Conserved Kinetics at the Reducing Side of Reaction-Center II in Photosynthetic Organisms; Changed Kinetics in Triazine-Resistant Weeds

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The decay of chlorophyll variable fluorescence after a "single turnover" flash is generally assumed to represent the reoxidation of the reduced quinone Q_a. We have observed that the kinetics of this decay are very similar in a wide variety of species. Comparing 28 different species, we found an average half decay time of 314 ± 46 µsec. No systematic correlations were found between the decay rate and biochemical or physiological specializations such as C2, C4 or CAM. This indicates that structural as well as functional factors controlling photosystem II electron transfer between Q_a and Q_b are highly conserved. Apparently, the freedom for natural structural variations in this region is very limited.

Triazine resistant plants, characterized by an altered amino acid sequence of the D1 protein, have clearly decreased rates of Q_a/Q_b electron transfer. We found an average half decay time of 946 ± 100 µsec (5 species). However, this three-fold decrease is much less than previously reported. Therefore, if alterations of photosystem II electron transfer efficiency contributes to an often reported reduction of "ecological fitness", this contribution is smaller than was hitherto assumed.

Introduction

The core of the photosystem II (PS II) reaction center (RC II) consists of two polypeptides of 30–32 kDa, designated as D1 and D2 and in addition cytochrome b_{559}. This RC II core holds several pheophytin, chlorophyll, carotene and plastoquinone molecules as well as a non-haem iron [1, 2]. Detailed models for the higher plant RC II have been proposed [3] based on the structure of the bacterial reaction center [1].

In RC II a "special pair" of chlorophyll molecules catalyzes the reduction of a pheophytin. The resulting negative charge is subsequently transferred to a primary plastoquinone, Q_a, and to a secondary plastoquinone, Q_b. D2 and D1 are considered as apoproteins of respectively Q_a and Q_b [4]. This mechanism of electron transfer at the reducing side of PS II is basically identical in all photosynthetic organisms where it has been studied [5, 6].

The D1 protein, also known as the 32 kDa or Q_b protein, rapidly turning-over protein or *psb*A-gene product, has been intensively characterized in the last years [7, 8]. It is thought to carry the binding site of Q_b and various classes of herbicides [3, 9]. DNA cloning and sequencing revealed a remarkably high degree of homology in the derived amino acid sequence of the D1 protein from a variety higher plants, algae and photosynthetic bacteria *e. g. Amaranthus hybridus, Anabaena* str. 7120, *Chlamydomonas reinhardtii, Euglena gracilis, Zea mays, Nicotiana* spp, *Rhodopseudomonas* spp, *Sinapsis alba* and *Spinacia oleracea* [7, 10]. Thus one of the unique features of the D1 protein is its extraordinary high degree of evolutionary conservation. It is reasonable to assume that the essential function of the D1 protein has been optimized during evolution. The fact that only minor changes are found in the primary structure of the D1 protein from a wide variety of photosynthetic organisms might indicate that structural changes are unfavourable for the essential functions of this protein. This implies that while the structural properties of the D1 polypeptide have been strongly conserved also functional properties must have been conserved. So, the kinetics of electron transfer mediated by the D1 protein should be very similar in different photosynthetic organisms.

Abbreviations: F_{max}, maximal fluorescence; F_v, variable fluorescence; PS II, photosystem II; RC II, reaction center of photosystem II.

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We compared the kinetics of the decay of chlorophyll fluorescence after a "single turnover" flash, in a variety of higher and lower plants, using a new pulse fluorimetric technique. These kinetics are considered to be an indicator for the functional properties of the D 1 protein or, more specifically, for the reoxidation of reduced Q_a. This kinetic analysis does not distinguish between an alteration of the Q_a-/Q_b equilibrium constant, and a change in the rate constant of the Q_a-/Q_b to Q_a/Q_b-transition [11].

