Location of photosystem I and photosystem II reaction centers in different thylakoid regions of stacked chloroplasts

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1. INTRODUCTION

Stacked chloroplast thylakoids are structurally differentiated into the appressed regions of the grana (partitions), and the non-appressed regions of grana end membranes, margins and stroma lamellae. This structural differentiation is also accompanied by differences in function (cf. [1–3]). In early fractionation studies small stroma lamellae vesicles highly enriched in photosystem (PS)1 were separated from rapidly sedimenting grana containing both PS1 and PS2 [4]. This suggested that the grana were the site for linear electron transport by PS1 and PS2 in close physical association while stroma lamellae was mainly involved in PS1-mediated cyclic electron flow. This view of thylakoid organization has been challenged by the availability of a fraction enriched in inside-out vesicles shown to originate from the appressed grana partitions [5–8]. Analyses of this fraction with respect to photochemical activities [8] and content of chlorophyll(Chl)–proteins [9] revealed a pronounced PS1 depletion. Based upon this, it was postulated that PS1 is mainly excluded from the appressed grana regions into the non-appressed regions thereby separated from most of PS2 which is concentrated in the appressed regions [2,9,10]. This model implies linear electron transport between spatially separated photosystems involving lateral shuttling of electron carriers between the different thylakoid regions.

Here the amount of PS1 and PS2 reactions centres in the various thylakoid subfractions were directly determined by measuring the light-induced absorbance changes of P700 and Q, respectively. The results present experimental evidence for the suggested, extreme lateral heterogeneity in the location of the two photosystems, particularly by demonstrating virtually no P700 in the appressed grana partitions.

2. MATERIALS AND METHODS

Spinach leaves obtained either from a growth chamber or from the local market were used to prepare stacked chloroplast thylakoids as in [8]. The thylakoids, suspended in 150 mM NaCl – 50 mM sodium phosphate buffer (pH 7.4), were fragmented by 2 passages through a Yeda press [5,8] and centrifuged at 40 000 × *g* for 30 min. The supernatant material was spun down at 100 000 × *g* for 60 min yielding the Y-100 fraction. The pellet (Y-40) was suspended in 100 mM sucrose – 10 mM sodium phosphate buffer (pH 7.4) – 5 mM NaCl and passed twice through the press. These membrane fragments were fractionated by aqueous polymer 2-phase partition as in [5], yielding the T2- and B3-fractions.

For measurements, the vesicles were suspended in 50 mM Tricine–NaOH buffer (pH 7.5) – 10 mM KCl – 5 mM $MgCl_2$. Absorbance changes of Q induced by Xe-flashes (t_{10-90} = 6 μs) of saturating intensity were monitored at 335 nm (bandwidth 4 nm FWHM) with a photomultiplier (Type 9818 QB), digitalized in a transient recorder (Biomation) and averaged in a signal processor

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(Tracor). The wavelength of 335 nm was chosen rather than that at the maximum for $Q^{\cdot -}$ (320 nm) to minimize superimposed plastoquinone absorbance changes [11]. The differential flattening factor at 335 nm determined as in [12] ranged between 1.07–1.16 for the various particle preparations. The molar ratio of Chl/Q was estimated by using an extinction coefficient of 10.7 $mM^{-1} \cdot cm^{-1}$ for $Q^{\cdot -}/Q$ at 335 nm [13]. Absorbance changes of P700 were measured as in [14]. An extinction coefficient of 64 $mM^{-1} \cdot cm^{-1}$ was used for P700 at 703 nm. Plastocyanin from spinach had a ratio of $A_{278}/A_{597} = 1.1$. All measurements were carried out in a 1 × 1 cm cuvette at room temperature.

3. RESULTS

The stoichiometry of the primary acceptor of PS2 (Q) and the PS1 reaction centre chlorophyll (P700) was determined in 5 different thylakoid fractions. The starting Yeda press homogenate (YPH) was separated by centrifugation into a grana stack fraction (Y-40) and a stroma lamellae vesicle fraction (Y-100). The latter fraction contained around 5–10% of the chlorophyll. After further press treatment, the Y-40 fraction was separated by phase partition into two distinct membrane populations, designated T2 and B3 [5,8]. The latter was enriched in inside-out thylakoids shown to originate from the grana partitions, and comprised around 20% of the Y-40 material while the remaining material (T2) consisted of right-sided thylakoids from both appressed and non-appressed regions [5–8].

