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LOCALIZATION OF PRENYLQUINONES IN THE ENVELOPE OF SPINACH CHLOROPLASTS

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Summary

The isolated and purified chloroplast envelope of spinach leaves contains, besides carotenoids, several prenylquinones as basic constituents: plastoquinone-9, phylloquinone K_1, α-tocoquinone and the chromanol, α-tocopherol. The relative quinone and carotenoid composition of the envelope differs distinctively from that of the thylakoid membranes. The possible role of prenylquinones in metabolic envelope activities and the mediator function of the envelope in prenylquinone biosynthesis are discussed.

Introduction

The chloroplasts of algae and higher plants contain several prenylquinones which are potential photosynthetic electron carriers. These are the benzoquinone derivatives, plastoquinone-9, α-tocoquinone (α-tocopherylquinone) and its chromanol, α-tocopherol, as well as the naphthoquinone derivative, phylloquinone K_1 [1—4].

The reduced prenylquinone forms (α-tocopherol, plastohydroquinone) may also serve as lipid antioxidants [5]. The chloroplast prenylquinones are bound to the photochemically active thylakoid membranes, which perform the photosynthetic electron transport reactions [3,4]. Plastoquinone-9 is present in thylakoid membranes in a much higher concentration (5—10-times) than phylloquinone or α-tocoquinone [1,7].

The role of plastoquinone-9 as a photosynthetic electron carrier, which mediates proton translocation through the membrane (plastoquinone shuttle),

is well established [4,8]. The functional site of phylloquinone and α-tocoquinone is not yet fully understood. Inhibitor studies indicate, however, a position for phylloquinone in the photosynthetic electron transport chain just before plastoquinone [9]. In a recent model, it is proposed that phylloquinone controls the influx of electrons and protons into the large plastoquinone pool and α-tocoquinone the efflux [10]. A second localization site for prenylquinones within the chloroplast is the osmiophilic plastoglobuli of the plastid stroma [11—16]. Excess amounts of prenylquinones (mainly plastohydroquinone-9 and α-tocopherol), which are accumulated after the termination of the thylakoid synthesis phase [3,14,15], are deposited extra-thylakoidally together with other storage lipids in the plastoglobuli, the size and frequency of which increase with increasing age of chloroplasts [11,14,15].

A third structural component of chloroplasts is the envelope. The latter plays an important role in the transport control of metabolites into and out of the chloroplast [19,20]. The envelope is very active in galactolipid synthesis [17,21,22], contains several carotenoids [23—25], synthesizes together with a recombined stroma system geranylgeranyl pyrophosphate from isopentenyl pyrophosphate [26], and also possesses the capacity to transfer methyl groups from *S*-adenosylmethionine into the aromatic nucleus precursor of α-tocopherol [27].

Though the site of synthesis of the chloroplast prenylquinones within the cell is not fully known, it is clear that at least the final steps in chloroplast prenylquinone biosynthesis must proceed in or on the chloroplasts [3]. Some observations indicate that tocopherol precursors may be synthesized outside the chloroplast and are transported into plastids before or after the final methylation step [28,29]. This points to a specific mediative role of the chloroplast envelope in the uptake and biosynthesis of chloroplast prenylquinones.

It is therefore of great interest to determine whether or not the envelope contains chloroplast prenylquinones.

Materials and Methods

Spinach leaves (8-week-old, 3 kg) were cut into small pieces and placed directly in ice-cold chloroplast extraction medium (330 mM mannitol, 50 mM $Na_4P_2O_7$, pH 7.8, 2 mM EDTA and 0.1% defatted bovine serum albumin) with a tissue/medium volume ratio of 1 : 3 (w/v). The material was homogenized in three successive batches in a 1 gallon Waring blendor at low speed for 3 s. The brei was filtered through six layers of muslin and one layer of nylon blutex (50 μm aperture). The filtered suspension was then centrifuged (six bottles, 500 ml each batch) at $1500 \times g$ (max) for 10 min (GS3 rotor, Sorvall). To process the three batches (18 bottles, 500 ml each), three centrifuges (RC5, Sorvall) were used and were operated at 3°C. The supernatant was removed by aspiration and each pellet was resuspended, using a spatula, in 4 ml of extraction medium. In order to separate the aggregates, the suspension (total volume 90—120 ml) was filtered on a nylon blutex layer. The nylon was then washed with a minimal volume (approx. 10 ml) of washing medium to give a suspension containing 250—300 mg of chlorophyll. Intact chloroplasts were then purified by isopycnic centrifugation either

in a sucrose gradient [23] or Percoll TM gradient [18] as previously described.

Envelope and thylakoid membranes were prepared from intact purified chloroplasts [17]. The plastoglobuli were isolated from sonicated chloroplasts by differential centrifugation [16].

