CHLOROPHYLL FORMS AFFECTED BY 3(3,4-DICHLOROPHENYL)-1,1-DIMETHYLUREA AS SHOWN BY LOW TEMPERATURE FLUORESCENCE SPECTRA OF CHLOROPLASTS AND FRAGMENTS

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1. Introduction

According to the widely accepted view DCMU** acts as a specific inhibitor of Photosystem II by blocking electron flow between the primary and secondary acceptors Q and A [1]. As a parallel action inhibition of the reaction center P 680 has also been suggested [2]. The latter would imply that DCMU acts on a special assembly of chlorophyll molecules. If this effect extends to the antenna chlorophylls surrounding the reaction center, DCMU treatment may result in extensive changes of the molecular structure of the chloroplast membranes. By investigating the low temperature fluorescence spectra of chloroplasts and chloroplast fragments we have found that this actually occurs. In the low temperature fluorescence spectra DCMU induced characteristic changes: a strong enhancement of the fluorescence band F 695, a moderate increase of F 705 and a slight decrease in F 685, F 720 and F 735, respectively. This effect was not confined to Photosystem II as shown by the effect of DCMU on particle fractions enriched in Photosystem I.

2. Materials and methods

Seedlings of maize (*Zea mays* L. cv. MV 651) were grown in greenhouse for 8–10 days. Chloroplasts were isolated from the mesophyll of the first leaves as previously described [3]. Stroma membranes and grana were prepared from the chloroplasts by ultrasonication and differential centrifugation [4]. Grana were fragmented by digitonin treatment at 4°C for 30 min, with 0.3% digitonin, with a molar ratio of digitonin to chlorophyll adjusted to 40 : 1. The particle fraction enriched in Photosystem II (10 K) was collected between 1500 and 10 000 *g*. The particle fraction enriched in Photosystem I (144 K) was sedimented between 50 000 and 144 000 *g* [5].

Suspensions were incubated with DCMU at 4°C for 10 min in the dark. The final concentration of chlorophyll in the samples was 10^{-5} M. Concentration of the DCMU was adjusted to 10^{-4} M or 10^{-5} M, and the sample contained 2.0 or 0.2% ethanol, respectively. The same amounts of ethanol were added to the controls.

Fluorescence measurements were performed in liquid N_2 with a cell thickness of 0.2 mm.Excitation wavelength was set at 435 nm with a band width of 5 nm. The light was provided by a 1000 W Xenon arc lamp, and transmitted through an ISP-51 monochromator. Emission spectra were determined with a Zeiss SPM 2 grating monochromator (400 nm, 650 lines/mm, blazed at 570 nm). The slit was adjusted to a band width of 4 nm. The spectra were detected by an RCA 31034/a multiplier and were corrected for the response of the emission monochromator and photomultiplier. Relative intensities were monitored with the aid of a plexiglass containing Rhodamine G, which was set in one of the sample positions. Intensities were normalized on chlorophyll *a* content,

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

which was separately determined in ethyl ether solution by the two-wavelengths method [6].

Results were obtained from 3–6 independent experiments.

3. Results and discussion

Low temperature fluorescence spectra of chloroplasts and different subchloroplast fragments demonstrated that DCMU affected the spectral distribution of fluorescence (fig.1, left). The changes were relatively small, but always characteristic and reproducible. A closer inspection of the change is afforded by the ratio spectra calculated from the spectra of DCMU treated and control samples (fig.1, right). As shown by the series of the ratio spectra the most conspicuous change was an enhancement of the fluorescence band at 695 nm. Fluorescence emitted around 705 nm was also increased by DCMU but to a lesser extent, and the intensities of the F 685, F 720 and F 735 fluorescence bands decreased.

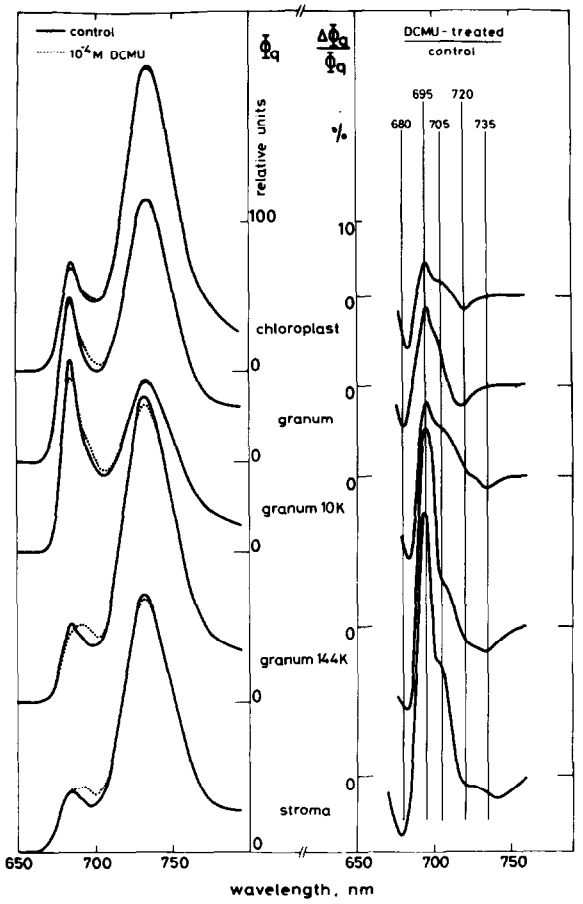

Fig.1. Fluorescence emission spectra at 77°K of untreated and 10^{-4} M DCMU treated chloroplasts and subchloroplast fragments isolated from the mesophyll of maize leaves (left column). Corresponding ratio spectra indicating the relative change in the emission due to 10^{-4} M DCMU treatment (right column). For details see Materials and methods.