3.1. *Distribution of Q*

Fig.1 shows absorbance changes at 335 nm of the Yeda press homogenate, Y-100 and B3 fractions in presence of the lipophilic electron acceptor phenyl-*p*-benzoquinone. The amplitude of the B3-material is much bigger compared to the starting material while the amplitude of Y-100 is close to the detection limit. In all fractions the absorbance increase in the flash is followed by decay kinetics with 2 fast components with respective half-times of ~ 100 and 600 μs and considerably slower components with 5 ms and longer halftimes. The 600 μs component represents Q reoxidation in intact PS2 [11], while the 100 μs component originates from PS2 with impaired water splitting where Q is reoxidized via cyclic electron flow provided an electron acceptor is present [15,16]. For quantification the slow components are extrapolated to the beginning at the time of the flash (fig.1) to give the amplitude of the 2 fast components representing total Q in the thylakoid fraction. The molar ratios of Chl/Q are given in table 1 after correcting the amplitudes for flattening effects [12]. The values of 380 and >8000 for the B3- and Y-100 fraction, respectively, indicate an uneven distribution of PS2 reaction centers with an

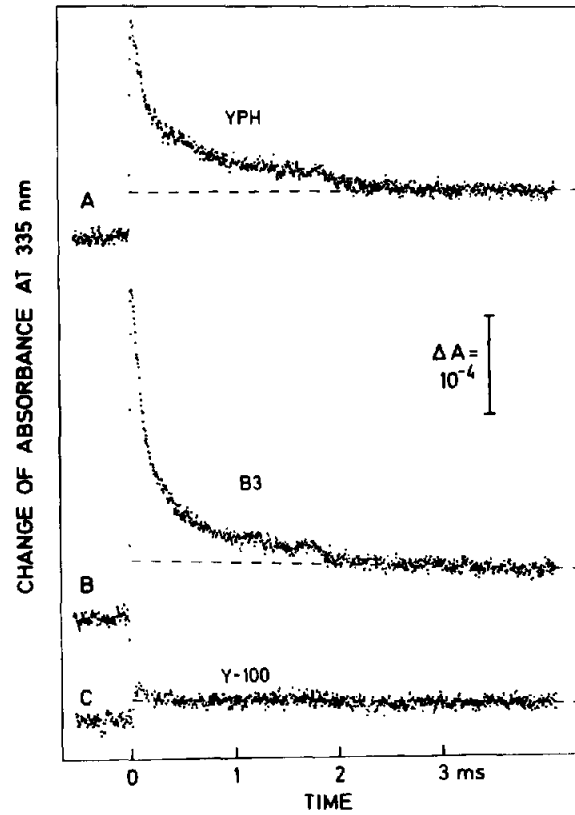

Fig.1. Absorbance change of Q at 335 nm induced by a short flash in different membrane fractions: (A) Yeda press homogenate; (B) B3-fraction enriched in appressed thylakoid regions; (C) Y-100 fraction from non-appressed thylakoid regions. The standard reaction mixture contained 10.1 μg Chl/ml and in addition 10 μM phenyl-*p*-benzoquinone. 200 signals were averaged at 5 μs/address with a repetition rate of 5 Hz. The dashed line extrapolates the slow components of the absorbance change monitored for 50 ms (not shown).

Table 1

Distribution of Q and P700 in thylakoid fractions derived from the different thylakoid regions

Fraction	Chl/Q	Chl/P700	Q/P700	Chl *a/b*
Yeda press homogenate	540	580	1.1	3.0
Y-40	510	665	1.3	2.9
T2	570	515	0.9	3.2
B3	380	1300	3.4	2.3
Y-100	>8000	240	<0.03	6.2

The molar ratios of chlorophyll (Chl) to Q and Chl/P700 were determined for all fractions from measurements as shown in fig.1 and 2, respectively

almost exclusive location of PS2 centers in appressed grana regions.

