

# Temperature Induced Changes in the Absorption Spectra of *Porphyridium cruentum* and *Anacystis nidulans*

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Cooling *A. nidulans* or *P. cruentum* from 26 °C to 4 °C results in progressive spectral changes. The most significant changes are increases in absorbance at 690 nm (photosystem II), 678 nm (antenna chlorophyll), 625 nm (phycocyanin), and 505 nm (electrochromic band). In the case of *P. cruentum* there are also increases in absorbance at 568 and 545 nm (B-phycoerythrin) and a decrease at 587 nm. The spectral changes in both organisms are accompanied by decreases at 703 nm (photosystem I or aggregated chlorophyll), 650 nm (allophycocyanin) and 486 nm (carotenoid).

Heating *A. nidulans* or *P. cruentum* from 4 °C to 44 °C results in increases in absorbance at 705 nm (photosystem I) and 486 nm (carotenoid), accompanied by decreases at 690 nm (photosystem II), 676 nm (antenna chlorophyll), 628 nm (phycocyanin), 507 nm (electrochromic band) and 469 nm (carotenoid). In the case of *P. cruentum* there are also decreases at 568 and 546 nm (B-phycoerythrin) and an increase at 587 nm. The possible origin of the spectral change at 587 nm. The possible origin of the spectral change at 587 nm is discussed.

The spectral changes of the chlorophyll bands (703, 690, 678 nm) and the electrochromic band (502 nm) are associated with phase changes of the lipid membrane. Lowering the temperature results in a decrease of aggregated chlorophyll or photosystem I, and vice versa. These spectral changes are also observed in green chloroplasts.

The spectral changes of the phycobilins may originate from a temperature dependent change of the ion balance of the thylakoid. A spectral change may result from the ensuring modification of the stacking or from an electrochromic effect.

## Introduction

Temperature induced changes in the absorption spectrum of barley chloroplasts were reported by Brody and Singhal [1]. As chloroplasts were cooled there were increases in absorption by chlorophyll at 675 and 436 nm plus an increase in absorption in the electrochromic region around 500 nm. As the chloroplasts were heated opposite changes in absorption were observed (*i. e.* absorption at 675, 500 and 436 nm decreased); in addition, there were increases in absorption at about 690 and 400 nm. The spectral changes of chlorophyll were interpreted as arising from modification in the state of chlorophyll aggregation resulting from a phase change of the membrane lipids. Upon heating there appeared to be an increase in chlorophyll aggregation.

The present work examines the absorption properties of a red and a blue-green algae as a function of physiological temperature. The blue green algae contain the pigments phycocyanin and allophycocyanin; red algae contain the same pigments plus B

(and b)-phycoerythrin [2]. The absorption maxima of these pigments in aqueous solution are 650, 620 and 545 nm, respectively [3]. These water soluble pigments are contained in structures known as phycobilisomes [4, 5]. The phycobilisome has an allophycocyanin core surrounded by a hemispherical layer of phycocyanin with phycoerythrin on the periphery. The unit contains over 100 chromophore molecules and has a diameter of 32–35 nm. The allophycocyanin core is associated with, and transfers excitation energy predominately to the reaction center complex of photosystem II [6, 7].

Murata and Fork [8] reported a temperature dependent variation of fluorescence yield in *A. nidulans*. Gantt *et al.* [9] reported that low temperature (4–10 °C) produced uncoupling of energy transfer and partial dissociation of isolated phycobilisomes. From a study of the fluorescence spectra of *A. nidulans*, Schreiber *et al.* [10] reported a decrease in transfer efficiency between phycobilisomes and chlorophyll as the temperature was lowered. They attributed the decrease in efficiency to a reversible, temperature dependent, detachment

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of the phycobilisome from the chlorophyll containing thylakoid membrane. They proposed that there was either a complete detachment of some of the phycobilisomes or a limited detachment of all the phycobilisomes as the temperature is lowered. On the basis of picosecond energy transfer in *A. nidulans* and *P. cruentum*, Brody and Tredwell [11] reported that lowering the temperature resulted in a decrease in the rise time of fluorescence from phycobilins. They suggested that the attachments between the phycobilisomes and the thylakoid membrane were not all the same. As the temperature was lowered it appeared that the population of the tightly attached phycobilisomes, which have a short distance to the chlorophyll, remain attached to the chlorophyll containing thylakoid while the "more" distant phycobilisomes may be detached. This could account for both the decrease in rise time of fluorescence for the phycobilins and the decrease of transfer efficiency as temperature was lowered.