The efficiency of DCMU in changing the spectral distribution of fluorescence of various preparations was estimated by the relative increase of the intensity of F 695 (table 1). Changes of the intensity of F 695 upon DCMU treatment were higher in grana and Photosystem II particles than in whole chloroplasts. This would be in agreement with the accepted view that DCMU has a specific action on Photosystem II. However, data obtained with Photosystem I particles of grana and stroma did not fit into this concept, since ΔF 695 of Photosystem I particles surpassed the values of Photosystem II particles. Thus, the DCMU effect reflected by the low temperature fluorescence spectra is not limited to Photosystem II but acts on the chlorophyll forms which influence the fluorescence at 695 nm. The increasing extent of the DCMU action with decreasing particle size of the chloroplast fragments can be explained by assuming a better access of DCMU to the small particles.

Support for the idea that DCMU acts specifically on some chlorophyll forms could be obtained also from experiments with stroma particles partially denatured by heat treatment (50°C, 30 min). The spectra of these samples revealed structural disorders (absorption maximum at 670 nm, chlorophyll *b* fluorescence at around 660 nm, F 720 domination in the long wavelength region). By applying DCMU these disorders were superimposed by the increase of F 695 (fig.2).

With lower concentrations of DCMU (10^{-5} M) we observed a less pronounced but reproducible effect amounting to about one third of the change obtained with 10^{-4} M DCMU. With 10^{-5} M DCMU the molar ratio of the inhibitor to chlorophyll was 1. This ratio might seem to be high but similar ratios can be found in the literature. A screening of the papers in the Biochim. Biophys. Acta, from 1974 has shown that among 17 works dealing with the fluorescence of

Table 1
The ratios of chlorophyll *a* to chlorophyll *b*, the ratios of relative intensities of long (F 735) and short wavelength (F 685) fluorescence at 77°K and the relative changes in the low temperature fluorescence due to 10^{-4} M DCMU treatment with chloroplasts and subchloroplast fragments obtained from the mesophyll of maize leaves.

	chlorophyll a / chlorophyll b	F 735 / F 685	ΔF 695 (%)
Chloroplast	3.37 ± 0.05	3.74 ± 0.47	6.3 ± 1.2
Granum	3.08 ± 0.06	1.82 ± 0.17	7.3 ± 1.1
Granum-10 K	2.68 ± 0.10	0.84 ± 0.03	7.8 ± 1.1
Granum-144 K	4.05 ± 0.29	3.92 ± 0.15	28.0 ± 2.1
Stroma	4.28 ± 0.06	5.77 ± 0.54	28.5 ± 3.5

DCMU-treated chloroplasts 12 papers used DCMU in the same or in a much higher ratio.

Changes in the spectral distribution of fluorescence in algae treated with 5×10^{-5} M DCMU have been observed at room temperature as well [7]. Although, this phenomenon has been explained by the block in the electron transport chain, we think, on the basis of the data presented in this paper that antenna chlorophyll could also be affected.

Our data are similar to the results obtained with phenanthroline derivatives [8]. These inhibitors act most probably as chaotropic agents inducing structural changes in the thylakoids. Such alteration in the structure (e.g. change in the orientation and/or distances) affects the energy transfer between the chlorophyll forms and can be reflected in the spectral distribution of low temperature fluorescence. These types of change in the emission and the inhibition of P-680 [9] can share a common basis: the DCMU-induced modification of chlorophyll forms. Nevertheless, it cannot be excluded that the effect reported here is another component in the complex action of DCMU: the uncoupling of phosphorylation [10], the removal of a fraction of Q [11,12] and inhibition at the donor side of Photosystem II [13].

In conclusion our results demonstrate an action of DCMU which (a) is independent of electron transport, (b) is not limited to Photosystem 2 but (c) is confined to some chlorophyll forms.

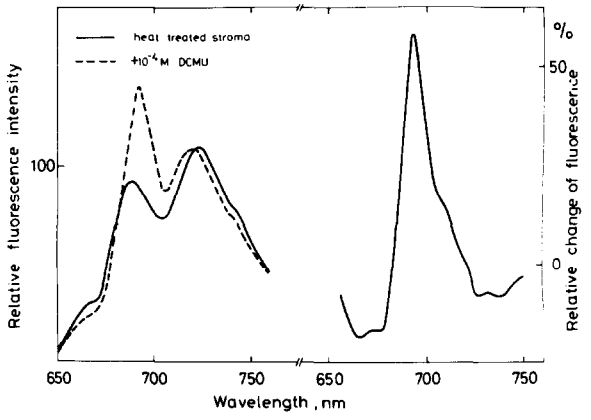

Fig.2. Fluorescence emission spectra at 77°K of heat treated stroma membranes before and after addition 10^{-4} M DCMU to the suspension (left). Ratio spectrum indicating the relative change in the fluorescence due to DCMU treatment (right). For details see Materials and methods.

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