The protein content of the envelope preparation was determined according to the method of Lowry et al. [30]. The lipid moiety of the freeze-dried envelope preparation was extracted with a mixture of hexane and acetone (10 : 4, v/v) until all carotenoids and prenylquinones were removed. By adding water the extract was washed free from acetone. The dried hexane was analyzed further for prenylquinones by thin-layer (TLC) [33] and by high-performance liquid chromatography (HPLC); both adsorption [31] and reversed-phase HPLC [32] were applied. The prenylquinones were partially identified by cochromatography (two-dimensional) with pure prenylquinones in the TLC and in the HPLC system, and more particularly by their chracteristic absorption spectra before and after reduction with $NaBH_4$ [33]. In the HPLC system, the ultraviolet detector was routinely set at 260 nm; in a second run the monochromator light of the detector system was set on the absorption maximum of the expected prenylquinone to enhance the sensitivity of detection and to allow a partial identification. In the case of α-tocopherol, oxidation with $FeCl_3$ yielded the expected α-tocoquinone. The prenylquinone amounts were determined spectrophotometrically using the extinction coefficients described [33]. The individual carotenoids were separated by TLC [34] and identified by their absorption spectra. For the quantitative determination an extinction coefficient $E^{1\%}_{1cm}$ of 2500 for the absorption maximum (λ_{max}) was applied. The λ_{max} values in ethanol are: β-carotene, 447 nm; violaxanthin, 441 nm; lutein, 445 nm; neoxanthin, 436 nm; antheraxanthin, 444 nm; and zeaxanthin, 448 nm. The values given in the tables represent mean values from three separately prepared envelope fractions (maximal deviation ±8%).

Results

Envelope membranes obtained from chloroplasts purified by isopycnic centrifugation in density gradients of silica (Percoll®) [18] or sucrose [23] differ strikingly from microsomal membranes and mitochondria in that they exhibit no oxidoreductase activities, no phosphatidylethanolamine and no *b* type cytochrome [23]. In addition, when examined in thin sections by electron microscopy, the purified envelope fraction shows relatively large vesicles or elongated profiles bordered by a single or a double membrane [19]. Neither thylakoid membranes nor plastoglobuli are trapped in the network of envelope membranes [19].

The absorption spectrum of a lipid extract of the spinach chloroplast envelope indicates the presence of carotenoids, as seen from the typical absorption maxima between 400 and 500 nm (Fig. 1). The lipid extract also exhibits light absorption in the ultraviolet range from 230 to 290 nm, in which the prenylquinones exhibit absorption maxima. The four typical chloroplast prenylquinones were detected in the envelope by the use of TLC and HPLC. The main component is α-tocopherol, followed by plastoquinone-9. α-Tocoquinone and phylloquinone are also present, but in much lower concentrations (Table

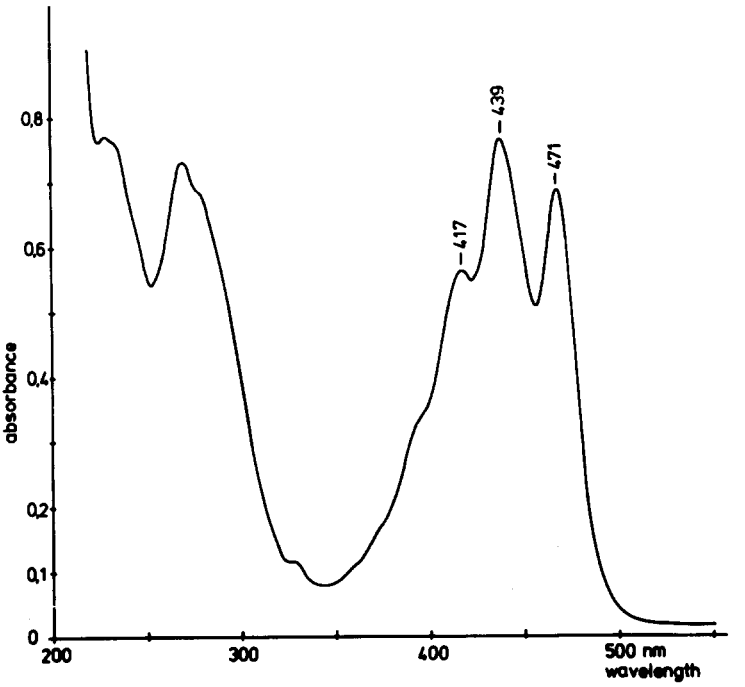

Fig. 1. Absorption spectrum of a lipid extract of the envelope from spinach chloroplasts in hexane. The absorption maxima in the blue region indicate the presence of carotenoids. The strong absorption in the ultraviolet region between 240 and 280 nm is mainly due to quinones.

TABLE I

PRENYLQUINONE AND CAROTENOID CONTENT OF THE SPINACH CHLOROPLAST ENVELOPE AND OF ISOLATED PLASTOGLOBULI-FREE SPINACH THYLAKOID MEMBRANES

Values are expressed as μg per mg protein.

