Effect of Preheating Intact Algal Cells on Pigments Revealed by Absorption and Fluorescence Spectra

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Received August 13, 1979 · Accepted March 16, 1981

Summary

Preheating intact cells of the green alga *Chlorella pyrenoidosa*, blue-green alga *Anacystis nidulans*, and the red alga *Porphyridium cruentum*, in the temperature range of 50 to 60C for 10 minutes, produces a 2–3 nm blueshift in the «red» absorption band of chlorophyll *a* (Chl *a*) at 298K; the sensitivity of these cells (in the order of increasing heat tolerance) is: *Porphyridium*, *Chlorella* and *Anacystis*. Preheating also causes considerable loss of absorption by phycobilins in the red and blue green algae. Only a little loss of Chl *a* is observed in all the three algae.

At 77K, preheated *Chlorella* cells show a new emission band at 710 nm (at 77K) in contrast to three bands at 687, 698 and 725 nm in untreated cells. Preheated *Anacystis* and *Porphyridium* cells when excited with 440 nm light (absorbed mainly by Chl *a*) show an increase in 685 nm emission band at 298K. At 77K, however, an increase at 717 nm *(Anacystis)* or 692 nm *(Porphyridium)* is observed. A decrease in excitation energy transfer from the remaining phycobilins to Chl *a* of system II, both at 298K and 77K (emission at 685 nm), is suggested from emission spectra obtained with 560 nm excitation (absorption in phycobilins). In *Anacystis*, an increased energy transfer from phycobilins to Chl *a* of System I (emission at 717 nm at 77K) also takes place upon preheating of cells.

Excitation spectra of Chl *a* fluorescence, both at 298 and 77K, in *Chlorella* show a relative increase in the bands for long wavelength absorbing forms of Chl *a* in preheated cells. Excitation spectra of Chl *a* fluorescence at 298K in *Porphyridium* and *Anacystis* suggest that either the energy transfer from the weakly fluorescent form of Chl *a* to the highly fluorescent form of Chl *a* is increased or the former is converted into the latter upon preheating the cells, explaining the increased emission from Chl *a* noted above; a decreased energy transfer from remaining phycobilins to Chl *a* of system II is also confirmed. In *Porphyridium*, a relative decrease of Chl *a* 680–685 excitation band in contrast to a relative decrease of Chl *a* 670 excitation band in *Chlorella*, is observed. This suggests that, perhaps, the physicochemical nature of the same spectral form of Chl *a* in different algae is different.

Key words: Anacystis, Porphyridium, Chlorella, fluorescence, phycobilins, chlorophyll a, heating effects.

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Introduction

Several spectral forms of chlorophyll *a* (Chl *a*) exist in thylakoids of green plants and cyanobacteria (Brown, 1972, Litvin and Sineshchekov, 1975). These spectral forms *in vivo* may originate as a result of different interaction of the chromosphore with different proteins and lipids (Thornber, 1975), and/or water and other chromophores (Katz and Norris, 1973). Physical treatments, such as dehydration (Cho, 1969), extraction with mixtures of water and organic solvents (Cederstrand et al., 1966), sonication (Das and Govindjee, 1967); and heat treatment; (Goedheer, 1970) of algal cells and chloroplasts alter the spectroscopic properties of Chl *a* *in vivo*. Such physical treatments are expected to perturb the chromophere from their natural environments, and thus, their effects may aid us in understanding their interactions. In this paper, we have examined the effects of preheating algal cells on their spectroscopic properties. Heat treatment not only inactivates the oxygen evolving system (Cheniae and Martin, 1970) but also denatures membrane proteins in general. If a thermal denaturation of proteins associated with pigments occurs, we shall observe changes in the spectroscopic properties of pigment-protein complexes *in vivo*.

