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Protection of reaction center II from photodamage by low temperature and anaerobiosis in spinach chloroplasts

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The rate of the irreversible damage to the reaction center II, caused by exposure of spinach thylakoids to high light was slowed down by anaerobic conditions and by lowering the temperature. The protective mechanisms of these conditions were different. In both cases F_{max} decreased more slowly than in control photoinhibition. A reversible intermediate step was only observed under anaerobic conditions. This state was inactive for oxygen evolution and it was characterized by an increase of F_o.

Photoinhibition; Photosystem II; D_1 protein; Anaerobiosis

1. INTRODUCTION

Photoinhibition is the decrease of photosynthetic activity induced by high light intensity. The primary site of photodamage is the reaction center of photosystem II (PSII) (reviewed in [1–3]). The molecular mechanism of photoinhibition is still controversial. It was proposed that the first step of photoinhibition may lead to damage of the Q_B [4,5] or Q_A site [6–8]; but the involvement of the donor side reactions was also suggested [9–11]. In living cells the repair process leading to reactivation of PSII activity involves de novo synthesis of thylakoids protein among which the most prominent is D_1 [12].

It is generally assumed that the damage of the photosynthetic apparatus produced in leaves by exposure to high light at chilling temperatures is larger than that observed at higher temperatures (reviewed in [13]). We have recently demonstrated that low temperature did not increase photoinhibition in *Synechocystis* 6714 (a cyanobacterium) or *Chlamydomonas reinhardtii* (a green alga) cells [14]. On the contrary, it had a protective effect from high light. The irreversible damage of the D_1 protein was slowed down in both species. However, in *Chlamydomonas* this damage was preceded by a reversible state, which was inactive for oxygen evolution as characterized by a high level of F_o. The existence of reversibly inactivated centers during photoinhibition treatments at low temperature could be misinterpreted as damaged reaction centers II (RCII). In reality, a large part of the observed inactivation of the PSII is not linked to any irreversible damage or degradation of the RCII [14].

In this work we show that during aerobic photoinhibition of spinach thylakoids there is no formation of a highly fluorescent reversible state even at low temperature. Nevertheless, the low temperature protected the RCII from irreversible photodamage in spinach thylakoids as it did in cyanobacteria. However, when chloroplasts were exposed to high light under anaerobic conditions, the reversibly inactivated high fluorescence state was generated.

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Abbreviations: Chl, chlorophyll; F_o, F_v, F_{max}, initial, variable and maximum fluorescence; PSII, Photosystem II; Pheo, pheophytin; Q_A and Q_B, primary and secondary quinone electron acceptors; RCII, reaction center II

2. MATERIALS AND METHODS

Spinach thylakoids were isolated according to [15]. Photoinhibitory treatment was performed at 20°C in a medium containing 50 mM Hepes pH 6.8, 5 mM $MgCl_2$, 10 mM NaCl and 100 mM sorbitol at a chlorophyll concentration of 0.15 mg/ml using two Atralux spots of 150 W (each giving an intensity of about 1000 $\mu E \cdot m^{-2} \cdot s$). Anaerobiosis was obtained by bubbling argon in the medium for 15 min and then above the sample for 5 min in a closed glass tube. After the photoinhibitory treatment the samples were incubated in the ice where they conserved the same characteristics for more than one hour.

Fluorescence kinetics were performed as in [16]. Thermoluminescence was measured as described in [17]. For B band measurements the samples were incubated 2 min in the dark, then a flash was given at −5°C and the sample was rapidly frozen. For the detection of the Q band, DCMU was added after dark adaptation and the flash was given at −20°C. All fluorescence and thermoluminescence measurements were done in aerobic cuvettes.

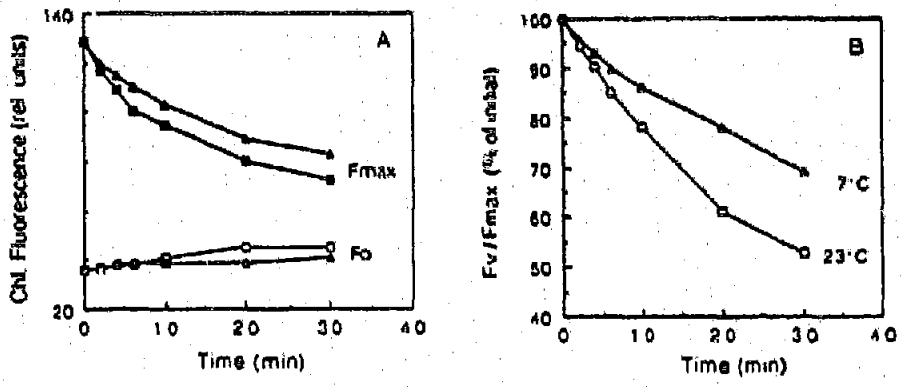

Fig. 1. Effect of temperature on F_{max} (closed symbols) and F_o (open symbols) under aerobic conditions (A) and on the decrease of variable fluorescence (B). Photoinhibition at 23°C (□—□ and ■—■); photoinhibition at 7°C (△—△ and ▲—▲).

