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Effect of chromate on photosynthesis in Cr(VI)-resistant *Chlorella*

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Abstract

Chromate-resistant *Chlorella spp*. isolated from effluents of electroplating industry could grow in the presence of 30 µM $K_2Cr_2O_7$. Since photosynthesis is sensitive to oxidative stress, chromate toxicity to photosynthesis was examined in this algal isolate. Chromate [Cr(VI)] up to 100 µM was found to stimulate photosynthesis, while 90% inhibition was found, when the cells were incubated with 1 mM Cr(VI) for 4 h. Photosystem (PS) II was inhibited by 80% and PSI by 40% after such Cr(VI) treatment. Thermoluminescence studies on cells treated with 1 mM Cr(VI) for 4 h showed that $S_2Q_A^-$ recombination peak (Q) was shifted to higher temperature, whereas $S_2/S_3Q_B^-$ recombination peak (B) was shifted to lower temperature. These shifts indicated alga stress response in order to overcome an excitation stress resulting from the inhibition of photosynthesis by Cr(VI). The nontreated *Chlorella* cells kept in the dark showed periodicity of four for the Q peak (4–8ºC) and B peak (34–38°C) after exposure to series of single, turnover, saturating flashes. This periodicity was lost in Cr(VI)-treated cells. Higher concentrations of Cr(VI) inhibited mainly the electron flow in the electron transport chain, inactivated oxygen evolving complex, and affected also Calvin cycle enzymes in the Cr(VI)-resistant isolates of *Chlorella*.

Additional key words: algae; chromate-resistant; photosynthesis; thermoluminescence.

Introduction

Chromium (Cr) is a highly toxic metal to all living organisms. It is known to induce oxidative stress. The widespread industrial use of Cr has resulted in making it a serious pollutant of aquatic bodies. Bioaccumulation of Cr and its effect on photosynthetic pigments or lipid peroxidation has been reviewed in plants by Shanker *et al*. (2005). Algae are known to grow in the most inhospitable environments (Kalin *et al.* 2004). Several heavy metal–polluted and Cr-contaminated sites can support growth of algae (Cervantes *et al.* 2001). Effective concentration for 50% survival (EC_{50}) for $K_2Cr_2O_7$ have been shown to range from ~4 × 10^{-3} mol m^{-3} in *Selenastrum* to ~25 × 10^{-3} mol m^{-3} in *Senedesmus* (Takami *et al*. 2012). We isolated unicellular, nonmotile green alga belonging to genus *Chlorella* from an effluent site of electroplating industry. This isolate showed EC_{50} value around 62 × 10^{-3} mol m^{-3} for $K_2Cr_2O_7$ (Yewalkar *et al.* 2007).

Since Cr(VI) is known to cause oxidative stress (Gorbi *et al.* 2006), it is important to investigate how the process of photosynthesis responds to Cr(VI) in algae. Wium-Andersen (1974) had shown that 0.5 ppm and 5 ppm Cr(VI) was responsible for 50% reduction of the photosynthetic CO_2 fixation in diatoms (*Nitzschia palea*) and green algae (*Chlorella pyrenoidosa*). In *Scenedesmus obliquus*, PSII was shown to be the major site of action of Cr(IV) and other heavy metals (Mallick and Mohn 2003). Ali *et al.* (2006) showed that in *Lemna gibba* Cr(VI)

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Abbreviations: BBOT – 2,5-bis(5'-tert-butyl-2-benzoxazol-2-yl)thiophene; Chl – chlorophyll; DCFH-DA – 2′,7′-dichlorodihydrofluorescein diacetate; Cr(VI) – chromate; DCMU – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP – 2,6-dichlorophenolindophenol; DPC – diphenylcarbazide; PSI – photosystem I; PSII – photosystem II; R-5-P – ribose-5-phosphate; ROS – reactive oxygen species; TL – thermoluminescence; T_m – temperature maxima of the thermoluminescence peak; EC_{50} – effective concentration for 50% survival.

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inhibited water oxidation complex and Q_a of PSII. In case of *C. pyrenoidosa*, it was shown that the D1 protein of the PSII reaction center was destroyed by Cr(VI) (Horcsik *et al.* 2007). Popovic *et al.* (2008) observed quenching of the variable fluorescence in *Chlamydomonas reinhardtii* after exposure to Cr(VI). When xanthophyll-deficient mutant of *C. reinhardtii* was exposed to dichromate and higher light intensity, 80% of the energy dissipation occurred through the nonregulated mechanisms, making mutant algae more sensitive to the dichromate toxicity (Ali *et al.* 2008). Further investigations of Cr(VI) interaction with photosynthetic machinery in *C. reinhardtii* showed inhibition of PSII, PSI, and electron transport sink beyond the photosystems (Perreault *et al.* 2009). In *Euglena gracilis*, PSII was found to be more susceptible to Cr(VI) and Cu (II) than the respiratory system (Rocchetta and Kupper 2009). In case of the freshwater green alga, *Monoraphidium convolutum*, the short-term exposure to Cr(VI) increased the electron transport rate and photosynthetic quantum yield, which was ultimately reduced after long-term exposure of 48 h (Takami *et al.* 2012). Recently, Volland *et al.* (2012) have shown that prolonged exposure of the unicellular alga, *Micrasterias*, to 5–10 × 10^{-3} mol m^{-3} Cr(VI) resulted in a strong inhibition of photosynthesis because of reduction in chlorophyll (Chl), alterations in ultrastructure, and inhibition of PSII as indicated by Chl fluorescence measurements.

