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Multiple action sites for photosystem II herbicides as revealed by delayed fluorescence

ILYA R. VASIL'EV, DMITRY N. MATORIN, VLADIMIR V. LYADSKY & PAVEL S. VENEDIKTOV

Department of Biophysics, Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow 119899, USSR

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Abstract. DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) at concentrations higher than 10 μM suppresses the second time range delayed fluorescence (DF) of pea chloroplasts, due to inhibition of the oxidizing side of photosystem II (PS II). The inhibition of the reducing side of PS II resulting in the suppression of millisecond DF takes place at much lower ($\sim$ 0.01 μM) DCMU concentrations. The variation in the herbicide-affinities of the reducing and oxidizing sides of PS II is not the same for DCMU and phenol-type herbicides. The DCMU-affinity of the oxidizing side considerably increases and approximates that of the reducing side upon mild treatment of chloroplasts with oleic acid. Probably this is a result of some changes in the environment of the binding site at the oxidizing side. At DCMU concentrations higher than 1 mM, the chaotropic action of DCMU leads to the generation of millisecond luminescence which is not related to the functioning of the reaction centres.

Abbreviations: D-1 – The 32 kDa 'herbicide-binding' intrinsic polypeptide of PS II, the apoprotein of Q_B; D-2 – The 32-34 kDa intrinsic polypeptide of PS II, probably the apoprotein of Z; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DF – Delayed fluorescence; Dinoseb – 2,4-dinitro-6-*sec*-butylphenol; DNOC – 4,6-dinitro-o-cresol; F_m – Maximal fluorescence yield (when all traps are closed); F_o – Constant fluorescence yield (when all traps are open); PS – Photosystem; Q_A and Q_B – The primary and secondary plastoquinone acceptors of PS II, correspondingly; Z – A plastoquinol electron donor, presumably associated with the D-2 protein.

Introduction

DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) and many other herbicides act by blocking the electron transfer from the primary (Q_A) to secondary (Q_B) plastoquinone acceptor of photosystem II (PS II) (Van Assche and Carles 1982, Vermaas et al. 1984). The DCMU-type herbicides were shown to bind to the 32 kDa Q_B-protein (D-1) (Mullet and Arntzen 1981). The phenol-type herbicides are less efficient inhibitors of electron

transfer both on the reducing and oxidizing sides of PS II (Van Assche and Carles 1982, Mathis and Rutherford 1984, Pfister and Schreiber 1984, Hideg and Demeter 1986). While most of the authors have not mentioned DCMU effect on the PS II oxidizing side (Van Assche and Carles 1982, Delrieu 1984, Mathis and Rutherford 1984, Pfister and Schreiber 1984, Hideg and Demeter 1986), there are also the results indicating the DCMU effect at higher concentrations on this side of PS II (Etienne 1974, Carpentier et al. 1985). We investigated the action of DCMU and two phenol-type herbicides, dinoseb (2,4-dinitro-6-*sec*-butylphenol) and DNOC (4,6-dinitro-*o*-cresol), on the oxidizing and reducing sides of PS II by measuring their effects on the delayed fluorescence (DF) of chloroplasts.

Materials and methods

Class C chloroplasts were isolated from 15-day-old pea leaves as described elsewhere (Whatley and Arnon 1963) using a medium containing 50 mM Tris-HCl buffer (pH 7.8), 400 mM sucrose and 10 mM NaCl and stored at − 196 °C in the medium containing 10% (v/v) glycerol. The PS I chlorophyll-protein complexes were isolated from spinach chloroplasts by the method of Bengis and Nelson (1975) modified as described in Vashakmadze et al. (1983). Before measurements the samples were diluted in the medium to a chlorophyll concentration of 30 μg/ml and incubated for 2 min in the presence of a herbicide at room temperature. When mentioned, the chloroplasts were incubated for 5 min with different concentrations of the oleic acid (Serva). The ethanol content in the samples, upon the addition of the herbicides and oleic acid solutions, did not exceed 2% (v/v), a concentration that causes no changes of the measured parameters in the control samples. The herbicides used were recrystallized from ethanol after purification with the activated carbon.

