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Changes in fast chlorophyll *a* fluorescence transient in heat-treated *Chlamydomonas reinhardtii* thylakoid membranes

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ABSTRACT

On illumination of *Chlamydomonas* thylakoid membranes after a period of darkness, the intensity of Chl *a* fluorescence passes through a characteristic sequence changes termed OIDPS transient. Addition of 2,5-dimethyl-p-benzoquinone quenched the DP rise only while 2,6-dichloro-p-benzoquinone addition quenched both the DP rise and the OI phase. The different changes in the OID phase upon the addition of both quinones demonstrate the predominance of the reduced Q_A^- component in the inactive photosystem II centers. However, the effect of both quinones is a concentration dependence and DCBQ is more effective in intercepting the electrons from the inactive PS II centers than DMQ. In *Chlamydomonas* thylakoid membranes, heat-treatment enhanced the initial Chl *a* fluorescence (F_I) and plateau (F_P) levels reflecting the reduction of Q_A to Q_A^-. The heated thylakoid membranes are sensitive to the addition of DMQ and DCBQ. Addition of DCBQ decreased the fluorescence to a level close to the steady-state of the control sample reflecting an effective electron flow. This in contrast to the DMQ treatment which did not reach (F_0) of the control. This leads to the assumption that heat-treatment converts the active centers of PS II into inactive centers in *Chlamydomonas* thylakoid membranes. The inactive centers are known to have an inefficient electron transfer to the plastoquinone pool and a poor water oxidation potential.

Abbreviations: DCBQ: 2,6-dichloro-p-benzoquinone; DMQ: 2,5-dimethyl-p-benzoquinone; PS II: photosystem II; PS I: photosystem I; Q_A: primary electron acceptor of photosystem II; Q_B: secondary electron acceptor of photosystem II. Chl *a*: chlorophyll *a*. ms: millisecond; *PQ*: plastoquinone; *F*: rate of emission; I_a: light flux, K_F: rate constant of fluorescence.

INTRODUCTION

Fluorescence is an important tool in basic and applied plant physiology research. The photosynthetic activity is measured by chlorophyll *a* fluorescence emitted by leaves from higher plants or algae. The rate of the emission (F) is proportional to the absorbed light flux (I_a) and to the quotient of the rate constant of fluorescence (K_F) over the sum of rate constants (ΣK_i) of all competing reactions that result in a return of chlorophyll molecule to the ground state as mentioned by Krause & Weis (1991). Thus, the generation equation for fluorescence yield will be:

