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Heat Induced Reversible Increase in Photosystem 1 Emission in Algae, Leaves and Chloroplasts: Spectra, Activities, and Relation to State Changes*

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Abstract

Low temperature fluorescence spectrum of *Chlorella* cells heated to 45—48 °C for 2 min was characterized by a high F_{715}/F_{685} ratio indicating increased fluorescence from photosystem 1 (PS 1). This effect was reversible as cooling the heated cells to room temperature decreased this ratio to a value obtained in control cells. The heated cells at elevated temperatures retained the ability to undergo state changes and irradiation by "light I" (710 nm) during heating prevented increased fluorescence from PS 1. The reversible increase in PS 1 fluorescence by heating was also observed in *Canna* leaf and isolated spinach chloroplasts. On the basis of changes in fluorescence and associated electron transport activities of chloroplasts, it is suggested that heating favours development of state II whereas cooling reverses it.

Temperature is an important factor in controlling the biochemical and biophysical reactions of photosynthesis. Under natural conditions several plants experience heat stress and it is important not only to study the effects of high temperature on photosynthetic reactions but also the mechanisms that permit the plant to endure the heat-stress (for a review of environmental regulation of photosynthesis see Berry and Downton 1982). Heat treatment (40—45 °C for 2 min) blocks the oxygen evolution without affecting electron transport through photosystems (PS) 1 and 2 (Katoh and San Pietro 1967).

The physical state of the membrane is affected by heating (Murata *et al.* 1975, Murata and Fork 1976) causing changes in its activities (Nolan and Smillie 1976). Heating also causes displacement of integral protein components of the membrane (Armond and Staehelin 1979). Also the spectroscopic properties of the pigments *in vivo* charge (Schreiber and Armond 1978, Bhardwaj and Singhal 1981, Singhal *et al.* 1981).

Many of the past studies have been made at temperatures that cause irreversible changes. Such irreversible effects may be related to heat stress and ultimate death of such systems. However, reversible effects may provide clues to the phenomenon of heat tolerance. In the present communication we show that heat induced increase in PS 1 emission is reversed by cooling the sample to room temperature in *Chlorella, Canna* leaves and spinach chloroplasts. We further demonstrate that in *Chlorella*, the heat induced increase in PS 1 emission can be prevented by exposure of cells to light I during heating. These changes have been suggested to be due to membrane conformational changes that are similar to state I/II changes.

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MATERIALS AND METHODS

Chlorella cells were grown autotrophically at room temperature (25 °C) under irradiation supplied by four fluorescent tubes of 20 W each and by bubbling air through the culture. The algal cells in the log phase (24 h old) were used in all the experiments. The state I/II changes were monitored using the procedure described by Sane *et al.* (1982). For this purpose, algal cells (3—5 cm^3) were exposed to "light I" (obtained by passing the "white light" from a 500 W projector lamp through a *Schott* interference filter, 710 nm, 47.5% transmission) or "light II" (using a 649.2 nm interference filter; 39.5% transmission) for 5 min. The treated sample (absorbance 0.05, in 10 mm path length, at 678 nm) was taken in a 3 mm diameter glass tube open at both the ends and quickly frozen to liquid nitrogen temperature. The low temperature emission spectra were recorded using an *Aminco-Bowmann* spectrophotofluorometer with a low temperature accessory. The ratio of F_{715} to F_{685} (uncorrected) was used as a measure of the development of state I/II (Sane *et al.* 1982).

The low temperature emission spectra of samples pretreated with different temperature regimes were recorded in a similar way except freezing to liquid nitrogen temperature of the sample was done under room irradiance.

In experiments on a *Canna* leaf, the leaf pieces cut to the size of 8 × 20 mm were used. After the temperature treatment the leaf piece was frozen to liquid nitrogen temperature quickly and fluorescence spectra were monitored. A special aluminium device to hold the leaf piece was employed.

Chloroplasts were isolated from spinach (*Spinacia oleracea* L.) leaves by the procedure of Sane *et al.* (1970). Electron transport from water to methyl viologen and from reduced DCIP (2,6-dichlorophenol indophenol) to methyl viologen was monitored using a *Clark* type oxygen electrode and reaction conditions as used by Sane *et al.* (1979). DCIP reduction with water as the donor was monitored at 600 nm using the *DW 2A* spectrophotometer.

