subcellular distribution of polyprenyl quinones and α-tocopherol leaves from 6-week-old shoots of *Calendula officinalis* were stored for 24 hr in the dark before processing to reduce the quantity of plastoquinone. Cellular subfractions were obtained by a modified method for plants [13]. It was found that purification of the chloroplast and mitochondrial fractions by centrifugation through dense sucrose exerted no definite effect on the content of different quinones in these subfractions. The differences in the amount of quinones between the purified and non-purified fractions varied from 7 to 14% (Table 1). These results suggest that quinones present in the chloroplast and mitochondrial fractions are probably so strongly incorporated into the structure of the organelles that centrifugation through dense sucrose fails to separate them. Also the crude chloroplast and mitochondrial fractions contain only a small amount of broken fragments which during centrifugation through 'dense sucrose' remain in the upper layer. On the basis of these observations, in the preparation of the chloroplast and mitochondrial subfractions the stage of purification by centrifugation through 'dense sucrose' was omitted. The consequent shortening of the preparation time also had the advantage of reducing the possibility of degradation of any polyphenyl quinones. In the resulting subfractions polyprenyl quinones were isolated by the method of Griffiths, Threfall and Goodwin [4–6]. The mean results of twelve experiments are presented in Table 2. The results indicated that the phylloquinone content is largest in the chloroplasts, and 3 times less in the mitochondrial fraction and postmitochondrial supernatant; α-tocopherol was localized in the chloroplasts and occurred in a somewhat smaller amount in the mitochondria. whereas plastoquinone and ubiquinone were found only in the chloroplasts or mitochondria, respectively.

The participation of different precursors in the biosynthesis of chloroplastic and exochloroplastic polyprenyl quinones and α-tocopherol was investigated with $^{14}CO_2$, acetate-[1-^{14}C] and mevalonate-[2-^{14}C]. The changes of the sp. act. of the investigated polyprenyl quinones and α-tocopherol labelled with $^{14}CO_2$ and acetate-[1-^{14}C] in the period from 1 to 24 hr are presented in Figs. 1 and 2, respectively. Incorporation of acetate, as compared with CO_2, was higher (× 6) in the case of total quinones and tocopherols in the cell, higher (× 39) for exochloroplastic compounds and lower (× 0·5) for chloroplastic ones probably because acetate is a nearer precursor for mevalonate than CO_2. $^{14}CO_2$ labelled quinones more effectively (× 5) in the chloroplast fraction than in the mitochondrial and supernatant fractions; acetate-[1-^{14}C] labelled more effectively (× 13) extrachloroplastic than chloroplastic compounds. This result was evident since CO_2 penetrates primarily into the chloroplasts where it can rapidly be utilized for the biosynthesis of chloroplast compounds, whereas the biosynthesis of exochloroplast quinones and α-tocopherol from this precursor takes place only after translocation of its metabolites to the cytoplasm. Acetate exhibits an opposite behaviour, as it

Table 1. The effect of purification of cellular subfractions of *Calendula officinalis* leaves by centrifugation in 1·6 M sucrose soln on their content of α-tocopherol and different polyprenyl quinones (incubation time 3 hr, measurements taken in triplicate)

Subfraction	Quinone	Content of quinones (in μg/10 g of tissue) in subfractions purified	non-purified
Chloroplasts	Plastoquinone	59·8	55·9
	Phylloquinone	99·6	92·7
	α-Tocopherol	28·3	26·3
Mitochondria	Ubiquinone	45·6	49·5
	Phylloquinone	26·3	29·4
	α-Tocopherol	19·3	21·0
Supernatant	Phylloquinone	34·1	37·0

Table 2. Content of α-tocopherol and polyprenyl quinones (from 12 determinations) and of protein in various cellular subfractions of *Calendula officinalis* leaves

Subfraction	Quinone	Content of quinones (μg/10 g of tissue) (and range).	Content of protein in mg/10 g of tissue	Quinone content per 1 mg of protein
Chloroplasts	Plastoquinone	62·2 (70·1–56·7)	4·5	11·2
	Phylloquinone	96·0 (102·3–87·5)		16·6
	α-Tocopherol	25·5 (27·5–21·9)		4·2
Mitochondria	Ubiquinone	42·2 (48·3–37·5)	3·9	9·0
	Phylloquinone	30·7 (32·3–26·9)		6·6
	α-Tocopherol	19·2 (21·7–18·1)		4·1
Supernatant	Phylloquinone	33·6 (38·1–30·0)	15·6	1·8

penetrates first into the cytoplasm and only later into the chloroplasts.

The changes of the sp. act. after labelling with both precursors were very similar. Maximum radioactivity incorporation into α-tocopherol occurred both in the chloroplasts and mitochondria 3 hr after the uptake of precursor and into polyprenyl quinones 12 hr after this uptake.

The labelling curves of α-tocopherol and polyprenyl quinones exhibited a parallel course between the 1st and 3rd hr of incubation, irrespective of the quinone localization. After this time the radioactivity of α-tocopherol rapidly dropped, while in the polyprenyl quinones it continually increased and then fell slightly after 12 hr. These results show that α-tocopherol is metabolized more rapidly than the polyprenyl quinones both in the chloroplast and mitochondrial fractions.