In the present paper, we report absorption, emission and excitation (action) spectra of Chl *a* fluorescence (298K, 77K) in control and heat-treated cells of three algae (*Chlorella*, *Anacystis* and *Porphyridium*). Drastic changes in emission and excitation spectra of fluorescence, reflecting changes in the spectral forms of Chl *a* as well as in the mode and extent of excitation energy transfer among various pigment-protein complexes are observed.

Methods

Algal cells were grown in continuous light as described by Govindjee and Rabinowitch (1960). Cells were harvested by centrifugation and washed either in the fresh culture medium without any micronutrients, or in 0.02 M phosphate buffer (pH 7.8). Cell suspensions were diluted to have an absorbance between 0.3 and 0.4 for 1 cm path length at the red peak of Chl *a* absorption band. The suspension was heated in a constant temperature bath (ranging from 45 to 60 °C) for 10 minutes, and then cooled to room temperature. These samples are referred to as preheated or heat-treated cells.

Absorption spectra of algal suspensions were recorded in a Bausch and Lomb spectrophotometer (Spectronic 505) equipped with an integrating sphere. The emission and the excitation spectra of Chl *a* fluorescence were measured in a spectrofluorometer described by Shimony et al. (1967). The procedures for 77K measurements were as described by Cho (1969). The half-band width of all the slits of the monochromators were 3.3 nm for 298K measurements. At 77K, narrower slits (the detecting slits in the case of emission spectra and the excitation slits in the case of excitation spectra) of 1–2 nm were used. Emission spectra are presented after corrections for the spectral sensitivity of the photomultiplier and the transmission characteristics of the observation monochromater and the excitation spectra after correction for the number of the incident photons.

Results

1. Absorption Spectra

The absorption spectrum of the intact cells of *Chlorella* was fairly resistant to heat treatment; there was no change with preheating upto 50 °C. However, preheating at 55 °C caused a 2 nm blue shift, and at 60 °C, a 3 nm blue shift of the red peak of Chl *a* absorption band. In addition, a slight decrease in absorbance due to Chl *a* and carotenoids was observed. The absorption spectrum of *Anacystis* (Fig. 1 A) was stable upto a preheating temperature of 55 °C. However, preheating at 60 °C caused a major loss of phycocyanin absorbance; this loss was much higher at 65 °C. In addition, a 2 nm blue shift of the red absorption band of Chl *a* from 676 to 674 nm was observed (see inset of Fig. 1 A). *Porphyridium* cells were relatively more susceptible to preheating. At 45 °C, some loss of phycoerythrin (absorption peak at 540 nm) was observed. Upon preheating at 55 °C, a major decrease in absorbance is observed in the phycoerythrin region. In addition, the Chl *a* absorption peak in the red region shows a 2 nm blue shift from 676 to 674 nm (Fig. 1 B).

2. Fluorescence Spectra

In contrast to small changes in the absorption spectra of Chl *a* bands, large changes were observed in the emission bands of Chl *a*, both at 298 and 77K. In *Chlorella*, preheating at 60 °C caused a considerable decrease in the main emission band at 685–687 nm, at 298K (excitation, 480 nm; see Fig. 2 A); qualitatively, the same results were obtained upon excitation with 440 nm. When the same samples were measured at 77K, control cells showed the usual 3 peaks (F_{687}, F_{698}, and F_{725}; Cho and Govindjee, 1970 a), but the preheated cells had a new band at 710 nm (Fig. 2 B).

Preheating *Anacystis* at 60 °C caused a reduction in allophycocyanin emission at 660 nm at 298K (excitation, 560 nm; Fig. 3 A) as there was less absorption due to the loss of phycobilins; a comparison with the absorption spectra (Fig. 1 A) suggests that there was no significant loss of the quantum yield of phycobilin fluorescence. However, there was a large suppression of Chl *a* emission yield at 685 nm indicating a decreased energy transfer from the remaining phycobilins to Chl *a* of system II (F_{685}). This seems to be accompanied by an increased transfer to Chl *a* of system I ($F_{710-720}$). Excitation with 440 nm leads to a relatively large absorption in the weakly fluorescent forms of Chl *a* and thus the fluorescence yield of Chl *a* emission when Chl *a* is directly excited should be low (see Duysens, 1952). Preheating *Anacystis* at 60 °C caused an increased yield of Chl *a* emission at 685 nm (Fig. 3 A) when the wavelength of excitation was 440 nm as only a very small change in absorbance at 440 nm was noted (Fig. 1 A). This may be due either to the conversion of some of the weakly fluorescent Chl *a* to strongly fluorescent Chl *a* or to an increased energy transfer from the former to the latter. The