3.2. *Distribution of P700*

Fig.2 shows the absorbance changes at 703 nm of the YPH, Y-100 and B3 material. During the long flash P700 is fully oxidized. After the flash, reduced 2,6-dichlorophenolindophenol reduces total $P700^+$ independently of the sidedness of the membranes or the presence of plastocyanin [17,18]. Thus, the amplitudes can be used to calculate the Chl/P700 molar ratios of the various thylakoid fractions (table 1). The high P700 content in the Y-100 fraction is in agreement with previous measurements [4,8] and in combination with the very low content in the B3 fraction indicative of a predominant location of PS1 to non-appressed regions.

A PS1 enrichment is always accompanied by a PS2 depletion and an increase in the Chl *a/b* ratio (table 1). The Q/P700 ratios of the subfractions (table 1) deviate significantly from the ratio of 1.1 in the starting material, a value normal for chloroplasts [19].

The actual content of P700 in the grana partition cannot be directly determined from the B3-fraction since not only inside-out vesicles from appressed regions are present but also some 25% contaminating right-side out material, as shown by freeze-fracturing [6]. To understand the extent of PS1 exclusion from the grana partitions it is crucial to know whether the low P700 content of the B3-fraction (1 P700:1300 Chl) represents a native PS1

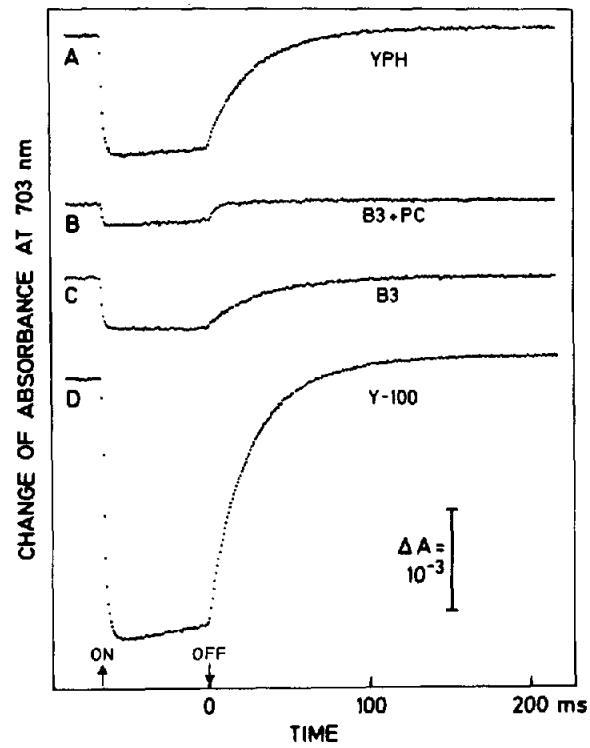

Fig.2. Absorbance change of P700 at 703 nm induced by a long flash in different membrane fractions: (A) Yeda press homogenate; (B) B3-fraction in the presence of plastocyanin (PC); (C) B3-fraction; (D) Y-100 fraction. The standard reaction mixture with 9.9 μg Chl/ml contained in addition 20 μM diuron (DCMU), 0.1 mM 2,6-dichlorophenolindophenol (except for trace B), 1 mM sodium ascorbate and 50 μM methylviologen. In (B) 2,6-dichlorophenolindophenol was replaced by 2 μM plastocyanin. 5 signals were averaged with a repetition rate of 0.2 Hz. The illumination period of 67 ms is marked by arrows.

content in the appressed regions or is due to contamination by right-sided non-appressed material. These two possibilities were discriminated by addition of plastocyanin which reduces only P700 in inside-out but not in right-side out vesicles [17]. The amplitudes in fig.2B,C show that only a minor portion (av. 27%) of total P700 in the B3-fraction is accessible to added plastocyanin. Knowing that inside-out vesicles comprise ~75% of total B3 material [6], the amplitude observed in presence of plastocyanin (fig.2B) gives a Chl/P700 molar ratio as high as 3500 for the appressed grana partitions.

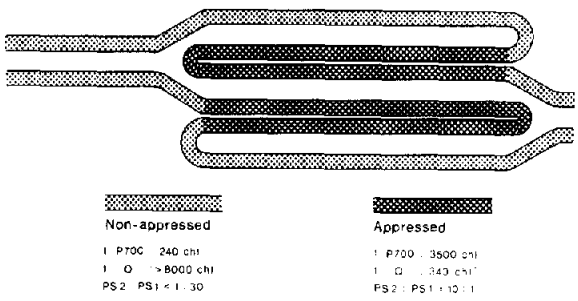

Fig.3. Schematic illustration of a cross-section through chloroplast thylakoids with appressed and non-appressed membrane regions. The values summarize the different stoichiometry for PS1 and PS2 reaction centers in the two thylakoid regions in support of an extreme lateral heterogeneity; calculated value.