The Cr(VI)-resistant *Chlorella* species isolated by us was able to withstand chronic Cr(VI) stress mainly by reduction of Cr(VI) in the medium and also by intracellular accumulation up to 10% of the externally added Cr(VI) (Yewalkar *et al.* 2007). However, the growth was sensitive to the presence of Cr(VI) above 200 µM in the medium. Therefore, we decided to investigate the effect of higher concentration of Cr(VI) on photosynthetic parameters, such as CO_2 fixation, oxygen evolution, and partial reactions of electron transport, charge recombination in PSII, and Calvin cycle enzymes in this isolate.

Materials and methods

Algal culture: The Cr(VI)-resistant isolates of *Chlorella* (Yewalkar *et al.* 2007) were grown in axenic condition in Tris-acetate-glycerophosphate (TAgP) medium containing 30 µM potassium dichromate and under continuous illumination of fluorescent lamps with 24 W m^{-2} of light intensity at room temperature (Collard and Matagne 1990). In all experiments, cells in the early log phase were used.

CO_2 fixation: The cell suspension in the growth medium was treated with 0, 10, 30, 50, 70, 100, 200, 500, 800, or 1,000 µM $K_2Cr_2O_7$. 100 µl of the cell suspension were withdrawn and added to 100 µl of the growth medium containing 20 mM $NaH^{14}CO_3$ (specific activity of 18.5 MBq mol^{-1}). $NaH^{14}CO_3$ was obtained from Board of Radiation and Isotope Technology, Mumbai, India.

The reaction mixtures were incubated for 10 min in the light (21 W m^{-2}) at room temperature and the reaction was stopped by addition of acetic acid. The vials were dried and the acid-stable ^{14}C-labeled product was resuspended in 3 ml of alcohol and mixed in 10 ml of 0.4% 2,5-bis(5'-tert-butyl-2-benzoxazol-2-yl) thiophene (BBOT) in toluene. Radioactivity was measured using a scintillation counter (*Tri-carb 2100 TR Liquid Scintillation Analyzer Packard, Canberra Company,* USA). In another experiment, cells were preincubated in the light with 1 mM $K_2Cr_2O_7$ for different times before measuring light-dependent CO_2 fixation. The rates were expressed as µmol(CO_2) fixed mg^{-1}(Chl) s^{-1}.

O_2 evolution: The cell suspension in the growth medium was treated with 1 mM $K_2Cr_2O_7$ for different time intervals under light intensity of 24 W m^{-2}. The light intensity during the measurement was 254 W m^{-2}. Oxygen evolution was monitored polarographically in the light by using a Clarke type oxygen electrode of *Gilson Oxygraph* (*Gilson Medical Electronics*, USA) and expressed as µmol(O_2) evolved mg^{-1}(Chl) s^{-1}.

Reactive oxygen species (ROS) estimation: ROS production was detected as described by He and Häder (2002) by using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). Various concentrations of $K_2Cr_2O_7$ were added to cell suspensions and incubated for 30 min in the light. DCFH-DA of 3.5 µM was added immediately to the reaction mixture, which was subsequently kept on shaker in the dark at room temperature for 20 min. The relative amount of ROS in samples was monitored at room temperature with *Hitachi F-4010* spectrofluorometer (*Hitachi*, Tokyo, Japan) by measuring fluorescence excited at 485 nm and emitted at 520 nm.

Thermoluminescence (TL) measurements: The cells were incubated with and without 1 mM $K_2Cr_2O_7$ for 4 h in the light (24 W m^{-2}). The cells were then allowed to relax in the dark for 10 min at room temperature. The samples containing 100 µg of Chl were exposed to light (210 W m^{-2}) for 6 min at room temperature and then frozen in liquid nitrogen under the same illumination. Glow curves were recorded as described by Desai (1992) using TL instrument built in *Bhabha Atomic Research Centre*, Mumbai, India equipped with far-red sensitive photomultiplier tube *R669* (*Hamamatsu*, Japan). The rate of heating was 16–18ºC min^{-1}. The TL glow peaks were recorded using the TL setup having a microcontroller-based data recording system, acquiring data at half-second interval. To study the flash induced oscillation patterns of $S_2Q_A^-$ (Q) and $S_2/S_3Q_B^-$ (B) recombination peaks, the cells (250 µg of Chl) were incubated with or without 1 mM $K_2Cr_2O_7$ in the light for 4 h at room temperature, allowed to relax in the dark for 10 min, and exposed to sequences of single turnover saturating flashes (3 µs, 0.5 J, white light ~13,264 W m^{-2} at 4°C and treated with 10 µM DCMU before quick freezing in liquid nitrogen to study the oscillation pattern of the Q peak (Demeter and Vass 1984). B peak oscillation pattern was studied similarly in cellsnontreated with DCMU.