### Materials and Methods

*P. cruentum* and *A. nidulans* were cultured as described by Porter *et al.* [12] and Gantt and Lipschultz [13]. Chlorophyll a was prepared as described previously [14].

A Beckman spectrophotometer (model DW-2) was used to measure absorption spectra. The absorbance (O.D.) of the red absorption band of the algae samples was between 1.3 and 1.6. Difference spectra were measured with the spectrometer set to give a full scale absorbance of 0.1. Spectral changes were determined by measuring the difference absorption spectrum between two identical algal suspensions. The reference sample was held at room temperature (26 °C) while the temperature of the sample was varied between 4 °C and 44 °C. The difference in absorption spectra indicated as  $OD(t\text{ °C}) - OD(26\text{ °C})$ , is the difference between the reference sample held at room temperature (26 °C) and the sample at same other temperature (t °C). When both samples are at the same temperature (26 °C) the spectrophotometer records a small difference in absorption. This difference spectrum is subtracted from  $OD(t\text{ °C}) - OD(26\text{ °C})$ . The resulting change in absorbance at a wavelength  $\lambda$  is indicated as  $\Delta OD(\lambda)$  in this work.

The sample cuvette was cooled or heated by circulating water from a constant temperature bath

through the cuvette holder. Temperature of the sample was measured with a calibrated copper-constantan thermocouple immersed in the algal suspension. In case of *P. cruentum* the rates of cooling and heating were 0.6 °C and 0.5 °C/min, respectively. For *A. nidulans* the rates of cooling and heating were 1.6 °C and 1.3 °C/min, respectively. The temperature of the algae was cycled as follows: cooled from room temperature 26 °C (or 29 °C) to 4 °C, then heated from 4 °C to 40 °C (or 44 °C), then cooled to 4 °C and held at 4 °C for 3 to 6 min.

The spectral changes in particular regions of the spectrum may be identified with specific pigment systems. In this work the  $\Delta OD(703)$  is identified with photosystem I or aggregated chlorophyll a; the  $\Delta OD(690)$  is identified with photosystem II; the  $\Delta OD(680-676)$  with antenna chlorophyll; the  $\Delta OD(433)$  with chlorophyll a. The  $\Delta OD(516-503)$  is identified with an electrochromic band. The  $\Delta OD(486-460)$  is identified with carotenoids. The  $\Delta OD(650)$  and  $\Delta OD(628-620)$  are identified with allophycocyanin and phycocyanin, respectively. The  $\Delta OD(568, 545)$  is identified with B-phycoerythrin.

### Results

#### *Anacystis nidulans*

Cooling *A. nidulans* from 26 °C to 4 °C results in progressive spectral changes. The spectral change observed at 4 °C is indicated as  $OD(4\text{ °C}) - OD(26\text{ °C})$  in Fig. 1. The most significant spectral changes are increases in absorbance at: 690 nm (photosystem II), 678 nm (antenna chlorophyll), 625 nm (phycocyanin), and 516 nm (electrochromic band). There are also decreases in absorbance at: 703 nm (photosystem I or aggregated chlorophyll), 650 nm (allophycocyanin) and 486 nm (carotenoid). The band at 690 nm appears as a shoulder on the band for antenna chlorophyll.

