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The modification of chlorophyll fluorescence of *Chlamydomonas reinhardtii* by photoinhibition and chloramphenicol addition suggests a form of photosystem II less susceptible to degradation

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The effect of photoinhibition and chloramphenicol addition on room-temperature chlorophyll fluorescence induction parameters exhibited by *Chlamydomonas reinhardtii* has been studied. The observed changes induced by these different treatments suggest the presence of a photosystem II (PS II) reaction centre having an abnormal turnover rate and which gives rise to the fast initital variable fluorescence rise (F_i–F_0) in the absence of DCMU. It is proposed that a pool of PS II with a modified D_1 protein exists which is less susceptible to degradation. Furthermore, it is shown that the fluorescence quenching associated with photoinhibition is not directly linked to PS II reaction centre degradation.

Chlorophyll fluorescence induction; Chloramphenicol; Photoinhibition; (*Chlamydomonas*)

1. INTRODUCTION

It has been shown that the turnover rate of the D1 protein (32 kDa polypeptide which is the *psb*A gene product) is faster than those of other PS II-associated proteins [1–3]. As damage to this protein necessarily precedes degradation, replacement or repair, one might expect the presence, in vitro, of a proportion of PS II reaction centres where Q_A photoreduction still occurs but Q_A reoxidation is inhibited or drastically slowed down. This situation could be envisaged if the proposed damage which initiates proteolysis is on the Q_B niche. Indeed, studies by Lavergne [4] on chlorophyll fluorescence induction have shown the existence of PS II centres with slow Q_A reoxidation kinetics and which correspond to the intermediate fluorescence level (F_i) reached upon illumination of dark-adapted cells or thylakoids with low light intensity. This F_i fluorescence yield is the same as the yield which is reached within a few milliseconds after a brief microsecond saturating flash. The return from F_i to the initial fluorescence level (F_o) requires several seconds [5]. It is also the level which is maintained when thylakoids are illuminated in the presence of ferricyanide, which cannot oxidize directly Q_A [6]. It has been suggested that this fast rise in fluorescence comes from PS II β-centres [6]. Graan and Ort [7] and Chylla et al. [8] have also shown that in spinach thylakoids a large proportion of PS II centres (30–40%) are not connected to the plastoquinone pool although they are active in the reduction of halogenated benzoquinones.

In order to verify the above hypothesis that F_i could depend upon the relative turnover rate of the D1 protein compared to the other PS II proteins, or more precisely on their relative rate of degradation, chloramphenicol (CAP) has been added to *Chlamydomonas reinhardtii* grown under moderate light intensities. This type of experiment, on the effect of CAP on Chl fluorescence and protein

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Abbreviations: CAP, chloramphenicol; PS, photosystem; Chl, chlorophyll; F_o, initial Chl fluorescence; F_i, intermediate Chl fluorescence; F_m, maximal Chl fluorescence (+ DCMU); F_v, $F_m - F_o$; Q_A, primary quinonic acceptor of PS II; Q_B, secondary quinonic acceptor of PS II; PFD, photon flux density

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degradation, has already been carried out but under high light conditions which induce photoinhibition. This led to the idea that it was the imbalance of D1 degradation and synthesis which produces the inhibition of PS II electron flow seen during photoinhibition [2,9]. However, high light conditions cannot be used to answer the question of the origin of 'F_i centres' because photoinhibition is accompanied by a quenching of Chl fluorescence, the mechanism of which is still unknown.

Furthermore, in order to determine whether PS II degradation and turnover depend upon the intensity of functioning of the PS II machinery, the effect of CAP has been investigated at different light intensities using photoautotrophic cultures.

2. MATERIALS AND METHODS

C. reinhardtii was grown at 25°C starting from approx. 10^5 cells/ml under two different conditions. (A) In TAP medium [10], consisting of 20 mM Tris, 17 mM acetate, 1 mM phosphate (pH 7); the bubbling of air, giving a CO_2 concentration of $\simeq 10^{-5}$ M; illumination of algae with a low-intensity white light (PFD 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) as in [11]. (B) In a mineral medium as in [12], consisting of 6.5 mM phosphate, 7.5 mM NH_4Cl, 0.45 mM $CaCl_2$, 0.85 mM $MgSO_4$ (pH 7); bubbling of air enriched in CO_2 (3%) imposed a CO_2 concentration of approx. 10^{-3} M; algae were illuminated with white light at an intensity of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The two media contained Hutner solution diluted 1000-fold.