Preparation of cell-free extracts: Seven ml of the cell suspension containing about 6.5 × 10^{10} cells ml^{-1} (treated with and without 1 mM $K_2Cr_2O_7$ for 4 h in the light 24 W m^{-2}) were subjected to sonication in pulse mode for 18 min in 50 mM Tricine-KOH buffer (pH = 7.6). The cell lysate was centrifuged at 500 × *g* for 3 min to remove unbroken cells and large cell debris. Cell free extracts were used for estimation of PSII and PSI activities and Calvin cycle enzymes.

Assay of photosynthetic electron transport in cell-free extracts: PSII and PSI activities were determined essentially according to Izawa (1980). The samples were illuminated with white light (254 W m^{-2}) during assay. For PSII assay, the reaction mixture contained cell free extract having 60 µg of Chl, 50 mM Tricine–KOH (pH 7.6), 5 mM KCl, 5 mM $MgCl_2$, and 3 mM $K_3Fe(CN)_6$ in the volume of 2 ml. Oxygen evolution was monitored polarographically in the light by using a Clarke-type oxygen electrode of *Gilson Oxygraph* and expressed as nmol(O_2) evolved mg^{-1}(Chl) s^{-1}.

PSI activity was also monitored polarographically by following the oxygen consumption in the light and the dark by using a Clarke-type oxygen electrode. The reaction mixture consisted of cell free extract containing 60 µg of Chl in 2 ml of 50 mM Tricine–KOH buffer, pH 7.6, containing 5 mM $MgCl_2$, 50 mM KCl, 50 µM DCPIP, 2 mM sodium ascorbate, 2 mM NaN_3, 5 mM NH_4Cl, 50 µM DCMU, and 50 µM methyl viologen in 2 ml. The assay mixture for PSII and PSI, excluding water oxidation complex, consisted of 50 mM Tricine–KOH (pH 7.6), 2 mM NaN_3, 5 mM NH_4Cl, 0.5 mM DPC as electron donor, 50 µM methyl viologen as an electron acceptor and cell-free extract containing 60 µg of Chl in 2 ml (Hind 1993). O_2 consumption was monitored and expressed as nmol(O_2) consumed mg^{-1}(Chl) s^{-1}.

Ribose-5-phosphate (R-5-P) and ATP-dependent CO_2 fixation: Cell-free extracts were used to measure R-5-P and ATP-dependent CO_2 fixation activity as described previously by Sainis *et al.* (2003). The assay mixture contained 50 mM Tris-EDTA (pH = 7.4) with 10 mM $MgCl_2$, 10 µM DTT, 2 mM ATP, and 10 mM $NaH^{14}CO_3$ (specific activity of 18.5 MBq mol^{-1}). The reaction mixture was incubated for 15 min. The reaction was started by adding R-5-P to make a final concentration of 2 mM and it was terminated after 6 min by transferring 100 µl of the reaction mixture to scintillation vial containing 200 µl of 6 N acetic acid. The acid stable, ^{14}C-labeled product was measured using scintillation counter as described earlier and expressed as nmol(CO_2) fixed mg^{-1}(Chl) s^{-1}.

Chl estimation was done as described by Wintermans and De Mots (1965). Briefly, 1.5 ml of culture was centrifuged and 1.5 ml of 95% ethanol was added to the pellet and mixed well for 5 min. The extract was centrifuged and absorbance (A) of supernatant was measured at 649 and 665 nm. Total Chl was calculated by using the formula:

$$\text{Total Chl } [\mu g\ ml^{-1}] = 6.10 \times A_{665} + 20.04 \times A_{649}$$

Intracellular Cr was determined as described by Yewalkar *et al.* (2007) using ^{51}Cr labeled (sodium chromate). Cells were incubated in TAgP medium containing 1 mM potassium dichromate with specific activity ~6.3 Bq µmol^{-1} for 4 h in the light. Concentration of ^{51}Cr was determined in cell pellets with scintillation counter.

Results

The *Chlorella* isolate used in the present work was found to grow in presence of 30–100 µM Cr(VI) (Yewalkar *et al.* 2007). Since Cr(VI) produces oxidative stress, we measured the effect of Cr(VI) on the production of ROS in the Cr(VI)-resistant algal cells. ROS was detected in all Cr(VI)-treated cells even at lower concentrations (Fig. 1*A*). There was an asymptotic increase in ROS concentration up to 0.8 mM $K_2Cr_2O_7$. The highest ROS production was observed in cells incubated with 1 mM $K_2Cr_2O_7$ (Fig. 1*A*).