latter suggestion is unlikely due to the uphill nature of the transfer. At 77K, upon excitation with 560 nm light, the well known bands at 660 nm (mainly allophycocyanin), 687 nm (Chl *a* II), 696 nm (Chl *a* II) and at 717 nm (Chl *a* I) are ob-

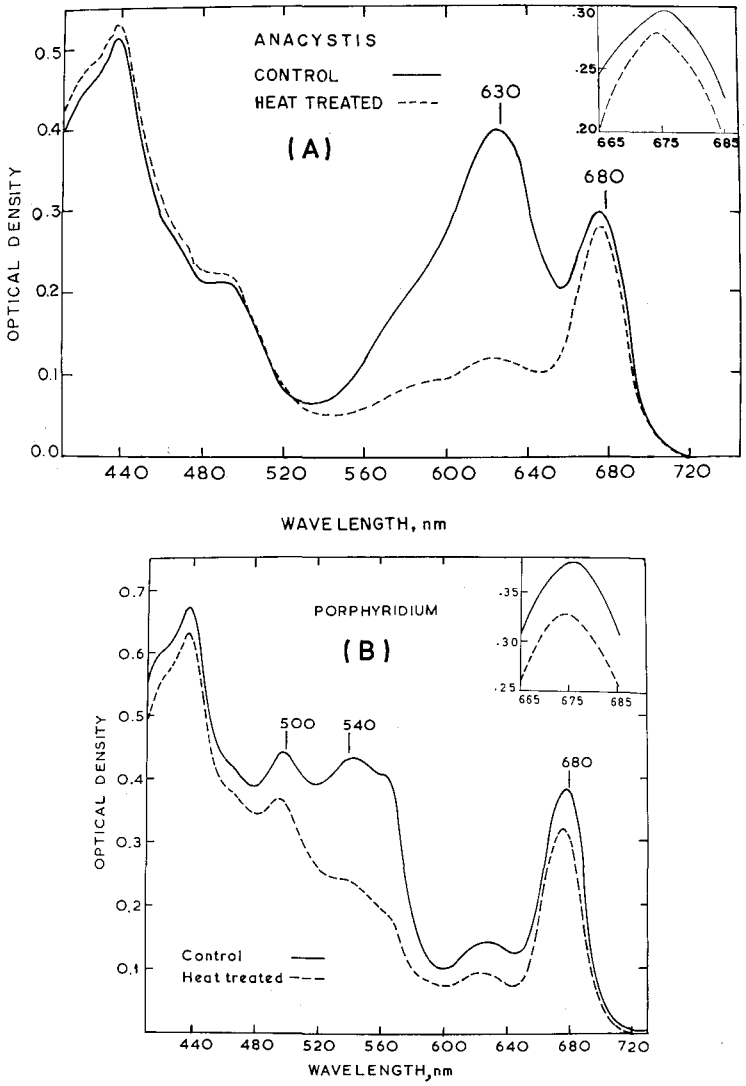

Fig. 1: Absorption spectra of normal and preheated algal cells (A: *Anacystis nidulans*; B: *Porphyridium cruentum*) at 298K. Heat treatment, 60 °C for 10 minutes (A), 55 °C for 10 minutes (B): Optical density means absorbance. Inserts show the replots of red absorption band.