Subsequent heating from 4 °C to 44 °C results in a different set of spectral changes. The spectral change observed at 44 °C is indicated as  $OD(44\text{ °C}) - OD(26\text{ °C})$  in Fig. 1. The most significant changes are increases in absorbance at 703 nm (photosystem I) and 486 nm (carotenoid). There are also decreases in absorbance at: 690 nm (photosystem II), 678 nm (antenna chlorophyll), 628 nm (phycocyanin), 507 nm (electrochromic band) and 433 nm (chlorophyll). For comparison purposes the

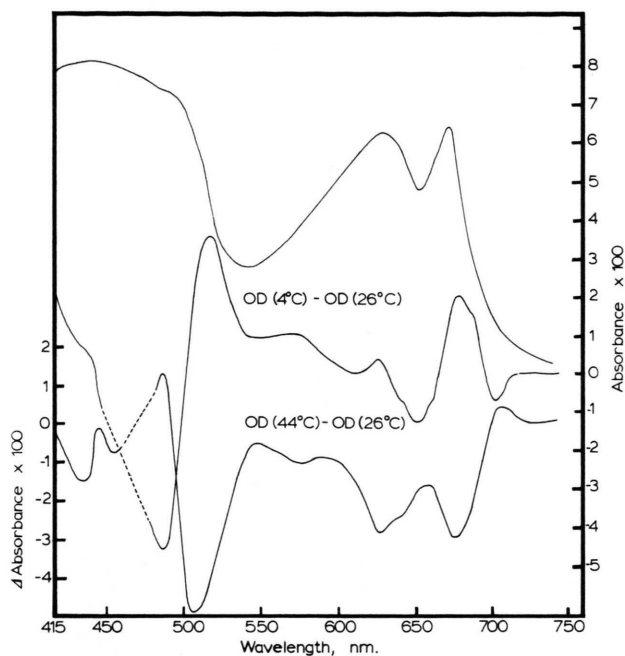


Fig. 1. The absorption spectrum of *A. nidulans* is shown by the uppermost graph, to obtain the true absorbance multiply the scale shown on the right hand axis by 20. The difference in absorbance between two identical samples, one at 4°C and another at 26°C is indicated by OD (4°C)–OD (26°C). The difference in absorbance is shown by the scale on the right hand axis. The difference between two identical samples one at 44°C and another at 26°C is indicated by OD (44°C)–OD (26°C). The difference in absorbance is shown by the scale on the left hand axis.

Table I. Summary of spectral changes in *A. nidulans* and *P. cruentum* upon cooling and heating.

Pigment	nm	Cooling		Heating	
		A.	P.	A.	P.
Photosystem I	703	—	—	+	+
Photosystem II	690	+	*	—	*
Antenna Chl	680	+	+	—	—
Allophycocyanin	650	—	—	*	*
Phycocyanin	625	+	+	—	—
b-phycoerythrin	587	*	—	*	+
B-phycoerythrin	568, 545	*	+	*	—
Electrochromic	516–502	+	+	—	—
Carotenoid	486	—	—	+	+

A indicates *A. nidulans*.

P indicates *P. cruentum*.

— indicates increase in absorbance.

— indicates decrease in absorbance.

\* indicates pigment not present or not resolved.

adsorption spectrum of *A. nidulans* is also shown in Fig. 1.

In general there is a reversal of the temperature dependent spectral changes. The spectral changes of the various pigment systems upon heating and cooling are summarized in Table I.

The changes in absorbance as a function of temperature at various wavelengths are shown in Fig. 2. At all wavelengths there is a time dependence for the spectral changes. The  $\Delta OD$  (703) (photosystem I) and  $\Delta OD$  (486) (carotenoid) vary in an approximately linear fashion with temperature (results for 486 nm are not shown). In addition the changes in OD appear to be reversible with respect to temperature. That is, with increasing and decreasing temperature the same path is followed with respect to changes in absorbance, at 703 nm (and 486 nm) see Fig. 2.

At 690, 678, 625 and 516 nm the absorbance varies inversely with temperature. The temperature dependent spectral changes at these wavelengths seem to be associated with a “hysteresis-like effect”,

