Regular paper

Polyphasic rise of chlorophyll a fluorescence in herbicide-resistant D1 mutants of Chlamydomonas reinhardtii

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Abstract

Chlorophyll (Chl) a fluorescence transient, a sensitive and non-invasive probe of the kinetics and heterogeneity of the filling up of the electron acceptor pool of Photosystem II (PS II), was used to characterize D1-mutants of Chlamydomonas reinhardtii. Using a shutter-less system (Plant Efficiency Analyzer, Hansatech, UK), which provides the first measured data point at 10 μs and allows data accumulation over several orders of magnitude of time, we have characterized, for the first time, complete Chl a fluorescence transients of wild type (WT), cell wall less (CW-15) C. reinhardtii and several herbicide-resistant mutants of the D1 proteins: D1-V219I*, A251V, F255Y, S264A G256D and L275F. In all cases, the Chl a fluorescence induction transients follow a pattern of O-J-I-P where J and I appear as two steps between the minimum Fo (O) and the maximum Fmax (P). The differences among the mutants are in the kinetics of the filling up of the electron acceptor pool of PS II (this paper) in addition to those in the re-oxidation kinetics of QA to QA, published elsewhere (Govindjee et al. (1992) Biochim. Biophys. Acta: 110h 353-358; Strasser et al. (1992) Archs. Sci. Genève 42: 207-224) and not in the ratio of the maximal fluorescence Fm to the initial fluorescence Fo. The value of this experimental ratio is Fm/Fo = 4.4 ± 0.21 independent of the mutation. At 600 W m⁻² of 650 nm excitation, distinct hierarchy in the fraction of variable Chl a fluorescence at the J level is observed: S264A > A251V ~ G256D > L275F ~ V219I > F255Y ~ CW-15 ~ WT. At 300 and 60 W m⁻² excitation, a somewhat similar hierarchy among the mutants was observed for the intermediate levels J and I. Addition of bicarbonate-reversible inhibitor formate did not change the O to J phases, slowed the I to P rise, and in many cases, slowed the decay of fluorescence beyond the P level. These observations are interpreted in terms of formate effect being on the acceptor rather than on the donor side (S-states) of PS II. The formate effect was different in different mutants, with L275F being the most insensitive mutant followed by others (V219I, F255Y, WT, A251V and S264A). Further, in the presence of high concentrations of DCMU, identical transients were observed for all the mutants and the WT. The quantum yield of photochemistry of PS II, calculated from 1 – (Fo/Fm), is in the range of 0.73 to 0.82 for the WT as well as for the mutants examined. Thus, in contrast to differences in the kinetics of the electron acceptor side of PS II, there were no significant differences in the maximum quantum yield of PS II, among the mutants tested. We suggest that earlier photochemistry yield values were much lower (0.4–0.6) than those reported here due to either higher measured values of Fo by instruments using camera shutters, or due to the use of cells grown in less than-optimal conditions.

Introduction

A variety of herbicides of agricultural importance inhibit photosynthesis by displacing QA, the secondary
plastoquinone acceptor of Photosystem II (PS II) (see Velthuys 1981; Trebst 1991; Oettmeier 1992). Different amino acid substitutions that confer resistance to a variety of PS II herbicides are clustered in the QB-binding region between helices IV and V from residue 211 to 275 of the D1 subunit of PS II (see a review by Diner et al. 1991). One of the mutants (D1-S264A) of Chlamydomonas reinhardtii markedly increases tolerance (5000 times) towards the herbicide metribuzine, where D1 serine 264 is changed to alanine. The loss of the D1 serine 264 is highly significant in herbicide tolerance, indicating that it plays an important role in herbicide binding. In addition to affecting herbicide binding, these alterations also modify, to different degree, certain functional properties of PS II (Erickson et al. 1989) including sensitivity to bicarbonate-reversible formate on chlorophyll a (Chl a) fluorescence transients (Govindjee et al. 1991), and on the kinetics of Chl a fluorescence decay yield, after single-turnover flashes (Govindjee et al. 1992; Strasser et al. 1992; Crofts et al. 1993). Govindjee et al. (1992) suggested that the amino acid substitutions at S264 and at G256 in C. reinhardtii altered the equilibrium for $Q_A$ $Q_B = Q_A Q_B^{-}$ reaction and the ratio of slow to the fast PS II centers showing a modification at the $Q_B$ binding site. By the analysis of the electron transfer kinetics, Crofts et al. (1993) showed a marked decrease in the rate of reduction of bound plastoquinone to the bound semiquinone in A251V and S264A mutants. In the S264A mutant, the second electron transfer was also slower but was normal in the A251V mutant. However, the G256D mutant showed normal electron transfer after the first flash but slower after the second flash. These results suggest differential involvement of S264 and G256 with protonation events (Crofts et al. 1993). On the other hand, other amino acids like D1 V219, F255, L275 were of marginal importance for the reactions at the $Q_A Q_B$ complex except that these reactions in the mutant D1 L275F are insensitive to bicarbonate-reversible formate effect (Govindjee et al. 1991, 1992; Strasser et al. 1992).