Prenyl lipid	Envelope	Thylakoid membranes
Plastoquinone-9 *	1.2	3.9 (3.0—4.6) **
Phylloquinone K_1	0.1	0.34 (0.3—0.4)
α-Tocoquinone	0.2	0.24 (0.2—0.3)
α-Tocopherol	2.8	1.1 (0.9—1.4)
Sum of prenylquinones	4.3	~5.5 (4.3—6.7)
β-Carotene	0.6	6.6
Violaxanthin	2.6	3.1
Lutein	1.8	7.8
Neoxanthin	0.3	1.3
Antheraxanthin	0.3	0.2
Zeaxanthin	0.8	0.5
Total carotenoids	6.4	19.5
Chlorophyll *a* + *b*	0	143

* The envelope only contained the oxidized quinone form, plastoquinone-9. The values given for thylakoid membranes represent the sum of plastoquinone and plastohydroquinone.

** Mean value (and range) of prenylquinone levels found in five different thylakoid fractions.

TABLE II

PERCENT COMPOSITION (WT.%) OF PRENYLQUINONES IN DIFFERENT SUBFRACTIONS, ISOLATED FROM SPINACH CHLOROPLASTS

Prenylquinone	Thylakoid membranes	Envelope	Plastoglobuli
Plastoquinone-9 *	70	28	56
Phylloquinone-K_1	6	3	4
α-Tocoquinone	4	5	6
α-Tocopherol	20	64	34
Total	100	100	100

* The values given for thylakoid membranes and plastoglobuli represent total plastoquinone-9 (sum of quinone and hydroquinone forms). Spinach thylakoid membranes contain the quinone to 30—50%, and plastoglobuli to 40—70% in the reduced form.

I). On a protein basis, the total prenylquinone content of isolated thylakoid membranes, which varies depending on the growth conditions [3], is similar to or only a little greater than that of the envelope. The level of α-tocopherol is higher in the envelope than in the thylakoid membranes. The ratio of plastoquinone-9/α-tocopherol of the envelope (0.4) is practically reversed to that of thylakoid membranes (approx. 3.5). The minor components α-tocoquinone and phylloquinone appear in both membrane fractions in lower proportions (Table I).

Plastohydroquinone-9, which occurs in the isolated spinach thylakoid membranes at 30—50% of the concentration of plastoquinone-9, was not detected in the envelope fraction. Consequently, it is possible that the envelope contains plastoquinone only in the oxidized form; however, the possibility that any endogenous plastohydroquinone is oxidized to plastoquinone during isolation of the envelope cannot be excluded.

In contrast to thylakoid membranes and the envelope, the osmiophilic plastoglobuli do not contain protein [11—15]. The relative prenylquinone composition of the envelope also differs from that of isolated spinach plastoglobuli as can be seen from the percent composition of the different chloroplast fractions (Table II).

The envelope exhibits a typical carotenoid composition as described before [23—25]. On a protein basis, it contains a much lower level of β-carotene, and also a lower content of lutein and neoxanthin than thylakoid membranes (Table I). That the envelope possesses a unique chemical composition, which is quite different from that of thylakoid membranes, is also seen from the much higher xanthophyll-to-carotene ratio (envelope, 9.7; thylakoid membranes, 1.9) and the lower ratio of carotenoids to prenylquinones (envelope, 1.5; thylakoids, 4.5).

Discussion

The results of this investigation show that prenylquinones are found at three sites in chloroplasts: (1) the photochemically active thylakoid membranes, which contain the prenylquinones needed for performance of the

photosynthetic electron transport, (2) the osmiophilic plastoglobuli, which store the excess prenylquinones (mainly plastohydroquinone and α-tocopherol) and (3) the chloroplast envelope.

In thylakoid membranes the chloroplast prenylquinones, plastoquinone-9, phylloquinone and α-tocoquinone, function as electron carriers between the two photosystems [7,8], whilst α-tocopherol is thought to function as a lipid antioxidant protecting thylakoid lipids from photodegradation [5]. Since the plastoglobuli serve as a storage site for excess chloroplast lipids [11,14—16], their prenylquinones represent a metabolic reserve but do not seem to play any physiological role. The relative prenylquinone composition of the envelope differs from that of the thylakoid membranes (functional quinones) and that of the plastoglobuli (storage quinones). With α-tocopherol as the main component the quinone composition of the envelope resembles that of etiolated leaf tissue [35].

The exact function of α-tocopherol and plastoquinone-9 in the envelope is not known. One recent observation indicates that the envelope is the site of prenylquinone synthesis, where homogentisic acid is prenylated to yield α-tocopherol and plastoquinone-9 [36]. Its prenylquinone would then reflect the biosynthetic pool size. Whether the prenylquinones might have a physiological function in the envelope (hydrogen carriers for desaturation of fatty acids or carotenoid precursors? lipid antioxidant?) needs much further research.

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