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Analysis of plastoquinone-9 levels in appressed and non-appressed thylakoid membrane regions

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Using direct chemical measurement, the level of plastoquinone-9 in chloroplast thylakoids of *Pisum sativum* (pea) was found to be 21 mol per 1000 mol chlorophyll, a value in close agreement with previous estimates made by direct kinetic and optical methods. Mechanical fragmentation of isolated thylakoids ensured removal of a major source of plastoquinone-9 contamination, the plastoglobuli, and provided samples containing predominantly appressed and non-appressed membranes. The level of plastoquinone-9 was 0.6–0.7% of the acyl-lipid matrix (mol/mol) in both thylakoid regions, a result consistent with the role of plastoquinone-9 as a long-range mobile electron/proton carrier.

Since the discovery that chloroplasts contain plastoquinone [1–3] there have been several attempts to quantify the concentration of this class of electron carrier in the thylakoid membrane. Early analyses suffered the problem of contamination of the membrane samples by plastoglobuli and greater levels of plastoquinone were estimated than appeared to be involved in intersystem electron flow [4]. The determination of plastoquinone levels and distribution within the thylakoid has recently taken on a renewed importance because of likely lateral separation of the photosystems between appressed and non-appressed regions of this membrane system in higher plant chloroplasts [5,6]. In this model quinones, probably molecules of plastoquinone-9 (PQ), act not only to facilitate proton transport across the membrane, but also as long-range redox carriers able to diffuse within the hydrophobic interior of the membrane between appressed and non-appressed regions [7,8]. With this concept in mind Jennings et al. [9] have attempted to determine, by means of analysing chlorophyll fluorescence signals, the plastoquinone level in different membrane regions. They concluded that plastoquinone is distributed in both the appressed and non-appressed membrane regions with a somewhat higher concentration in the latter. Previous to this, chemical analyses of membrane fragments enriched in photosystem two or photosystem one yielded variable results, probably due to plastoglobuli contamination [4,10]. In addition, some problems with biophysical determination have been identified [11]. In all cases the plastoquinone content has been directly or indirectly related with chlorophyll levels. However, since the mobile plastoquinone pool is 'dissolved' in the hydrophobic matrix of the membrane its concentration should be given in terms of the content of polar lipids which constitute the bilayer.

In this communication we report analyses of the levels of plastoquinone-9 and polar lipids in mechanically prepared granal and stromal frag-

Abbreviation: PQ, plastoquinone-9.

ments in order to gain a direct chemical estimate of the level of this intersystem redox carrier in the appressed and non-appressed regions of the thylakoids.

Chloroplast thylakoids were isolated from pea plants (*Pisum sativum*), fragmented using a Yeda press and separated by differential centrifugation [12] to give granal and stromal enriched fractions with recoveries of total thylakoid chlorophyll of 80% and 20%, respectively. The plants were grown in a green-house with natural lighting supplemented by mercury-vapour lamps operating on a 16 h photoperiod [13]. The fractionation techniques used in this work are known to remove plastoglobuli from the membranes [14,15] and indeed, we observed that the thylakoid fractions had a much lower PQ content than the intact thylakoid preparations (data not shown). Total lipids were extracted with chloroform/methanol (2 : 1, v/v) and quantities of acyl lipid measured by gas chromatography of fatty acid methyl esters as previously described [16]. The PQ levels were determined by spectrophotometric detection at a wavelength of 255 nm after reverse-phase high-performance liquid chromatography of lipid extracts [17]. Chlorophyll levels were determined according to Arnon [18].

The PQ content of the thylakoid fractions are given in Table I. When expressed on a chlorophyll basis the granal lamellae contained only half the level found in the stromal fractions. Interestingly, however, when the PQ concentration is calculated in terms of acyl-lipid levels there is no difference. This is because the lipid-to-chlorophyll ratio for granal membranes is significantly lower than for the stromal lamellae [12]. The even distribution of PQ, relative to acyl lipids, is consistent with the existence of a pool of PQ within the lipid phase which is continuous throughout the appressed and non-appressed regions of the membrane. Such a conclusion is also in line with the idea that PQ can act as a mobile redox carrier between the laterally segregated protein complexes which constitute a functional whole-chain electron-transport system [5–8,19].

The size of the inter-system PQ pool has been determined many times from various types of kinetic and optical measurements and most estimates are between 10 and 20 electron equivalents (e.g., Ref. 20) or between 5 and 10 PQ molecules per electron-transport chain. Our chemical analysis seems to agree with this consensus, since for the whole membrane we calculate that there are 21 mol PQ per 1000 mol chlorophyll (see Table I) and therefore about nine PQ molecules per chain. This calculation takes into account the relative recoveries of chlorophyll in the two fractions and assumes there are between 400 and 500 chlorophylls per chain. Bearing in mind the possibility that two PQ molecules are likely to be firmly bound to the Photosystem II complex, as Q_A and as Z [21–23], then our calculated pool size reduces to seven molecules. The figures given in Table I also show that the concentration of PQ throughout the membrane corresponds to one PQ molecule per 140 molecules of acyl lipid. This is 0.7% of the lipid phase and contrasts with earlier estimates [4] which would be as high as 3% if calculated on this basis. If allowance is made for PQ molecules tightly associated with PS II then the free PQ is about 0.6% of the lipid matrix.

Overall, the results presented in this short com-

TABLE I

CHLOROPHYLL AND PLASTOQUINONE CONTENT OF THYLAKOID FRACTIONS PREPARED BY HIGH-PRESSURE FRAGMENTATION

Average values and standard errors are given for analyses of membranes isolated from four separate batches of plants.

Membrane fraction	% Yield Chl	Chl *a*/*b* ratio	PQ/Chl (mmol/mol)	PQ/acyl lipid (mmol/mol)
Granal	80	2.7 ± 0.04	16.3 ± 0.8	6.8 ± 0.3
Stromal	20	5.3 ± 0.3	33.3 ± 4.4	7.8 ± 0.6
Whole membrane (calculated)	100	3.2	20.7	7.1

munication clarify the apparent anomaly that the functional pool of PQ appeared to be significantly lower than the chemical pool of PQ within the thylakoid membrane. It seems that the previous analyses of intact membranes were hampered by contamination from closely associated plastoglobuli. Removal of this contaminant leads to chemical pool sizes equivalent in magnitude to those determined by kinetic and optical measurements. Moreover, as suggested by the biophysical determinations of Jennings et al. [9], placing the PQ levels in terms of acyl-lipid concentrations indicates its even distribution between appressed and non-appressed lamellae reflecting its probable role as a long range mobile electron/protein carrier.

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