In contrast to Chl a fluorescence decay measurements, that measure $Q_A^{-}$ to $Q_B^{-}$ kinetics, Chl a fluorescence induction transients measure the kinetics of the filling up of the plastoquinone (PQ) pool and the heterogeneity associated with it (see reviews by Govindjee and Papageorgiou 1973; Papageorgiou 1975; Lavorel and Etienne 1977; Govindjee and Satoh 1986). During Chl a fluorescence rise, the electron acceptor pools ($Q_A$, $Q_B$ and PQ, etc.) of PS II are filled with electrons originating from water on the donor side of PS II; its kinetics represent all the PS II steps. The inflections in the transients represent 'quasi-steady states' not only related to reduced $Q_A$ but related as well to the heterogeneity in the PQ (a fast versus slow reducing) pool. Three different rise components have been distinguished when Chl a fluorescence induction transients are measured in vivo (Schreiber and Neubauer 1987; Neubauer and Schreiber 1987; Strasser and Govindjee 1991, 1992; Strasser et al. 1995). The O to J phase, that reflects mainly the reduction of $Q_A$ to $Q_A^{-}$ (photochemical phase), also includes the influence of the S-states (Delosme 1967; Schreiber and Neubauer 1987; Hsu 1993). The J to I and I to P phases are due to the filling up of the heterogeneous fast and slow PQ-pool (Strasser et al. 1995). P is followed by a decay, via a level S, to the terminal steady state T. The entire phenomenon is, as expected, dependent upon the intensity of excitation light as shown for plants and cyanobacteria (Strasser et al. 1995).

In this paper we present (i) for the first time, the characterization of complete fluorescence transients of several (F255Y, V219I, A251V, L275F, S264A, G256D) D1-herbicide resistant mutants, (ii) the maximum quantum yield of PS II ($Phi_{max}$), calculated from the ratio of variable to maximal fluorescence yield $((F_p-F_{o})/F_{p})$, and (iii) the characterization of the Chl a fluorescence transients of the D1 mutants in the presence of DCMU and formate which differently block electron transfer on the electron acceptor side of PS II (see e.g. Blubaugh and Govindjee 1988; Govindjee and Van Rensen 1993).

Our work has revealed a high quantum yield of PS II but differences in the kinetics of the filling up of the electron acceptor pool of PS II among the six D1-herbicide mutants of C. reinhardtii. Fluorescence transient data presented here allows one to distinguish the different sites of action of inhibitors on the acceptor side of PS II.

Materials and methods

Growth conditions of the algae

Wild type Chlamydomonas reinhardtii 137C and nine mutants ((Dr-2 (V219I #1), DR-18 (V219I #2); Ar-207 (P255Y); MZ-2 (A251V); DCMU-4 (S264A); AR-204 (G256D #1); BR-24 (G256D #2); Br-202 (L275Y) and CW-15 (a cell wall less)) were grown mixotrophically in tris-acetate phosphate medium at pH 7 (Gorman and Levine 1965) on agar plates. Cultures were grown in