Photosystem II Heterogeneity in Triazine-Resistant and Susceptible Biotypes of *Chenopodium album*

Jack J. S. van Rensen and Leon E. E. M. Spätjens

Laboratory of Plant Physiological Research, Agricultural University Wageningen, Gen. Foulkesweg 72, 6703 BW Wageningen, The Netherlands

Z. Naturforsch. **42c,** 794–797 (1987); received November 13, 1986

Chloroplasts, Triazine Resistance, α and β Centers, Chlorophyll *a* Fluorescence, Herbicides

The heterogeneity of photosystem II with respect to α and β centers was investigated in triazine-resistant and susceptible biotypes of *Chenopodium album*. In both biotypes the light harvesting antenna sizes of photosystem II α centers was larger than those of β centers. In the resistant biotype the antenna size of the α centers was smaller than those in the susceptible one. There was not much difference in the antenna sizes of the β centers. The proportion of β centers was larger in the resistant biotype compared with the sensitive one.

Introduction

In the chloroplasts of higher plants photosystem II is not homogeneous. Both on the oxidizing and reducing sides of the reaction center chlorophyll, P_{680}, differences have been found. For a recent review on PS II heterogeneity, see Black *et al.* [1]. One type of heterogeneity relates to the chlorophyll fluorescence rise seen upon continuous illumination of dark-adapted chloroplasts in the presence of DCMU. Measurement of chlorophyll fluorescence induction at room temperature of chloroplasts in which electron transfer from Q_A to Q_B has been inhibited by DCMU produces a curve which is not explainable by the kinetics of a single first order reaction. The major part of the curve is sigmoidal; it is followed by an extended slow phase. A quantitative description of this biphasic kinetics has been presented by Melis and Homann [2, 3]. It is based upon the observation that the area above the fluorescence induction curve is proportional to the number of quanta utilized by the reaction center of PS II. In the presence of DCMU, the area growth during the fluorescence rise directly reflects the progress of photochemical charge separation. By analyzing the normalized area growth above the curve two phases can be detected. The first phase displays non-linearity on a semilogarithmic plot, corresponds to the sigmoidal phase and is termed PS II α centers. The second phase is slower than the first one, shows a straight line in a semilogarithmic plot, corresponds to the slow exponential tail of the induction curve and is termed PS II β centers. The rate constants of PS II α and PS II β are determined from their respective slopes in the semilog plot, and the relative proportion of PS II β is determined by extrapolation to zero time.

The differences between the two PS II centers have been widely investigated. One of the differences relates to thylakoid stacking. The thylakoid membranes of the chloroplast are partly exposed to the stroma (non-appressed thylakoids), and partly closely appressed in grana stacks. This structural differentiation is accompanied by a functional heterogeneity. Most of the PS II including its light-harvesting chlorophyll *a*/*b* antenna complex is located in the appressed thylakoid regions and has been ascribed to PS II α. A smaller fraction of PS II is found in non-appressed thylakoids; this type of PS II has been ascribed to PS II β [4].

Resistance to triazine herbicides is caused by a strongly lowered affinity of the herbicide binding site for triazine herbicides [5]. Many herbicides bind to a 32 kDa protein (Q_B-protein) which is part of the PS II reaction center complex and regulates electron transport between PS II and the plastoquinone pool [6, 7]. In triazine-resistant biotypes a small alteration of one single amino acid is observed in the Q_B-protein [8]. In addition, several other differences between resistant and susceptible biotypes have been found: alterations in lipid composition [9, 10], photosynthetic unit size, chlorophyll *a*/*b* ratio and starch

Abbreviations: Amax, normalized area over induction curve; atrazine, 2-chloro-4-(ethylamino)-(isopropylamino)-*s*-triazine; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DNOC, dinitro-*o*-cresol; PS II, photosystem II; Q_A, primary quinone electron acceptor of photosystem II; Q_B, secondary quinone electron acceptor of photosystem II.