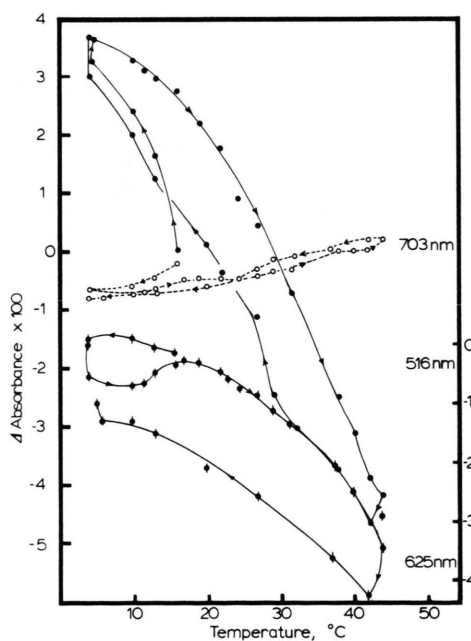


Fig. 2. A change in absorbance at 703, 625 and 516 nm as a function of temperature in *A. nidulans*. The direction of heating and cooling is indicated by arrows. The spectral change at 703 nm is identified with aggregated chlorophyll or photosystem I. The spectral changes at 625 and 516 nm are identified with phycocyanin and an electrochromic band, respectively.

*i.e.* with increasing and decreasing temperature the change in absorbance follows different pathways. In all cases the "hysteresis-like loop" closes at the end of the temperature cycle, at 4 °C. Data for 625 and 516 nm are shown in Fig. 2. Closure of the "hysteresis loop" usually requires that the temperature be held at 4 °C for 3 to 6 min.

By plotting the spectral change at one wavelength against the spectral changes at other wavelengths one can see which spectral changes are related. A linear relationship between the two wavelengths would indicate that both changes in absorbance originate from the same pigment or from pigment changes which are a function of the same phenomena.

The changes in absorbance at 703, 690, 625 and 516 nm are plotted as a function of change in absorbance at 678 nm (Fig. 3). Only the results of the heating cycle are plotted so that the various wavelengths may be compared without the complication of the hysteresis effect. A nonlinear relationship is noted between phycocyanin (625 nm) and antenna chlorophyll (678 nm). Approximately linear relationships are noted between antenna chlorophyll (678 nm) and both photosystem II (690 nm) and the electrochromic band (516 nm).

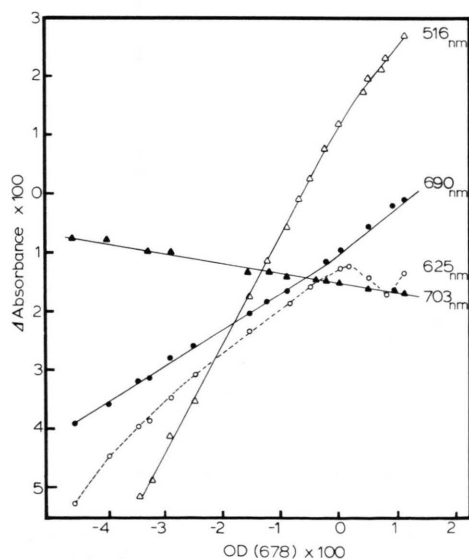


Fig. 3. Temperature induced change of absorbance at 703, 690, 625 and 516 nm as a function of the change of absorbance of antenna chlorophyll at 678 nm, in *A. nidulans*. The spectral change at 690 nm is identified with photosystem II.

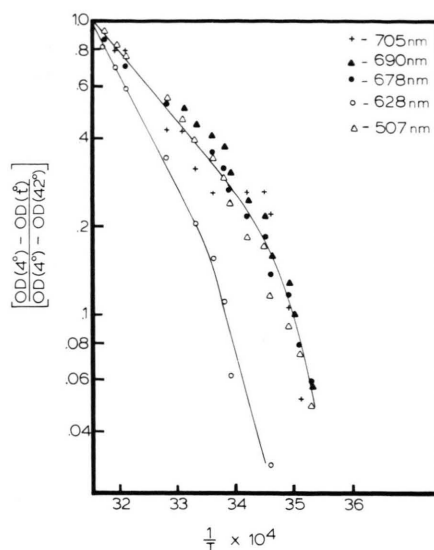


Fig. 4. Spectral changes at 705, 690, 678, 628 and 507 nm in *A. nidulans* as a function of the reciprocal of temperature. The spectral changes are identified, with photosystem I, photosystem II, antenna chlorophyll, phycocyanin, and an electrochromic band, respectively. The algae are heated from 4 °C to 42 °C.