RESULTS AND DISCUSSION

Fluorescence emission spectra at 77 K of three samples of *Chlorella* cells that were initially at 25 °C (*A*) or 45 °C (*B*) or at 25 °C after pretreatment with 45 °C for 2 min (*C*) before being frozen to 77 K (Fig. 1, *top*) show that heat induced an increase in F_{715}/F_{685} ratio. The changes observed in the spectra from *A* to *B* are similar to those obtained on exposure of the sample to "light II" (primarily absorbed by PS 2 pigments) leading to the development of state II (see Barber 1982 for a review on state changes). The heating effect was reversible, *i.e.*, cooling the heated sample (*C*) to room temperature (25 °C) restored the ratio of F_{715}/F_{685} to that observed in the 25 °C. Similar effects were obtained with a *Canna* leaf (Fig. 1, *middle* — spectra normalised to 685 nm) and isolated spinach chloroplasts (Fig. 1, *bottom*). However, with chloroplasts the heat treatment had to be carried out at 38 °C for 120 s rather than at higher temperatures, when the fluorescence changes were irreversible.

Earlier studies on adaptation of *Chlorella* cells to lights I and II have shown that these cells develop state I and state II, respectively, with characteristic changes in the ratio of F_{715}/F_{685} (Sane *et al.* 1982). The reversibility of the spectra suggested that possibly heating lead to such changes in the membrane conformation which were observable on exposure of cells to "light II". Cooling of the sample would result in reversing the changes and the membrane would acquire a conformation similar to state I. If this is true, we can predict that heating of the cells to high temperature in the presence of "light I" will not allow a change in the spectra by preventing development of state II by higher temperatures. These predictions were confirmed (Fig. 2):

State I was developed by 710 nm irradiation at 25 °C (Fig. 2*B*), while heat induced the development of state II with a characteristic high ratio of F_{715}/F_{685} (Fig. 2*C*). The exposure of the sample to 710 nm during incubation at an elevated temperature (Fig. 2*D*) decreased the ratio of F_{715}/F_{685} restoring a level obtained in Fig. 2*A*. Thus "light I" prevents the development of state II

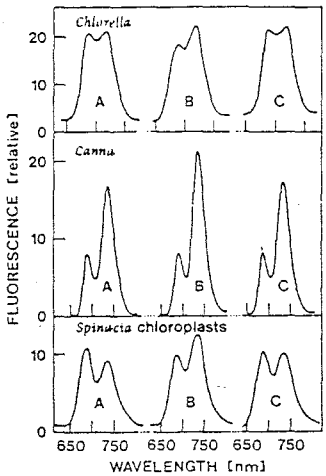

Fig. 1.

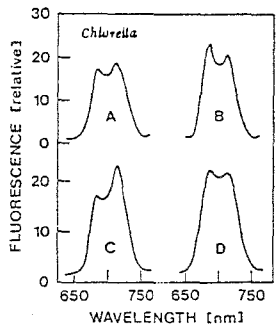

Fig. 2.

Fig. 1. Uncorrected fluorescence spectra at liquid nitrogen temperature of *Chlorella* cells (*top*), *Canna* leaf (*middle*), or isolated spinach chloroplasts (*bottom*) that were initially at (*A*) 25 °C, (*B*) 45 °C (38 °C for chloroplasts), or (*C*) 25 °C, but the sample was heated to 45 °C (38 °C for chloroplasts) for 120 s before slowly cooling to 25 °C.

Fig. 2. Uncorrected fluorescence spectra at liquid nitrogen temperature of *Chlorella* cells that were initially at (*A*) 25 °C, (*B*) 25 °C, but exposed to 710 nm for 5 min at 25 °C prior to freezing, (*C*) 45 °C, and (*D*) 45 °C, but exposed to 710 nm for 5 min at 45 °C prior to freezing. — *Chlorella* cells exposed to 650 nm for 5 min at 25 °C gave fluorescence spectra at liquid nitrogen which were similar to (*C*).

at elevated temperatures indicating that temperature induced changes may be similar to state changes induced by "light'I" and "light II". A comparison of Figs. 2*B* and 2*D* shows that the extent of state I developed by "light I" at an elevated temperature is not as high as at room temperature. This points to a possibility that state changes are dependent on the membrane fluidity. At elevated temperatures the fluidity is increased and the membrane has a tendency to acquire a relaxed conformation. Our earlier studies (Sane *et al.* 1982) on *Chlorella* have shown that a dark adapted relaxed sample acquires a state identical to state II. Thus the extent of development of state I by "light I" at an elevated temperature will be controlled by the ability of the membrane to acquire a relaxed state (state II) due to increased fluidity of the membrane. The change in the ratio F_{715}/F_{685} in Fig. 2 from *A* to *B* was similar to the change of the ratio from *C* to *D* brought about by "light I". Thus the reversible heat effects in the fluorescence spectra of algal cells brought about by temperature are probably caused by membrane conformational changes similar to or identical to state changes. High temperature induces the state II. The membrane of *Chlorella* at room temperature is in an intermediate state and can be driven to extreme state II either by heating or by "light II", or to extreme state I by "light I". The ratio F_{715}/F_{685} measures the extent of state change.