3. RESULTS

Recently, it was shown that oxygen evolution in spinach thylakoids was less sensitive to photoinhibition at low temperatures [18]. Here we confirmed by fluorescence measurements that low temperature protects the RCII from damage in spinach thylakoids. In addition, we demonstrated that in chloroplasts the decrease of PSII activity (which is concomitant with the decrease of F_v/F_{max}) at any temperature was correlated with a decrease of F_{max} whereas F_o remained constant (Fig. 1). The rate of F_{max} decrease slowed down when lowering the temperature (Fig. 1). Similar phenomenons were observed in cyanobacteria [14,17]. We did not observe any reversible state like that detected at low temperature in *Chlamydomonas* cells.

T. Hundall et al. [19] observed a reversible photoinhibition of spinach thylakoids in anaerobic conditions. We wanted to compare this phenomenon to that we observed in *Chlamydomonas* cells at low temperature [14]. When isolated spinach thylakoids were photoinhibited in anaerobic conditions, the decrease of F_v/F_{max} was accelerated (Fig. 2B). The faster inhibition of the PSII activity was due to a large increase of F_o in anaerobic conditions (Fig. 2A). In contrast, the decrease of F_{max} was slower under anaerobic than under aerobic conditions (Fig. 2A). The high fluorescent state was not stable at room temperature. The high level of F_o disappeared after 1 h incubation of the sample in the dark at room temperature (Fig. 2A). The F_o decay was concomitant with an increase of the F_v/F_{max} ratio (PSII activity) (Fig. 2B).

Charge recombination gives rise to thermoluminescence which enables the detection and quantitation of the $S_2Q_B^-$ (B band) and $S_2Q_A^-$ (Q band) states and measurement of their stability during photoinhibition [20]. Fig. 3 shows the B band of samples photoinhibited under anaerobic conditions compared to that of control thylakoids. The B band was not shifted. It decreased with the same kinetic as that of F_v/F_{max} decrease. This photoinduced decrease of the thermoluminescence band was reversible (Fig. 3, inset). The Q band was not shifted and its amplitude also decreased during photoinhibition (data not shown). By these measurements it is

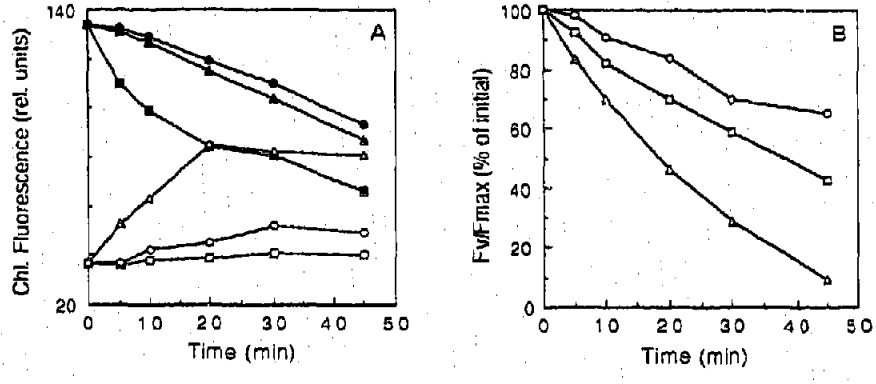

Fig. 2. Effect of anaerobiosis on F_{max} (closed symbols), F_o (open symbols) (A) and on F_v/F_{max} (B) during photoinhibition. The measurements were done about 10 min after photoinhibitory treatment under aerobic (□—□) or anaerobic conditions (△—△) and also after one hour of dark incubation at room temperature of the photoinhibited samples under anaerobic conditions (○—○).

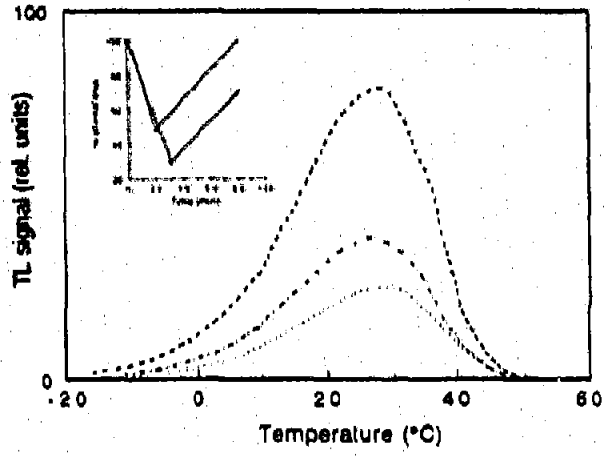

Fig. 3. The B band of thermoluminescence in the control sample (– – –) and in photoinhibited samples under anaerobiosis for 20 (– · –) and 30 min (· · · · ·). Inset: decrease of the areas of the B band during photoinhibitory treatment and their following increase after about one hour of dark incubation at room temperature of the samples photoinhibited 20 min (□—□) and 30 min (■—■) under anaerobic conditions.

demonstrated that the Q_B site was not modified in the active centers. The centers with a high level of F_o were not detected by thermoluminescence.