The incubation of *Chlorella* at lower concentration of $K_2Cr_2O_7$ (30–100 µM) was found to stimulate CO_2 fixation (Fig. 1*B*). CO_2 fixation was inhibited only by 20% at 200 µM Cr(VI). In all these cases, the time of incubation was lesser than 30 min including the assay time. The percentage of inhibition remained similar even when the concentration of $K_2Cr_2O_7$ increased up to 1 mM. Although ROS production was the highest at 1 mM Cr(VI), the inhibition of photosynthesis was time-dependent; it was *ca.* 20%, when examined within 30 min after the treatment. However, the inhibition increased with prolonged time of the incubation. Around 90% inhibition was observed after 4 h (Fig. 2*A*). The inhibition of light-dependent oxygen evolution paralleled the effect of Cr(VI) on photosynthesis (Fig. 2*B*) as expected. It was observed that *Chlorella* cells became brittle and showed 144.85 ± 24.46 × 10^{-7} nmol of Cr(VI) per cell, when incubated with 1 mM $K_2Cr_2O_7$ for 4 h in the light.

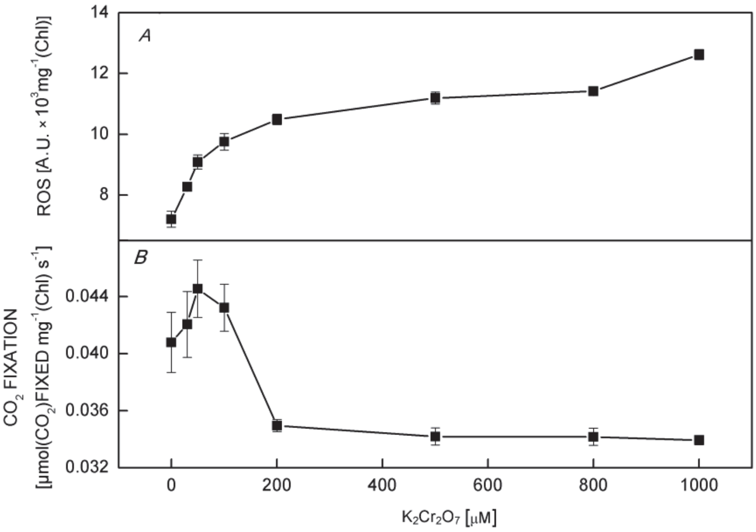

Fig. 1. Effect of Cr(VI) on *A*: ROS production (mean ± SE, $n = 6$) and *B*: CO_2 fixation (mean ± SE, $n = 3$). Cells in the log phase of *Chlorella* isolate were treated with various concentrations of $K_2Cr_2O_7$. ROS produced is represented in arbitrary units (A.U.) of fluorescence (λ = 485 and 520 nm for excitation and emission, respectively).

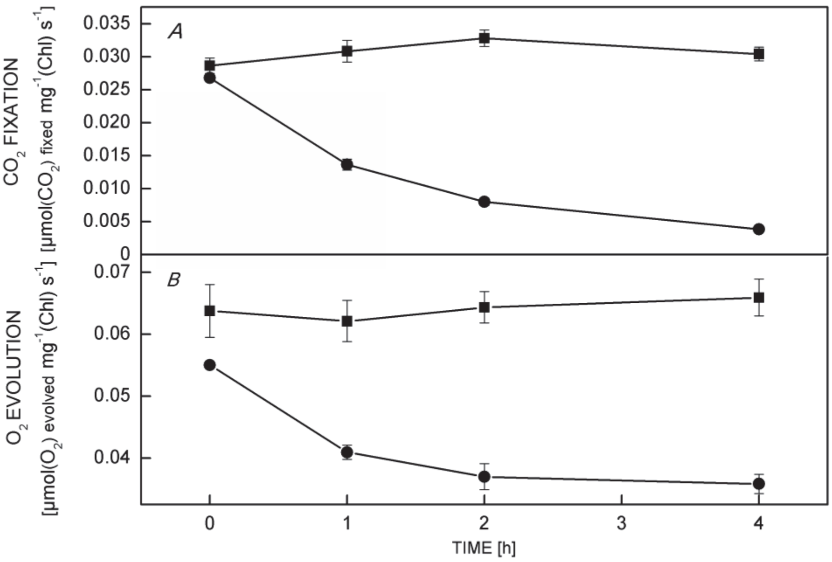

Fig. 2 Effect of Cr(VI) on *A*: CO_2 fixation (mean ± SE, $n = 4$) and *B*: O_2 evolution (mean ± SE, $n = 6$). Cells in the log phase of *Chlorella* were incubated in the light with and without 1 mM $K_2Cr_2O_7$ 0–4 h. ■ control cells, ● treated cells.