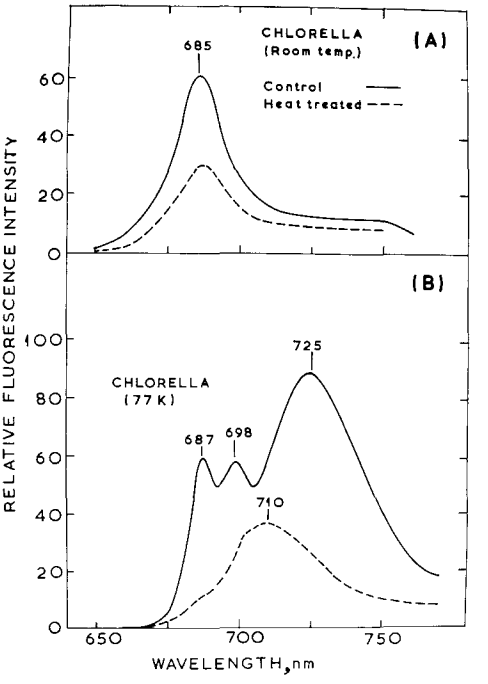

Fig. 2: Fluorescence emission spectra of normal and preheated *Chlorella* cells (A, at 298K; B, at 77K). Heat treatment, 60 °C for 10 minutes. Excitation, 480 nm. Since change in absorbance at 480 nm due to heat treatment is small, fluorescence intensities are approximately equivalent to yields; it is, however, difficult to compare yields in the two samples at 77K due to the inability to control exact path lengths.

served in normal samples (cf. Cho and Govindjee, 1970 b); the ratio of Chl *a* II and Chl *a* I emission is large as expected (Fig. 3 B). Preheating at 60 °C decreased the energy transfer from allophycocyanin to Chl *a* II, and increased the transfer to Chl *a* I as the 685 and 717 nm bands were decreased and increased, respectively. This confirms clearly the conclusion reached from 298K spectra. The 77K emission spectra, obtained upon excitation with 440 nm light, showed that preheating caused an increase in the ratio of Chl *a* I (F_{717}) to Chl *a* II (F_{685}) emission. This observation would argue against an increased system I to system II transfer at 298K but would be consistent with the suggestion that some weakly fluorescent Chl *a* were converted into strongly fluorescent Chl *a* upon preheating. The shift of phycobilin emission from 655 nm to 660 nm, observed in both Figs. 3 A and 3 B, upon preheating *Anacystis*, could be due to several heat-induced effects: (a) a greater loss of phycocyanin emitting at about 645 nm; (b) an increased transfer from phycocyanin to allophycocyanin fluorescing at 660 nm; and (c) change in the pigment-protein complex leading to a spectral shift.

