Biochimica et Biophysica Acta, 934 (1988) 151–155
Elsevier

BBA 40213

BBA Report

Minimum photosynthetic unit size in System I and System II of barley chloroplasts

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(Received 19 January 1988)

Key words: Chlorophyll antenna size; Intermittent light plastid; Chloroplast development; Photosynthetic unit; Chlorina f2; (Barley)

The minimum functional chlorophyll antenna size of Photosystem I and of Photosystem II was measured in the chlorophyll *b*-less chlorina f2 mutant of barley grown under intermittent light conditions. In spite of the severely limiting rate of chlorophyll biosynthesis, the chloroplasts assembled functional Photosystem I and Photosystem II complexes containing 95 and 37 chlorophyll *a* molecules, respectively. This may constitute the smallest functional chlorophyll antenna that can be stably assembled in the thylakoid membrane of developing chloroplasts.

The photochemical reaction center of PS I (P-700) is closely associated with Chl *a*-binding proteins which, under denaturing conditions, migrate in the 65–70 kDa region [1–4]. It is believed that, in addition to P-700, these '68 kDa' polypeptides collectively bind about 130 ± 10 Chl *a* and 16 carotenoid molecules. The 68 kDa supramolecular complex, along with the 130 Chl *a* molecules, may form the Chl *a* 'core' antenna of PS I. This PS I-core configuration is evident in cyanobacteria [3,5] and is approximated in the Chl *b*-less chlorina f2 mutant of barley where PS I contains 150 Chl *a* in its antenna [6].

The photochemical reaction center of PS II (P-680) is contained in the D1/D2 (32/34 kDa) heterodimer which also contains the primary electron acceptor pheophytin and primary quinone acceptor Q_A [7]. Closely associated with the photochemical reaction center of PS II are two polypeptides, with apparent molecular masses of 47 and 43 kDa [8], that collectively contain from 35 to 50 Chl *a* molecules. This PS II-core configuration is present in the thylakoid membrane of cyanobacteria [5] and in the Chl *b*-less chlorina f2 mutant of barley [6]. It is believed that of the two polypeptides the 47 kDa forms the inner core antenna; it is directly associated with P-680, so that excitation energy from the antenna Chl *a* of the 43 kDa polypeptide must go through the Chl *a* molecules of the 47 kDa polypeptide in order to reach the photochemical reaction center [8].

We report here the results of a developmental study on the Chl antenna size of PS I-core and PS II-core complexes in the Chl *b*-less chlorina f2 mutant of barley. Chloroplasts in this mutant fail to synthesize Chl *b* [9] and lack all polypeptides and pigments associated with LHC II and LHC I

Abbreviations: PS, Photosystem; Chl, chlorophyll; LHC, light-harvesting Chl *a*/*b* protein complex; IML, intermittent light; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

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[6,9–12]. Consequently, the mutant does not assemble the accessory light-harvesting Chl *a/b* protein complexes, a fact that simplifies the study of the assembly and organization of the PS I and PS II core antenna. This developmental study was undertaken in order to measure the smallest functional Chl antenna size of PS I and PS II in the chlorina f2 mutant of barley that would be assembled in the thylakoid membrane under conditions of limited Chl biosynthesis (growth under intermittent light (IML) regime). Using these light conditions, Chl synthesis and chloroplast development are limited [13,14] allowing characterization of the early stages in the development of PS I and PS II. We were specifically interested in determining whether a photosynthetic unit size smaller than those found in the chloroplasts of fully developed chlorina f2 barley could be functional in the thylakoid membrane.