Materials and Methods

Leaf material was collected in the Botanical Garden, University of Basle, Switzerland, and used within 2 h after harvesting. Triazine susceptible and resistant weeds were collected at various locations in Switzerland. *Chlamydomonas reinhardtii* Br 24 was obtained by courtesy of Dr. Rochaix, University of Geneva, Switzerland. For detailed characterization of this mutant see ref. [12]. All experiments were performed on intact material dark adapted for at least 30 min.

The decay of variable chlorophyll fluorescence (in a strict sense we mean decay of fluorescence yield) following a 8 µsec saturating Xenon flash (EG & G FY 611, Salem, M.A.) was studied using modulated measuring light (660 nm) of very low intensity from a pulse fluorometer (PAM 101, Fa. Walz, Effeltrich, F.R.G.), operated at 100 kHz pulse frequency. This technology is described in detail in ref. [13]. The actinic flash was considered short enough to induce only a single charge separation ("Single turnover flash"). Measuring light and flash light were directed on the leaf using fibre optics. The common end of the 4-branched fiber optic system was positioned about 1 mm above the surface of the plant material. Decay curves (4,096 points in 4 msec) were stored temporarily in a digital Nicolet Explorer III (Madison, W.I.) oscilloscope. Analysis of the data was performed on a Hewlett Packard 9920 computer. Several transients were averaged with a 10 sec dark interval between actinic flashes. The reaction rate constant and the half-time of the fluorescence decay were calculated by regression analysis from the first 150 µsec of the first-order plot of $\ln (F_{max}/F_v)$ *versus* time.

Results

The decay curves of variable chlorophyll fluorescence were monitored following a saturating flash. Two typical decay curves and their corresponding first order plots are shown in Fig. 1 and 2. At least two kinetic phases can be visualized in the first order plots (Fig. 2a, b). A first linear phase is arbitrarily defined to start at the beginning of the curve and to last for 150 µsec, followed by a second slower phase. Both phases can be blocked entirely by 10 µM diuron and thus reflect electron transfer reactions involving Q_a and Q_b. A much slower diuron insensitive phase with a time scale in seconds might reflect other mechanisms such as the backflow of electrons to the oxidizing site of PS II (data not shown). The decay of chlorophyll fluorescence following the first 150 µsec phase, is not strictly first order. Probably this reflects more complex and heterogeneous PS II-dependent reactions [14]. Therefore, only the first phase (< 150 µsec) is taken as a direct indicator for the Q_a/Q_b electron transfer kinetics.

The half-times of fluorescence decay were calculated from linear regression analysis of the first phase of each curve and are listed in Table I. The derived half decay times can be clearly grouped into two categories. The first group of plants shows a mean half decay time of 314 µsec. For the second group, consisting exclusively of "triazine resistant" plants, a mean half decay time of 969 µsec was calculated.

The half decay times within the first group are very similar among all species. No systematic de-

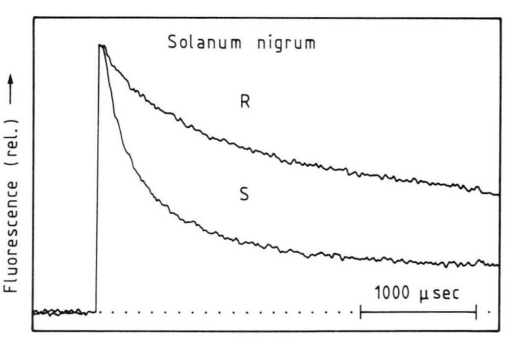

Fig. 1. Decay curves of chlorophyll fluorescence following an actinic "single turnover" flash. Material: intact, fresh leaves of *Solanum nigrum*, susceptible- (S) and triazine-resistant (R) biotype. 16 traces were averaged and normalized to the same maximum fluorescence.