DF was measured with a rotating-cylinder phosphoroscope. The duration of the excitation period was 1.3 ms and DF was measured for 1.3 ms starting from 2 ms after the end of the excitation period. The actinic light provided from a 300-W halogen lamp was screened by 5-cm of water and a red cut-off filter ($\lambda > 630$ nm). The light intensity at the surface of the cuvette was 250 W/m^2. The samples were illuminated for about 1 min to allow DF reach a stationary level and then the illumination was ceased with a shutter within 2 ms. The stationary intensities of the millisecond and long-lived (determined 2 s after the cessation of the ilumination) DF components were estimated from the amplified DF signals displayed on a line recorder. The constant fluorescence (F_o) was measured with a fluorimeter by illuminating

the dark-adapted samples with series of probe flashes in the presence of DCMU. The maximum level of fluorescence (F_m) was measured the same way after the photoreduction of Q_A under constant actinic illumination with blue light (350 nm $< \lambda <$ 450 nm, 5 W/m^2).

Results and discussion

As seen from Fig. 1, the millisecond and long-lived DF components behave in different ways with increasing DCMU concentration. The millisecond DF is suppressed by 0.01 μM DCMU. Increasing the concentrations above 10 μM results in the suppression of long-lived DF. The concentration dependence of the extent of inhibition of millisecond DF by DCMU has a less steep pattern than that of the long-lived DF. This may be explained in terms of the heterogeneity of DCMU-affinity of the PS II centers (Black et al. 1986) or may be a result of the heterogeneous nature of the precursors of the millisecond components of DF (Malkin 1977). The suppression of millisecond DF by low DCMU concentrations is known to be associated with the inhibition of electron flow on the reducing side of PS II (Lavorel 1975), occurring due to DCMU binding to the D-1 protein (Mullet and Arntzen 1981). The suppression of the long-lived DF by DCMU has not been reported in the literature. It cannot be ascribed to the inhibition of the electron transfer from Q_A to Q_B because of the high concentration of DCMU required. Rather, it may be due to the inactivation of the oxygen-evolving system, since in the presence of DCMU the long-lived DF with $t_{1/2} \simeq 2$ s arises from the reoxidation of Q_A^- in a reaction with the S_2-state of the oxygen-evolving system (Rutherford et al. 1984). In this case, the suppression of the long-lived DF must be accompanied by the inhibition of Q_A^- dark reoxidation, like with other inhibitors of the PS II oxidizing side (Bennoun

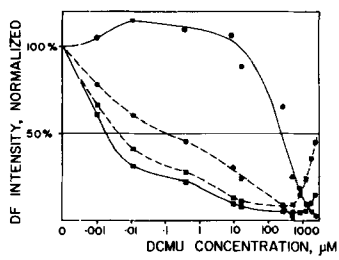

Fig. 1. The intensity of the long-lived (●) and millisecond (■) DF of pea chloroplasts as a function of DCMU concentration (solid lines). Dashed lines—chloroplasts treated with the oleic acid, 0.35 μmols/mg chlorophyll. The curves are normalized to the DF intensities in the absence of DCMU.

1970). In fact, DCMU, at concentrations above 10 μM, was observed to increase the contribution of the slow component of the dark recovery of F_m to the F_0 level (Fig. 2), a process which reflects the dark reoxidation of Q_A^- (Bennoun 1970).

We investigated the dependence of the DF intensity on the concentrations of DNQC and dinoseb, the phenol-type herbicides, which act both on the reducing and the oxidizing sides of PS II. These herbicides, like DCMU, suppress the two components of DF at different concentrations, but the difference between the inhibitory concentrations for the long-lived and millisecond DF is smaller than in the case of DCMU, especially for dinoseb (Fig. 3).