Barley seeds (chlorina f2) were germinated and grown in moist vermiculite in the dark for 9 days. The etiolated seedlings were subsequently illuminated with IML from incandescent bulbs at a photon flux density of 3 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The IML regime consisted of repetitive cycles of 1 min light followed by 120 min darkness for up to two days. Of several different IML regimes tested, we found that this light regime (1 min L, 120 min D) provided close to the minimum dosage of light required for a sustained chloroplast development. Seedlings were harvested in dim light and chloroplast membranes were immediately isolated by homogenization in 50 mM Tricine (pH 7.8), 10 mM NaCl, 5 mM $MgCl_2$, 400 mM sucrose. The homogenate was filtered through nylon mesh and centrifuged at $1000 \times g$ for 2 min (4 °C) to remove cell debris. The supernatant was then centrifuged at $7500 \times g$ for 8 min. The resulting chloroplast pellet was resuspended in the isolation buffer for subsequent analyses (please see figure legends for specific conditions). The Chl *a* content of the thylakoid membranes was determined in 80% (v/v) acetone extracts from the absorbance at 663 nm using an extinction coefficient of 84 $mM^{-1} \cdot cm^{-1}$ [15]. The Chl content of leaf tissue was determined from absolute methanol extracts using the absorbance at 665 nm and an extinction coefficient of 74 $mM^{-1} \cdot cm^{-1}$ [16]. The concentrations of PS I and PS II complexes and the functional Chl antenna size of PS I and PS II were determined as previously described [17].

The light conditions employed in this work were chosen to provide the plants with enough light to allow synthesis of Chl and protein but prevent complete development of the chloroplasts. Under these conditions the Chl content of chlorina f2 leaves was about 6% of the Chl content of leaves from plants grown under continuous illumination (37 μg of Chl/g fresh weight vs. 660 μg Chl/g fresh weight, respectively.

The effect of the reduced Chl content in the leaf on the Chl antenna size of PS I and PS II was investigated. The functional Chl antenna size of PS I and PS II was estimated from the known ratio of total chlorophyll to a reaction center (Chl/PS I and Chl/PS II) and from the relative rates of light absorption by each photosystem. According to this approach, Chl was assigned to each reaction center in direct proportion to the measured rate. In the past, this method predicted a PS II_α antenna of 230 Chl $(a + b)$ and a PS I antenna of 210 Chl $(a + b)$ molecules in spinach [17,18]. This was subsequently verified upon the isolation of resolved membranes from the grana partition regions (BBY particles [19]) and of a 'native' PS I complex [20]. Moreover, a PS I antenna size of 140 Chl *a* molecules was measured in *Synechococcus* 6301 thylakoids [5], and was later confirmed in isolated PS I complexes from cyanobacterial thylakoids [3,21].

In order to estimate the absolute sizes of the Chl antennae of the photosystems, it was necessary first to determine the total Chl to reaction center ratios (Chl/PS I and Chl/PS II) in the thylakoid membrane of the chlorina f2 grown under the IML and continuous illumination. The results of such measurements revealed a Chl/P-700 = 250 : 1 and $Chl/Q_A = 61 : 1$ in IML grown seedlings (Table I). Both ratios were lower than the corresponding ones in continuous light grown seedlings (Table I), suggesting a smaller photosynthetic unit size for both PS I and PS II in IML plastids. The results of the quantitation of PS I and PS II revealed that the PS II/PS I stoichiometry in IML plants was 4.1 compared with a PS II/PS I = 3.0 in chlorina f2 under continuous illumination (Table I).

The relative Chl antenna sizes of PS I and PS II

in IML-grown chlorina f2 seedlings were estimated from the rates of light absorption by the photosystems using broad-band green light of limiting intensity [17]. In Fig. 1A the kinetics of PS II fluorescence induction are shown for chlorina f2 chloroplasts from plants grown under IML. The semilogarithmic plot of the area growth over the fluorescence induction curve as a function of time is shown in Fig. 2. This kinetic analysis revealed that the fluorescence induction is a single exponential function of time. The rate of light absorption by PS II was defined by the slope of the semilogarithmic plot. The rate of light absorption by PS II (K_{II}) in the IML chlorina f2 chloroplasts was $3.4 \pm 0.2\ s^{-1}$ (Table I).