After administration of mevalonate-[2-^{14}C] the plants were processed after 3 hr that is at the time when α-tocopherol and polyprenyl quinones had already been well labelled with $^{14}CO_2$ and acetate-[1-^{14}C]. The results are recorded in Fig. 3. According to Goodwin [7], mevalonate penetration through the chloroplast membrane is limited; consequently, after this time it should label

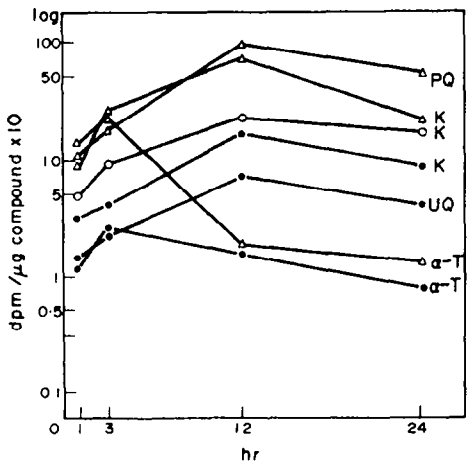

Fig. 1. $^{14}CO_2$ incorporation into α-tocopherol and polyprenyl quinones in different cellular subfractions of *Calendula officinalis* leaves. PQ—plastoquinone; K—phylloquinone; UQ—ubiquinone; αT—α-tocopherol; △—chloroplast fraction; ●—mitochondrial fraction; ○—supernatant fraction.

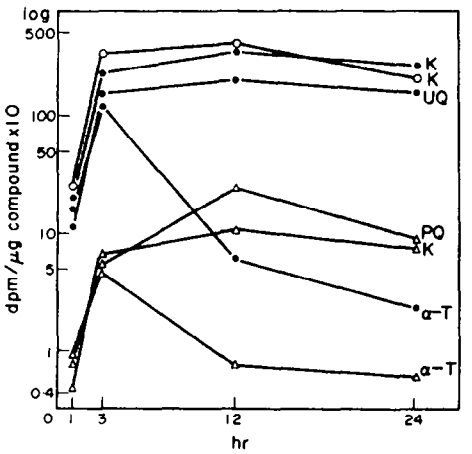

Fig. 2. Acetate-1-^{14}C incorporation into α-tocopherol and polyprenyl quinones in different cellular subfractions of *Calendula officinalis* leaves. Abbreviations as Fig. 1.

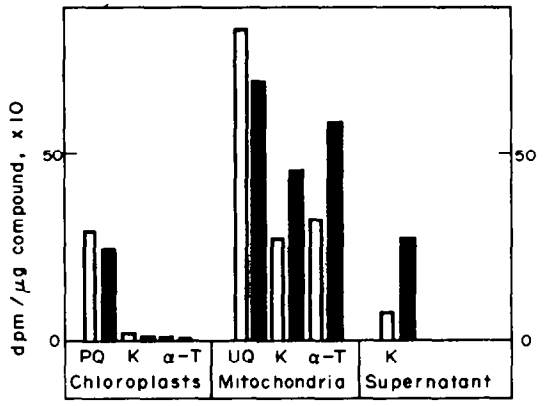

Fig. 3. Mevalonate-2-^{14}C incorporation into α-tocopherol and polyprenyl quinones in different cellular subfractions of *Calendula officinalis* leaves. Abbreviations as Fig. 1. □—total radioactivity dpm/10 g of fresh tissue; ■—Specific radioactivity dpm/μg of compound.

mainly the exochloroplast quinones. Nevertheless, considerable radioactivity was detected in chloroplast quinones, with as much as 87% of this radioactivity localized in plastoquinone. Incorporation of radioactive mevalonate into α-tocopherol and phylloquinone present in the chloroplasts was very low.

The aromatic ring of α-tocopherol and the *p*-benzoquinone ring of plastoquinone are synthesized in a common metabolic pathway; thus, the occurrence of radioactivity in plastoquinone probably indicates that it is labelled in the side chain but not in the ring. This suggests that one of the metabolites of mevalonate, probably solanesol pyrophosphate, being a precursor of the side chain of plastoquinones, is transported to the chloroplasts from the cytoplasm which most probably is the site of its synthesis.

EXPERIMENTAL

Material. Six-week-old shoots of *C. officinalis* L. var. Radio, were obtained from phytotron culture [9]. Eight shoots, fr. wt 16 g ± 15%, were taken for each experiment after 24 hr storage in the dark.

Precursor administration. Acetate-[1-^{14}C]/200 μCi, sp. act. 19·7 mCi/mmol and mevalonate-[1-^{14}C]/100 μCi, sp. act. 36 mCi/mmol were administered through the cut stem and $^{14}CO_2$/200 μCi, sp. act. 15·4 mCi/mmol in a special chamber as previously describes [10]. During precursor administration and incubation the plants were exposed to light of 20000 lx; they were processed 1, 3, 12 and 24 hr after precursor uptake.

Fractionation of material. Leaves were ground with Si gel in a 0·3 M sucrose soln (0·5 g gel + 10 ml sucrose soln per 1 g. of leaves) for 40 sec. This treatment was repeated ×3 and the resulting homogenate was filtered through 4 layers of cheesecloth. The filtrate was centrifuged at 600 *g* for 5 min; the pellet was discarded and the supernatant was centrifuged at 3000 *g* for 10 min. The subsequent supernatant was centrifuged at 10000 *g* for 20 min. Pellets obtained by these centrifugations were purified by filtration through a 1·6 M sucrose soln, this yielding the chloroplast and mitochondrial fractions, respectively. The 10000 *g* supernatant was also used for study.

Isolation of polyprenyl quinones. From each subfraction the different quinones were obtained by extraction, column chromatography and TLC under conditions described in refs. [4–6]. Identification of quinones and determination of their content in the different subfractions were performed by spectrophotometry [5,6].

Radioactivity measurement was carried out in a scintillation counter with 70% efficiency. Compounds were dissolved in 5 ml of scintillator soln containing PPO (5 g/l.) and POPOP (0·5 g/l.) in toluene. *Protein content* of the cellular subfractions was assayed by the Lowry method [11].

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