The ratio of the change in absorbances give an indication of the stoichiometry for the reactions which appear to be related linearly. The ratio of  $\Delta OD$  (516), electrochromic band to  $\Delta OD$  (678), antenna chlorophyll is 10 to 6 or about 2 to 1. The ratio of  $\Delta OD$  (690), photosystem II to  $\Delta OD$  (678), antenna chlorophyll is about 2 to 3.

There is an inverse relationship between  $\Delta OD$  (703), aggregated chlorophyll and  $\Delta OD$  (678), antenna chlorophyll; the ratio of the change is about 1 to 6.

The changes in absorbance at 703, 690, 670, 625 and 516 nm plotted logarithmically as a function of the reciprocal of temperature are shown in Fig. 4. Only data obtained during the heating cycle, from 4 °C to 42 °C, are plotted. Data fall into two groups, one containing 625 nm, the other group containing 703, 690, 678 and 516 nm.

#### *P. cruentum*

Cooling a suspension of *P. cruentum* from 29 °C to 7 °C causes progressive changes in the absorption spectrum. The spectral changes observed at 7 °C are indicated in Fig. 5 as  $OD(7^\circ C) - OD(29^\circ C)$ . There are decreases in absorption at 702 (photosystem I), 650 (allophycocyanin), 587, 431 (chloro-



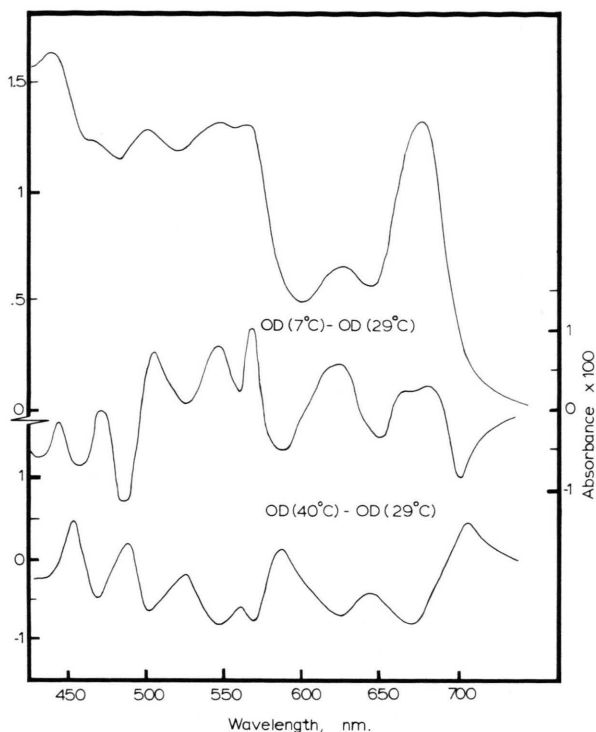


Fig. 5. The absorption spectrum of *P. cruentum* is shown by the uppermost graph, the absorbance is given by the upper scale on the left hand axis. The difference in absorbance between two identical samples, one at 7 °C and another at 29 °C is indicated by OD (7 °C)–OD (29 °C). The difference in absorbance is shown by the scale on the right hand axis. The difference in absorbance between a sample at 40 °C and another at 29 °C is indicated by OD (40 °C)–OD (29 °C). The difference in absorbance is shown by the lower scale on the left hand axis.

phyll), 484 and 456 (carotenoid) nm, accompanied by increases in absorption at 680 (antenna chlorophyll), 624 (phycocyanin), 505 (electrochromic band), 568 and 545 (B-phycoerythrin) and 469 (carotenoid) nm. By recording and comparing the change in absorption every few degrees it seems that there are isobestic points at 574 and 687 nm.

Following cooling to 7 °C the *P. cruentum* is heated to 44 °C. The absorption at 44 °C relative to the absorption at 29 °C shows decreases in absorption at 670 (antenna chlorophyll), 625 (phycocyanin), 568 and 546 (B-phycoerythrin), 502 (electrochromic band) and 469 (carotenoid) nm. There are also increases in absorption at: 706 (photosystem I), 587, 487 (carotenoid) and 450 nm (carotenoid or aggregated chlorophyll). The spectral changes at 44 °C are

indicated in Fig. 5 as OD (40 °C)–OD (29 °C). There is an isobestic point at 687 nm. For comparison purposes the absorption spectrum of *P. cruentum* is also shown in Fig. 5.