$$F = I_a \cdot K_F / \Sigma K_i$$

and

$$\Phi F = F/I_a = K_F/\Sigma K_i$$

As proposed by Duysens & Sweers (1963), fluorescence yield is minimal (ΦF_0) when all reaction centers are opened, i.e. the primary plastoquinone Q_A is in the oxidized state (ZP_{860} *pheo* $Q_A\,Q_B$). The maximum fluorescence yield (ΦF_M) is obtained when Q_A is fully reduced i.e. all reaction centers are closed (ZP_{680} *pheo* $Q_A^-\,Q_B$). The typical fluorescence induction signal; which represents a complex polyphasic process of fluorescence chloroplasts of higher plants or algae, is known as Kautsky phenomenon (Briantais *et al.* 1986). The minimum fluorescence emission originates from antenna chlorophyll *a* molecules as suggested by many workers (e.g Butler 1977, 1978) whereas viable fluorescence F_v (which is the difference between the maximum F_M and minimum F_0 fluorescence) may be originated in the antenna (Butler 1977, 1978) or due to the back transfer of excitation energy from the closed reaction centers (Klimov *et al.* 1977 and Klimov & Krasnovskii 1981). As electrons are passed on from Q_A via Q_B to the plastoquinone pool (*PQ*), the fluorescence rise is also related to the reduction of these electron carriers and has been used to determine the size of the *PQ* pool (Briantais *et al.* 1986). Oxidized *PQ* has been shown to act as a fluorescence quencher (Vernotte *et al.* 1979). Thus, the true F_M can only be reached when the *PQ* pool becomes reduced. Thus, the rate of Q_A reoxidation causes fluorescence quenching depending on electron transfer via photosystem *I* and on the final consumption of reducing equivalents in carbon metabolism and other metabolic reactions (Krause & Weis 1991). The typical fluorescence induction signal exhibit a relatively fast rise from F_0 to F_I (*I* is inflection) followed by a plateau or dip (F_D) and a slower rise to the peak (F_P) or fluorescence maximum (F_M). F_P is lower than F_M when full reduction of Q_A is not achieved. Chylla & Whitmarsh (1989) proved the existence of inactive PSII centers in intact leaves. Melis *et al.* (1988) and Melis & Homann (1976) postulated the presence of two types of PSII units: PSIIα centers which are supposed to be in the appressed grana membranes and PSIIβ centers which are located in the nonapprosed membranes. Cao & Govindjee (1990) demonstrated the quenching of variable fluorescence of thylakoids to F_I level upon the addition of 2,5-dimethyl-p-benzoquinone. Addition of 2,6-dichloro-p-benzoquinone lowered the variable fluorescence to F_I then to F_0 reflecting the enhancement of photosynthetic oxygen evolution as DCBQ accepts electrons directly from Q_A. Larcher *et al.* (1990) showed the changes in the fluorescence quenching kinetics using thylakoid membranes heated up to 50°C. The current investigation was undertaken to study the changes of chlorophyll *a* fluorescence transient in heat-treated *Chlamydomonas* thylakoid membranes. DMQ and DCBQ were used as exogenous acceptors to demonstrate the relationship between the nature of the OID phase which is affected by the reduction of Q_A to Q_A^- and the active and inactive PSII centers in heated thylakoid membranes. The differential effects of both quinones on fast chlorophyll *a* fluorescent yield at different temperatures were studied.

MATERIALS AND METHODS

Chlamydomonas reinhardtii cells were originally grown in sterilized plates containing Tris-Acetate-Phosphate (TAP) culture media mixed with 15% agarose. The algae were transferred to the growing flasks containing (TAP) culture media at 25°C and

pH 7.3 as described by Gorman and Levine (1965). The growing cultures were shaken on a gyratory shaker and exposed to continuous illumination from fluorescent lamps for 96 hours before fluorescence measurements. Isolation of chloroplasts was carried out according to the method described by Hyunsuk *et al.* (1989). Chloroplasts were suspended in a reaction media containing 20 mM MES, 2 mM $MgCl_2$, 2 mM EDTA (disodium salt) and 0.1% Bovin Serium Albumin in (BSA). Chloroplasts transferred at Teflon pestle tissue homogenizer to be homogenated and adjusted to 2 mg Chl/mL. Chlorophyll content was determined according to the equation described by Arnon (1949). Heat-treatment was carried out in a water bath at different temperatures for 3 minutes. Dimethyl quinone (DMQ) or dichlorobenzoquinone (DCBQ) were added to the chloroplasts directly before the measurements were undertaken. Chlorophyll *a* fluorescence transient were carried out by the home-built spectro fluorimeter. Illumination was provided with a Kodak Carousel 4200 slide projector filtered with a blue filter CS4-76 and a yellow filter 3-13. The fluorescence was filtered with 2 Corning CS2-61 red filter before entering the monochromator. The slit widths on the monochromator were set to 4 mm. Chlorophyll *a* fluorescence transient can be displayed on an oscilloscope or an output of the trace on a digital plotter.

Three ml of *Chlamydomonas* chloroplasts containing 20 μg/ml were placed into the bottom of the glass-bottomed Dewar flask which formed the sample chamber.