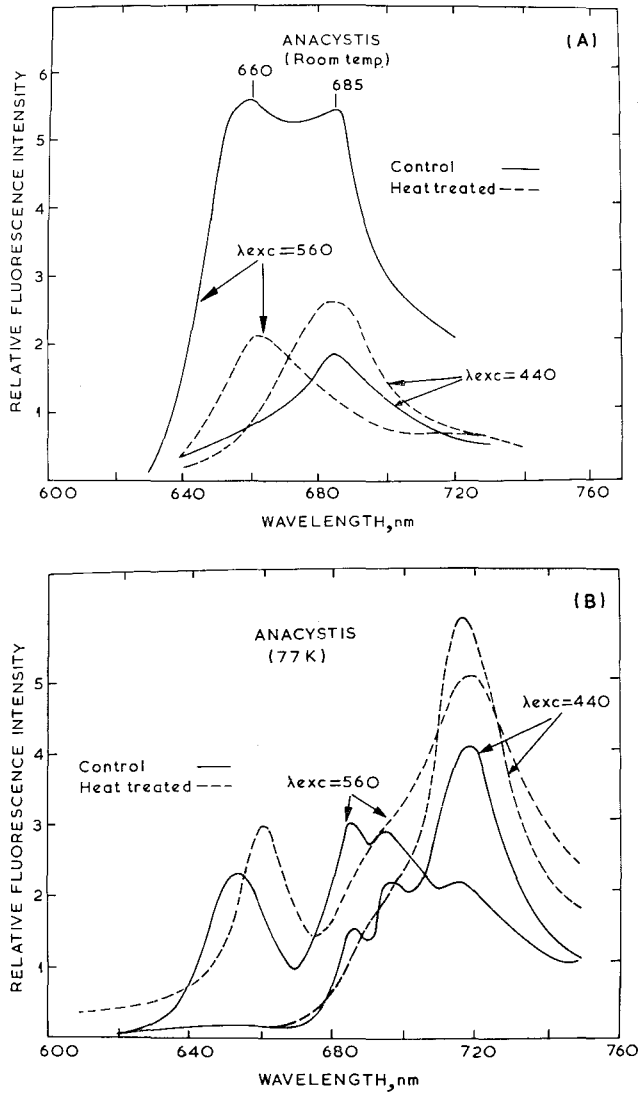

Fig. 3: Fluorescence emission spectra of normal and preheated *Anacystis* cells (A, at 298K; B, at 77K). Heat treatment, 60 °C for 10 minutes. Measurements with excitation at both 440 and 560 nm. Fluorescence intensities for 440 nm excitation curves are equivalent to yields as only a very small change in absorbance at 440 nm was observed. Fluorescence curves for 560 nm excitation in preheated cells should be multiplied by 2.2 for comparison on the basis of yields; however, 440 and 560 nm curves cannot be compared with each other on the basis of yields; also, it is difficult to compare 77K curves on the basis of fluorescence yields.