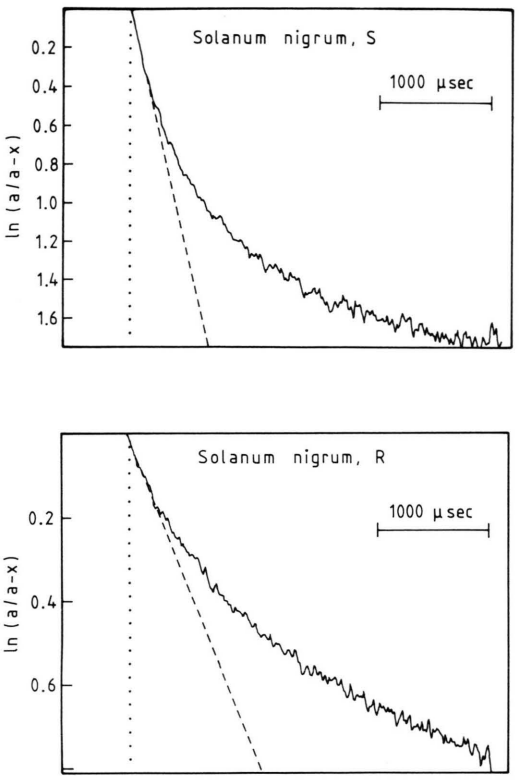

Fig. 2a, b. First-order transformation (ln $[F_{max}/F_v]$) of the decay curves from Fig. 1. A: susceptible biotype, B: triazine-resistant biotype. F_{max} (or a): maximum fluorescence at $t = 0$, F_v (or [a−x]): variable fluorescence at time t. A regression line was fitted to the first 150 µsec of each trace. This phase is considered to reflect the reoxidation of reduced Q_a. Note: both traces are normalized to different scales.

viation from the average half decay time was observed, precluding a correlation with biochemical or physiological specializations like C3, C4, CAM or succulence. Also no correlation became obvious with the evolutionary age of taxonomically diverse species including algae, mosses, ferns, gymnosperms, monocots and dicots.

Clearly, the "triazine resistant" plants showed much longer half-decay times with an average value of 969 µsec (Table I). The decay of fluorescence in resistant biotypes is on the average 3.1 times slower than in sensitive wild types. This change in the Q_a/Q_b reaction kinetics of triazine resistant plants also affects the fluorescence induction transients, in which it had been initially observed [15] and was subsequently studied in detail [16].

Table I. Half-times (µsec) of chlorophyll fluorescence decay curves representing the kinetics of Q_a/Q_b electron transfer in various plant species. The curves were recorded following a 8 µsec saturating flash. The values of the half decay times were calculated by linear regression from the first 150 µsec of the first-order plot (regression coefficients generally $\geqslant 0.99$). Each value is calculated from at least 20 decay curves measured at intervals of 10 sec on two or more leaves (or samples). Material: intact, freshly harvested plants.

Species	Type	$t_{1/2}$ [µsec]
Abies concolor	Gymnosperm	401
Amaranthus hybridus	Dicot	356
Ananas comosus	Monocot	352
Avena sativa	Monocot	325
Brassica napus	Dicot	275
Ceropegia sandersonii	Dicot	328
Chenopodium album	Dicot	269
Chlamydomonas reinhardtii	Green algae	294
Clusia flava	Dicot	260
Colletia cruciata	Dicot	302
Crassula rossularis	Dicot	261
Hypogymnia physodes	Lichen	375
Nephrolepis exalta	Fern	348
Passiflora cinnabarina	Dicot	393
Pinguicula moreansis	Dicot	343
Pisum sativum	Dicot	241
Polytrichum commune	Moss	308
Psilotum nudum	Fern	356
Ruscus aculeatis	Monocot	396
Saccharum officinarum	Monocot	295
Saxifraga stolonifera	Dicot	277
Scolopendium vulgare	Fern	305
Selaginella wallichii	Moss	297
Senecio crassissimus	Dicot	241
Solanum nigrum	Dicot	284
Vanilla plantifolia	Monocot	342
Xerosiyos danguiyi	Dicot	255
Zea mays	Monocot	306
		mean: 314 ± 46 (S.D.)
Triazine-resistant biotypes		
Amaranthus hybridus		1020
Brassica napus		881
Chenopodium album		843
Chlamydomonas reinhardtii (mutant Br 24)*		1050
Solanum nigrum		1054
		mean: 969 ± 100 (S.D.)