The rate of PS I photoactivity was determined in KCN-poisoned chloroplasts in which electron donation from plastocyanin to P-700 is inhibited [22]. The kinetics of P-700 photooxidation (under the same limiting green light conditions used for fluorescence induction) are shown in Fig. 1B. The semilogarithmic plot of the ΔA_{700} as a function of time characterized the PS I kinetics as an exponential function of time and defined the rate of light absorption by PS I (K_I) in the IML-grown chlorina f2 barley as $8.8 \pm 1.0\ s^{-1}$ (Table I). These kinetic measurements reveal that PS I and PS II in the chlorina f2 mutant grown with IML are each characterized by a single kinetic component and that the rate of light absorption by PS I is greater than by PS II in the chlorina f2 plants. The results suggest the presence of uniform antenna size for all PS II centers (lack of PS II heterogeneity) and

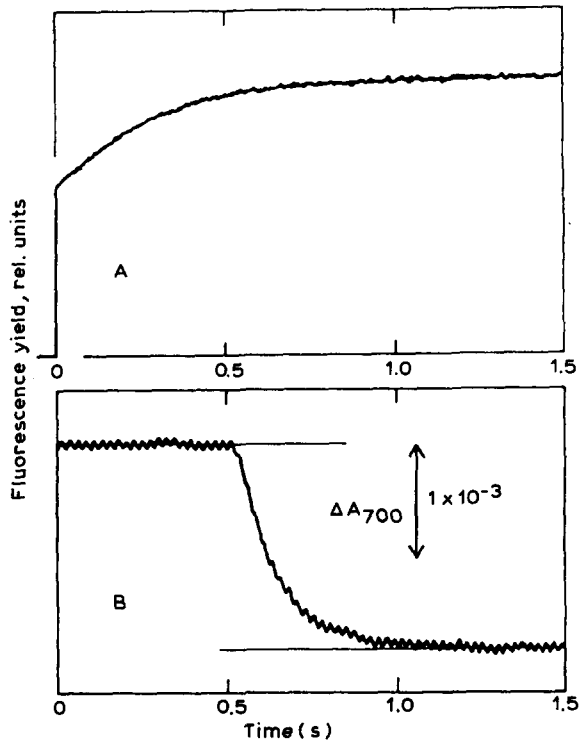

Fig. 1. (A) Room-temperature fluorescence induction kinetics of thylakoids from chlorina f2 barley grown under IML and measured in the presence of 20 μM DCMU. The thylakoid membrane sample contained 10 μM Chl and was supplemented with 1 mM $MnCl_2$. The sample was excited with broad-band green light (Corning CS 3-70 and CS 4-96 filters) at a photon flux density of 75 μmol $m^{-2} \cdot s^{-1}$. (B) Kinetics of P-700 photooxidation in KCN-poisoned thylakoids from chlorina f2 barley seedlings grown under IML. The reaction mixture contained 50 μM Chl, 200 μM methyl viologen, 20 μM DCMU. Illumination as in Fig. 1A. The actinic light was turned on at 0.5 s.

TABLE I

PHOTOSYSTEM CONCENTRATION, RATE OF LIGHT ABSORPTION AND CHLOROPHYLL ANTENNA SIZE IN THE THYLAKOID MEMBRANES OF CHLORINA F2 BARLEY GROWN WITH INTERMITTENT AND CONTINUOUS LIGHT

K_I and K_{II} are the rates of light absorption by PS I and PS II, respectively; N_I and N_{II} are the functional chlorophyll antenna sizes of PS I and PS II, respectively. Numbers are shown with standard deviations ($n = 4$).