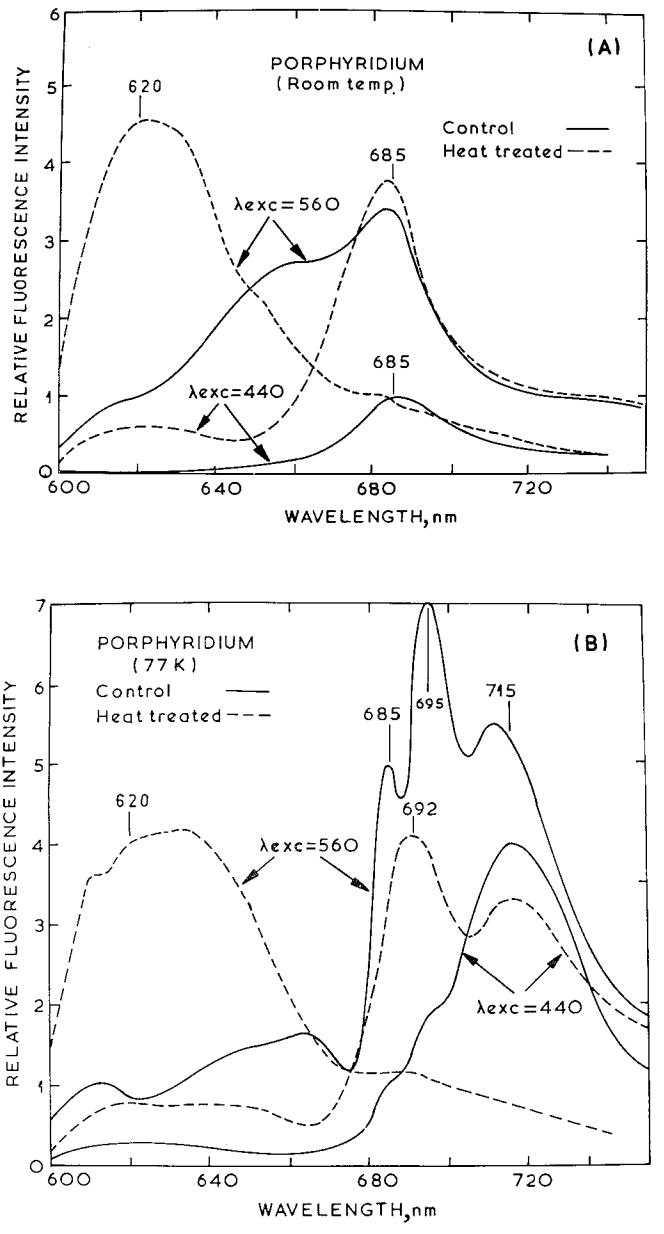

Fig. 4: Fluorescence emission spectra of normal and preheated *Porphyridium cells* (A, at 298K; B, at 77K). Heat treatment, 60 °C for 10 minutes. See legend of Figure 3 for other details.

Preheating of *Porphyridium* at 60 °C caused at 4 fold increase in the 685 nm fluorescence band at 298K (excitation 440 nm; Fig. 4 A). As in the case of *Anacystis,* this could be interpreted as the conversion of weakly fluorescent Chl *a* into strongly fluorescent Chl *a*; these changes are indeed in the quantum yield of fluorescence as no significant change in absorbance at 440 nm was observed (Fig. 1 B). Excitation spectra obtained with 560 nm light suggest that preheating caused a decreased energy transfer from the remaining phycobilins to Chl *a* II; increased transfer to Chl *a* I, however, was not documented here; the ratio of phycobilin to Chl *a* I emission increased just as in *Anacystis.* In addition to a band at about 660 nm (due mainly to allophycocyanin), we observe an additional band at 620 nm that is due to some other phycobilin. Whether heating caused the formation of some solubilized phycobilins giving high phycobilin emission in both *Anacystis* and *Porphyridium* remains a valid possibility. At 77K, normal sample shows the well known emission bands, a preponderantly large emission band at 715 nm upon excitation with 440 nm light (Chl *a* I) and the usual triple peaks at 685, 695 and 715 nm upon excitation with 560 nm light (absorption mainly in phycoerythrin, pigment system II). Preheating at 60 °C caused a drastically reduced energy transfer from phycobilins to Chl *a* II as the ratio of phycobilin to Chl *a* II emission increased dramatically. This confirms the conclusion obtained at 298K. In contrast to *Anacystis*, preheating *Porphyridium* did not cause an enhancement of the 715 nm band (excitation 440 nm), but instead a 692 nm band was enhanced as if preheating caused a decreased transfer from Chl *a* II to Chl *a* I; this would explain the enhancement of 685 nm band at room temperature as Chl *a* II is more fluorescent than Chl *a* I at 298K. Thus, the preheating effects on *Porphyridium*, although similar to *Anacystis* in general terms is somewhat different.

3. Action Spectra of Chlorophyll a Fluorescence

Fig. 5 shows the action spectra fo Chl *a* fluorescence in *Chlorella* at 720 nm at 77K. Preheating at 55 °C does not seem to change energy transfer from Chl *b* (650 nm) to Chl *a*; it remains as high (almost 100 %) as in untreated cells. Preheating causes a relative decrease in Chl *a* 670 excitation band, and a relative increase in Chl *a* 680–690 band inspite of the 2 nm blue shift in the absorption spectra. However, the peak of excitation is at 675 nm at 298K in both cases, but it is at 680 nm in preheated samples at 77K.

Preheating *Porphyridium* at 60 °C either converts the weakly fluorescent to strongly fluorescent form or induces energy transfer from the former to the latter explaining the large 670 nm Chl *a* band in the excitation spectrum of preheated samples at room temperature (data not shown). Excitation spectra of chlorophyll *a* fluorescence at 740 nm, at 77K, in the 600–700 nm range show that preheating at 60 °C increases the Chl *a* 680 excitation band and the 625 nm excitation band (due to phycocyanin; Fig. 6). A decrease in excitation band at 680 nm in *Porphyri-*