Most of the temperature dependent spectral changes are reversible. The spectral changes observed upon cooling and heating are summarized in Table I.

The maximum, temperature induced, change in absorbance is about 0.02. Therefore, for a sample that has an absorbance of 1.3 at the red absorption maximum, the maximum change in absorbance is of the order of 1%.

The changes in OD at various wavelengths as a function of temperature are shown in Fig. 6. The curves are shifted vertically so that they may be clearly seen. As with *A. nidulans*, a “hysteresis-like effect” is also observed with *P. cruentum*. At several of the wavelengths the temperature dependences are similar. The temperature dependent spectral changes of the phycoerythrin maxima at 568 and 545 nm are quite parallel to each other. Both absorbancies vary in an inverse fashion with respect to temperature. The spectral changes of phycocyanin at 625 nm are similar to, but not the same as the changes for

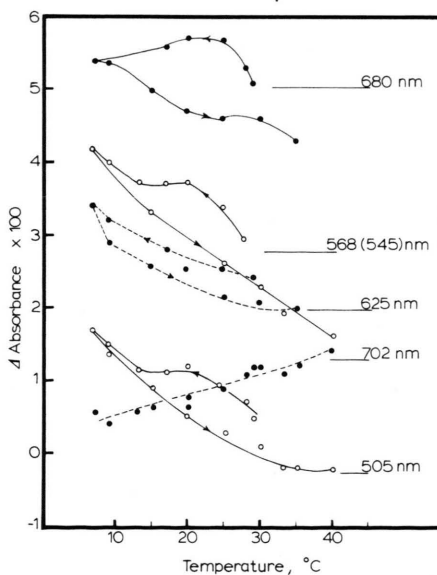


Fig. 6. Change in absorbance at 702, 678, 625, 568 and 505 nm as a function of temperature in *P. cruentum*. The direction of heating and cooling is indicated by arrows. The curves are displaced vertically from one another to improve clarity.

phycoerythrin. The change of absorbance for phycocyanin is smaller than that for phycoerythrin.

In contrast to the "hysteresis-like" effects observed at 680, 625, 568, 545 and 436 nm, for both photosystem I (or aggregated chlorophyll) at 702 nm and carotenoid at 487 nm no significant hysteresis is observed. In addition,  $\Delta OD(702)$  varies linearly with temperature, in contrast to the inverse relationships observed at the other wavelengths.

The changes in absorbance at 702, 680, 624, 545 and 505 nm are plotted as a function of the change in absorbance of phycoerythrin at 568 nm, in Fig. 7. A linear relationship is observed for  $\Delta OD(545)$  as a function of  $\Delta OD(568)$ . The ratio of  $\Delta OD(545)$  to  $\Delta OD(568)$  is close to 1:1. The  $\Delta OD(624)$  (phycocyanin) and  $\Delta OD(505)$  (electrochromic band) as a function of  $\Delta OD(568)$  is almost a linear response. The ratio of  $\Delta OD(624)$  to  $\Delta OD(568)$  is 4 to 5.

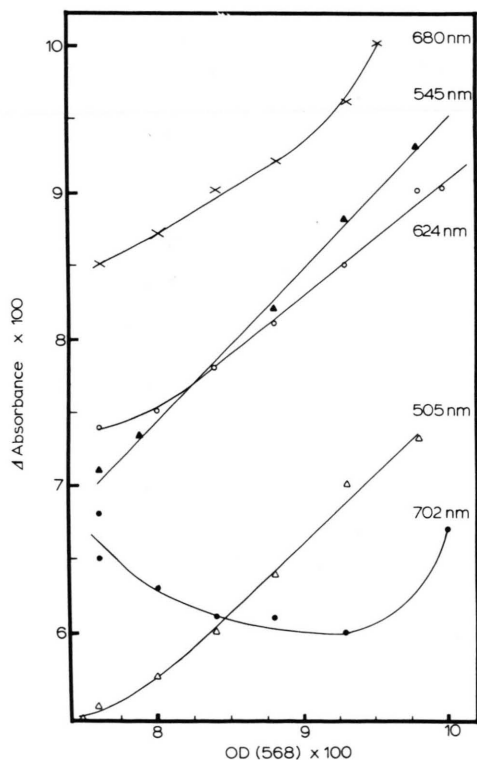


Fig. 7. Temperature induced change of absorbance at 702, 678, 624, 545 and 505 nm as a function of the change of absorbance of phycoerythrin at 568 nm in *P. cruentum*. The spectral change are identified with photosystem I, antenna chlorophyll, phycocyanin, phycoerythrin and electrochromic band, respectively. Curves are displaced vertically to improve clarity.