Since CO_2 fixation and oxygen evolution were inhibited by 90% after incubation with 1 mM $K_2Cr_2O_7$ for 4 h in the light (24 W m^{-2}), partial reactions of photosynthesis were measured in cell-free extracts of *Chlorella* treated for 4 h with 1 mM $K_2Cr_2O_7$ in the light. It was observed that under these conditions, PSII activity was inhibited by ~87%, whereas the activity of PSI was inhibited by ~40%. The activity of electron transport including PSI and PSII, but excluding water oxidation complex, was inhibited by ~49% (Table 1). Activities of partial reactions of Calvin cycle were also examined in the cell-free extracts of treated cells. The linked activity

Table 1. Effect of 1 mM Cr(VI) on partial reactions of photosynthesis. The experiments were repeated 4 times. The data represent means of 4 readings with ± SE. R-5-P + ATP-dependent CO_2 fixation activity shows the linked activity of 3 sequential enzymes of Calvin-Benson cycle, *viz*. phosphoriboisomerase, phosphoribulokinase, and RuBP carboxylase.

	Control	Treated	Inhibition [%]
PSII activity including water oxidation complex (ferricyanide reduction) [nmol(O_2) evolved mg^{-1}(Chl) s^{-1}]	4.556 ± 0.583	0.583 ± 0.167	87
PSII and PSI activity excluding water oxidation complex (DPC → MV) [nmol(O_2) consumed mg^{-1}(Chl) s^{-1}]	4.056 ± 0.667	2.083 ± 0.611	49
PSI activity (Reduced DCPIP →MV) [nmol(O_2) consumed mg^{-1}(Chl) s^{-1}]	21.058 ± 0.778	12.539 ± 0.972	40
R-5-P + ATP-dependent activity [nmol(CO_2) fixed mg^{-1}(Chl) s^{-1}]	28.53 ± 0.067	18.14 ± 0.028	36

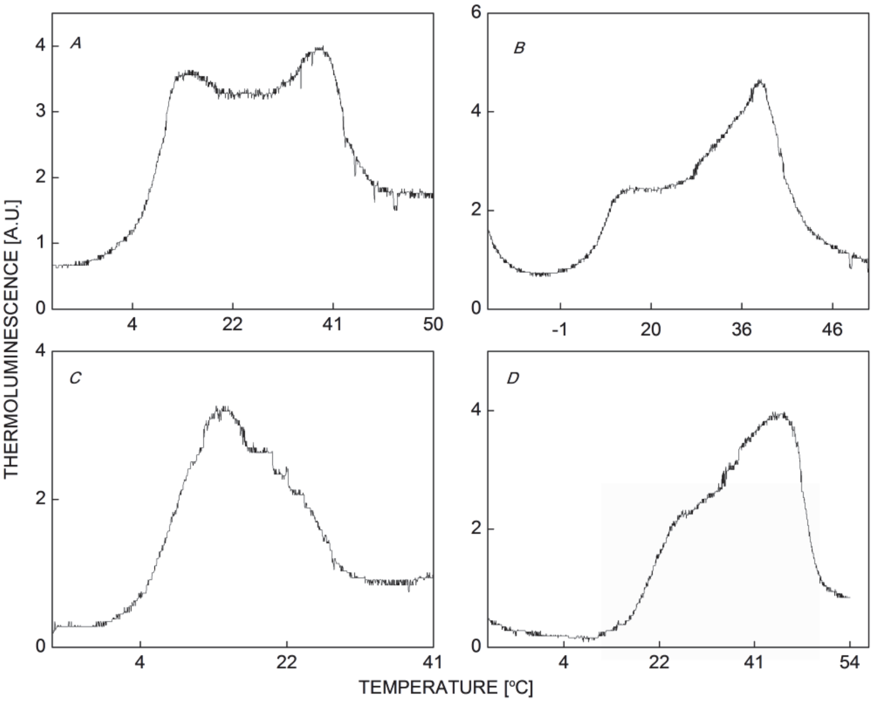

Fig. 3. Effect of Cr(VI) on thermoluminescence. Cells of *Chlorella* were treated with 1 mM $K_2Cr_2O_7$ in the light for 4 h and glow curves were monitored as described in Materials and methods. Thermoluminescence without DCMU (*A,B*) and with DCMU (*C,D*) in control and $K_2Cr_2O_7$ treated cells, respectively. A.U. – arbitrary units.

of three sequential enzymes of Calvin–Benson cycle, *i.e.* phosphoriboisomerase, phosphoribulokinase, and ribulose-1,5-bisphosphate (RuBP) carboxylase was measured as R-5-P+ATP dependent CO_2 fixation activity. This linked activity was inhibited by ~36% in Cr(VI)-treated cells (Table 1).

