Short Communication

3-(3,4-Dichlorophenyl)-1,1-dimethylurea Effect on Cytochrome \( b_{559} \) Photooxidation and Q Reduction at Temperatures Near 0 C

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The position and interaction of cytochrome \( b_{559} \) with the electron transport chain in green plant photosynthesis has remained obscure. Levine et al. (13) and Levine and Gorman (12) first demonstrated the presence of cytochrome \( b_{559} \) in the photosynthetic electron transport chain. Their results (12, 13), showing that photosystem II reduced cytochrome \( b_{559} \) but that it was oxidized by PS I in a DCMU-insensitive reaction at room temperature, suggest that cytochrome \( b_{559} \) is closely associated with PS II. Vernon et al. (14), Boardman and Anderson (3) and Huzisige et al. (9) have isolated particles having PS II properties which have cytochrome \( b_{559} \) as an integral part. Our recent findings (8) showing that cytochrome \( b_{559} \) is oxidized by PS II (PS II reaction center) in a temperature-insensitive reaction at low temperature point clearly to the conclusion that cytochrome \( b_{559} \) can interact with PS II under certain conditions. Knaff and Arnon (11) discovered a PS II driven decrease in absorbance with a peak at 550 nm. Erixon and Butler (6) recently observed, using potential poising, a component having a peak at 550 nm. The midpoint potential of this component was \(-50 \text{ mV} \) and prompted them (6) to suggest that the 550 nm component was \( Q_b \), the acceptor of PS II. I report here that in chloroplasts maintained near 0 C, cytochrome \( b_{559} \) photooxidation by 680 nm light is inhibited by DCMU with the concomitant appearance of a 550 nm component. These results can be explained by cytochrome \( b_{559} \) photooxidation and Q reduction by PS II reaction center.

Materials and Methods

Chloroplasts were isolated from spinach (Spinacia oleracea) as described by Cramer and Butler (5). Absorption changes were determined on a dual wavelength type apparatus as described by Chance (4). The reference wavelength was 540 nm. The temperature of the chloroplast solution, while photo-induced absorbance changes were being recorded, was kept near 0 C (approximately 2 C) by dipping the aluminum tongue of the cuvette in an ice-water solution. The cuvette had a total volume of 0.225 cm³ with a path length of 1 mm. Chlorophyll was determined by the method described by Arnon (1).

Results

Figure 1 presents the light minus dark difference spectra of spinach chloroplasts maintained at or near 0 C. Strong 680 nm light was the actinic source. In the control chloroplasts the peak at 554 nm corresponds to cytochrome \( f \) photooxidation, whereas the shoulder at 560 nm indicates photooxidation of cytochrome \( b_{559} \). There is also a shoulder at 550 nm which is apparently due to partial reduction of Q (see below). The addition of DCMU caused the elimination of cytochrome \( b_{559} \) photooxidation but a concomitant increase in the 550 nm component, which, following Erixon and Butler (6), is apparently due to the reduction of Q. DCMU did not exhibit the photooxidation of cytochrome \( f \). The computed difference spectrum between the control and DCMU-treated chloroplast data of Figure 1 is shown in Figure 2. The major differences between the two occur at 550 and 560 nm, thus indicating that DCMU inhibited the photooxidation of cytochrome \( b_{559} \) with a concomitant reduction of Q.

Discussion

The observations that DCMU inhibits cytochrome \( b_{559} \) oxidation and the appearance of a 550 nm component can be explained by the scheme presented in Figure 3. In this scheme it is expected that electron transfer from PS II to PS I is blocked or considerably diminished at 0 C and hence cytochrome \( b_{559} \) will be fully reduced and will be oxidized by the PS II reaction center. In this respect, it is competing with \( H_2O \) as the electron donor of PS II. As temperature is lowered from 25 C to 0 C it would be expected that \( O_2 \) evolution would decrease by 2-fold since the \( Q_b \) is 1.4 (7), whereas the photon peak of 545 nm.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Spectra of control and DCMU (5 \( \mu \text{M} \)) treated chloroplasts. The reference wavelength was 540 nm. Temperature was 0 C. Actinic light was 680 nm (bandwidth halfheight 10 nm, intensity 0.55 \( \times 10^{-4} \text{nano einstein/cm}^2\text{-sec} \)). Chlorophyll content was 250 \( \mu \text{g/sample} \). Pathlength was 1 mm. Each point is the average of three determinations on one sample. The values were taken 10 sec after the actinic light was switched on. A new sample was taken for each point.
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reduction of Q, it is possible to see cytochrome $b_{559}$ oxidation only if most of the electrons Q accepts are passed on to components between Q and cytochrome $b_{559}$ without reducing the $b$ cytochrome. The small band at 550 nm in the control chloroplasts (Fig. 1) indicates there is some reduced Q in the absence of DCMU, suggesting that the steps between Q and cytochrome $b_{559}$ are to some extent slower at 0 C.

The results of this study indicate that cytochrome $b_{559}$ is functionally closely associated with PS II and thus corroborate our previous observation (8) and those of Knaff and Arnon (10) that PS II can oxidize cytochrome $b_{559}$. The results also corroborate the findings of Knaff and Arnon (11) that the 550 nm component is best explained by a component involved in the primary process of PS II and specifically as Erixon and Butler (6) suggested as being due to Q, the acceptor of PS II.

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LITERATURE CITED
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