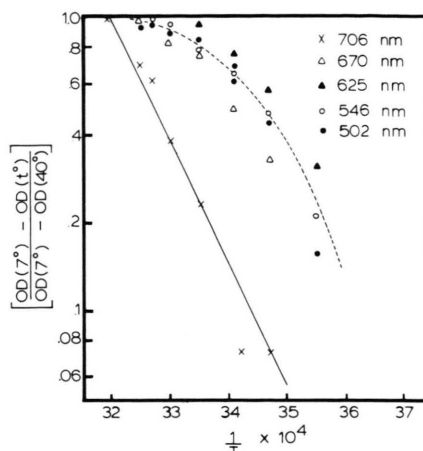


Fig. 8. Spectral changes at 706, 676, 625, 568, 546 and 502 nm in *P. cruentum* as a function of the reciprocal of temperature.

Similar data are obtained for the relationship between  $\Delta OD(505)$  and  $\Delta OD(568)$ .

A non-linear relation is observed for  $\Delta OD(680)$  (antenna chlorophyll) and  $\Delta OD(702)$  (photosystem I) as a function of  $\Delta OD(568)$ . A linear relationship obtains when  $\Delta OD(702)$  (photosystem I) is plotted as a function of  $\Delta OD(680)$  (antenna chlorophyll) (the graph is not shown). The ratio of  $\Delta OD(702)$  photosystem I to  $\Delta OD(680)$  antenna chlorophyll is about 1 to 2.

The spectral changes at 502, 546, 625, 670 and 706 nm, plotted as a function of  $1/T$  are shown in Fig. 8. Only the data obtained during the heating cycle are plotted. The data fall into two groups, one containing 706 nm, the other group containing all other spectral changes.

### Chlorophyll a

When a solution of pure chlorophyll a in chloroform is subjected to cooling and heating, it is necessary to correct for the relatively large temperature coefficient of expansion for chloroform. After this correction is made, it is observed that there is no temperature dependent change in the spectrum of chlorophyll in solution.

### Discussion

In green chloroplasts, and in the red and blue green algae similar temperature dependent spectral

changes and wavelength shifts are observed at certain wavelengths. Of particular interest are the spectral changes that occur in the red region of the spectrum. The changes in absorbance of the red absorption band are attributed to different forms of chlorophyll at 680 and 690 nm, accompanied by opposite changes in absorbance of a long wavelength form of chlorophyll with a band at 703 nm. The increase in absorbance at 680 nm and decrease in absorbance at 703 nm, on cooling, observed in green chloroplasts. *A. nidulans* and *P. cruentum* are readily interpreted as shifts in population of aggregated and monomeric forms of chlorophyll. The isobestic point observed at 687 nm in *P. cruentum* could well be associated with a transformation between antenna chlorophyll and aggregated form of chlorophyll. If an aggregated form of chlorophyll "A" containing "*n*" monomers reverts to monomeric chlorophyll "M" this may be described as  $A_n \rightleftharpoons nM$ . The absorption coefficient of the chlorophyll dimer is about 20% greater than that of the chlorophyll monomer [15]. From the ratio of the  $\Delta OD$  680 (M) to  $\Delta OD$  703 (A) it is possible to estimate "*n*". The value of "*n*" is an average value since a range of aggregate sizes may be involved rather than a single specific size. With *P. cruentum* a value of about 2 was found for "*n*". With *A. nidulans* a value of about 6 was found for "*n*". The difference in these two values may indicate a difference in the molecular architecture between the two classes of algae.