Reprint requests to Dr. J. J. S. van Rensen.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/87/0600–0794 $ 01.30/0

accumulation [10]. With respect to chloroplast structure larger and more abundant grana thylakoids were found in the resistant biotypes, compared with the susceptible biotypes [10, 11].

When the amount of grana thylakoids in resistant plants is larger than in the susceptible ones, it can be expected that the proportion of PS II α centers is larger and consequently the proportion of β centers lower. Therefore, we have investigated the relative proportion of PS II β centers in chloroplasts of a triazine-resistant and susceptible biotype of *Chenopodium album.*

Materials and Methods

The origin and growth of the triazine-resistant and susceptible plants of *Chenopodium album* L. was described earlier [12]. Broken chloroplast thylakoid membranes were isolated from the leaves according to a previously published procedure [13]. For the measurement of chlorophyll *a* fluorescence induction the chloroplasts were suspended in a medium containing in 0.7 ml: 50 mM tricine-NaOH (pH 7.6), 0.3 M sorbitol, 5 mM $MgCl_2$; the chlorophyll concentration was 14 $\mu g \cdot ml^{-1}$. The chloroplasts were dark-adapted for 8 min, then DCMU was added at a final concentration of 12.5 μM and after another 2 min dark the measurement was started.

Chlorophyll *a* fluorescence was measured with a Walz PAM fluorescence apparatus. Fluorescence was excited with a LED (type USBR, Stanley) having a broad band at 650 nm, in combination with a DT Cyan filter. Fluorescence was detected by a PIN photodiode (type S 1723, Hamamatsu) screened by a Schott RG9 filter. The signal was stored in 4K words at 12 bit resolution in a Nicolet digital oscilloscope and plotted on a X-Y recorder.

The fluorescence induction curves were analyzed according to the procedure of Melis and Homann [2, 3]. For a more detailed analysis of the area growth kinetics in the presence of DCMU a semilogarithmic plot was constructed of Amax-At/Amax against time. Shown is one pair of representative results out of four experiments.

Results and Discussion

Our resistant and susceptible biotypes of *Chenopodium album* were collected in the same geographical area and are visually indistinguishable from each other when grown in the growth chamber. Resistance to atrazine was frequently checked by measuring the effect of atrazine on the Hill reaction in isolated chloroplasts. Electron flow between Q_A and plastoquinone has a lower activity in the resistant thylakoids. However, the rate of whole chain electron transport is the same in both biotypes. The resistant chloroplasts show a large resistance to triazine herbicides, are slightly less susceptible to DCMU and are more susceptible to DNOC [12]. This type of cross resistance has been found in several triazine-resistant weeds and was correlated with an alteration of serine to glycine at position 264 in the Q_B-protein [14].

We have been comparing several aspects of the photosynthetic process in our triazine-resistant and susceptible *C. album* biotypes [12]. Here, we report on differences in PS II heterogeneity. In Fig. 1 the