The growth rate was determined by cell counting. Under the two trophic conditions, algae grew during the exponential phase with doubling times of 10 h in heterotrophy (TAP medium) and 8 h in phototrophy with a PFD of 350 $\mu mol \cdot m^{-2} \cdot s^{-1}$. When a steady rate had been reached (constant concentration of cultures on a Chl basis), the culture was divided into equal batches and crystalline chloramphenicol, at a final concentration of 1 mg/ml, was added to one flask. Cultures were maintained under the same growth conditions as described above, aliquots set aside and fluorescence induction and chlorophyll content measured. Fluorescence induction was determined with a fluorimeter described in [13]. Aliquots of the cultures were diluted 5-fold in water in a stirred cuvette. Algae, corresponding to 2.5 μg Chl/ml, were dark-adapted for 5 min before measurement of the fluorescence induction. The F_m level was determined after addition of DCMU (500 ms after the beginning of illumination). Suspensions were excited with blue light (Corning 4.96 filter) at 170 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (low enough to obtain an F_i level corresponding to photoreduction of centres where Q_A reoxidation is slow). Fluorescence was selected with a combination of Corning 2.64 and Schott RG5 filters. Chlorophyll content was determined spectroscopically on acetone (80% in water) extracts, utilizing the equations of MacKinney [14].

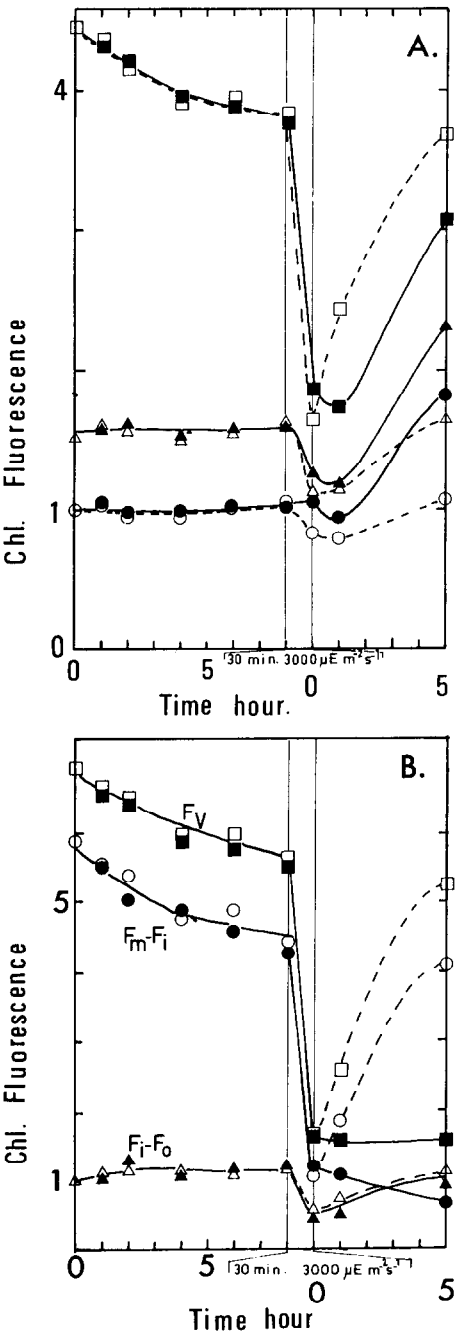

Fig.1. (A) Modification of the fluorescence levels F_o (○,●), F_i (△, ▲) and F_m after addition of 10^{-5} M DCMU (□, ■) of *C. reinhardtii* grown heterotrophically as a function of time after CAP addition. Open symbols, without CAP; closed symbols, plus CAP. (B) Changes in total variable fluorescence F_V (□, ■), and the two components $F_i - F_o$ (△, ▲) and $F_m - F_i$ (○,●) calculated from the data of panel A. Open symbols, without CAP; closed symbols, plus CAP.

3. RESULTS

In the steady-state cultures, the $(F_i - F_o)$ variable fluorescence fraction represents 12–17% of the total variable fluorescence (F_m + DCMU – F_o). If $(F_i - F_o)$ is influenced as F_v by exciton transfer between PS II units, then the complementary area over fluorescence corresponding to $F_1 - F_o$ represents 29–37% of the total complementary area of fluorescence induction obtained in the presence of DCMU. Indeed, from fluorescence induction in the presence of DCMU a transfer probability of 0.65 was calculated.

As shown in fig.1A,B under heterotrophic growth conditions, CAP addition had no effect on the fluorescence level even after 8 h of incubation with CAP. Nevertheless, as shown in [9], high light induced a quenching of fluorescence with the $(F_m - F_i)$ fraction being the most sensitive (fig.1B). When put back under low light, a slow relaxation of the fluorescence quenching occurred which was different in the presence and absence of CAP. In the absence of CAP, complete restoration of the fluorescence levels before the photoinhibitory treatment occurred as previously shown [9]. In the presence of CAP restoration of F_m was observed but an increase in F_o was seen, only the $(F_i - F_o)$ variable fluorescence fraction was restored as in the absence of CAP while the $(F_m - F_i)$ variable fluorescence remained quenched.

