BRIEF COMMUNICATION

The antenna size of Q_B-reducing photosystem 2 complexes in different fractions of subchloroplast particles

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Abstract

The antenna sizes of Q_B-reducing photosystem 2 (PS2) complexes in two different fractions of the subchloroplast particles were compared by measuring time corresponding to the second maximum of the first derivative from induction curve of chlorophyll fluorescence as a function of actinic irradiance. The Q_B-reducing PS2 complexes in the fraction of particles that originated from inner parts of grana thylakoids had smaller antennae than those in the fraction from non-appressed regions of thylakoid membranes.

Additional key words: appressed and non-appressed thylakoids; chlorophyll fluorescence induction; *Pisum sativum*.

Chloroplasts contain two types of PS2 complexes which differ in properties of their acceptor sides (Q_B-reducing and Q_B-non-reducing) (Melis 1991). Since the Q_B-reducing PS2 complexes transport electrons from the primary acceptor, Q_A, to the secondary acceptor, Q_B, these complexes provide the linear non-cyclic transport of electrons. Therefore it is important to study their structural and functional properties. This paper compares the antenna sizes of Q_B-reducing PS2 complexes and estimates the pool size of electron acceptors for these complexes using the analysis of chlorophyll (Chl) fluorescence induction curves. The study of functional properties of Q_B-reducing PS2 complexes in the two fractions of subchloroplast fragments was taken as an example of applying these approaches.

Chloroplasts were isolated as described by Volovik and Kochubeï (1989) from upper well-developed leaves of 2-week-old pea plants grown in a greenhouse. Subchloroplast particles of the fraction 1 (FR1) were obtained by 0.3 % digitonin

fragmentation and centrifugation at 20 000×g. Pellets of the fraction 2 (FR2) were sedimented at 70 000×g from the supernatant obtained through 20 000×g centrifugation. The time of centrifugation was 30 min for both fractions.

Chl fluorescence induction curves were recorded using a laboratory assembled device. Fluorescence measured at 685 nm was excited by a blue radiation of mercury arc. The time of shutter opening was 3 ms. Values were stored by means of a personal computer with a frequency of 9.09 kHz. F_0 (ground level) and F_{pl} [the level of fluorescence at the intermediate plateau on the induction curve; F_{pl} corresponds to F_I in the nomenclature of van Kooten and Snel (1990)] were determined from calculations of the second and first derivatives, respectively, as described by Korneev (1997). Since the fluorescence rise caused by the shutter opening is more rapid than that caused by the reduction of Q_A, the second derivative has a minimum in the time of full shutter opening. The fluorescence level at this moment approaches F_0. The intermediate plateau on the induction curve is the result of a temporary slowing down the fluorescence rise. Thereby, the first derivative has a minimum in the time of reaching the plateau. The fluorescence level at this moment is equal to F_{pl}. Derivatives from the curves were calculated with a digital method (Savitzky and Golay 1964). Chl concentration in the samples was 0.2 kg m^{-3}.

Table 1. Parameters of chlorophyll fluorescence induction curves of two subchloroplast fractions. The actinic irradiance was 5 W m^{-2}. Each value is the mean ± S.E. based on the three independent experiments. For explanation see text.

Fraction	F_v/F_m	$(F_{pl}-F_0)/F_v$	T_{2max1d}	R
FR1	0.77 ± 0.02	0.17 ± 0.02	0.20 ± 0.04	11.2 ± 1.3
FR2	0.55 ± 0.11	0.49 ± 0.11	0.63 ± 0.07	6.9 ± 2.5

The analysis of parameters of the induction curve showed differences between FR1 and FR2 in values of F_v/F_m and $(F_{pl}-F_0)/F_v$ (see Table 1). The $(F_{pl}-F_0)/F_v$ ratio was used for the estimation of the relative amount of Q_B-non-reducing PS2 complexes. The value of F_v/F_m was lower and the value of $(F_{pl}-F_0)/F_v$ was higher for induction curves of FR2 in comparison to FR1. A low F_v/F_m ratio and a high relative amount of Q_B-non-reducing PS2 complexes are distinguishing features for the stroma-exposed thylakoid membranes (Henrysson and Sundby 1990, Melis 1991, Neale and Melis 1991, Wollenberger et al. 1995). Thereby, we suggest that particles of FR2 originate from the non-appressed regions of thylakoid membranes, probably from grana margins. Since the induction curves of FR1 had a higher value of F_v/F_m and a lower value of $(F_{pl}-F_0)/F_v$ than those of FR2, FR1 probably contained fragments from the inner parts of grana thylakoids. This suggestion is also based on the procedure of fragmentation and on the other characteristics of the fractions (77 K fluorescence spectra and results of SDS-polyacrylamide gel electrophoresis are not shown).