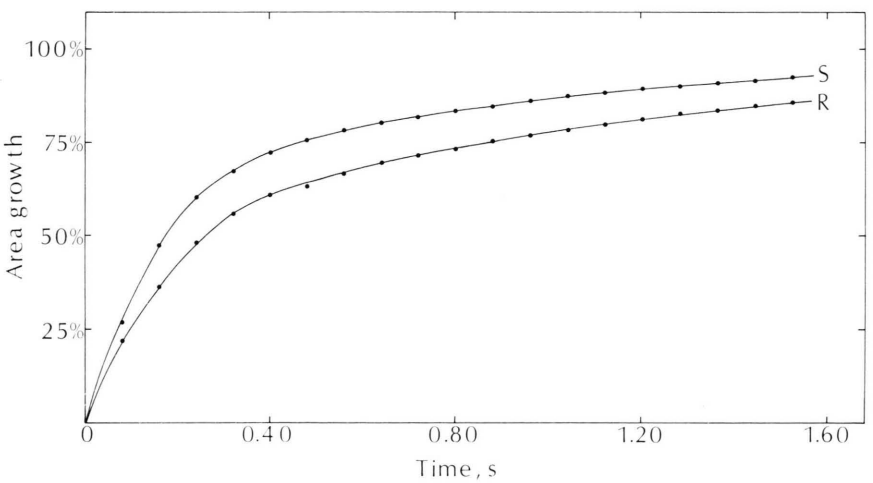

Fig. 1. Kinetics of the growth of the area of the chlorophyll *a* fluorescence induction curves at room temperature in the presence of DCMU of triazine-resistant (R) and susceptible (S) chloroplasts of *Chenopodium album.*

kinetics of the growth of the normalized area over room-temperature fluorescence induction curves in the presence of DCMU are illustrated for chloroplasts of both biotypes. The semilogarithmic plots of these results are shown in Fig. 2. From this figure it can be concluded that the proportion of PS II β centers in the susceptible biotype is 37%, while it is 53% in the resistant one. From the slopes of the curves the rate constants (kα and kβ) for the closure of the traps in PS II α and PS II β can be determined. These rate constants relate to antenna sizes of the centers. For both biotypes the slopes of the fast α components are larger than those of the slow β components. This indicates that the light harvesting antenna sizes of the α centers are larger than those of the β centers which is generally observed. Since the slope of the α component of the resistant biotype is smaller, the antenna size of PS II α is smaller in the resistant biotype compared with the sensitive one. There is not much difference in the slopes of the slow β components suggesting that the antenna sizes of the PS II β centers are almost the same in the two biotypes.

The finding that the proportion of PS II β centers is larger in the resistant biotype is difficult to interprete in the light of the observations that resistant plants have more grana thylakoids [11] and that PS II β centers are located in the stroma thylakoids [3, 4]. However, the functional and structural aspects of PS II α and β centers are still a matter of debate. Hodges and Barber [15] presented data which suggest that the biphasic nature of the induction curves may not simply be described as two distinct forms of PS II located in different membrane regions. Furthermore, there are several observations on α and β centers which may explain our result.

(a) It has been reported that PS II β centers are not associated with the two-electron gate quinone Q_B [16].
(b) Photosystem II β centers have been observed to be less sensitive to DCMU [17]; this might explain the cross resistance to DCMU of our triazine-resistant biotype.
(c) Photosystem II α and β centers have different primary quinone electron acceptors; those of the β centers have a higher midpoint redox potential [18].

It seems important to extend our observation of a larger proportion of PS II β centers in the resistant