Light conditions	Chl / P-700 (mol/mol)	Chl / Q_A (mol/mol)	Q_A / P-700 (mol/mol)	K_I (s^{-1})	K_{II} (s^{-1})	N_I	N_{II}
Intermittent	250±21	61±12	4.1	8.8±1.0	3.4±0.2	95±16	37±2
Continuous	313±11	104±19	3.0	13.1±1.0	4.4±0.1	150±15	50±2

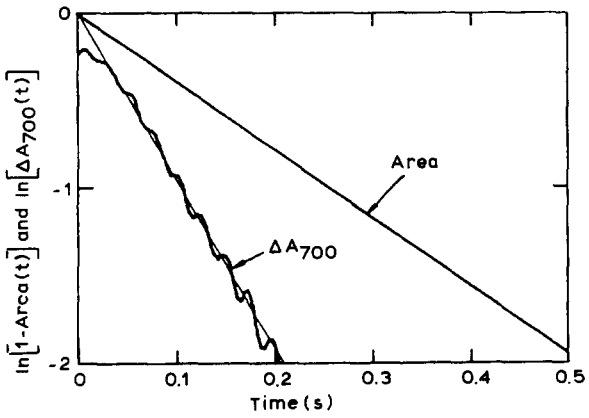

Fig. 2. Semilogarithmic plots of the growth of the area over the fluorescence curve and the 700 nm absorbance change kinetics from the traces shown in Fig. 1A and B, respectively. The slopes of the straight lines defined the rates of light absorption by PS II (K_{II}) and PS I (K_I) in the chlorina f2 chloroplasts.

a functional PS I antenna size that is considerably greater than that of PS II. From the Chl/PS I and Chl/PS II ratio and from the experimentally determined rates of light absorption K_I and K_{II}, the absolute number N_I and N_{II} of Chl molecules transferring excitation energy to the reaction centers of PS I and PS II, respectively, were determined [17,23]. The results of Table I present numerical values of the antenna size for the chlorina f2 mutant grown under IML and under continuous illumination. The Chl antenna of PS I was 95 Chl *a* molecules in the IML-grown plants (vs. 150 Chl molecules in plants grown under continuous light) while the PS II antenna was 37 Chl *a* molecules in IML (vs. 50 Chl molecules under continuous light).

The results presented clearly suggest smaller Chl antenna size for PS I and PS II in chlorina f2 plants grown under IML than continuous illumination. An explanation of the results could be provided by postulating specific changes in the organization of the Chl-proteins such that fewer Chl molecules are associated with each Chl-binding polypeptide. The implication from this hypothesis is that IML-treatment regulates the stoichiometry of Chl and protein within the Chl-binding core polypeptides of PS I and PS II. On the other hand, the lack of Chl in the IML plastids could prevent the stabilization and/or insertion of non-essential Chl-protein components in the core antenna of PS I and PS II, resulting in a smaller Chl antenna size. For example, assuming a 68 kDa tetramer as the basic PS I-core complex [2,3], we noted that elimination of one of the 68 kDa polypeptides would reduce the PS I-core antenna from 130 ± 10 to 97 ± 8 Chl *a* molecules. Alternatively, it is possible that the PS I-core configuration contains a 68 kDa dimer [1,4], containing 95 Chl *a* molecules. In this case, one would have to postulate a PS I complex, both in *Synechococcus* 6301 and in the Chl *b*-less chlorina f2 mutant of barley, which contains an as yet unidentified auxiliary Chl *a* LH antenna. There is precedence in the literature for a PS I-core Chl antenna of 90–95 Chl molecules in developmental mutants of tobacco [18] and in IML-grown wild-type pea plants [23]. In addition, detergent-derived PS I complexes have been isolated with antenna size of about 95 Chl *a* molecules [20,24].

The PS II antenna in the IML-grown chlorina f2 plants is smaller than the PS II antenna of plants grown under continuous light by about 13 Chl *a* molecules. This smaller antenna size was correlated with the absence of a 29 kDa polypeptide from the thylakoid membranes of chlorina f2 when grown under IML conditions (results not shown). In chlorina f2 plants grown under continuous illumination, this 29 kDa polypeptide was identified recently as CP29 [25,26]. Thus, it would appear that CP29 in the chlorina f2 mutant binds approximately 13 Chl *a* molecules.

Finally, the results presented in this work suggest that under conditions of severe limitation in the rate of Chl biosynthesis, the chloroplast will assemble a minimum Chl antenna size containing about 95 Chl *a* molecules for PS I and about 37 Chl *a* molecules for PS II.

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