The spectral changes of the chlorophyll bands and the electrochromic band (516–502) are not related to the phycobilins since they are also observed in green chloroplasts which lack phycobilins.

The existence of what might be a hysteresis loop in Fig. 2 and 6 may be related to the involvement of transformation of macromolecules (proteins) in the spectral changes. The area of a hysteresis loop would be proportional to the work required to induce configurational or orientational changes of the macromolecule. The changes at 703 (photosystem I) and 486 (carotenoid) nm which exhibit no significant hysteresis are related to transformations of small molecules (such as chlorophyll aggregates and lipids) which can undergo relatively fast reactions. Phase changes of the lipids are presumably the origin of the spectral changes of the chlorophyll and electrochromic bands. There is no discernable temperature dependent change in absorption of pure chlorophyll in solution. Specific interaction be-

tween chlorophyll and lipids are described by Katz [16].

There is a large difference in the temperature dependent change in absorption of phycocyanin (625 nm) compared to that of the chlorophyll (705, 690, 678 nm) and electrochromic bands (507 nm) (Fig. 4). This suggests that the latter spectral changes originate from a system that is different from that of phycocyanin. The fact that the spectral changes at 705, 690, 678 and 507 nm have the same temperature dependence indicates that these spectral changes could have the same origin. The spectral change at 628 nm is definitely associated with phycocyanin which is a protein. While the spectral changes at the other wavelengths might be related to a protein, they are more likely related to lipid phase changes.

The spectral changes of the phycobilins are not interpretable in terms of a spectral shift. A spectral shift would not give an isobestic point as observed in *P. cruentum* at 574 nm, for the bands at 587 and 568, 545 nm (B-phycoerythrin). The isobestic point is readily interpreted as a transformation between monomeric and aggregated forms of phycoerythrin, or a transformation between different forms of phycoerythrin. In the latter case the isobestic point at 574 nm may represent a transformation between b and B-phycoerythrin. This interpretation would require that the band at 587 nm be the position of b-phycoerythrin *in vivo*. (*In vitro*, b and B-phycoerythrin have absorption maxima at the same wavelength.) If the difference between the spectral properties of b and B-phycoerythrin were the difference in the composition of their proteins, then a transformation between them would not be possible. However, if the difference between b and B-phycoerythrin were a difference in protein conformation then a temperature dependent conformational change could result in an isobestic point.

One might account for the spectral changes of the phycobilins in several ways. In the case of phycoerythrin the bands get sharper (on cooling), perhaps the phycobilisomes contract on cooling thereby increasing the pigment-pigment interaction. Narrowing of the absorption bands could result from an increase in exciton interaction. Another possibility is that there is an electrochromic effect on the phycobilin spectra. This possibility is supported by the observation that the change in absorbance of the phycobilins and the electrochromic band (in the

502–516 nm region) almost parallel one another in *P. cruentum* (Fig. 6).

The increase in absorbance observed in the region 502–516 nm upon cooling may be related to the electrochromic effects observed by Witt [17]. Upon heating, opposite changes in absorbance are observed. A pronounced spectral shift is noted for this band in *A. nidulans*. At 4 °C, in *A. nidulans*, there is a maximum at 516 nm, while at 44 °C there is a minimum at 507 nm. In *P. cruentum* the maximum is at 505 nm and the minimum is at 502 nm.

The possible origin of the relationship between temperature and the electrochromic effect should be considered. Phase changes of the various lipids could result in transformations of the state of chlorophyll, as well as modification of the properties of the phycobilins. As the lipids undergo a temperature dependent phase change, the orientation of the charged lipids in the membrane could also change. This might lead to a change in membrane potential, which could in turn give rise to an electrochromic

effect. Consequently, the change in lipid phase could influence the ion balance of the thylakoid. The flux ratio of various ions across a membrane is a function of both temperature and membrane potential. As the flux ratio changes the ion balance within the thylakoid is modified. A change in ion concentration in the thylakoid would modify the surface charge and the potential of the membrane [18]. Such a change in surface charge could lead to unstacking of thylakoids or partial detachment of phycobilisomes from the membrane surface. In either case the surface charge would govern the extent of interaction between thylakoids or between thylakoid and phycobilisomes.

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