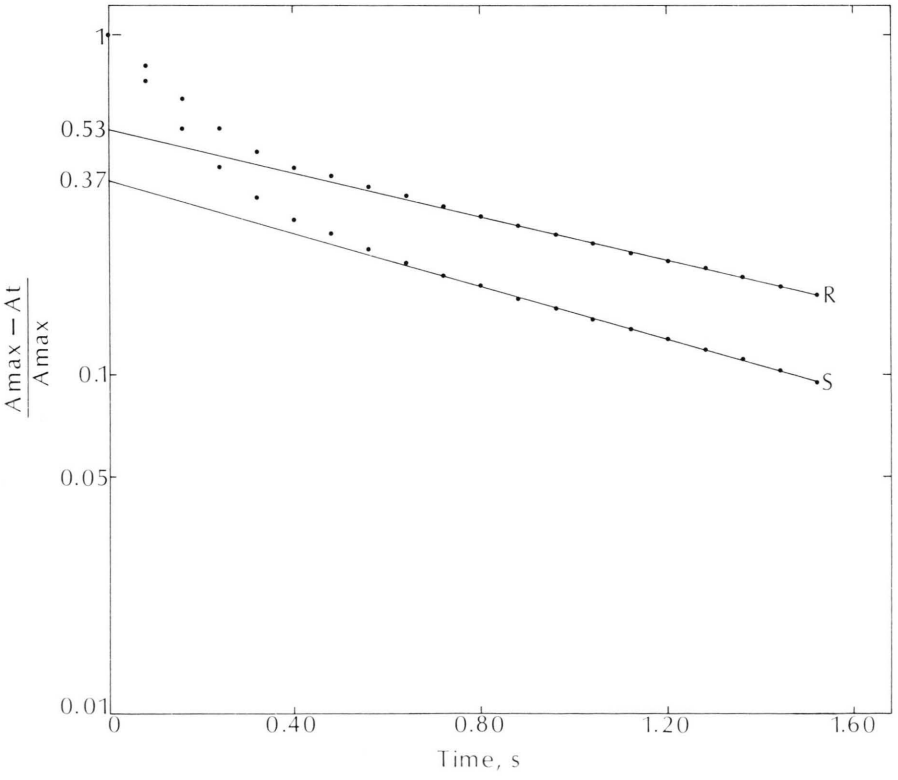

Fig. 2. Semilogarithmic plots of Amax-At over Amax against time of the area growth over the fluorescence induction curves of triazine-resistant (R) and susceptible (S) chloroplasts.

biotype of *C. album* to other triazine-resistant weeds, especially in the light of indications that the 32 kDa Q_B-protein together with a 34 kDa protein may be the reaction center protein of photosystem II [19, 20].

Acknowledgements

We thank Drs. W. F. Buurmeijer, J. F. H. Snel and W. J. Vredenberg for their helpful discussions and comments.

[1] M. T. Black, T. H. Brearly, and P. Horton, Photosynth. Res. **8,** 193–207 (1986).
[2] A. Melis and P. H. Homann, Photochem. Photobiol. **21,** 431–437 (1975).
[3] A. Melis and P. H. Homann, Photochem. Photobiol. **23,** 343–350 (1976).
[4] J. M. Anderson and A. Melis, Proc. Natl. Acad. Sci. USA **80,** 745–749 (1983).
[5] K. Pfister and C. J. Arntzen, Z. Naturforsch. **34c,** 996–1009 (1979).
[6] J. J. S. van Rensen, Physiol. Plant **54,** 515–521 (1982).
[7] J. J. S. van Rensen and J. F. H. Snel, Photosynth. Res. **6,** 231–246 (1985).
[8] J. Hirschberg and L. McIntosh, Science **222,** 1346–1349 (1983).
[9] P. Pillai and J. B. St. John, Plant Physiol. **68,** 585–587 (1981).
[10] J. J. Burke, R. F. Wilson, and J. R. Swafford, Plant Physiol. **70,** 24–29 (1982).
[11] K. C. Vaughn and S. O. Duke, Physiol. Plant **62,** 510–520 (1984).
[12] M. A. K. Jansen, J. H. Hobé, J. C. Wesselius, and J. J. S. van Rensen, Physiol. Vég. **24,** 275–284 (1986).
[13] J. J. S. van Rensen, D. Wong, and Govindjee, Z. Naturforsch. **33c,** 413–420 (1978).
[14] J. Hirschberg, A. Bleecker, D. J. Kyle, L. McIntosh, and C. J. Arntzen, Z. Naturforsch. **39c,** 412–420 (1984).
[15] M. Hodges and J. Barber, Biochim. Biophys. Acta **848,** 239–246 (1986).
[16] A. P. G. M. Thielen and H. J. van Gorkom, Biochim. Biophys. Acta **635,** 111–120 (1981).
[17] U. Schreiber and K. Pfister, Biochim. Biophys. Acta **680,** 60–68 (1982).
[18] A. Melis, FEBS Lett. **95,** 202–206 (1978).
[19] J. Deisenhofer, O. Epp, K. Miki, R. Huber, and M. Michel, Nature **318,** 618–624 (1985).
[20] A. Trebst, Z. Naturforsch. **41c,** 240–245 (1986).