* This mutant is slightly triazine-resistant and carries a mutation at codon 256. For a detailed characterization see ref. [12].

Discussion

Similar kinetics of fluorescence decay in a wide variety of species indicate that PS II electron transport between Q_a and Q_b is controlled by very simi-

lar structural and functional factors. This has been demonstrated at the molecular level, where polypeptides making up the PS II complex show a high degree of homology in amino acid sequence in different species [7]. In addition to structural evidence, our functional data showing conserved kinetics, also indicate that the RC II region involved in binding the primary and secondary quinones and in mediating electron transport between these quinones has been extremely conserved during evolution. Apparently, the freedom for structural variations in this region is very limited. This could mean for example that most or all mutations in the quinone binding region of the D 1 protein decrease the efficiency of the electron transfer and thus constitute a disadvantage for plant survival. This view can be supported by studies of "triazine resistant" plants which have an altered amino acid sequence of the D 1 protein [3, 7, 17, 18] and clearly decreased rates of Q_a/Q_b electron transfer. These plants are often reported to show decreased photosynthetic performance, biomass production and ecological "fitness" [19, 20].

Our results indicate that the rate of electron flow between Q_a and Q_b in the resistant biotypes is much less decreased than previously described. Pfister and Arntzen [15] reported a ratio of >10 for the decay times of resistant *versus* susceptible plants. Similar data were also published by Bowes *et al.* [16]. These data were obtained using isolated thylakoids. These decay times included also the slower kinetic phases. A recalculation of the published data (first-order analysis of the first 150 μsec) reveals half decay times comparable to our data. Thus, the contribution of an altered PS II efficiency to an often reported reduction of "fitness" may be smaller than previously assumed. It should also be noted, that not all mutations near the quinone binding niche at D 1 lead to an equally strong impairment of electron transfer kinetics [12].

It has been argued that several steps in photosynthesis are kinetically much slower than the Q_a/Q_b reaction kinetics and are thus more significant in limiting the overall rate of photosynthetic electron transport. The rate limiting step in intersystem electron transport is the oxidation of plastoquinol ($t_{1/2}$ = 10 msec) [21]. A turnover time of 3.5 to 14.5 msec has been estimated for the entire electron transport from water to CO_2 [22] and a turnover time of around 5 msec for the linear electron transport [23]. From this it has been argued, that a decreased rate of Q_b reduction in triazine-resistant plants will not significantly affect overall photosynthetic electron transport or plant growth. In contrast to observations of decreased ecological fitness [19, 20] several groups have noted a lack of difference between resistant and sensitive biotypes with respect to photosynthetic electron transport and biomass production [11, 24, 25]. Furthermore, there is firm evidence, that biomass production and crop yield are not limited by primary photosynthetic performance but by CO_2-fixation, assimilate partitioning and sink strength [22, 26, 27]. This is also supported by our data showing almost identical values of fluorescence decay times in high performance crops auch as corn (306 μsec) or sugarcane (295 μsec) and of slow growing plants like mosses (297 μsec) or lichens (375 μsec) (Table I).

The lack of a direct correlation between Q_a/Q_b reaction kinetics, primary photosynthetic performance, biomass production and ecological "fitness" provides no evidence, as to why the rate of Q_a/Q_b reaction has been kept kinetically very constant during evolution. So far, the "triazine-resistant" plants are the only case, where a significant change in the Q_a/Q_b reaction kinetics has been observed and where the change is often reported to be correlated with a loss of productivity and ecological "fitness". We suggest that the change in the D 1 protein, found in triazine-resistant weeds, and visualized as changed kinetics of the Q_a/Q_b reaction does not simply decrease primary photosynthetic efficiency and biomass productivity *via* a direct effect on photosynthetic electron flow. We speculate that the mutation in the D 1 protein affects also some other functional properties of the RC II complex, important for regulating photosynthesis and biomass production, *e.g.* the turnover of the D 1 protein.

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