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DICHLOROPHENYLUREA-RESISTANT OXYGEN EVOLUTION IN *CHLORELLA* AFTER CERULENIN TREATMENT

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Summary

Fluorescence spectra at 77 K, oxygen evolution at 30°C and delayed fluorescence at 25°C were measured in *Chlorella pyrenoidosa* cultures with and without cerulenin and subsequent 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) treatment, respectively. In pure algal cultures the oxygen evolution was inhibited by DCMU and the long-time component of fluorescence was highly influenced by DCMU, as expected. In contrast, both oxygen evolution and delayed fluorescence became DCMU-resistant in cerulenin-treated cultures. The DCMU-resistance is correlated with a change in the fatty acid distribution of the thylakoid membrane, which also leads to changes in the prompt fluorescence. Cerulenin appears to be a promising new tool of diagnostics for the hitherto unsatisfactorily understood processes of oxygen evolution in photosynthesizing organisms.

In whole algal cells, subcellular preparations, chloroplasts and chloroplast fractions of higher plants, the reduction of Hill acceptors and the oxygen evolution in general have been found to be inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU).

Diner and Mauzerall [1] were the first to report a DCMU-resistant oxygen evolution in cell-free preparations of *Phormidium luridum* in the presence of one-electron oxidants, a reaction which proceeds faster in the presence of Ca^{2+} [2]. Such effects can be seen with artificial acceptors, such as Hg^{2+}, silicomolybdate, etc. [3—9]. In all these cases shunted electron transport was assumed to operate. According to Regitz and Ohad [10] and Renger [11—12], trypsinization of chloroplasts removes the DCMU-inhibition site of Photosystem II, without destroying the oxygen-evolving system.

Abbreviation: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

Several properties of synchronized *Chlorella pyrenoidosa* cultures treated by cerulenin*, a specific inhibitor of fatty acid biosynthesis [13—16], were studied with the aim of revealing the role of lipids in the function of Photosystem II. In the course of these investigations an unexpected phenomenon was observed: cerulenin induced profound structural and functional changes in cells, but the oxygen-evolving system remained intact. Even more surprising, it was found to have lost its normal sensitivity to the Photosystem II inhibitor. This appears to be the first report in the literature of a cellular system capable of evolving oxygen at normal rates in the presence of DCMU and the absence of an artificial electron acceptor.

Chlorella pyrenoidosa algal cells were grown as synchronized cultures at 25°C under high light conditions in normal air without additional CO_2. 22.5 μM cerulenin was added to cell suspensions. After 72 h light absorption, prompt fluorescence, delayed fluorescence, oxygen evolution, light-microscopic and electron-microscopic structures and some other characteristics of cerulenin-treated and normal cell suspensions were compared. Cerulenin treatment arrested cell division and caused dramatic changes in the structure (plasmolysis, fragmentation of the pyrenoid grains, alteration in the organization of the chloroplast).

The absorption spectrum (measured at 20°C with integrating sphere) shows practically no change on cerulenin treatment other than a small increase in the region of carotenoid absorption. The ratio of chlorophylls to carotenoids decreases from 4.48 to 3.11, whilst the amount of chlorophyll per cell remains practically unchanged.

The spectral distribution of prompt fluorescence exhibits much greater changes after cerulenin treatment: a decrease in the 680—700 nm region and an increase at longer wavelengths (Fig. 1). Since the fluorescence bands are as-

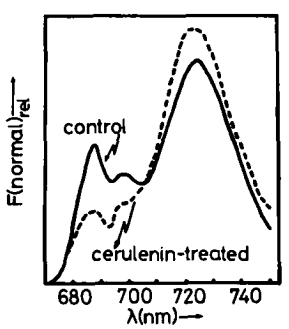

Fig. 1. Prompt fluorescence spectra of *Chlorella* at 77 K normalized to unit area, measured with a Perkin-Elmer MPF-44A spectrofluorimeter. Excitation at 480 nm; reabsorption is less than 5%.

sociated with the integration of the different spectral forms of chlorophylls and the thylakoid membrane [17], the changes of the fluorescence spectrum can be attributed to changes in the membrane structure in qualitative correlation with the changes in chloroplast structure brought about by cerulenin treatment. According to preliminary measurements the ratio of saturated to unsaturated fatty acids is about 1 in untreated, control suspensions. The total

*Cerulenin, (2*S*),(3*R*),2,3-epoxy-4-oxo-7,10-dodecadienoyl amide, is an antibiotic produced by the fungus *Cephalosporium caerulens*.

amount of fatty acids in cerulenin-treated cultures decreases to 40—60% of the controls, but the ratio of saturated to unsaturated fatty acids increases up to about 3 or more. This leads to changes in chlorophyll-chlorophyll, chlorophyll-lipid, chlorophyll-carotenoid and chlorophyll-protein interactions, energy distribution and migration processes; the alterations, e.g. in fluorescence properties, are thus understandable.