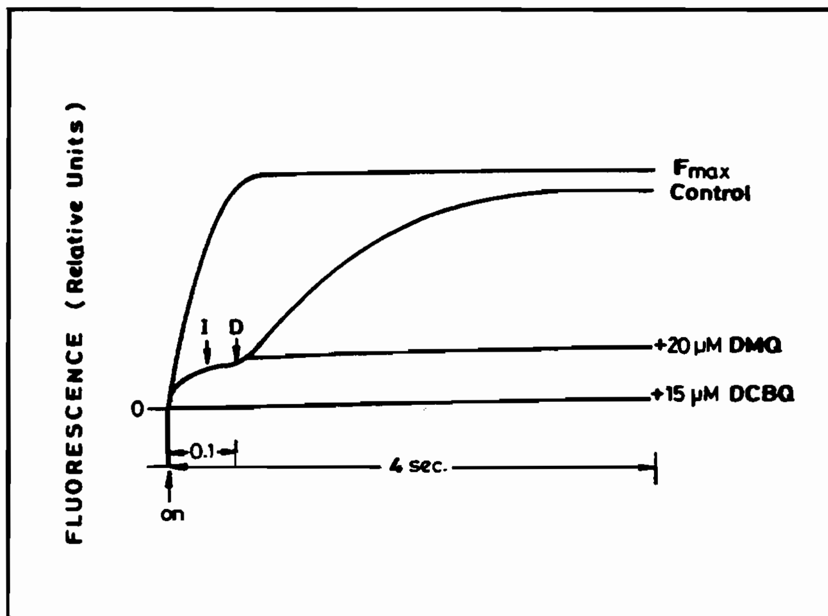

Fig. 1. Chlorophyll *a* fluorescence transient of *Chlamydomonas reinhardtii* at 20°C with 20 μMDMQ, 15 μMDCBQ as quinone acceptors and without quinones. Chloroplasts were incubated in the reaction media contained: 20 mM MES, 15 mM $MgcL_2$, 2 mM EDTA and 0.1% Bovin serium Albumen. Chlorophyll concentration was 20 μg Chl/mL. The samples were left in the dark for 10 minutes before the fluorescence measurements. O is the constant level, I is the intermediate fluorescence, D is a dip and P is the maximum fluorescence yield.

RESULTS AND DISCUSSION

CHANGES IN CHLOROPHYLL *a* FLUORESCENCE AT 20°C:

Analyses of fluorescence induction curves help in understanding the organization and function of the photosynthetic apparatus. Holzwarth (1990) demonstrated the existence of two PSII pools namely PSIIα and PSIIβ centers which are characterized by having components with different lifetimes. Melis & Anderson (1983) described PSIIα to posses a bigger antenna size compared to PSIIβ centers. Moreover, the efficiency of the electron flow was found to be 3 times higher in PSIIα than PSIIβ centers as proposed earlier. Thus, PSIIα centers can be considered the main pool of electrons needed for NADP reduction in photosynthesis as recommended by Melis (1985). Chylla & Whitmarch (1989) showed that the exponential fluorescence rise from F_0 to F_I can be attributed to inactive centers and conditions of smaller antenna system of inactive units. Thus, the two types of PSII reaction centers control the initial fluorescence rise from its constant level F_0 to its maximum level F_M. Figure 1 shows chlorophyll *a* fluorescence transient in *Chlamydomonas* chloroplasts affected by different concentrations of DMQ (A) and DCBQ (B). The control sample (without exogenous quinones) shows a typical chlorophyll *a* fluorescence induction. O-I-D-P points are clearly observed; I level appears at 50 ms and P level at 3 s after the start of illumination. It is clear that chlorophyll *a* fluorescence transient is affected differently by the two acceptors and the effect depends on the quinone concentration. However, DMQ quenches only the DP rise, while DCBQ quenches both the DP rise and the OI phase. Figure 2 represents the effect of quinone concentrations on chlorophyll *a* fluorescence at both I and P levels (after 50 ms and 3 seconds of illumination)