The fluorescence rise from F_{pl} to F_m reflects the reduction of Q_A in the Q_B-reducing PS2 complexes (Melis 1991). According to Hsu (1992), kinetics of this

phase depends on the pool size of acceptors and radiant energy influx that is in its turn influenced by cross-section of the light-harvesting antennae and by the actinic irradiance. At a particular moment when the rate of fluorescence increase from F_{pl} to F_m is maximal, plot of the first derivative from the induction curve has the second maximum (Korneev 1997). The time corresponding to this maximum is termed as T_{2max1d}. Level of the T_{2max1d} parameter was higher for induction curves of FR2 in comparison with FR1 (see Table 1). To verify the suggestion that this observation cannot be applied to the difference between fractions in the pool size of electron acceptors, the ratios (R) of complementary areas over the induction curves in the absence and presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (10^{-5} M) were determined (Table 1) according to Zankel and Kok (1972).

Since a part of PS2 complexes is not able to reduce Q_B and has a short chain of electron acceptors, the value of R depends on the relative amount of these complexes that can be calculated from $(F_{pl}-F_0)/F_v$. The ratio of complementary areas over induction curves in the absence and in the presence of an inhibitor must be 1 for a sample containing only Q_B-non-reducing complexes. If only the Q_B-reducing PS2 complexes are presented in a sample, the ratio of complementary areas over induction curves in the absence and presence of an inhibitor is termed X. If the relative amount of Q_B-reducing complexes is equal to $1-(F_{pl}-F_0)/F_v$, then R may be expressed as:

$$R = (F_{pl}-F_0)/F_v + X[1-(F_{pl}-F_0)/F_v].$$

The value of X can be used for estimation of the average amount of electron acceptors per Q_B-reducing PS2 complex. It was calculated by means of the equation:

$$X = [R - (F_{pl}-F_0)/F_v]/[1-(F_{pl}-F_0)/F_v].$$

Values of X for FR1 and FR2 were similar (13.3 and 12.6, respectively). Therefore, the difference of T_{2max1d} can be explained by different antenna sizes of Q_B-reducing PS2. The higher value of T_{2max1d} in the case of FR2 indicates the lower rate of fluorescence increase which is seemingly caused by a smaller antenna size of Q_B-reducing PS2 in the fraction FR2 in comparison with FR1. To confirm this conclusion, the relationship between $1/T_{2max1d}$ and actinic irradiance (I) was studied (Fig. 1). Under the irradiances used, the plot of $1/T_{2max1d}$ against I was close to a line. The linear regression equations were:

$1/T_{2max1d} = 0.26 + 0.35\ I\ (r^2 = 0.96)$ for FR1;
$1/T_{2max1d} = 0.63 + 0.02\ I\ (r^2 = 0.84)$ for FR2.

The coefficient of regression for FR1 was higher than that for FR2. This result can be explained by a relatively larger antenna size of Q_B-reducing PS2 complexes in this fraction.

The time when fluorescence level is equal to $F_{pl} + (F_m - F_{pl})/2$ (the time of half the fluorescence increase from F_{pl} to F_m) may also be used for comparative studies of the antenna size of Q_B-reducing PS2 complexes in cases when the determination of T_{2max1d} is difficult.
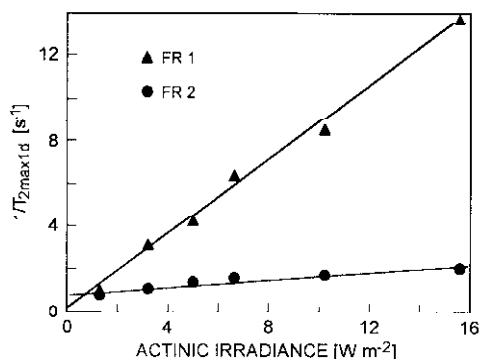
Fig. 1. Changes in value of $1/T_{2max1d}$ for fractions FR1 and FR2 as a function of actinic irradiance.

Moreover, if the suggestion that pellets of FR2 originate from non-appressed regions of thylakoid membranes is true, the results described above are in accordance with certain statements of the PS2 repair cycle hypothesis (Melis 1991), which postulates the existence of Q_B-reducing PS2 complexes with small antenna sizes in this region of the thylakoid membrane. Similar results were gained by Wollenberger *et al.* (1995), who used measurements of PS2 electron transport activity and other procedure of fragmentation.

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