Low Temperature Reactions in Chloroplasts

SEYMOUR STEVEN BRODY

Photobiology Laboratory, Dept. of Biology, New York University, Washington Square, New York, N.Y. 10003, U.S.A.


Some photochemical reactions of photosynthetic materials (corn chloroplasts, spinach chloroplasts and subchloroplast particles rich in System II) are examined at 77 °K. The fluorescence yield of chlorophyll is observed to decrease reversibly upon irradiation at 77 °K. The presence of ferricyanide in the chloroplast preparations increases the rate of decline of fluorescence yield while the presence of benzylophenol and phenazine methosulfate inhibits the decline of fluorescence yield. Extracts of Rricinus communis leaf (castor bean) also inhibits the decline of fluorescence yield. Data is given showing the effect of benzylophenol, extracts of castor leaf, ferricyanide, benzylphenol and phenazine methosulfate on the ratio of fluorescence intensities at 725 and 700 nm.

Studies at low temperature eliminate all diffusion controlled reactions so that primary photochemical processes may be observed in the absence of most restoring transformations. By eliminating diffusion one may determine which materials (additives) are directly involved with the chlorophyll fluorescent centers of System I and II.

It is becoming quite apparent that there are many photochemical reactions in photosynthetic materials which can proceed in the frozen state at temperatures of 77 °K and 4 °K. For example there are reports of light induced changes in absorption spectra, electron spin resonance, separation of charge and fluorescence induction. For such reactions to occur at low temperatures the reactants must either be complexed with one another or there must be semi-conductor properties whereby an electron can be transferred over a distance between the pigment and an electron donor or acceptor.

Materials and Methods

Intensity of fluorescence was measured at 700 and 725 nm by front surface excitation with the aid of an RCA 6217 (S 20 response) photomultiplier tube and a galvanometer (10⁻⁹ amps/mm. sensitivity). Emission was sensitized with monochromatic blue light (436 nm)

4 N. MURATA, Biochim. biophysica Acta [Amsterdam] 162, 106 [1968].
7 J. M. BLUMENTHAL, Photochem. Photobiol. 6, 155 [1967].
Results

In the course of this work two parameters of chloroplast fluorescence were measured. One was the ratio of the fluorescence intensities measured at 725 and 700 nm, referred to as $F_{725}/F_{700}$, after a few minutes illumination at 77 °K. The second was the rate at which the fluorescence yield at 725 nm decreased when irradiated, referred to as $\Delta\Phi_R$. All the results are tabulated in Table I.

<table>
<thead>
<tr>
<th>Subject</th>
<th>$\Delta\Phi_R \times 10^3$</th>
<th>$F_{725}/F_{700}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spin. (1) + FERRI</td>
<td>218</td>
<td>3.0</td>
</tr>
<tr>
<td>Board. (2) + FERRI</td>
<td>220</td>
<td>1.8</td>
</tr>
<tr>
<td>Corn (3) + FERRI</td>
<td>220</td>
<td>3.0</td>
</tr>
<tr>
<td>Spin. + FERRI + RLE</td>
<td>85</td>
<td>2.2</td>
</tr>
<tr>
<td>Board. + FERRI + RLE</td>
<td>82</td>
<td>1.8</td>
</tr>
<tr>
<td>Corn + FERRI + RLE</td>
<td>96</td>
<td>2.5</td>
</tr>
<tr>
<td>Board. + FERRI + LINO</td>
<td>193</td>
<td>1.9</td>
</tr>
<tr>
<td>Corn + FERRI + LINO</td>
<td>181</td>
<td>2.9</td>
</tr>
<tr>
<td>Spin. + PMS + BV</td>
<td>67</td>
<td>2.5</td>
</tr>
<tr>
<td>Board. + PMS + BV</td>
<td>107</td>
<td>1.7</td>
</tr>
<tr>
<td>Corn + PMS + BV</td>
<td>47</td>
<td>3.0</td>
</tr>
<tr>
<td>Board. + PMS + BV + RLE</td>
<td>168</td>
<td>1.9</td>
</tr>
<tr>
<td>Corn + PMS + BV + RLE</td>
<td>74</td>
<td>3.0</td>
</tr>
<tr>
<td>Corn + PMS + BV + LINO</td>
<td>29</td>
<td>3.2</td>
</tr>
<tr>
<td>Corn + BV</td>
<td>84</td>
<td>2.7</td>
</tr>
<tr>
<td>Corn + BV + LINO</td>
<td>29</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Table I. Summary of results.
1 = Spinach chloroplasts, 2 = Boardman particles rich in System II, 3 = Corn chloroplasts.

Fluorescence Ratio $F_{725}/F_{700}$: For subchloroplast particles (referred to as Boardman particles), which are rich in System II, the fluorescence ratio is quite low (between 1.7 and 1.9) indicating much more fluorescence from System II than System I chlorophyll. In this case neither the addition of RLE nor LINO have much effect on $F_{725}/F_{700}$.