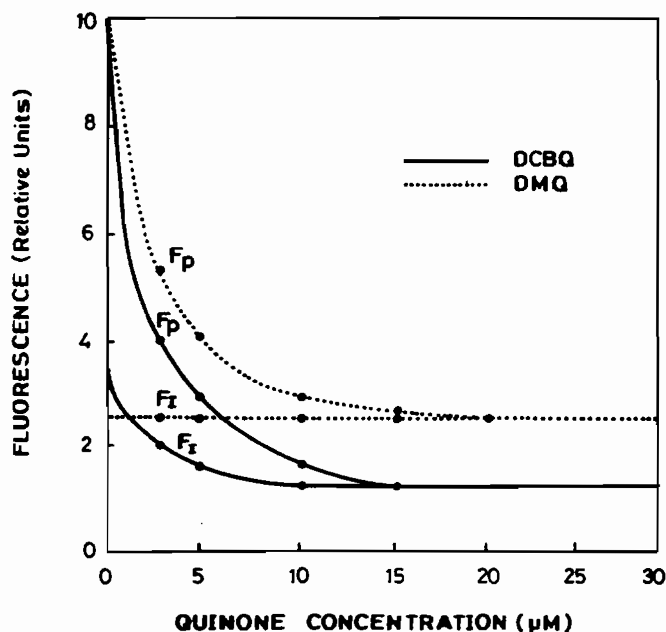

Fig. 2. Effects of quinone concentration on chlorophyll *a* fluorescence yield at the initial (F_I) and the maximum (F_P) fluorescence levels. (......) represents the effect of DMQ and (____) represents the effect with DCBQ.

respectively. F_P is quenched by both quinones while F_I is quenched only by DCBQ and the quenching is saturated at 20 μMDMQ and 15 μMDCBQ. Moreover, only DCBQ is able to quench P level to a level that is close to the constant level F_0. The different effects of different concentrations of the two electron acceptors DMQ and DCBQ on F_I quenching (Figs. 1 & 2) demonstrate the predominance of Q_A^- component in the inactive PSII centers. This result is in agreement with that obtained by Cao & Govindjee (1990) using thylakoid isolated from soybean plants. However, Guenther *et al.* (1988) mentioned the presence of PSIIβ centers in both active and inactive centers of green algal cells *Dunaliella salina*. The interconversion between PSIIα and PSIIβ is considered as a dynamic property of the thylakoid membrane as remarked by many workers (Sundby *et al.* 1986, Gunther & Melis 1990).

CHANGES IN CHLOROPHYLL *a* FLUORESCENCE TRANSIENT AT 55°C:

Heat-stress was found by Timmerhaus & Weis (1990) to convert PSII centers into its β-form. Bilger *et al.* (1988) showed that high temperature)over 40°C) caused an impairment of photosynthesis in heated thylakoid membranes. F_I level can serve as an indicator of inactive PSII centers as mentioned by Krause & Weis (1991). The dependence of the initial fluorescence level F_I (measured at 50 ms of illumination); reflecting the conversion of active centers into inactive, on temperature is demonstrated in Fig. 3. Increasing temperature caused an increase in the fluorescence level suggesting that heat-treatment keeps the primary electron acceptor in the reduced state Q_A^-. Figure 4 shows the changes in fast chlorophyll *a* fluorescence transient in *Chlamydomonas* chloroplasts at 55°C compared to 25°C. A fast initial fluorescence