It has been shown that the modification of the PS II proteins by detergent treatment during the isolation of subchloroplast particles results in an increased DCMU affinity of the PS II oxidizing side (Carpentier et al. 1985). We observed a considerable increase of the DCMU inhibitory effect on the long-lived DF in chloroplasts treated with oleic acid in concentrations, which do not affect the long-lived DF. In such preparations the DCMU affinity of the oxidizing side approximates that of the reducing side (Fig. 1). The nature of the protein responsible for the herbicide effect on the oxidizing

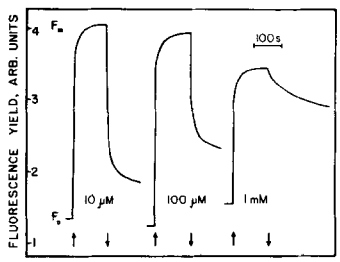

Fig. 2. Kinetics of the dark recovery of the maximum fluorescence yield (F_m) of pea chloroplasts in the presence of different DCMU concentrations (↑ — actinic light on, ↓ — actinic light off).

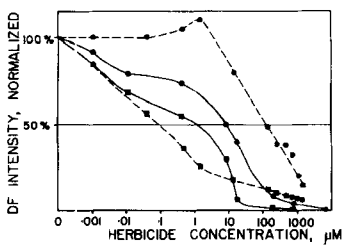

Fig. 3. The intensity of the long-lived (●) and millisecond (■) DF of pea chloroplasts as a function of dinoseb (solid lines) and DNOC (dashed lines) concentrations. The curves are normalized to the DF intensities in the absence of herbicide.

side of PS II is not known. Carpentier et al. (1985) have proposed that this may be the D-2 protein in which the segments of the polypeptide chain containing the plastoquinone-binding site are by 50% homologous to those in the D-1 protein (Murphy 1986). On other hand, recent data indicate that the 'herbicide-binding' D-1 protein has a dual function at both the reducing and oxidizing sides of PS II (Metz et al. 1986, Ikeuchi and Inoue 1987). Whichever the case, we suppose that the herbicide-binding sites in the oxidizing and reducing sides are rather similar, but the herbicide-affinity of the oxidizing side is lower. Presumably, this is a result of some differences in the environment of the binding sites. The variation in their affinities is not the same for DCMU and different phenol-type herbicides. The treatment with oleic acid probably changes the native conformation of the binding site at the oxidizing side such that its affinity approaches that of the reducing side.

As DCMU concentration in the chloroplast suspension was increased to 2 mM we also observed the appearance of a new millisecond luminescence. DCMU does not remain in water solution at concentrations higher than 0.5 mM. The appearance of luminescence at higher DCMU concentrations is apparently associated with the accumulation of DCMU in the thylakoid membranes as a consequence of its redistribution between the medium and the lipid phase of the membrane (Izawa and Good 1965). The accumulation of DCMU is manifested by the slow, light-independent development of the light emission after the addition of the herbicide (not shown). This luminescence is not related to reaction centres activity, since it is not suppressed by treatments inhibiting the millisecond DF, such as heating to 65 °C or treatment with a high concentration of oleic acid (Fig. 4). We also see the appearance of this luminescence at high concentrations of DCMU in native and thermally inactivated chlorophyll-protein complexes of PS I (Fig. 4). The luminescence rise may be related to changes in the state of chlorophyll

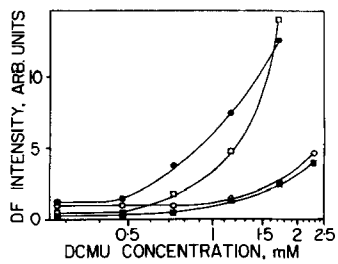

Fig. 4. The intensity of the millisecond DF as a function of DCMU concentration: (○)—pea chloroplasts heated for 5 min at 65 °C; (●)—pea chloroplasts treated with the oleic acid, 2 μmols/mg chlorophyll; (□)—spinach PS I chlorophyll-protein complexes; (■)—PS I chlorophyll-protein complexes heated for 5 min at 70 °C.

due to the chaotropic action of high DCMU concentrations (Garab et al. 1975, Horvath et al. 1984), inducing the chlorophyll-sensitized photochemical reactions, probably of the same origin as in the case of chlorophyll incorporated in liposomes (Krasnovsky and Semenova 1982).

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