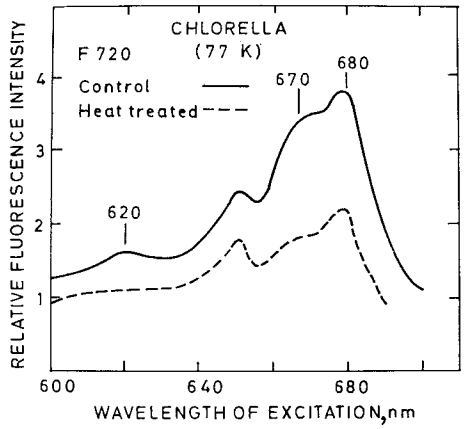

Fig. 5: Action spectra of Chlorophyll *a* fluorescence in *Chlorella* at 77K for F_{720}.

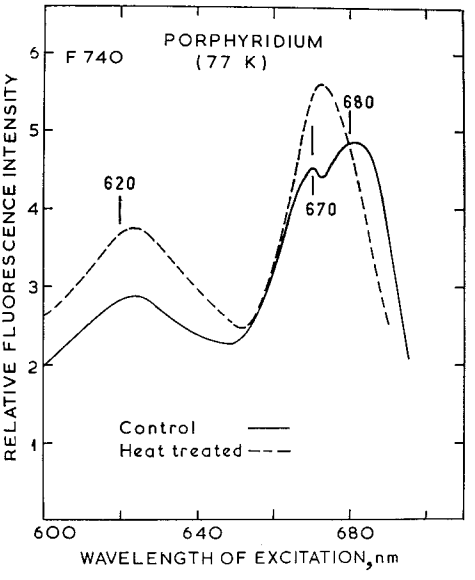

Fig. 6: Action spectra of chlorophyll *a* fluorescence in *Porphyridium* at 77K for F_{740}.

dium, and a decrease in a band at 670 nm in *Chlorella* upon preheating suggests their similar sensitivity to heating.

Fig. 7 shows room temperature excitation spectra of Chl *a* fluorescence at 715 nm in *Anacystis*. As is already known, Chl *a* fluorescence is excited mainly by absorp-

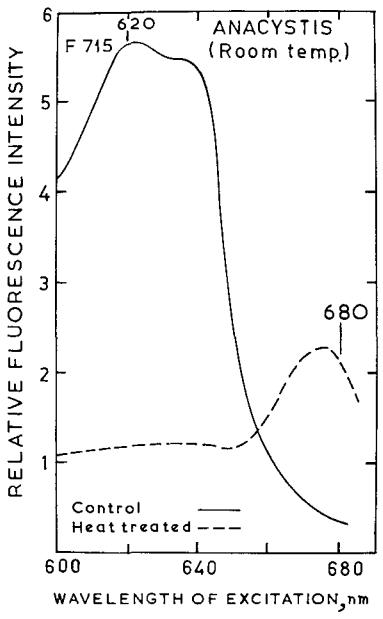

Fig. 7: Action spectra of chlorophyll *a* fluorescence in *Anacystis* at 298K for F_{715}.

tion in phycobilins (620 nm and 640 nm), (Duysens, 1952; Cho and Govindjee, 1970 b). However, preheating leads to the appearance of a Chl *a* band confirming the conclusion from emission spectra (excitation, 440 nm) that either weakly fluorescent Chl *a* forms are converted into strongly fluorescent Chl *a* forms or energy transfer from the former to the latter increases as in *Porphyridium*. As noted earlier, the last possibility is less likely due to the uphill nature of this energy transfer. At 77K, both phycobilin (625 nm, 630 nm) and Chl *a* (675 nm) bands are present, but preheating increases the ratio of Chl *a*/phycobilin bands confirming the above conclusions from 298K spectra.