In contrast to the above changes, the oxygen evolution (measured with a Clark electrode at 30°C) after cerulenin treatment remains intact (Table I). In control cultures the oxygen evolution is inhibited by 10 μM DCMU, as expected. In cerulenin-treated cultures, however, no DCMU-inhibition of oxygen evolution appears, in spite of the absence of any artificial electron acceptor. This is an astonishing, unique phenomenon in the behavior of Photosystem II.

TABLE I

THE EFFECTS OF DCMU ON THE OXYGEN EVOLUTION AND DELAYED FLUORESCENCE OF *CHLORELLA* FROM SYNCHRONIZED CULTURES

Cerulenin treatment was for 72 h in growth medium containing 22.5 μM cerulenin. Oxygen evolution was measured at 30° C and delayed fluorescence at 25° C. Reproducibility was within 10%. Tr, traces.

C. pyrenoidosa	O_2-evolution in μmol O_2 / nmol chlorophyll per h	Time constants of the components of delayed fluorescence (ms)		
Control	0.62	0.93	3.0	20
Control + DCMU	Tr		42.0	
Cerulenin treatment	0.69	0.95	3.2	16
Cerulenin treatment + DCMU	0.65	1.80	8.6	27

Delayed fluorescence also being peculiar mainly to Photosystem II, its response to cerulenin treatment was investigated too.

Cerulenin treatment (22.5 μM) alone has practically no influence on the time course of delayed fluorescence (Fig. 2A and B). 10 μM DCMU-addition induces a great change in the control (Fig. 2A), but in cerulenin-treated samples addition of DCMU leaves the general appearance of the time course of delayed fluorescence essentially unchanged (Fig. 2B). Similar conclusions can be drawn if the time courses are analysed in terms of exponential components (Table I). DCMU in control samples quenches two short-life components and extends the lifetime of the one long-living component (42 ms time constant). Cerulenin-treated samples keep the original three components even in the presence of DCMU, though with somewhat longer time constants (1.80, 8.6 and 27 ms, respectively). Since delayed fluorescence emitted at times in the millisecond range after the excitation is presumably correlated with the deactivation of S_2 - and S_3 -states and the reduction level of the plastoquinone pool [18], the slight influence of DCMU on the delayed fluorescence of cerulenin-treated samples in this time range shows that Photosystem II activity in this region of events is not much affected.

The DCMU-resistance of the oxygen evolution and the partial DCMU-resistance of the delayed fluorescence of cerulenin-treated *Chlorella* cultures show that cerulenin treatment induces profound changes in the Photosystem II without influencing its main role. According to the current concepts, DCMU, either in the dark or under illumination, leads to conformational changes

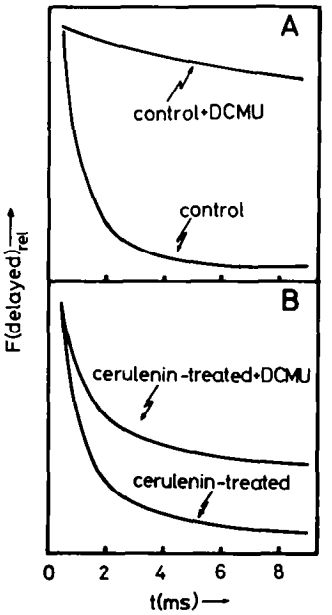

Fig. 2. Decay of delayed fluorescence (total light beyond 680 nm) of *Chlorella*, measured at 25°C from 500 μs. Excitation with Xe-lamp at 10 W·cm^{-2} intensity; measurement with a Becquerel-phosphoroscope ICA-70 multichannel analyser providing 4 μs resolution time and, optionally, glass light filters.

which break down the function of Photosystem II [19]. Cerulenin treatment definitely suspends the effect of DCMU, indicating that alteration of membrane composition and structure alters the site of DCMU-inhibition in Photosystem II, so as to make it inaccessible to DCMU. An alternative explanation of the DCMU-resistance of oxygen evolution caused by cerulenin: the treatment opens a new path for the electron transport, avoiding the site of DCMU-inhibition [20].

In any case, cerulenin seems to be a promising new tool in the study of the hitherto not satisfactorily known process of oxygen evolution, and in addition it may lead to a better understanding of the mode of DCMU-action.

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