TL of the Cr(VI)-resistant *Chlorella* cells kept with and without 1 mM $K_2Cr_2O_7$ was monitored (Fig. 3). After the exposure to $K_2Cr_2O_7$ for 4 h in the light, the cells were kept in the dark for 10 min to allow the excited states to relax. TL glow peaks were monitored after the exposure of cells to continuous white light for 6 min (210 W m^{-2}). In the nontreated cells, Q band (9–14ºC), originating from $S_2Q_A^-$charge recombination, and B band (34–37ºC), originating from $S_2Q_B^-$ charge recombination, were observed (Fig. 3*A*). In presence of DCMU, intensity of Q band increased, whereas B band was lost (Fig. 3*C*). The deconvolution analysis of the peaks indicated that the intensity of Q band increased 2.5 times after DCMU treatment (Fig. 3*A,C*). In cells treated with 1 mM $K_2Cr_2O_7$, where photosynthesis was inhibited by 90%, Q peak was observed at 8–15ºC and B peak was observed at 31–37ºC (Fig. 3*B*). After DCMU treatment, there was a significant change in the glow curves of $K_2Cr_2O_7$-treated cells. The Q peak was shifted to higher temperatures in this case and appeared at 22–25ºC (Fig. 3*D*), while in the nontreated cells it was observed in the range of 10–17ºC (Fig. 3*C*). TL showed an additional peak at 50°C to Q peak. The deconvolution analysis of the Cr(VI)-treated cells revealed that although Q peak appeared in the DCMU-treated cells, the intensity was reduced 1.4 times (Fig. 3*B,D*).

Effect of 1 mM $K_2Cr_2O_7$ on water oxidation complex was checked by studying the periodicity of the Q and B peaks of the cells kept in the dark for 4 h. The flash induced peaks also provide more accurate temperature maxima of the thermoluminescence peak (T_m) of both Q (S_2Q_A) and B (S_2Q_B) peaks as compared to peaks obtained from continually illuminated samples. The nontreated cells showed periodic oscillations of B and Q peaks when exposed to single turnover saturating flashes of white light (Fig. 4*A,B*). Such a periodicity was not observed in the cells treated with 1 mM $K_2Cr_2O_7$ as described above (Fig. 4*C,D*). The T_m for the Q peak was found to be shifted from about 4–8ºC in control to about 12–17ºC in Cr(VI)-treated cells, while the B peak was shifted to a lower temperature, *i.e.* from 34–38ºC in the control cells to 32–35ºC in the treated cells (Table 2). Similar shift of temperature was also observed in the continually illuminated cells (Fig. 3). The temperature gap between the T_m of Q and B peaks narrowed from 30ºC in the control cells to 18–20ºC in Cr(VI)-treated cells.

Discussion

Cr is known to be toxic mainly because of a production of ROS. The membrane functions are considered to be more susceptible to oxidative stress (Leyko and Bartosz 1986). We isolated a unicellular, nonmotile algae belonging to *Chlorella*, which showed EC_{50} around 62 µM. This isolate showed the stimulation of light-dependent CO_2 fixation in the presence of up to 100 µM of $K_2Cr_2O_7$ in the medium. We also measured the effect of Cr(VI) on

CO_2 fixation in the standard culture of *Chlorella pyrenoidosa* (NCIM 2738) obtained from the National Chemical Laboratory, Pune. Although this culture was sensitive to Cr(VI) (EC_{50} was 3.2 μM), the effect of Cr(VI) on CO_2 fixation was similar to Cr(VI)-resistant isolate (data not shown). This was in contrast to observations of Wium-Andersen (1974), who had shown that 5 ppm of Cr(VI) was inhibitory for the photosynthetic CO_2 fixation in *C. pyrenoidosa*. The incubation of cells with higher concentration of $K_2Cr_2O_7$ up to 1 mM did not substantially reduce the rate of light-dependent CO_2 fixation immediately after the treatment with Cr(VI), although there was the significant increase in ROS production in the presence of Cr(VI). When the cells of Cr(VI)-resistant strain of *Chlorella* were incubated with 1 mM $K_2Cr_2O_7$, the accumulation was 144.85 ± 24.46 × 10^{-7} nmol of Cr per cell in 4 h. This intracellular Cr might affect the algal cells, as we could observe the substantial reduction in the rate of light-dependent CO_2 fixation as well as O_2 evolution. Therefore, we analyzed partial reactions of photosynthesis in these Cr(VI)-treated cells. PSII activity including water oxidation was found to be inhibited to a greater extent than PSI activity suggesting that PSII was more susceptible to Cr(VI). When PSII activity was measured excluding water oxidation complex with DPC as the electron donor, the inhibition was lesser. Since the DPC to methyl viologen reaction uses the entire electron transport of both PS excluding the water oxidation complex, it was apparent that water oxidation complex was the most sensitive to Cr(VI).

We used TL, which is an effective tool to monitor PSII *in vivo* (Sane 2004). The data with the continuous light exposure of treated and nontreated cells showed that Q peak (in presence of DCMU) originating from the recombination of the $S_2Q_A^-$ charge pair, shifted to the higher temperature in Cr(VI)-treated cells suggesting an increased activation energy and stabilization of the $S_2Q_A^-$ charge pair compared with the nontreated cells. Similar change in redox potential of Q_A was reported in *Arabidopsis*, acclimatized to cold temperatures (Sane *et al.* 2003).

The Tms of TL peaks are known to be related to the redox potentials of the recombining species involved. Therefore, it appeared that the Cr(VI) treatment influenced the redox properties of the recombining species responsible for both Q and B peaks.