In the same way, the contaminating right-side out vesicles can be calculated to have Chl/P700 ratio of ~450 which is similar to the corresponding value in the T2-fraction.

In contrast to P700, the actual amount of Q in the grana partitions cannot be determined directly. However, assuming the Chl/Q ratio of the T2-fraction (table 1) for the contaminating right-side out vesicles (25%) in the B3-fraction we estimate for inside-out vesicles a ratio of 340 Chl/Q. The molar ratio of Q/P700 is therefore as high as 10 in appressed thylakoid regions, while <0.03 in the non-appressed thylakoids (fig.3).

A total recovery of both Q and P700 was obtained during all fractionation steps considering the amount of chlorophyll in each fraction. This excludes that the observed differences in P700 and Q contents are due to selective inhibition. Moreover, a calculation based on the molar ratios found for the 2 thylakoid regions (fig.3) and around 40% of total chlorophyll in non-appressed thylakoids [9] gives Chl/Q and Chl/P700 ratios of 550 and 545, respectively, for total chloroplasts. These values are in striking agreement with our experimental ratios for the starting material (table 1) thereby demonstrating the reliability of the present approach.

4. DISCUSSION

The distribution of the 2 photosystems in the thylakoid membrane was investigated by combining 2 selective techniques, the separation of vesicles from appressed and non-appressed regions and direct quantitative determinations of the amounts of PS2 and PS1 reactions centres from the amplitudes of Q and P700, respectively. Fig.3 illustrates the stoichiometry of PS1 and PS2 reaction centres in the non-appressed and appressed thylakoid regions: < 1 Q/30 P700 was determined for the non-appressed thylakoids. In contrast, the appressed grana partitions contain as much as 10 Q/P700. The nearly complete exclusion of PS1 from the grana partitions could be experimentally demonstrated by determining the P700 associated with inside-out vesicles in the B3-fraction. The ratios provide experimental evidence for the postulated extreme lateral asymmetry in the distribution of the 2 photosystems between the 2 thylakoid regions postulated in [2,9,10,20].

Earlier, the concept of extreme lateral heterogeneity was based on characterization of the thylakoid subfractions by rates of photochemical activities [8] and relative contents of chlorophyll–protein complexes [9]. Quantification of the photosystems by electron transport rates under continuous illumination may be obscured by effects of the rate-limiting step. An accurate stoichiometry based on chlorophyll–proteins is hampered by the release of chlorophyll from the reaction center complexes which is a particular problem for the sensitive CPa complex [9]. Moreover, the assignment of the CPa complex to the reaction center of PS2 is so far only indirect. Although, in support for the lateral heterogeneity, these studies could not correct for the contaminating P700 reaction centers deriving from right-sided vesicles in the B3-fraction and verify the great extent of PS1 exclusion from the grana partitions.

Melis and Brown [21] have reported PS2/PS1 ratios ranging between 0.25–2 in sub-thylakoid fractions using a spectrophotometric method. However, in that study a grana stack fraction still possessing non-appressed grana margins and end membranes was compared with a stroma lamellae fraction with a low Chl *a/b* ratio which explains why much less pronounced differences were observed as compared to this study. Tiemann et al. [22] could not detect any differences in the relative content of the photosystems in a B3-fraction and intact thylakoids, respectively. Whether this was due to sub-optimal measuring conditions or to the

preparation cannot be judged since no other parameters such as Chl *a*/*b* ratios were given.

The depletion of Q in the Y-100 fractions is more pronounced than that previously observed for the PS2 chlorophyll–protein complex (CPa). Whether this is in support of suggested heterogeneities on the acceptor side of PS2 [23] cannot be judged at present.

The spatial separation of the two photosystems has important consequences for the mechanism of linear electron transport, since most P700 is functionally coupled to PS2 [19]. Thus, electron transport in chloroplasts of higher plants must proceed from the appressed grana region containing almost exclusively PS2 to non-appressed regions with PS1 [2,9] and not between closely associated entities in the grana.

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