The anaerobiosis was created by argon bubbling in the sample. This treatment may produce a bicarbonate depletion. In order to differentiate the effect of anaerobiosis from that of depletion of bicarbonate, we photoinhibited the sample in the presence of bicarbonate (which was added after argon bubbling) or in an oxygen enriched atmosphere after argon bubbling. The results are described in Fig. 4. We observed an increase of F_o level only in anaerobic conditions plus or minus bicarbonate. It was clear that the reversible state was produced by the anaerobiosis and not by the depletion of bicarbonate.

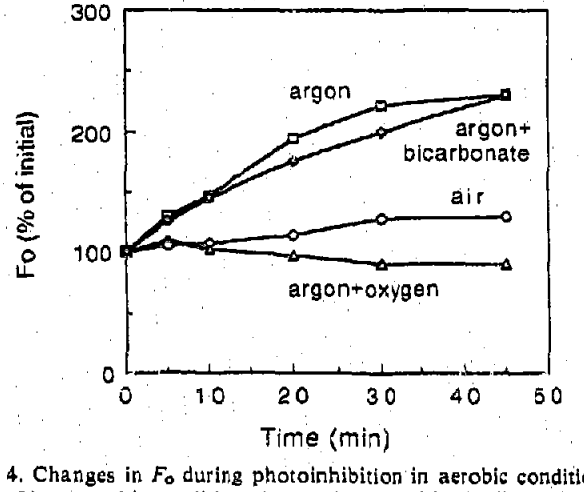

Fig. 4. Changes in F_o during photoinhibition in aerobic conditions (○—○); anaerobic conditions (□—□); anaerobic conditions in the presence of bicarbonate (◇—◇); anaerobiosis and then oxygen atmosphere during photoinhibition (△—△).

4. DISCUSSION

It has been shown that the protection from an irreversible photodamage of the CRII by low temperature occurs in all the organisms which perform the oxygenic photosynthesis: cyanobacteria [14], green algae [14] and higher plants ([18] and this article). This protection was characterized by a slower decrease of F_{max}. Low temperature may protect the reaction center by slowing down the rate of the chemical reactions resulting in D_1 damage.

In anaerobic conditions the rate of irreversible damage in spinach thylakoids was also slowed down. It was preceded by a high fluorescent state. This state corresponded to closed centers inactive in oxygen evolution. The closed centers can be rapidly reactivated without replacement of the D_1 protein. The existence of the reversible inactive state may explain the general observation of a faster photoinhibitory effect under anaerobiosis [7,21,22]. Recently, Sundby [23] has proposed that a faster inhibition of oxygen evolution is produced by the absence of bicarbonate, rather than by the absence of oxygen. We did not observe a clear difference between the rates of F_v/F_{max} decrease under anaerobic conditions in the presence or absence of bicarbonate (data not shown), but we have detected the high fluorescent state under these two conditions.

The increase of F_o was also observed during photoinhibition at low temperatures of *Chlamydomonas* cells [14] and higher plant leaves [24]. We have previously demonstrated that the high level of F_o characterized a reversible state which is due to modified closed centers in which the reduced primary acceptor (Q_A) is less accessible to reoxidation. The reversible states which appeared in *Chlamydomonas* cells at low temperature and in spinach thylakoids under anaerobiosis seem to have the same characteristics.

The reversible state detected by Hundall et al. [19] and by us seems to be different from that described by Ohad et al. [25] which is characterized by a shift of the B band of thermoluminescence to lower temperatures. Under anaerobic conditions, we observed only a decrease of the B band, which was reversible but we have not detected any shift of the peak.

A fast and a slow phase of photoinhibition were defined by Setlik et al. [26]. The characteristics of the fast phase, where D_1 is not degraded [27], may relate with those of the reversible state described here. We think that the fast phase may represent formation of a state which is relatively insensitive to damage by high light rather than increased effect of photoinhibition. In the presence of Q_A^- the radical pair P_{680}^+-Pheō recombines in a few nanoseconds. This prevents the formation of long-lived strongly oxidizing components which may damage the D_1 protein. At the same time, in the closed centers the occupancy of the site Q_B by Q_B^- is reduced. This situation was also proposed to protect the reaction center II from degradation.

We have clearly demonstrated that low temperature and anaerobiosis do not increase the effect of photoinhibition. They protect the reaction center II from photodamage by two distinct mechanisms.

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