In flash experiments conducted on cells relaxed in the dark, the nontreated cells showed periodicity of four with maxima at 2nd flashes as expected for Q (T_m 4–8ºC) and B peak (T_m 34–38ºC). Similar periodicity of B peak was recently reported by Ducruet *et al.* (2011) in mixotrophically and autotrophically grown cells of *Chlorella* sp. and in *C. reinhardtii*, when cells were exposed to xenon or far-red light single turnover flashes. The cells treated with 1 mM Cr(VI) did not show typical oscillation pattern for either Q or B peaks supporting the contention

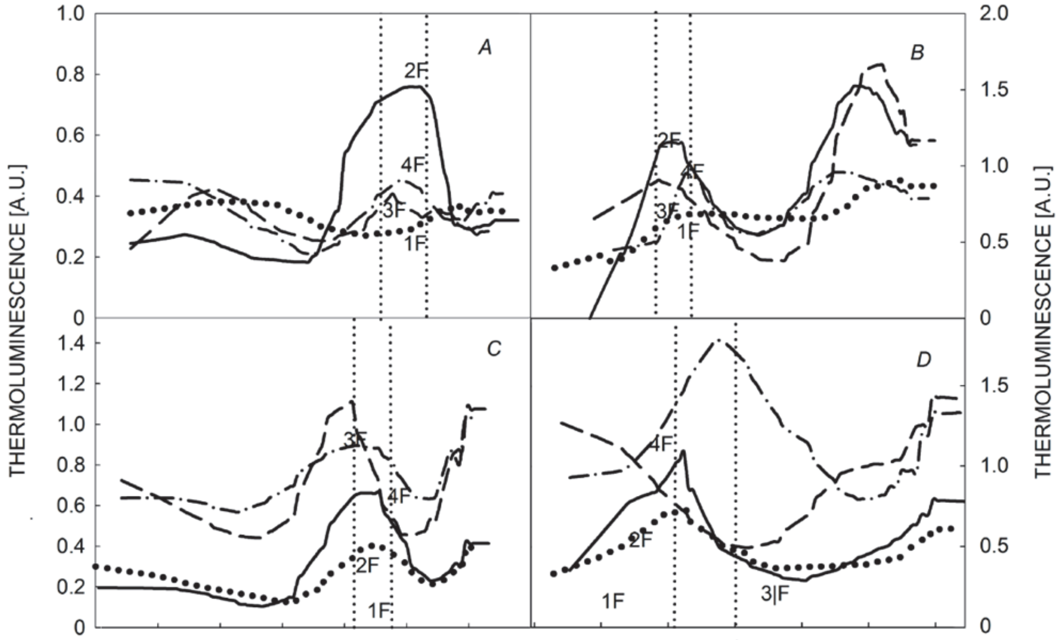

Fig. 4. Effect of Cr(VI) on B and Q peak periodicity. Cells of *Chlorella* were kept in the light for 4 h and relaxed in the dark for 10 min. Thermoluminescence of cells containing 250 μg of total Chl was monitored after exposing to different number of single saturating turnover flashes of white light. 1F, 2F, 3F, and 4F – TL yield in arbitrary units (A.U.) after 1, 2, 3, and 4 flashes, respectively. *A*,*B*: B and Q peaks in control cells. *C*,*D*: B and Q peaks in treated cells. *A*,*C*: Cells not treated with DCMU; *B*,*D*: Cells treated with 10 μM DCMU.

Table 2. Effect of Cr(VI) on Q and B peaks of thermoluminescence in flash experiments. The cells were treated with 1 mM $K_2Cr_2O_7$ for 4 h in the light, relaxed in the dark and exposed to sequences of single turnover saturating flashes (3μs, 0.5 J, white light) at 4°C. The data represent means of 6–8 readings with SE of T_m of TL peaks.

Type of sample	Q peak [T_m ºC]	B peak [T_m ºC]
Control	6.55 ± 0.8	36.51 ± 1.00
Cr(VI)-treated	15.49 ± 2.3	32.65 ± 1.17