It is interesting to note that when the same photoinhibitory treatment was carried out on a *Chlamydomonas* mutant lacking PS II reaction centres, which does not exhibit variable fluorescence and has a fluorescence yield similar to F_m in the wild type [15], the same high light treatment did not induce any quenching of fluorescence (not shown).

Under photoautotrophic growth conditions, as shown in fig.2A,B, where the PFD was 350 μmol·m^{-2}·s^{-1}, CAP addition induced an increase in F_o and only a slight increase in F_m, consequently a decrease in F_v occurred. However, as shown in fig.2B the decrease in F_v was due to the diminution of the $(F_m - F_i)$ fraction. In contrast, a slight transitory increase in $(F_i - F_o)$ occurred but the amplitude of this enhancement does not compensate the $(F_m - F_i)$ decrease.

In the presence of CAP, in parallel to the increase in $(F_i - F_o)$ amplitude with incubation time

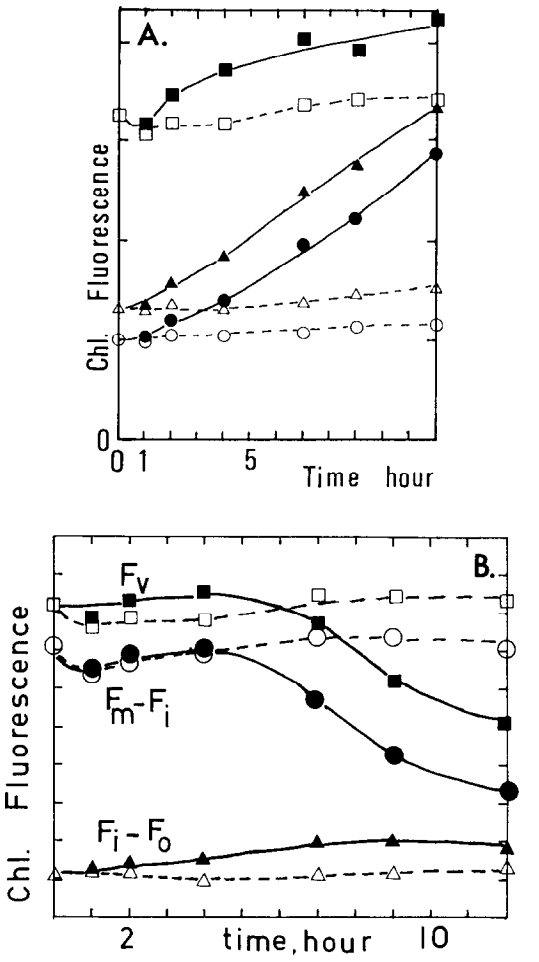

Fig.2. (A) Modifications of fluorescence parameter as a function of time after CAP addition to *C. reinhardtii* grown photoautotrophically under a PFD of 350 μmol·m^{-2}·s^{-1}. Symbols as in fig.1A. (B) Changes in total variable fluorescence and its two components deduced from the data presented in panel A. Symbols as in fig.1B.

with inhibitor, a decrease in rise time from F_o to F_i is observed. This is shown in fig.3 where $(F_i - F_o)$ and 1/half-time of F_o to F_i induction vs F_o are plotted for various times after CAP addition to the photoautotrophic culture.

20 h after CAP addition, F_v was totally abolished without significantly changing F_m.

In the absence of CAP the fluorescence levels stayed constant.

Fig.4 reports the inhibition of variable fluorescence, 18 h after CAP addition to steady-state algae which were illuminated with different

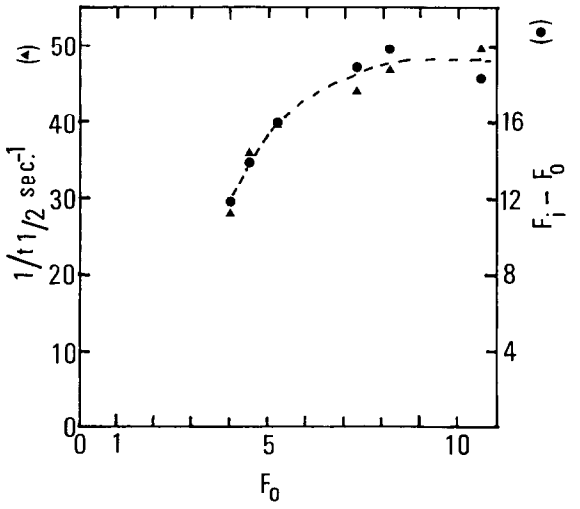

Fig.3. Variations of ($F_i - F_o$) amplitude (●) and of the rate of rise from F_o to F_i (▲) (measured by the inverse of the rise half-time) vs F_o enhancement which occurs with time after CAP addition to the photoautotrophically grown *C. reinhardtii*.