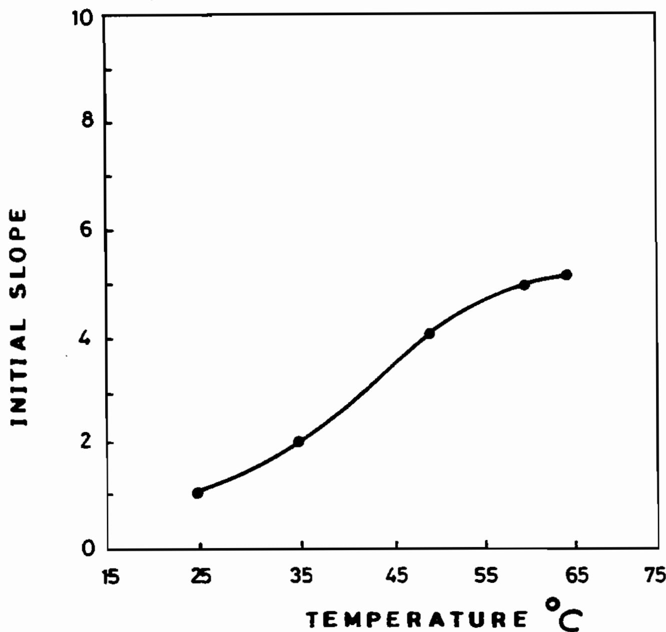

Fig. 3. The initial slope of chlorophyll *a* fluorescence after 50 ms of illumination in heat-treated membranes (up to 65°C). Chloroplasts membranes were prepared as described in Materials and methods.

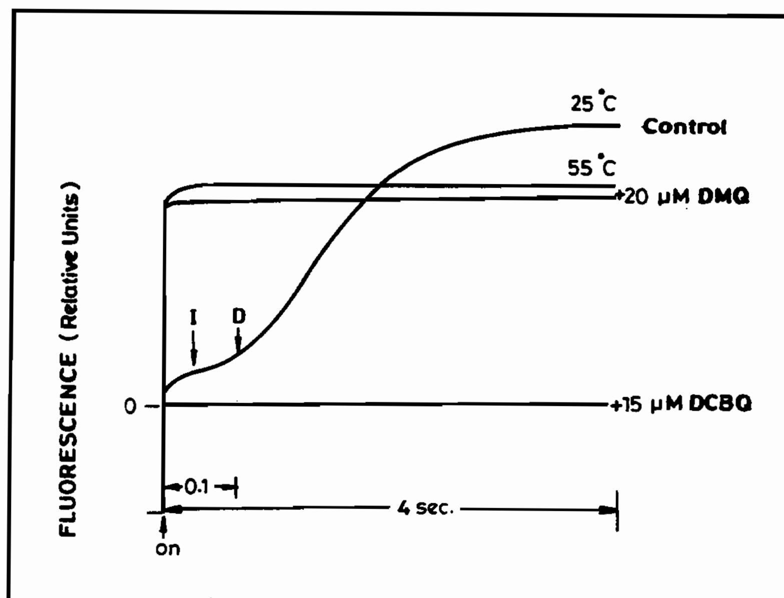

Fig. 4. Chlorophyll *a* fluorescence transient of heat-treated membranes (at 25°C and 55°C) and the effects of DMQ and DCBQ on the fluorescence yield. The membranes were incubated in the reaction media and heated in a water bath for 3 minutes. O, I, D and P were explained in the legend of Fig. 1.

rise appears and the OID phase disappears in heat-treated sample. However, addition of DCBQ to the heat-treated samples quenched the fluorescence level as in chloroplasts at 20°C (Fig. 1). It should be noticed that addition of 20 μMDMQ quenched the fluorescence level to its constant level (F_0) at 20°C but not at 55°C and the quenching is less effective compared to DCBQ. Figure 5 shows the dependence of the initial fluorescence (F_I) which reflects the reduction of Q_A to Q_A^- on DCBQ concentration at 55°C. It is clear that increasing quinone concentration quenched the initial level in heated thylakoid membranes. Addition of 15 μMDCBQ quenched the fluorescence level to its minimum value F_0 reflecting the efficiency of the electron flow by converting the inactive centers; affected by heat treatment, into active centers. The high ability of DCBQ, compared to DMQ, to draw electrons from inactive PSII centers; as it quenches the heat-induced fluorescence rise, is also demonstrated in Fig. 4.