that Cr(VI) inactivated the water oxidation complex. The data on flash induced generation of Q and B peaks provided a clearer picture of the T_m of the peaks. It showed that in Cr(VI)-treated cells, Q peak shifted to a higher temperature (12–17°C), while B peak appeared at a lower temperature (32–35°C) (Fig. 4*C,D*). T_m of the TL peaks was directly proportional to the activation energies of the charge pairs involved in the recombination. Increasing T_m in the Cr(VI)-treated cells suggested that the activation energy of the $S_2Q_A^-$ redox pair yielding Q peak increased, while the activation energy of the $S_2Q_B^-$ redox pair decreased in the Cr(VI)-treated cells. Thus, the gap between the activation energy of the two charge pairs was reduced in the treated cells. Since the activation energies are directly related to the redox potentials of the participating species (DeVault and Govindjee 1990), it is apparent that the redox potentials of the participating species should have changed. In the production of Q and B peaks, the participating species involved are the S_2 state and the two quinone molecules, Q_A and Q_B, on the acceptor side of PSII. Thus, the redox potential of either the S_2 state or Q_A and Q_B or both must have changed. We believe that the redox potential of the S state did not change as it should have either increased or decreased the activation energy of both recombinations. However, the activation energy of S_2Q_A increased, while that of S_2Q_B was reduced. This could happen only if the redox potential of Q_A became more positive and that of Q_B became more negative. The net result was the reduction in the redox potential difference between these two quinone acceptors. A number of authors noted such changes in the redox potentials of Q_A and Q_B under various stress and acclimation conditions. Cold acclimation or growth at low temperature resulted in the downshift of the $S_2Q_B^-$ band (Briantais *et al.* 1992, Janda *et al.* 2001, Sane *et al.* 2002, 2003; Ivanov *et al.* 2006). Heavy metals, such as copper (Horvath *et al.* 1998) and mercury (El-Seekh and Mustafa 1999) have been observed to downshift $S_2Q_B^-$ besides decreasing its intensity. Exposure to ozone (Skotnica *et al.* 2003), desiccation (Skotnica *et al.* 2000) or water stress (Metwally *et al.* 1998) resulted in the downshift of S_2Q_B and in some cases, an upshift of S_2Q_A peak. As pointed out by Ivanov *et al.* (2003), such shifts appear to be a general response of plants under stress conditions. Sane *et al.* (2002, 2003) and Ivanov *et al.* (2003) have suggested that the shifts of S_2Q_A and S_2Q_B in the opposite direction narrows the redox potential difference between the immobile one electron acceptor quinone, Q_A, and the mobile two electron acceptor, Q_B, resulting in the changes in the rate constants of electron flow between these two acceptors in a way that the electron has a higher probability of being retained on Q_A. The prominent peak observed in Fig. 3*D* at 50–52°C, in 1 mM $K_2Cr_2O_7$-treated cells, after incubation with DCMU might be a peak C, which is enhanced by DCMU or by a damage. It might originate from dysfunctional electron donor of PSII, $TyrD^+$ and Q_A^-, as suggested by Ducruet and Vass (2009). Horcsik *et al.* (2007) showed that in *C. pyrenoidosa* cells treated with 20–40 g m^{-3} of $K_2Cr_2O_7$ for 8–72 h, PSII function was inhibited due to enhanced photodestruction of the reaction centers. However, although no change in redox potential of Q_A and Q_B was observed, a decrease in intensity of TL peak and increase in lipid peroxidation was reported. The lethal concentration of Cr(VI) for the standard culture of *C. pyrenoidosa* was 20 g m^{-3} (Horcsik *et al.* 2006). The Cr(VI)-resistant *Chlorella* isolate under study, exposed to 1 mM $K_2Cr_2O_7$ showed the decrease in temperature gap between $S_2Q_A^-$ and $S_2Q_B^-$ recombination. It was possible that this Cr(VI)-resistant isolate might acclimatize to the Cr stress. Thus, it showed this shift of redox potentials due to the acclimatization to the Cr(VI) ions, because the isolate was always maintained in the medium containing 30 μM $K_2Cr_2O_7$.

Previous studies by Minagawa *et al.* (1999) showed that narrowing the gap in the T_m of S_2Q_A and S_2Q_B by 6–8ºC in a temperature range of 15 to 30ºC resulted in a calculated redox potential difference of 22 mV between these two acceptors in *Synechocystis*. The temperature gap between S_2Q_A and S_2Q_B in our studies was around 30ºC for controls and it was reduced to 18–20ºC in the Cr(VI)-treated cells. Sane *et al.* (2002, 2003) and Ivanov *et al.* (2006) have argued that this confers photoprotection to PSII by an increased nonradiative recombination of $P680^+Q_A^-$, which does not generate the Chl triplets responsible for a production of ROS. It appeared that Cr(VI)-treated cells of Cr(VI)-resistant *Chlorella* responded by this mechanism to protect themselves from an increased excitation pressure resulting from the inhibition of electron transport. This quenching mechanism appears to be a general response of plants under stress (Huner *et al.* 2008), including the stress associated with the presence of Cr(VI) as it was shown in our experiments. In our study, the treatment of Cr(VI)-resistant *Chlorella* isolate with 1 mM $K_2Cr_2O_7$ showed shifts in the redox potentials of Q_A and Q_B that might confer photoprotection through reaction center quenching mechanism in the resistant alga.

In summary, we observed that the photosynthetic machinery of Cr(VI)-resistant *Chlorella* was stimulated by lower concentrations (up to 100 μM) of $K_2Cr_2O_7$ in the medium. Most of the unicellular algae showed deleterious effects of Cr(VI) at this concentration. However, the incubation with 1 mM of $K_2Cr_2O_7$ for 4 h resulted in 90% inhibition of photosynthesis. In these cells, Cr(VI) affected electron transport systems and water-oxidation complex to a considerable extent as well as Calvin cycle enzymes to some extent. The data also explained the possible mechanism by which the Cr(VI)-resistant *Chlorella* may prevent damage due to the excitation pressure resulting from loss of photosynthetic activity due to the presence of Cr(VI).

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