Bhardwaj and Singhal (1981) have previously observed an increased emission from PS 1 at high temperature and suggested increased efficiency of photosynthetic energy transfer from light harvesting chlorophylls to PS 1. However, the reversibility was not studied.

Table 1

Effect of temperature on electron transport in chloroplasts. Isolated spinach chloroplasts, equivalent to 20 μg chlorophyll per 2 cm^3 of 0.05 M phosphate buffer, pH 7.5, containing 50 mM NaCl, were treated at given temperatures for 120 s and assayed at required temperatures after addition of necessary reagents. The reaction mixture in a total volume of 2 cm^3 contained phosphate buffer, pH 7.5, 50 mM; NaCl, 50 mM; and chloroplasts equivalent to 20 μg chlorophyll. In addition, either methyl viologen (MV), 0.1 mM; NaN_3, 0.3 mM ($H_2O \rightarrow MV$); or dichlorophenolindophenol (DCIP), 0.1 mM ($H_2O \rightarrow DCIP$) or ascorbate 3 mM; DCIP, 0.01 mM; DCMU, 10 μM; MV, 0.1 mM and NaN_3, 0.3 mM ($DCIPH_2 \rightarrow MV$) were added. The control rate of electron transport with H_2O as the donor was 20 μmol O_2 kg^{-1}(chl) s^{-1} and for $DCIPH_2 \rightarrow$ $\rightarrow$ MV 60 μmol O_2 kg^{-1}(chl) s^{-1}. Irradiance provided by a 300 W tungsten lamp from a projector was passed through a water filter and a *Corning* red cut off filter *2—63*. Irradiance was not saturating, but limiting.

Pre-treatment for 2 min at [°C]	Temperature of assay [°C]	Activity [%]		
		$H_2O \rightarrow MV$	$H_2O \rightarrow DCIP$	$DCIPH_2 \rightarrow MV$
25	25	100	100	100
38	38	75	53	153
38	25	84	73	111

We argue that if heat induces the development of state II which can be reversed by cooling then these state changes should be reflected in electron transport activities. Although electron transport cannot be measured in algal cells or *Canna* leaf pieces, we have determined the electron transport from H_2O to methyl viologen, H_2O to DCIP and reduced DCIP to methyl viologen in chloroplasts under conditions that produce reversible fluorescence changes in them. Table 1 shows that if chloroplasts were heated to 30 °C for 120 s prior to measuring activity at that temperature the $H_2O \rightarrow MV$ and $H_2O \rightarrow DCIP$ rates (PS 2 activity) declined to 75 and 53%, respectively. As against this, the PS 1 activity (reduced $DCIP \rightarrow MV$) increased by over 50% at elevated temperatures. If the chloroplast sample heated to 38 °C for 120 s was brought back to room temperature, then electron transports $H_2O \rightarrow MV$ and $H_2O \rightarrow DCIP$ improved to 84 and 73%, respectively. The PS 1 rate also reversed back to almost the original level. These data suggest that associated with the development of state II at an elevated temperature, there was the expected increase in PS 1 activity and decrease in PS 2 activity. This trend was reversed on cooling the chloroplasts to room temperature. Thus the postulated state changes on heating and cooling were reflected in the changes in electron transport activities of the two photosystems. The incomplete reversal of the PS 2 activity suggested that some irreversible damage, although small, was done to PS 2 activity.

The question of why chloroplasts or algal cells acquire state II at elevated temperatures is difficult to answer. However, one explanation could be that state II is a relaxed state and is therefore connected with increased fluidity of the membrane at elevated temperatures. There is also an attractive possibility that development of state II permits delivery of exciton energy to PS 1

which is less sensitive to damage. If this exciton energy was retained by PS 2 possibly it may have suffered photooxidation damage also. If so, development of state II at an elevated temperature may serve as a protective mechanism against photooxidation damage to the photosynthetic apparatus.

Recent studies (Bennett *et al.* 1980, Allen *et al.* 1981) have shown that the development of state II is due to phosphorylation of the light harvesting chlorophyll-protein complex (LHCP). If so, one would expect LHCP phosphorylation at elevated temperature and dephosphorylation on lowering the temperature. The chloroplasts that are not supplied with ATP or substrates for phosphorylation cannot phosphorylate LHCP. Thus at elevated temperatures, fluorescence characteristics similar to state II could not have been induced by LHCP phosphorylation under our experimental conditions. This suggests that a state II identifiable by fluorescence changes (or electron transport activities) can also be developed independent of LHCP phosphorylation. Our earlier studies (Sane *et al.* 1982) had also questioned LHCP phosphorylation as a pre-requisite for the development of state II under all experimental conditions.

The present data show that increased PS 1 emission observed here (*cf.* also Sane *et al.* 1980, Downton and Berry 1982) at elevated temperatures may be due to the development of state II. This change is reversible and associated with changes in electron transport rates typical of state changes.

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