In summary, the differential effects of DMQ and DCBQ on the OID phase shown in (Figs. 1 & 2) was explained by Krause & Weis (1991) as the inactive centers possess a functional water oxidation system but do not significantly contribute to net electron as they do not have the ability to reduce PQ. The initial fluorescence level (F_I) was increased by increasing temperature (Fig. 3) showing the conversion of active centers into its β form. Addition of DCBQ to the heat-treated thylakoid membranes caused quenching of the fluorescence rise to its minimum level (Figs. 4 & 5). This is explained by the ability of DCBQ to convert the inactive centers (caused by heat-treatment) into active centers possessing an efficient electron flow. The inactive PSIIβ centers were recommended by Guenther *et al.* (1990) to function in keeping the assembly of active PSII centers of the thylakoid membrane by acting as a reserve pool. This function was

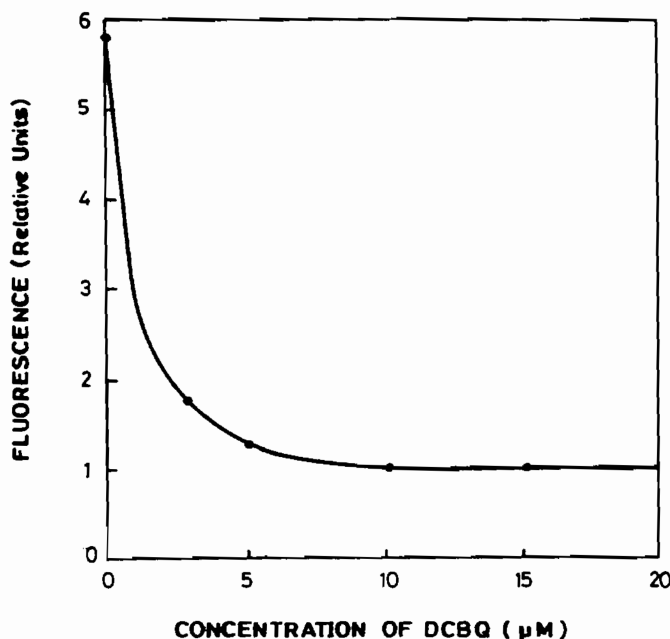

Fig. 5. The initial chlorophyll *a* fluorescence yield after 50 ms of illumination upon the addition of different concentrations of DCBQ to the heat-treated membranes.

suggested by Guenther and Melis (1990) to be related to development or turnover of the D1 protein in the light and repair of damaged centers (Sundby *et al.* 1986).

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دراســة التغـيرات التي تحــدث لمعـدل التفلور السريــع بـاستخــدام خـلايــا طحلب الكيلاميدوموناس المعاملة حراريا

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خلاصة

استخدم لهذا الغرض كل من ثنائي ميثيل كينون وثنائي كلوروبنزوكينون كمستقبلين صناعيين للألكترونات لبيان تأثيرهمـا على معـدل التفلور في المدى الأولي السريـع عند درجـة الحرارة العادية والمرتفعة.

وقد وجد أنه عند درجة حرارة ٢٥°م يعمل المستقبل الأول على تخفيض مستوى التفلور في جزء من المدى السريع فقط (OI) بينما يعمل المستقبل الثاني على تخفيض المستوى في كل المدى السريع (OIDP). وهذا يثبت سيادة المركب الأكتروني الأولي المختزل في المراكز غير النشطة في النظام الضوئي الثاني في عملية البناء الضوئي. وبصفة عامة يتوقف هذا التأثير على تركيز المستقبل الأكتروني المضاف ويتميز داي كلوروبنزوكينون بفاعلية أكثر في هذا المجال.

وقد نتج عند المعاملة الحرارية (00°م) لخلايا الطحلب زيادة في المدى السريع لمعدل التفلور مما يؤكد سيادة المركب الأكتروني الأولي المختزل. وقد اختفت هذه الزيادة تماما عند اضافة المستقبل الأكتروني ثنائي كلوروبنزوكينون. وهذا يؤكد أن المعاملة الحرارية لخلايا طحلب الكيلاميدوموناس تؤدي الى تحول المراكز النشطة في النظام الضوئي الثاني لعملية البناء الضوئي الى مراكز غير نشطة تتميز بعدم كفاءة الإنتقال الأكتروني الى مركز البلاستوكينونات.