Addition of RLE to a mixture of FERRI and corn or spinach chloroplasts decreases the ratio of $F_{725}/F_{700}$ from 3.0 to 2.2 and 2.5 respectively. The result is that RLE tends to make the fluorescence spectrum of chloroplasts resemble the spectrum obtained from Boardman particles which are rich in System II. It is to be noted that there is also a direct reduction of FERRI by the cytochrome present in RLE, thereby decreasing the concentration of acceptor. Addition of RLE or LINO to mixtures of PMS, BV and corn chloroplasts does not change the ratio to any extent. We have not yet determined the time dependence of $F_{725}/F_{700}$ when illuminated at 77 °K.

Rate of Decrease of Fluorescence Yield ($\Delta\Phi_R$): When illuminating Boardman particles, spinach or corn chloroplasts in the presence of FERRI the fluorescence yield at 725 nm decreases at its most rapid rate, i.e. the value of $\Delta\Phi_R$ is largest (see Fig. I). In the case of a mixture containing corn chloroplasts, PMS and BV the action of RLE and LINO are different; the value of $\Delta\Phi_R$ increases if RLE is added and decreases if LINO is added.

Interrupting the illumination stops the decrease in fluorescence yield; furthermore there is no recovery in the dark. When irradiation is resumed the fluorescence yield continues to decline as if there were no interruption in illumination. An experiment in which the illumination was interrupted is shown in Fig. I.

Fig. I. Relative fluorescence yields at 77 °K of corn chloroplasts plus additives as indicated in the figure as a function of time of irradiation. Fluorescence was measured at 725 nm and sensitized with high intensity ($1.6 \times 10^6$ ergs/cm² sec) monochromatic blue light (436 nm). The concentrations of FERRI, BV, PMS and LINO are given in the text. In the experiment depicted by the lowest line, irradiation was interrupted for 5 min. at the time indicated.

That the change in fluorescence yield brought about at 77 °K does not involve a photodestruction of chlorophyll is readily shown. By warming the chloroplast sample to about 233 °K then cooling again to 77 °K the initial fluorescence intensity is restored—demonstrating the reversible nature of this light induced decrease in fluorescence yield.

For Boardman particles in the presence of PMS and BV $\Delta\Phi_R$ is rather large; addition of RLE increases $\Delta\Phi_R$ still further.

Discussion

The large decrease in the ratio $F_{725}/F_{700}$ seems to result from an active component in RLE that spe-
specifically complexes with and quenches the fluorescent chlorophyll of System I (F 725). Addition of RLE to B o a r d m a n particles has a minimal effect on F 725/F 700 since the concentration of System I chlorophyll (F 725) is low and RLE has little apparent effect of System II chlorophyll (F 700) (see Table I). Addition of RLE to corn chloroplasts containing PMS and BV has no effect on F 725/F 700 perhaps indicating similar fluorescence quenching properties of RLE and these materials.

The linear relationship obtained with the log-log presentation of the data in Fig. I follows the empirical expression: $\Delta \Phi_R = 1/A \cdot (1 + t)^{-\phi_R}$. A constant (unity) is added to the time, $t$, to avoid a discontinuity at time zero.

From the long irradiation times required to bring about the change in fluorescence yield it is apparent that the quantum yield of the reaction is quite low. The mechanism for this reversible change in fluorescence is not yet clear. The decrease in fluorescence may arise from an oxidation or reduction of chlorophyll by either a naturally occurring or added substrate which is directly complexed to chlorophyll. It is also possible that there is a change in energy transfer efficiency to the fluorescence center by a "local "initial shock" to chlorophyll upon absorbing a light quantum. An increase in local temperature is brought about by conversion of the light energy into heating the chlorophyll so as to bring about a reorientation, deaggregation or decomplexation.

Since addition of LINO to chloroplasts containing FERRI has only a small effect on $\Delta \Phi_R$, LINO is tentatively assumed to interact with System II. This is in agreement with the findings of COHEN et al. 13.

The presence of FERRI in chloroplast mixtures always results in a large value for $\Delta \Phi_R$. Addition of RLE to a mixture of FERRI and chloroplasts results in a much smaller value of $\Delta \Phi_R$. It would appear that a component in RLE is blocking the interaction between FERRI and the chlorophyll giving rise to fluorescence at 725 nm.

This work is supported by N.S.F. research grant (GB 7244). The author was supported, in part, by U.S. Public Health Service Research Career Program Award K3-GM 17918.


Effects of Ricinus Leaf Extracts on Light Induced Changes in Absorption of Chloroplasts Associated with System I and System II

M. BRODY*, S. S. BRODY **, and G. DÖRING ***


Absorption changes associated with photosynthetic System I and System II are (differentially) inhibited by aqueous extracts of Ricinus leaf (RLE). The absorption changes associated with thylakoid effects (i.e. those at 515 nm) are also modified by RLE; the slow component ($>10^{-2}$ sec) is diminished and the fast component ($<10^{-2}$ sec) is eliminated.

Fluorescence and amperometric studies were made with Zea mays chloroplasts suspended in an aqueous of Ricinus leaf (RLE)1–5. Although the influence of the extract seems to be a complex one, it has been interpreted, in part, as resulting from sequential inhibitory action towards the System I and System II sides of electron transport4,5.

The present work describes the effects of RLE on light induced changes in absorption of chloroplasts and sub-chloroplast particles. In regard to these

3 S. S. BRODY, in preparation.

* Dept. Biol. Sci., City Univ. of New York, New York, N.Y.