Discussion

Although preheating of algal cells causes very small changes in the absorption spectra of Chl *a* *in vivo*, large changes in emission spectra both at 298 and 77K occur. One of the major effects is the disappearance or reduction of 77K emission band at 685 nm associated with pigment system II (Govindjee and Yang, 1966). A decreased Mn^{2+} content also leads to a suppression of 685 and 695 nm emission bands (Cheniae and Martin, 1966; confirmed by A. Kumar and P. Mohanty, unpublished observation). However, preheating at 55–60 °C for 10 minutes, beyond that needed for loss of O_2 evolving activity, causes additional changes in the pig-

ment-protein complexes; in *Chlorella*, a new emission band at 710 nm becomes predominant at 77K; in *Porphyridium* and *Anacystis*, 685 nm band increases with excitation at 440 nm, and it decreases with excitation at 560 nm at 298K; in *Porphyridium* at 77K an emission band appears at 692 nm with excitation at 440 nm. These and other changes are explained in terms of changes in the mode and extent of excitation energy transfer from one pigment-protein complex to another.

Most of the spectral changes, reported in this paper, may be due to thermal denaturation of proteins to which pigments are attached. In *Chlorella*, Chl *a* 670 is selectively reduced or energy transfer from it to other species is reduced, whereas in *Porphyridium*, this effect is on Chl *a* 680–690. The new emission band at 710 nm in *Chlorella* and a band at 692 nm in *Porphyridium* may be either due to alteration of an existing complex, or to their unmasking because of destruction of other Chl *a* complexes.

In both *Anacystis* and *Porphyridium*, preheating causes a loss of absorption due to phycobilins accompanied by a decrease in excitation energy transfer from the remaining phycobilins to chlorophyll *a* of pigment system II. An additional effect of preheating is either in the conversion of weakly fluorescent Chl *a* to strongly fluorescent Chl *a* or in an increase in energy transfer from the former to the latter. We do not favour the last possibility.

Usually proteins are denatured at 45 °C, but preheating at 45 °C for 10–16 minutes did not exhibit any alteration in the absorption or fluorescence characteristics of Chl *a* *in vivo*. Thus, it seems that membrane bound pigment-protein complexes are more stable to heating, and require higher temperatures to show effects.

Acknowledgements

This research was completed during an award (INT 7822353) to Govindjee by NSF (USA) and CSIR (India).

We are thankful to the NSF-CSIR program that permitted the completion of this paper at Jawaharlal Nehru University, New Delhi. We are grateful to Prof. P. N. Srivastava and Prof. Sivatosh Mookerjee, and the University Grants Commission for support during the completion of this investigation.

References

Brown, J. S.: Ann. Rev. Pl. Physiol. *23*, 73 (1972).

Cederstrand, C., E. Rabinowitch, and Govindjee: Biochim. Biophys. Acta *120*, 247 (1966).

Cheniae, G. and I. F. Martin: Brookhaven Symp. Biol. *19*, 406 (1966).

Cho, F.: Ph. D. Thesis in Biophysics, University of Illinois at Urbana-Champaign, 1969.

Cho, F. and Govindjee: Biochim. Biophys. Acta *205*, 371 (1970 a).

– – Biochim. Biophys. Acta *216*, 151 (1970 b).

Das, M. and Govindjee: Biochim. Biophys. Acta *143*, 570 (1967).

Duysens, L. N. M.: Ph. D. Thesis, the State University, Utrecht, the Netherlands, 1952.

GOEDHEER, J. C.: Photosynthetica *4*, 97 (1970).
GOVINDJEE and E. RABINOWITCH: Biophysic. J. *1*, 73 (1960).
GOVINDJEE and L. YANG: J. Gen. Physiol. *49*, 763 (1966).
KATZ, J. J. and J. R. MORRIS: Current Topics in Bioenergetics *5*, 41 (1973).
LITVIN, F. F. and V. A. SINESHCHEKOV: In: GOVINDJEE (Ed.): Bioenergetics of Photosynthesis, pp. 619–661. Academic Press, New York, 1975.
SHIMONY, C., GOVINDJEE, and J. SPENCER: Photosynthetica, *1*, 113 (1967).
THORNBER, J. P., Ann. Rev. Pl. Physiol. *26*, 127 (1975).