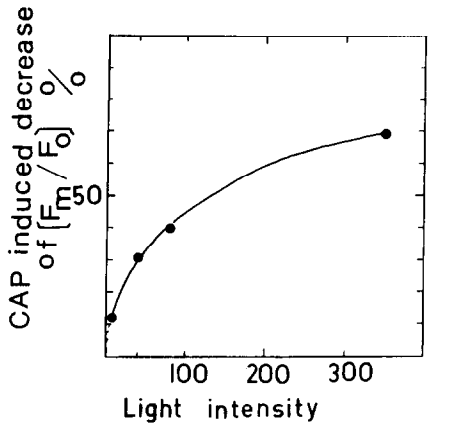

Fig.4. Effect of light intensity (PFD in $\mu mol \cdot m^{-2} \cdot s^{-1}$) during 18 h CAP treatment on the disappearance of F_v.

PFD. The amplitude of the CAP effect increased with light intensity in parallel with the photosynthetic activity.

4. DISCUSSION

Fluorescence induction in the absence of DCMU gives rise to an initial fast rise ($F_i - F_o$) which accounts for only 12–17% of the total variable fluorescence. However, when the non-linear relationship between fluorescence and Q_A is taken into consideration this corresponds to 30–40% of the total number of PS II reaction centres. This is in close agreement with the conclusions of Graan and Ort [17], who proposed that a certain number of PS II reaction centres are inactive in plastoquinone reduction. It has been reported [4] that the '$F_i - F_o$' centres are reoxidised very slowly ($t_{1/2} \simeq$ 10–15 s; unpublished) which also suggests that Q_A^- system II recombination is not possible and therefore strongly infers that these centres have a modified D_1 protein.

Our results suggest that these $F_i - F_o$ centres have a D_1 protein which is subject to a slower than normal turnover rate. This can be seen from fig.2 in which after 12 h of CAP addition the ($F_i - F_o$) variable fluorescence is hardly modified whereas the ($F_m - F_i$) component arising from 'normal' PS II has decreased by 50%. The slight increase in $F_i - F_o$ is probably due, as inferred from fig.3, to an increase in antenna size brought about by the loss of functional '$F_m - F_i$' centres arising from their degradation. This means that both normal PS II and inactive $F_i - F_o$ centres are located in the same antenna-bed and hence rules out the possibility that the $F_i - F_o$ centres correspond to PS II β.

Furthermore, it is observed that, after a photoinhibitory treatment (fig.1) in the presence of CAP, the $F_i - F_o$ fluorescence is completely restored when *Chlamydomonas* is transferred back to a low light regime. This suggests that this type of PS II is not damaged during the treatment and therefore does not have a degraded D_1. This could simply reflect the slow electron-transfer kinetics associated with this PS II or the fact that it does not reduce PQ. This would agree with the data of fig.4, that the turnover of the PS II reaction centre, as detected by the loss of F_v, is highly dependent upon the degree of PS II functioning.

A quenching of chlorophyll fluorescence is characteristic of photoinhibition although the origin of the mechanism is still not understood. If the degradation of D_1 plays a role in the fluorescence decrease, then the restoration of F_v(+ CAP) in fig.1 would not be expected. This conclusion, in fact, agrees with the kinetics of the loss of F_v and D_1 in which the half-time for F_v is faster than that of D_1 [16]. After relaxation in the presence of CAP a situation similar to that ob-

served in fig.2A for the effect of CAP after a 12 h incubation is reached in which F_V is lost (via $F_m - F_i$) and an increase in F_o is observed. This situation reflects the loss of active PS II reaction centres capable of reducing PQ. Therefore, a quenching appears to be superimposed upon a photoinhibition-induced increase in F_o. This quenching does not occur because of the degradation of the D_1 protein and therefore inactivation of reaction centre functioning because in the presence of CAP (at non-photoinhibitory light intensities) a loss of PS II is not associated with fluorescence quenching.

The fact that no transitory increase in $F_i - F_o$ is observed in the presence of PS II degradation (figs 1B,2A) shows that these PS II centres are not linked to the rapid degradation of D_1 producing functional centres capable of reducing Q_A but not PQ. This conclusion is expected when considering the proposed protein composition and organisation of the PS II reaction centre [17].

It therefore seems that the turnover of D_1 in normal PQ-reducing PS II centres depends upon the degree of functioning of the centre. However, there exists a pool of PS II which is inactive in PQ reduction that has a slower D_1 turnover, either because the changes necessary to signal proteolysis are lacking due to the low rate of electron-transfer functioning or because of a structural modification in D_1 which makes the protein less susceptible to protease action. Perhaps, these PS II centres reflect the presence of a poorly processed D_1 protein.

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